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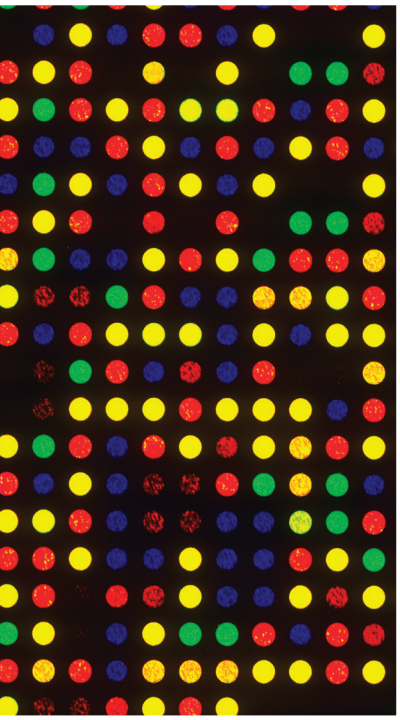
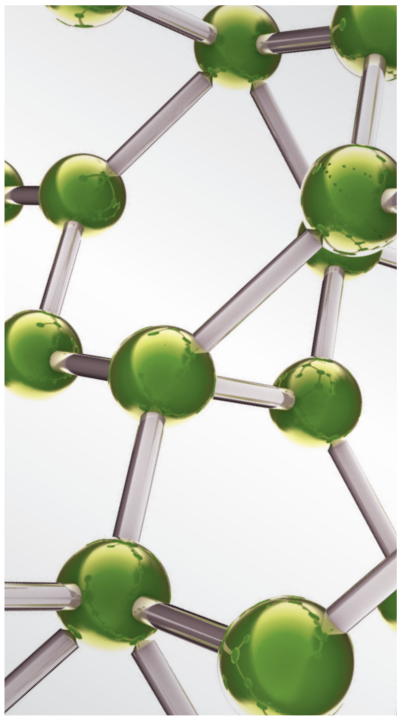
Guest Editors: Rahmatullah Qureshi, Shahina A. Ghazanfar, Hassan Obied, Viliana Vasileva, and Mohammad A. Tariq



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Editorial Board

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Editorial

Ethnobotany: A Living Science for Alleviating Human Suffering

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Since time immemorial, plants served as the first source of medicine to treat ailments. Man learnt about the therapeutic use of plants through trials and errors. This knowledge has been orally passed from generation to generation which led to the development of the traditional health care system, practiced in various countries of the world [1]. Ethnobotanical studies discover plant resources that can be used for targeting novel compounds leading to the development of new medicaments for treating especially complicated and minor diseases [2]. Today, ethnobotany and ethnopharmacognosy are being used for targeting new compound. Due to being rich in diversity, tropical regions may play key role in providing germplasm with new leads [1].

It is estimated that 80% of the world's population lives in developing countries and over 80% of the world's population rely on plant-derived medicines for their primary health care needs [3]. Based on the personal experience, people knew therapeutic potential of the medicinal plants without rationale of their efficacy. Because of advancement, we have a better understanding of the healing powers of plants due to presence of multifunctional chemical entities for treating complicated health conditions.

The ethnobotany provided significant information that led to isolation of active compounds from the recent past like morphine from opium, cocaine, codeine, digitoxin, and quinine [4–6]. It is worthwhile to mention that a dozen of effective valuable drugs are discovered during the last 40 years from higher plants. The very common ones are diosgenin derived from *Dioscorea deltoidea*; reserpine from *Rauwolfia serpentina*; pilocarpine from *Pilocarpus* spp.; vincristine/vinblastine from *Catharanthus roseus*; digoxin/digitoxin from *Digitalis* species [7]; arteether (trade name Artemotil), a recent antimalarial drug is obtained from artemisinin-a sesquiterpene lactone isolated from *Artemisia annua* [8]; galantamine (also known as galanthamine, trade name Reminyl) isolated from *Galanthus woronowii* [9, 10].

Drug discovery from plant lore and traditional medicines are reemerging. Ethnobotanical studies exposed various medicinal plants for discovering miraculous drugs which are still available in the market. Even today, various areas of the world have a unique tradition of plant lore for alleviating human suffering as well as their domesticated animals. There is a need to document such valuable information before it is permanently lost. Based on such data, new medicaments can be predicted through undergoing experimentation which may be of potential use to treat various complicated human diseases. The plant kingdom is an implicit gold mine of new chemical compounds which are still waiting to be explored. It is estimated that there are approximately 500,000 to 750,000 species of higher plants existing on earth and less than 10% of them are examined for their biochemical constituents [11].

Keeping the importance of ethnobotany, an interdisciplinary field of study, this special issue was dedicated to the integration of past and present use of plants reporting

traditional/folk medicinal use along with latest development for validation of such information through scientific studies. This special issue is a collection of seven articles portraying the use of medicinal plants and their therapeutic potential. The issue is mainly divided into two main themes; the first one describes the traditional knowledge of plants and the other one describes validation of such knowledge through *in vitro* assays.

From ethnobotanical perspective, four articles are selected. K. C. Chinsembu carried out an ethnobotanical study from Livingstone, Southern Province, Zambia. He reported 94 medicinal plant species which are used to treat HIV/AIDS-related diseases. He stressed to confirm the antimicrobial efficacies, pharmacological parameters, cytotoxicity, and active chemical ingredients of the discovered plants. In a study carried out by M. Meragiaw et al. reported ethnobotanical enumeration of Delanta (Ethiopia) to examine the use of medicinal plants and impacts of the 1984/85 resettlement program on the local people's knowledge on herbal medicine and its uses. They reported 133 species belonged to 116 genera and 57 families in treating 76 human and livestock ailments. Their analysis showed that the resettlement program has both positive and negative impacts on nature rehabilitation and local knowledge along with many human induced threats. S. F. Sabran et al. discovered ethnomedical knowledge of plants used for the treatment of tuberculosis by the Jakun community of Kampung Peta (Malaysia). They identified 23 plants which are used by the community for the same purpose. *Dipterocarpus sublamellatus* was recorded for the first time as novel species to treat tuberculosis. They urged that findings of this study are worth being further investigated for conservation strategies and are worthy of verifying their ethnomedical claims scientifically. A survey was conducted by M. A. Agbor and S. Naidoo to document ethnomedicinal use of plants by the traditional healers in treating oral health problems in Cameroon. They reported 52 plants which are being used for the management of toothache, sore throat, mouth sores, abscess, broken tooth and jaw, tooth sensitivity, mouth thrush, dental caries, gingivitis, sinusitis, tonsillitis, xerostomia, oral syphilis, oral cancer, TMJ pain, halitosis, tooth bleaching, and dental extraction.

From the bioactivity assessment point of view, four articles were selected. M. K. Swamy et al. investigated the effect of different solvents on the extraction of phytoconstituents of *Lantana camara* leaves and their antioxidant and antibacterial activities. They reported that the methanol solvent yielded the highest phenolic (92.8 mg GAE/g) and flavonoid (26.5 mg RE/g) content revealing antioxidant activity. Methanol extract had the highest inhibition activity against all the tested microbes. They identified major compounds such as hexadecanoic acid (5.197%), phytol (4.528%), caryophyllene oxide (4.605%), and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (3.751%) through GC-MS. A laboratory study carried out by N. Jayawardena et al. investigated antioxidant and starch hydrolase inhibitory activities of 10 spices through *in vitro* model of digestion mimicking the gastric and duodenal conditions. The total phenolic contents in all spice extracts had significantly increased following both gastric and duodenal digestion revealing a correlation with the antioxidant assays quantifying the water-soluble antioxidant capacity of the extracts. They concluded that the tested spices had a significant source of total phenolics, antioxidant, and starch hydrolase inhibitory activities. Finally, S. Baral et al. studied *in vivo* ameliorating effect of myrrh (AEM) on scopolamine-induced memory impairments using mice model. The AEM was estimated with (2E,5E)-6-hydroxy-2,6-dimethylhepta-2,4-dienal as a representative constituent through HPLC. The oral administration of AEM ameliorated the scopolamine-induced memory impairments and increased the phosphorylation of Akt and ERK in the hippocampus of mice brain.

We anticipate that this special issue will provide traditional knowledge of plants existing in various traditional communities to manage and treat various diseases as well as their scientific validation through bioassay assessment.

Acknowledgments

We express our great gratitude to all authors for their contributions and reviewers for their great help. We convey our sincere thanks to the Editorial Board of ECAM for their approval on this topic and continuous support in successful publication of this special issue. The lead guest editor would like to thank the guest editors for their enthusiastic assistance. We hope this special issue will bring readers a useful academic reference in their research.

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Research Article

Ethnobotanical Study of Plants Used in the Management of HIV/AIDS-Related Diseases in Livingstone, Southern Province, Zambia

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Faced with critical shortages of staff, long queues, and stigma at public health facilities in Livingstone, Zambia, persons who suffer from HIV/AIDS-related diseases use medicinal plants to manage skin infections, diarrhoea, sexually transmitted infections, tuberculosis, cough, malaria, and oral infections. In all, 94 medicinal plant species were used to manage HIV/AIDS-related diseases. Most remedies are prepared from plants of various families such as Combretaceae, Euphorbiaceae, Fabaceae, and Lamiaceae. More than two-thirds of the plants (mostly leaves and roots) are utilized to treat two or more diseases related to HIV infection. Eighteen plants, namely, *Achyranthes aspera* L., *Lannea discolor* (Sond.) Engl., *Hyphaene petersiana* Klotzsch ex Mart., *Asparagus racemosus* Willd., *Capparis tomentosa* Lam., *Cleome hirta* Oliv., *Garcinia livingstonei* T. Anderson, *Euclea divinorum* Hiern, *Bridelia cathartica* G. Bertol., *Acacia nilotica* Delile, *Piliostigma thonningii* (Schumach.) Milne-Redh., *Dichrostachys cinerea* (L.) Wight and Arn., *Abrus precatorius* L., *Hoslundia opposita* Vahl., *Clerodendrum capitatum* (Willd.) Schumach., *Ficus sycomorus* L., *Ximenia americana* L., and *Ziziphus mucronata* Willd., were used to treat four or more disease conditions. About 31% of the plants in this study were administered as monotherapies. Multiuse medicinal plants may contain broad-spectrum antimicrobial agents. However, since widely used plants easily succumb to the threats of overharvesting, they need special protocols and guidelines for their genetic conservation. There is still need to confirm the antimicrobial efficacies, pharmacological parameters, cytotoxicity, and active chemical ingredients of the discovered plants.

1. Introduction

Livingstone has the highest human immunodeficiency virus (HIV) prevalence level in Zambia. Although the average HIV prevalence rate in Zambia is about 13%, the HIV infection rate in Livingstone is about 25.3%, significantly higher than the national average [1]. During the period 1994–2002, Livingstone's HIV prevalence was stable at around 30% [2]. Located in Southern Province, Livingstone is the tourist capital of Zambia, home to the famous Victoria Falls. Therefore, many socioeconomic factors conspire to fuel the town's growing HIV epidemic. Transactional sex is very common in Livingstone, attributable to local or foreign tourists and high levels of poverty; receiving money or gifts for sex is the only means for vulnerable women to financially secure themselves and their families because other sources of income are not sufficient [3].

According to Byron et al. [4], women combine sex with the sale of material products to earn higher profits when they go to the market in town and end up contracting HIV. At 3.1%, women in Livingstone had the highest prevalence of sexually transmitted infections (STIs) in Zambia [1]. Migrant labourers, especially sugar cane cutters from Mazabuka (Zambian town with the second highest HIV prevalence at 18.4%), are often blamed for being high-risk transmitters of HIV. In several sites in Livingstone, there are designated venues where people meet new sexual partners [2]. These sexual venues are linked to high partner turnover and due to major challenges in on-site condom availability, unprotected

sex is common among guests [2]. Livingstone also has low rates of male circumcision at 11% [1].

In Livingstone, studies have reported sexual behaviours with a high potential for HIV transmission, yet there are few signs of HIV preventive interventions [3]. Despite the rollout of antiretroviral therapy (ART), Cataldo and others [5] stated that Zambian HIV-infected persons still seek treatment from traditional healers. Thus, although some western trained health care providers remain suspicious of traditional healers, most agree that traditional healers play an important and complementary role in the provision of effective HIV prevention or treatment [6]. Kaboru [7] also found that many biomedical health practitioners believe that Zambian traditional healers can help control HIV/AIDS.

Undoubtedly, several patients seek herbal remedies for conditions related to acquired immune deficiency syndrome (AIDS) before seeking care at health centres [8]. This is because there are many deficiencies in the provision of biomedical services for STIs and HIV/AIDS in Zambia [7]. Unlike hospital staff with poor attitudes, traditional healers are also kinder and more compassionate to patients [7]. According to Ndulo et al. [9], traditional healers attend to patients with sexually transmitted infections (STIs), in both rural and urban areas; therefore, efforts should be made to promote cooperation between traditional and biomedical health care providers, so that treatment of patients and their partners could be improved. Traditional management that concurs with biomedical practices could thus be a starting point for discussion and cooperation.

Moreover, traditional healers have good knowledge of STIs [9]. Most of them use herbal preparations in the form of roots or powders administered orally to induce diarrhoea, vomiting, and diuresis. Traditional healers also correctly cite symptoms associated with STIs such as urethral or vaginal discharge. Therefore, Makasa et al. [10] observed that about 15% of patients with genital ulcer disease seek treatment from traditional healers. Although the use of traditional medicine is associated with nonadherence to ART, health care providers at hospitals should open lines of communication with traditional healers [11]. Makasa et al. [10] also noted the need to increase awareness among traditional healers that handle patients presenting with STIs and to refer certain cases to health facilities, especially when patients do not respond to traditional medicines.

Traditional healers far outnumber modern health care providers in Zambia where the Traditional Health Practitioners Association has over 40,000 members compared to a paltry 1,000 conventional medical doctors that are practicing nationwide [11]. There were 1,390 medical doctors practicing in Zambia; the doctor to population ratio was 1 to 17,589 instead of the World Health Organization recommended ration of 1 to 5,000 [12]. At the entrance of Zambia's University Teaching Hospital (UTH), a signpost reads "kindly take note that members of staff at UTH work under very critical shortage of manpower," a stark reminder of the dearth of health staff and severe crisis facing the country. Given the glaring personnel shortages in many public health care facilities [13], involvement of traditional healers in the management of HIV/AIDS opportunistic diseases is a ubiquitous narrative.

Zambia is among the Sub-Saharan African countries with the most acute shortages of trained personnel in the health sector [14]. Predictably, use of traditional medicines was reported among 75% of inpatients at the UTH and among 68% of those seeking services for HIV counselling and testing [11]. Studies have shown that individuals that use traditional medicines are also associated with alcohol, have two or more sexual partners, engage in dry sex, and harbour STIs. Corollary, identification of persons who access traditional medicines may be an important target population for HIV prevention because many HIV risky behaviours are common among clients of traditional healers [11].

Besides, traditional healers are still consulted because they are deemed to provide client-centred and personalised health care that is customized to the needs and expectations of patients, paying special respect to social and spiritual matters [15]. Indeed, whilst the majority of HIV/AIDS patients that need treatment can access ART from local hospitals and health centres, several constraints of the ART programme compel many HIV-infected people to use traditional medicines to manage HIV/AIDS-related conditions [16]. Others use ethnomedicinal plants to offset side effects from ART [17]. The use of medicinal plants in Livingstone is also part of the medical pluralism whereby the introduction of allopathic medicines has not really dampened beliefs in indigenous diagnosis and therapeutic systems [18].

Even though there are some anecdotal reports regarding the traditional uses of ethnomedicinal plants to manage various diseases in Livingstone, knowledge on specific plant species used to manage HIV/AIDS-related diseases is still scanty and not well recorded. This paper is an inaugural and modest report on medicinal plants used in the management of HIV/AIDS opportunistic infections in Livingstone, Southern Province, Zambia. Documentation of putative anti-HIV plant species may help preserve this critical tacit indigenous knowledge resource. Plus, indigenous knowledge, coupled with a history of safe use and ethnopharmacological efficacy of medicinal flora, also presents a faster approach to discover new chemical compounds that may be developed into novel antiretroviral drugs.

2. Materials and Methods

2.1. Study Area. The study was carried out in Chibelenga, Burton, Dambwa, Hillcrest, Libuyu, Linda, Malota, Maramba, Ngwenya, and Zakeyo; these form the urban and rural settlements of Livingstone, provincial headquarters of Southern Province until 2012 (Figure 1). The geographical coordinates of Livingstone are $17^{\circ}51'0''$ south, $25^{\circ}52'0''$ east. The town is situated about 981 metres above sea level near the Victoria Falls on the Zambezi River close to the Zimbabwean border. Situated in agroecological region I, Livingstone has a humid subtropical climate. Its average annual rainfall is about 690–740 mm. The mean maximum temperature is high, 35°C in October, and the mean minimum temperature is low, 7°C in June. Recorded high (41.1°C) and low (−3.7°C) temperatures in November and June, respectively, have been documented. Rainy season occurs between November and March when it is wet, hot, and humid.

(a) (b)

Figure 1: (a) Map of Zambia showing the location of Livingstone. (b) Townships in Livingstone town.

According to the Holdridge life zones system of bioclimatic classification, Livingstone is situated in or near the subtropical dry forest biome. The terrain in Livingstone is well vegetated with over 1,000 plant species represented by riparian forests and woodlands, Kalahari woodlands, *Colophospermum mopane* (J. Kirk ex Benth.) J. Léonard woodlands, and deciduous woodlands mostly consisting of *Brachystegia glaucescens* Hutch. and Burtt Davy and the tall mahogany *Entandrophragma caudatum* Sprague. The vegetation is quite similar to that in adjoining Sesheke District as reported by Chinsembu [18] except for a few dominant plant species.

Between 1907 and 1935, Livingstone was the capital city of Northern Rhodesia, now Zambia. The town is named after David Livingstone who in 1840 as a young Scottish doctor and ordained minister sailed from Britain to the Cape to work as a medical evangelist with the London Missionary Society. In 1855, Dr. David Livingstone became the first European to see the Victoria Falls when he was taken there by Sekeletu, chief of the Subiya/Kololo people. Although contemporary life is a blend of values and traditions of more than 70 of Zambia's ethnically diverse people, the main tribes in Livingstone are the Tonga/Tokaleya and Lozi; many of them live in townships such as Maramba. Archaeological artifacts suggest the existence of the Tonga for at least 900 years in southern Zambia's Zambezi Valley. The Lozi migrated into Western Zambia from the Luba/Lunda Kingdom of Mwata Yamvwa in Zaire, present day Democratic Republic of the Congo.

After Zambia's independence in 1964, President Kenneth Kaunda's government built motor vehicle and radio assembly plants in Livingstone, attracting migrant workers. These manufacturing industries closed soon after President Frederick Chiluba became president of Zambia in 1991. In recent years, the town's economic fortunes have dwindled except for a slight influx of investment in the tourism sector characterized by the opening of modern hotel chains like Sun International. Commercial sex work is common among women; many of them are from neighbouring Zimbabwe where the socioeconomic situation remains dire.

2.2. Ethnobotanical Data Collection. Ethnobotanical data were collected using methods similar to that of [17–19]. Briefly, snowball sampling was applied during ethnobotanical surveys of thirty knowledge holders including 10 traditional healers that use plants to treat HIV/AIDS-related diseases. Before conducting interviews, the aim of the study was clearly explained and knowledge holders were asked for their consent. Then the knowledge holders were individually engaged in semistructured interviews supplemented with questionnaires. During the conversations, data on respondent characteristics and information related to medicinal uses of plants for the management of HIV/AIDS-related diseases were captured. All interviews were conducted in local languages, Tonga/Tokaleya, and Lozi. Research assistants acted as Tonga/Tokaleya/Lozi to English translators.

Data were collected during two stages consisting of primary and secondary samplings. The primary stage involved an exploratory and descriptive study of eight knowledge holders that manage HIV/AIDS-related infections. The focus of the exploratory study was to gain critical insights into the work of the knowledge holders, distil pertinent issues, and gauge whether a detailed ethnobotanical survey would be feasible. Knowledge holders were asked about the main symptoms of HIV/AIDS, their healing practices, and sources of ethnomedicinal knowledge. The following data in relation to the plants were also recorded: vernacular names (Tonga/Tokaleya/Lozi), plant habits, plant parts used, the HIV/AIDS-related conditions treated with the plants, and the modes of preparation and application of the plant remedies to the patient.

The secondary sampling stage was a follow-up and detailed descriptive study of 22 knowledge holders who verified prior ethnobotanical data obtained from others during the exploratory inquiry. To allow for triangulation of ethnomedicinal use, only plants mentioned by at least three knowledge holders in the descriptive study (for each disease condition) were eligible for documentation [20]. On-the-spot identification of familiar plant species was done in the field. Voucher numbers for plants were assigned and specimens for

plants were collected in herbarium plant presses for identification and confirmation. Botanical names were verified using the International Plant Name Index (IPNI).

2.3. Data Analysis. Quantitative analysis of ethnobotanical data was done by calculating percentage frequencies, familiarity index F_i, and factor informant consensus (F_{IC}). The F_i, a relative indicator of the familiarity of a plant species, is defined as the frequency a given plant species is mentioned as an ethnomedicine divided by the total number of knowledge holders interviewed in the study [21]. The F_i was calculated as follows:

$$F_i = \frac{N_a}{N_b} \times 100, \quad (1)$$

where N_a is the number of informants that mention a species as a medicine and N_b is the total number of respondents.

The F_{IC} was the number of use citations in each ailment category (N_{ur}) minus the number of species used (N_t), divided by the number of use citations in each category minus one [22]:

$$F_{IC} = N_{ur} - \frac{N_t}{(N_{ur} - 1)}. \quad (2)$$

F_{IC} values are low (near 0) if plants are chosen randomly or if informants do not exchange information about their use. Values are high (near 1) if there was a well-defined selection criterion among informants and/or if information was exchanged between informants. High F_{IC} values are also obtained when only one or a few plant species are reportedly used by a high number of knowledge holders to treat a particular disease, and low F_{IC} values imply that respondents disagree over which plant to use [23].

3. Results

Of all the thirty knowledge holders included in the study, only eight were female. This gender difference may be explained by the fact that male knowledge holders in the community were more comfortable to talk about STIs than female knowledge holders who face cultural restrictions when it comes to talking about matters related to sex, STIs, and HIV/AIDS. The average age of the healers was 48 years. About 70% of the knowledge holders received their medicinal plant knowledge from their older family members and the remainder from spiritual and supernatural powers such as ancestral spirits, dreams, and visions. Only six traditional healers had an apprentice under their tutelage; the rest did not train other people.

Medicobotanical data including the plants' scientific names, vernacular names, families, voucher numbers, habits, frequency indices, parts, HIV/AIDS-related diseases treated, modes of preparation, and application are described in Table 1. Overall, 94 plant species from 39 families were used by various knowledge holders to manage HIV/AIDS-related diseases in Livingstone, Southern Province, Zambia (Table 1). Their growth habits were as follows: almost half were trees (53.2%), about a quarter were shrubs (24.5%), and there were approximately equal proportions of climbers (11.7%) and herbs (10.6%).

The most used families were Fabaceae (22%), Combretaceae (9%), Euphorbiaceae (6%), and Lamiaceae (5%) (Figure 2). The most plant parts used were leaves (33%), roots (25%), bark (22%), and stems/stem barks (20%) (Figure 3). Pods/seeds (2%) and tubers (1%) were least used. Plant exudates in the form of sap were also harvested from 2% of the plants. Figure 4 presents the proportions of plant species used to treat various HIV/AIDS-related disease conditions: skin infections (16.4%), diarrhoea/dysentery (15.0%), gonorrhoea (12.7%), syphilis (10.0%), tuberculosis (TB)/pneumonia (8.6%), cough (8.2%), malaria (6.8%), and oral infections (5.0%).

Figure 5 illustrates that of all the plants that were used to ameliorate skin conditions, most of them were used to manage skin sores or ulcers (33.0%), rashes (28.0%), herpes zoster (15.0%), boils (10.0%), and abscesses (7.0%). About 5% of all plants used on skin conditions treated general infections. Of all the ethnomedicinal plants used to manage STIs, the majority of them were used for gonorrhoea (40.0%), syphilis (32.0%), and HIV (7.0%) (Figure 6).

Eighteen plants were utilized to treat four or more disease conditions: *Achyranthes aspera* L., *Lannea discolor* (Sond.) Engl., *Hyphaene petersiana* Klotzsch ex Mart., *Asparagus racemosus* Willd., *Capparis tomentosa* Lam., *Cleome hirta* Oliv., *Garcinia livingstonei* T. Anderson, *Euclea divinorum* Hiern, *Bridelia cathartica* G. Bertol., *Acacia nilotica* Delile, *Piliostigma thonningii* (Schumach.) Milne-Redh., *Dichrostachys cinerea* (L.) Wight and Arn., *Abrus precatorius* L., *Hoslundia opposita* Vahl, *Clerodendrum capitatum* (Willd.) Schumach., *Ficus sycomorus* L., *Ximenia americana* L., and *Ziziphus mucronata* Willd. Only 31% of the plants in this study were administered as monotherapies.

The F_i values are given in Table 1. Informants were more familiar with the medicinal uses of the following fourteen most frequently used plants: *Cassia abbreviata* Oliv., *Combretum imberbe* Wawra, *Diospyros mespiliformis* Hochst. ex A.DC., *Fockea angustifolia* K. Schum., *G. livingstonei*, *Kigelia africana* (Lam.) Benth., *Mimosa pigra* L., *Syzygium cordatum* Hochst., *Syzygium guineense* DC., *Terminalia prunioides* M. A. Lawson, *Peltophorum africanum* Sond., *Plumbago zeylanica* L., *X. americana*, and *Z. mucronata*. According to Table 2, F_{IC} values for the various disease conditions show that consensus was high over plants used to treat malaria, oral infections, and fever/flu/colds/headache.

4. Discussion

The highest proportion of plants in Livingstone was used to manage skin diseases, probably because they contain antimicrobial agents. A similar scenario was obtained in Sesheke (Zambia) and Rundu (Namibia). This speaks to the fact that skin infections are quite common during HIV infection. Many of the plants for skin diseases in Livingstone were used to manage skin infections in other geographical settings. For instance, Afolayan et al. [24] and Hedimbi and

Table 1: Plants used to manage HIV/AIDS related diseases in Livingstone, Southern Province, Zambia.

Family	Botanical name	Vernacular name	Growth form	Frequency index, voucher number	Diseases treated	Plant parts used, preparation, and mode of administration
Acanthaceae	*Barleria kirkii* T. Anderson	Chavani	Herb	13.3, L144	HIV/AIDS	Leaf decoction is drank
Amaranthaceae	*Achyranthes aspera* L.	Tantajulo	Herb	20.0, L186	Cancer, pneumonia, cough, diarrhoea, fungal infections of the skin, genital warts	Root infusion or whole plant decoction is drank; paste of plant is applied to skin
Anacardiaceae	*Lannea stuhlmannii* (Engl.) Eyles	Mungangacha, Mucheche	Tree	83.3, L245	Gonorrhoea, syphilis, herpes zoster, herpes simplex, skin infections, HIV/AIDS	Crushed leaves or roots are boiled in water, decoction is drank; stem bark decoction is used to wash affected skin
Anacardiaceae	*Lannea discolor* (Sond.) Engl.	Mungongwa	Tree	50.0, L235	Diarrhoea, gonorrhoea	Fruit pulp eaten to relieve diarrhoea; root infusion drank to relieve gonorrhoea
Annonaceae	*Friesodielsia obovata* (Benth.) Verdc.	Muchinga	Shrub	20.0, L216	Skin rashes	Pounded leaves rubbed into skin
Annonaceae	*Artabotrys brachypetalus* Benth.	Mulandabala	Climber	13.3, L230	Skin infections	Crushed leaves rubbed into skin
Apiaceae	*Steganotaenia araliacea* Hochst.	Mupelewa	Tree	60.0, L113	Headache	Root decoction is drank
Arecaceae	*Hyphaene petersiana* Klotzsch ex Mart.	Kakunka, Mapokwe	Tree	20.0, L217	Malaria, cough, tuberculosis, skin rashes, sores related to STIs	Palm fruit is eaten raw or boiled to treat malaria and cough; seeds are used to treat TB; sap is applied to heal skin rashes and STIs
Asclepiadaceae	*Fockea angustifolia* K. Schum.	Mutindika, Nanyama	Herb	83.3, L210	Cough	Tuber is eaten raw
Asparagaceae	*Asparagus setaceus* (Kunth) Jessop	Mutandamyoba	Climber	50.0, L207	Eczema	Whole plant is crushed and rubbed into affected skin
Asparagaceae	*Sansevieria deserti* N. E. Br.	Musombo, Mukonje	Herb	13.3, L360	Oral infections	Leaves are chewed and then spitted
Asparagaceae	*Asparagus racemosus* Willd.	Mutandamyoba, Ilutwa	Climber	20.0, L433	Pneumonia, cough, diarrhoea, syphilis	Whole plant is boiled; decoction is drank
Asteraceae	*Vernonia amygdalina* Delile	Musoboyo	Shrub	83.3, L481	Coughs, tuberculosis, malaria	Leaves are boiled; decoction is drank
Bignoniaceae	*Kigelia africana* (Lam.) Benth.	Muzungula	Tree	83.3, L485	Syphilis, herpes simplex, diarrhoea, boils, colds, flu	Stem bark and leaves are boiled
Burseraceae	*Commiphora mollis* Engl.	Muntyokela	Tree	50.0, L312	Treats swollen pancreas in patients on antiretroviral therapy	Stem bark is crushed and boiled; decoction is drank
Capparaceae	*Boscia salicifolia* Oliv.	Mulaba, Kabombwe	Tree	70.0, L406	Syphilis, HIV/AIDS	Roots are ground and left in water; infusion is drank
Capparaceae	*Capparis tomentosa* Lam.	Chonswe	Shrub	20.0, L329	Syphilis rashes; HIV/AIDS; cryptococcal meningitis, oral candidiasis, herpes zoster, herpes simplex, chronic diarrhoea	Roots are crushed and boiled and decoction is drank; crushed leaves are applied to sores or soaked in water used to wash the mouth
Capparaceae	*Cleome hirta* Oliv.	Mulangazuba, Kalungukachisiungwa	Herb	13.3, L303	Pneumonia, tuberculosis, fungal infection of the skin, malaria	Leaf infusion is drunk
Celastraceae	*Maytenus senegalensis* (Lam.) Exell	Mukuba	Shrub	50.0, LV235	Tuberculosis	Leaves are crushed and soaked in water; infusion is drank
Celastraceae	*Hippocratea africana* Loes. ex Engl.	Mulele	Climber	60.0, LV280	Malaria	Root decoction is drunk
Chrysobalanaceae	*Parinari curatellifolia* Planch. ex Benth.	Mubulabula, Mula	Tree	13.3, LV245	Toothache, diarrhoea	Fruit eaten raw
Clusiaceae	*Garcinia livingstonei* T. Anderson	Mutungwa, Mukwanaga	Tree	83.3, LV226	Cryptococcal meningitis, herpes zoster, herpes simplex, skin rashes Tuberculosis chronic diarrhoea	Fruit eaten raw or in porridge
Combretaceae	*Combretum collinum* Fresen.	Mukunza, Mulamana	Tree	43.3, LV187	Chronic diarrhoea, tuberculosis, cough	Roots are crushed and soaked in water overnight; filtrate is drank
Combretaceae	*Combretum imberbe* Wawra	Mubimba, Muzwili	Tree	83.3, LV238	General STIs, tuberculosis	Stem bark is crushed and soaked in water overnight; filtrate is drank
Combretaceae	*Combretum apiculatum* Sond.	Mukalanga, Kalanga, Nkalanga	Tree	50.0, LV220	General STI syndromes; tuberculosis	Stem bark is crushed and soaked in water overnight; filtrate is drank

Table 1: Continued.

Family	Botanical name	Vernacular name	Growth form	Frequency index, voucher number	Diseases treated	Plant parts used, preparation, and mode of administration
Combretaceae	*Combretum elaeagnoides* Klotzsch	Mukalanga, Mukupo	Tree	30.0, L576	Malaria, tuberculosis, diarrhoea	Fresh leaves are crushed and soaked in water overnight; filtrate is drank
Combretaceae	*Combretum hereroense* Schinz	Namazubo	Tree	53.3, L437	Gonorrhoea	Roots are crushed and soaked in water overnight; filtrate is drank
Combretaceae	*Terminalia prunioides* M. A. Lawson	Mutala, Mukonono, Mulumbu	Tree	83.3, L247	Gonorrhoea, syphilis, HIV/AIDS	Outer parts of roots are dried, crushed, and mixed with water overnight; infusion is drank
Combretaceae	*Combretum mossambicense* Engl.	Numinambelele, Silutombolwa, Mutombololo	Climber	36.7, LV475	Gonorrhoea, syphilis	Fresh leaves are crushed and soaked in water overnight; filtrate is taken orally
Combretaceae	*Combretum paniculatum* Vent.	Mutombolo	Shrub	50.0, L322	Malaria, diarrhoea	Leaves are pounded and soaked in water; infusion is drank
Connaraceae	*Byrsocarpus orientalis* Baill.	Kazingini	Shrub	13.3, L521	Skin abscesses and boils	Young stems are cut and boiled, and decoction is applied to skin
Dioscoreaceae	*Dioscorea cochleari-apiculata* De Wild.	Mpama	Climber	13.3, L511	Syphilitic sores, chancroid	Root decoction drank
Ebenaceae	*Diospyros mespiliformis* Hochst. ex A.DC.	Mchenja	Tree	83.3, L109	Malaria	Crushed roots are boiled; filtrate is drank
Ebenaceae	*Diospyros quiloensis* (Hiern) F. White	Musiaabwele	Tree	50.0, LV339	Gonorrhoea, syphilis, malaria	Crushed stem bark is boiled; filtrate is drank
Ebenaceae	*Euclea divinorum* Hiern	Munyansyabweli	Tree	20.0, LV140	Syphilis, gonorrhoea, genital herpes, oral candidiasis, abscesses, diarrhoea	Roots are ground and boiled in water and decoction is taken orally
Erythroxylaceae	*Erythroxylum zambesiacum* N. Robson	Mubalubalu	Tree	16.7, LV277	Malaria, headache	Roots are cut into small pieces and boiled, and decoction is administered orally
Euphorbiaceae	*Croton gratissimus* Burch.	Mungai, Kanunkila Mpati	Tree	66.7, LV399	Syphilis	Stem bark is boiled; decoction is drank and used to wash sores
Euphorbiaceae	*Croton megalobotrys* Müll. Arg.	Mutua, Mutuatua	Tree	60.0, S149	Gonorrhoea	Leaves are boiled and decoction is drank
Euphorbiaceae	*Pseudolachnostylis maprouneifolia* Pax	Mukunyu	Tree	23.3, S108	Diarrhoea, pneumonia	Stem bark decoction is drank
Euphorbiaceae	*Bridelia cathartica* G. Bertol.	Munyanyamenda	Shrub	13.3, S154	Oral infections, diarrhoea, gonorrhoea, malaria	Leaves and fruits are chewed raw to act as mouthwash; stem bark infusion is drank
Euphorbiaceae	*Margaritaria discoidea* (Baill.) G. L. Webster	Mulyankanga	Shrub	16.7, S119	Skin rashes and sores; headache	Stem bark
Euphorbiaceae	*Phyllanthus reticulatus* Lodd.	Mwichechele	Shrub	26.7, S260	Herpes simplex	Crushed leaves are rubbed to affected areas of skin
Fabaceae	*Acacia albida* Delile	Musangu, Muunga	Tree	50.0, S100	Syphilis sores, herpes zoster	Crushed leaves and stem bark are applied to heal sores
Fabaceae	*Acacia nigrescens* Oliv.	Mwabaa, Mukwena	Tree	60.0, S78	Herpes zoster	Leaves and stem bark are boiled, decoction used to
Fabaceae	*Acacia polyacantha* Willd.	Mumbu, Mukotokoto, Luntwele	Tree	50.0, S63	Gonorrhoea, herpes zoster	Leaves and stem bark are crushed and boiled; decoction is drank or used to wash affected skin
Fabaceae	*Afzelia quanzensis* Welw.	Mupapa, Mukamba	Tree	76.7, S27	General STIs	Leaves are pounded and soaked in water overnight and then drank
Fabaceae	*Albizia amara* (Roxb.) Boivin	Kankumbwila,Mukangola	Tree	60.0, S44	Gonorrhoea, diarrhoea	Stem bark infusion is drank
Fabaceae	*Lonchocarpus capassa* Rolfe	Mukololo	Tree	13.3, S31	Gonorrhoea, cough	Stem bark and leaves are mixed, crushed, soaked in water, and filtered and infusion is drank
Fabaceae	*Peltophorum africanum* Sond.	Muzenzenze	Tree	83.3, K203	General STIs, oral infections	Roots are soaked in water and infusion is drank to treat STIs; leaves are crushed and soaked in water; infusion is used to wash mouth
Fabaceae	*Pterocarpus antunesii* Harms	Mukambo	Tree	26.7, K166	Diarrhoea	Stem bark infusion is drank
Fabaceae	*Acacia goetzei* Harms	Mwaba	Tree	46.7, K88	Cough, pneumonia	Root decoction is drank
Fabaceae	*Acacia nilotica* Delile	Mukoka	Tree	66.7, K56	Tuberculosis, diarrhoea, gonorrhoea, dental caries	Twigs used as chewing sticks to treat dental caries

Table 1: Continued.

Family	Botanical name	Vernacular name	Growth form	Frequency index, voucher number	Diseases treated	Plant parts used, preparation, and mode of administration
Fabaceae	*Cassia abbreviata* Oliv.	Mululwe	Tree	83.3, K39	Gonorrhoea, diarrhoea	Root infusion is drank
Fabaceae	*Dalbergia melanoxylon* Guill. & Perr.	Musonkomo	Shrub	13.3, K17	Diarrhoea	Root decoction is drank
Fabaceae	*Piliostigma thonningii* (Schumach.) Milne-Redh.	Musekese	Tree	60.0, K12	Coughs, skin rashes, gonorrhoea, syphilis	Stem bark and roots are boiled; decoction is drank to heal coughs and STIs; leaf infusion is used to wash infected skin
Fabaceae	*Acacia ataxacantha* DC.	Lubamfwa	Shrub	50.0, K60	Gonorrhoea, syphilis	Roots are boiled and decoction is drank
Fabaceae	*Acacia schweinfurthii* Brenan & Exell	Lubua, Mokoka	Shrub	33.3, L34	Gonorrhoea, syphilis	Roots, stem bark, and leaves are mixed, pounded, and soaked in water and infusion is drank
Fabaceae	*Dichrostachys cinerea* (L.) Wight & Arn.	Katenge, Mugee	Shrub	66.7, L79	Gonorrhoea, syphilis; oral candidiasis, and skin rashes	Stem bark is boiled and decoction is drank, used to wash oral cavity, or used to disinfect skin by washing
Fabaceae	*Mimosa pigra* L.	Muchabachaba, Sichatubabi	Shrubs	83.3, L56	Diarrhoea, genital ulcers, gonorrhoea	Leaves and small and young stems are dried, pounded into powder, mixed with water, and filtered and extract is drank or used to wash genital ulcers
Fabaceae	*Sesbania sesban* Britton	Mbelembele	Shrub	13.3, L77	Malaria	Roots are boiled and decoction is administered orally
Fabaceae	*Indigofera colutea* (Burm. f.) Merr.	Kapalupalu	Herb	13.3, L59	Diarrhoea	Whole plant is pounded, soaked in water overnight, and filtered, and infusion is drank to alleviate diarrhoea
Fabaceae	*Abrus precatorius* L.	Musolosolo	Climber	60.0, KC280	Gonorrhoea, syphilitic ulcers, genital herpes; oral candidiasis, ulcer boils	Whole plant is crushed, boiled in water, and filtered; decoction is drank; crushed leaves soaked in water and used to wash mouth and syphilitic ulcers
Fabaceae	*Mucuna pruriens* (L.) DC.	Muyuyu	Climber	20.0, KC245	Weight loss, lack of libido in men	Pods and beans are boiled and consumed for body building and to act as an aphrodisiac
Flacourtiaceae	*Flacourtia indica* (Burm. f.) Merr.	Mutumbula	Shrub	13.3, KC350	Diarrhoea	Leaves chewed raw
Flacourtiaceae	*Oncoba spinosa* Forssk.	Mukumbuzu	Shrub	16.7, KC2	Dysentery	Roots and fruits are boiled and decoction is drunk
Kirkiaceae	*Kirkia acuminata* Oliv.	Musanta, Muzumina	Tree	13.3, KC18	Diarrhoea	Stem bark infusion is drank
Lamiaceae	*Vitex payos* (Lour.) Merr.	Mfudu, Muyankonga	Tree	30.0, KC36	Coughs	Fruit eaten raw or leaves burnt and smoke inhaled as cough medicine
Lamiaceae	*Hoslundia opposita* Vahl	Musombwani	Shrub	60.0, KC48	Coughs, flu, fever, loss of libido in men; skin wounds	Roots are boiled and decoction is drunk; sugary fruits are eaten raw to boost libido; crushed leaves are rubbed into skin to heal wounds
Lamiaceae	*Premna senensis* Klotzsch	Mumpika	Shrub	13.3, KC360	Syphilitic sores, skin ulcers	Leaf infusions applied to sores
Lamiaceae	*Vitex petersiana* Klotzsch	Mufulibulimbo, Mukoma	Shrub	23.3, KC312	Gonorrhoea	Leaf decoction is drank to treat gonorrhoea;
Lamiaceae	*Clerodendrum capitatum* (Willd.) Schumach.	Shamanya	Herb	60.0, L402	Gonorrhoea, erectile dysfunction, pneumonia, diarrhoea	Leaf and root decoctions drunk
Loganiaceae	*Strychnos potatorum* L. f.	Musisilombe	Tree	40.0, KC464	Syphilis	Infusions of leaves are drank
Loganiaceae	*Strychnos innocua* Delile	Mutimi, Muhuluhulu, Mwabo	Tree	20.0, KC216	Gonorrhoea, sore throat	Eat fruit pulp
Malvaceae	*Azanza garckeana* (F. Hoffm.) Exell & Hillc.	Makole, Munego	Tree	60.0, KC143	Malaria	Eat raw fruit, cook, and eat as relish
Malvaceae	*Sida alba* L.	Mulyangombe, Babani	Shrub	20.0, KC525	Gonorrhoea	Roots and leaves are boiled; decoction is drank
Malvaceae	*Hibiscus vitifolius* L.	Mubaluba	Herb		Chronic diarrhoea	Leaves are boiled and filtered through wire sieve and decoction is drank
Meliaceae	*Khaya nyasica* Stapf ex Baker f.	Mululu	Tree	43.3, KC113	Fever	Stem bark infusion is drank
Meliaceae	*Trichilia emetica* Vahl	Musikili	Tree	56.7, KC217	Fever, pneumonia; skin rashes	Root decoction is drunk; leaves rubbed onto skin

Table 1: Continued.

Family	Botanical name	Vernacular name	Growth form	Frequency index, voucher number	Diseases treated	Plant parts used, preparation, and mode of administration
Menispermaceae	*Cissampelos mucronata* A. Rich.	Itende	Climber	73.3, KC210	Syphilis, chancroid	Whole plant is cut into small pieces and boiled and decoction is drank after filtering
Moraceae	*Ficus capensis* Hort. Berol. ex Kunth & C. D. Bouché	Mukuyu	Tree	60.0, KC207	Diarrhoea, tuberculosis, skin sores, genital warts	Fresh leaves are boiled in water and decoction is drank or used to wash warts and skin sores
Moraceae	*Ficus sycomorus* L.	Mukuyu	Tree	76.7, KC360	Cough, tuberculosis, periodontitis, and oral candidiasis	Fresh leaves are boiled in water and decoction is drank or used to wash the mouth
Myrtaceae	*Syzygium cordatum* Hochst.	Katope	Tree	83.3, KC433	Diarrhoea	Stem bark decoction is drunk
Myrtaceae	*Syzygium guineense* DC.	Mutoya, Katope	Tree	83.3, KC481	Abscesses, skin rashes, diarrhoea	Fruits eaten raw; stem decoction applied to affected skin
Olacaceae	*Ximenia americana* L.	Munchovwa, Muchonfwa	Tree	83.3, KC318	Candidiasis, malaria, throat infection, tonsillitis, gonorrhoea, diarrhoea, skin rashes	Roots, leaves, and fruits are crushed while fresh and mixed with water overnight and taken orally
Pedaliaceae	*Sesamum angolense* Welw.	Bwengo	Herb	13.3, KC267	Skin rashes	Leaves are crushed and used as soap to bath skin
Plumbaginaceae	*Plumbago zeylanica* L.	Sikalutenta	Shrub	83.3, KC318	Generally treats all STI symptoms	Root infusion is drank
Ranunculaceae	*Clematis brachiata* Ker Gawl.	Kalatongo	Climber	20.0, KC354	Coughs, headache, fever	Leaves are boiled and drunk as a tea; sometimes honey is added as a sweetener
Rhamnaceae	*Berchemia discolor* (Klotzsch) Hemsl.	Mwiiyi, Mwinji	Tree	60.0, KC235	Cough	Use fruit in porridge
Rhamnaceae	*Ziziphus mauritiana* Lam.	Masawu, Masawu	Tree	40.0, KC226	Gonorrhoea, syphilis	Fruit eaten raw, apply to wound, and put into porridge
Rhamnaceae	*Ziziphus mucronata* Willd.	Muchechete, Mwichechete	Tree	83.3, KC144	Gonorrhoea, syphilis, boils, pneumonia, cough	Root or leaf infusion is drank
Sapotaceae	*Mimusops zeyheri* Sond.	Mukalanjoni	Tree	40.0, KC120	Oral candidiasis	Fruit is eaten or root infusion is used as a mouthwash
Tiliaceae	*Grewia flavescens* Juss.	Namulomo, Mukunyukunyu	Shrub	40.0, KC199	Diarrhoea	Eat fruit pulp to relieve diarrhoea
Tiliaceae	*Corchorus tridens* L.	Delele	Herb	13.3, KC101	Syphilitic ulcers, chancroid	Root infusion drank or applied to ulcers
Vitaceae	*Cissus quadrangularis* L.	Mulungalunga, Chamulungelunge	Climber	40.0, KC78	Malaria, gonorrhoea	Sap is drank; whole plant infusion is drank

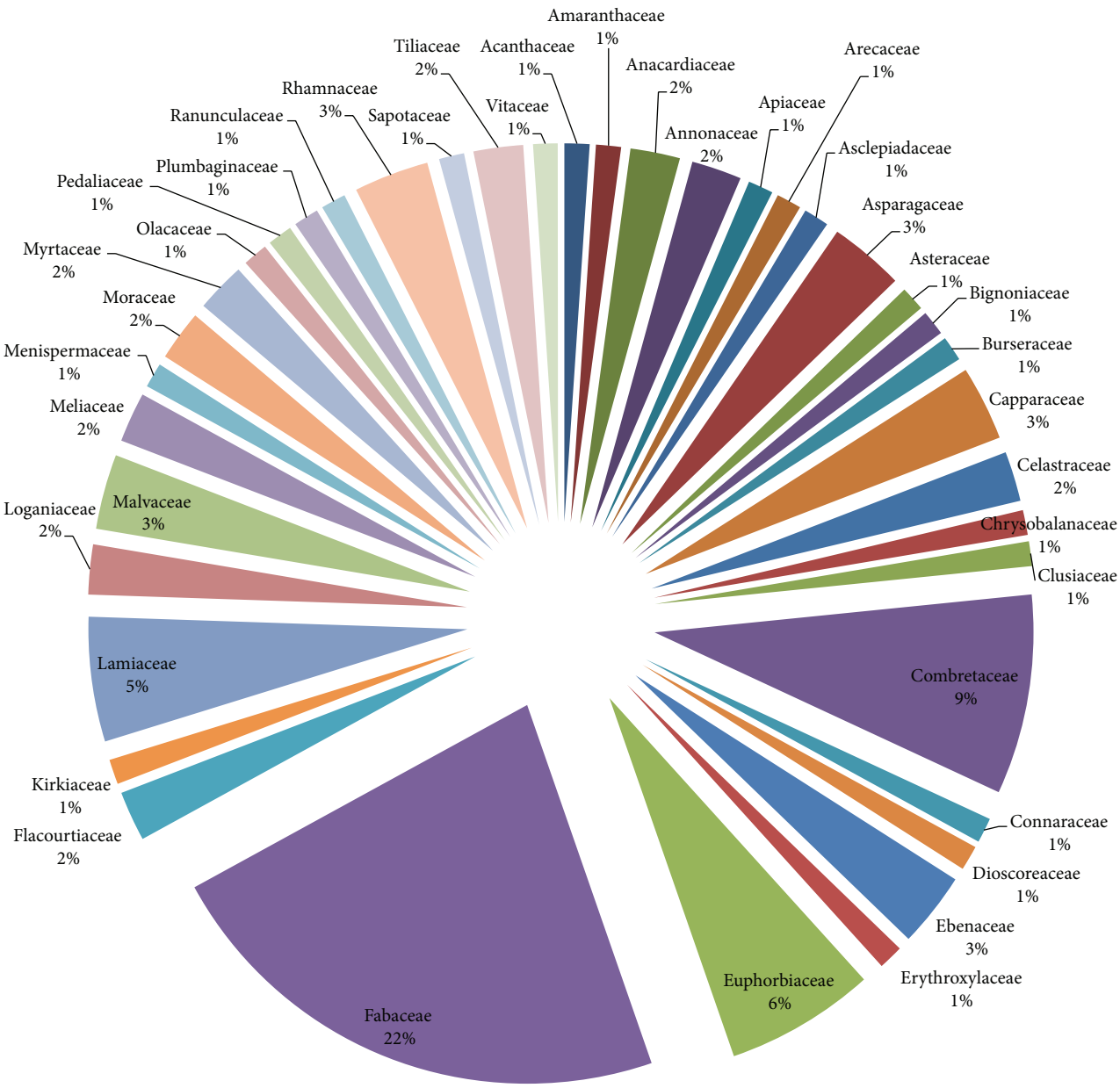

Figure 2: Percentage use of plant families.

Chinsembu [25] documented the use of *Asparagus* species in the treatment of eczema in South Africa and Namibia, respectively. *Friesodielsia obovata* was used to treat skin infections in the Zambezi Region of Namibia [26].

Capparis tomentosa is also used to treat skin rashes and herpes zoster in Katima Mulilo, Namibia [17]. Many *Acacia* species are also used to manage skin conditions in Southern Africa [27]. Kenyans use *Trichilia emetica* and *Syzygium guineense* to treat skin cancers [28]. Leaves of one of the fig trees, *Ficus capensis,* were also a remedy for skin sores. Skin diseases lie at the centre of both Christian and Islamic faiths. Indeed, the use of figs to treat skin diseases such as boils is well documented in the Bible; see 2 Kings 20:7 where a poultice of common figs (*Ficus* sp.) was applied to heal boils.

Lannea stuhlmannii was used to treat skin infections in Livingstone. The *Lannea* species were used to treat skin diseases in South Africa [27]. Chinsembu and Hedimbi [17] found that *Lannea zastrowina* was used as a remedy for skin rashes and herpes zoster in Katima Mulilo in Namibia. Elsewhere, *Lannea* species were known to have antibacterial [29] and antiviral [30] properties, making them good candidates for treating microbial skin infections.

The plant *Kigelia africana*, used in this study to manage boils, was also used in Ghana to treat skin ailments including fungal infections, boils, psoriasis and eczema, leprosy, syphilis, and cancer [31]. The plant is known to contain iridoids which confer antibacterial properties [32]. *Euclea divinorum* and *Ximenia americana,* skin remedies described

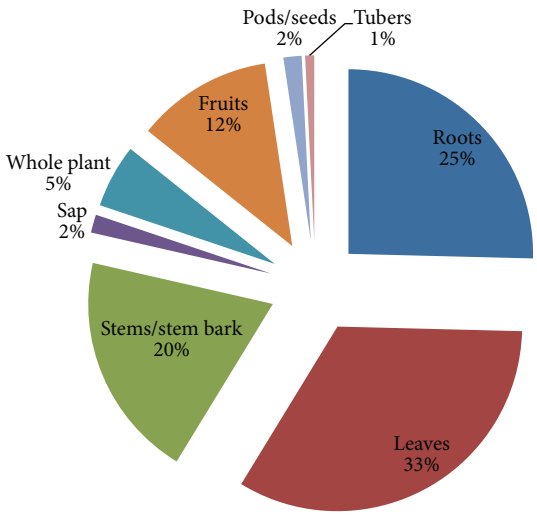

Figure 3: Percentage use of plant parts.

Table 2: Informant consensus factor (F_{IC}) for different ailments.

Ailment	Number of species	Number of citations	F_{IC}
Diarrhoea/dysentery	33	120	0.73
Skin infections	36	108	0.67
STIs	70	210	0.67
Malaria	15	77	0.82
TB/pneumonia	19	68	0.73
Oral infections	11	55	0.81
Cough	18	71	0.76
Fever/flu/colds/headache	10	45	0.80
Libido/erectile dysfunction	4	12	0.73
Meningitis	2	6	0.80
Weight loss	1	4	1.00
Cancer	1	4	1.00

in this study, were also documented as skin treatments by [27] in South Africa. Plants such as *Acacia, Kigelia africana,* and *Maytenus senegalensis* are used as ethnomedicine for skin infections [33].

Some of the plant taxa used to manage diarrhoea in this study have also been reported to treat diarrhoea in other studies. *Achyranthes aspera* L. is a known treatment for diarrhoea [34, 35]; *Asparagus racemosus* roots have been used traditionally in Ayurveda for the treatment of diarrhoea and dysentery [33]; *K. africana* is a known antidiarrhoeal remedy and *Parinari curatellifolia* attenuates diarrhoea [36, 37].

Oncoba spinosa is an antidote for diarrhoea in Ethiopia [38]. Studies in Nigeria showed that extracts of *Acacia nilotica* produced comparable antidiarrhoeal activity similar to loperamide, a drug widely employed against diarrhoeal disorders [39]. *Garcinia livingstonei* was a remedy for diarrhoea in KwaZulu-Natal Province, South Africa [40]; a decoction from the roots *Combretum collinum* was drunk for the treatment of diarrhoea [41]; *Bridelia cathartica*, *Flacourtia indica,* and *Kirkia acuminata* are prescriptions for diarrhoea in Zimbabwe [42, 43].

Members of the genus *Grewia* were used as a remedy for diarrhoea in Katima Mulilo [17] and *Mimosa pigra* was harvested to treat diarrhoea in Rundu [18]. Rakotomalala et al. [44] showed that *M. pigra* is rich in tryptophan, quercetin, and several phenolic compounds which confer antioxidant and anti-inflammatory properties. *Dalbergia melanoxylon* has antidiarrhoeal effects [45].

Studies in Tanzania found that *Indigofera colutea* has antimicrobial activities and hence can be used to manage diarrhoea. *Ficus capensis* has vibriocidal and antiamoebic actions [46] and therefore is used to treat diarrhoea in Lubumbashi in the neighbouring Democratic Republic of the Congo [47]. An anti-inflammatory bioflavonoid, gossypin, is found in *Hibiscus vitifolius*, a good remedy for diarrhoea [48]. *Syzygium cordatum* Hochst, due to its antibacterial properties, is an antidiarrhoeal remedy in Swaziland [49].

Many plants used to treat STIs in Livingstone were also used to manage STIs in Sesheke District, Zambia [18]. This is because inhabitants of both Livingstone and Sesheke mainly belong to the Lozi ethnic group. Therefore, they tap into a similar ancestral vein of indigenous knowledge. For example, in both Livingstone and Sesheke, gonorrhoea was treated with a couple of species of the genera *Lannea, Combretum*, *Terminalia*, *Diospyros, Ximenia,* and *Ziziphus*.

The Lozi people of Sesheke used 52 plant species in 25 families and 43 genera to treat gonorrhoea, syphilis, chancroid, chlamydia, genital herpes, and anogenital warts. STIs were frequently managed using the following plants: *Terminalia sericea*, *Strychnos cocculoides*, *Ximenia caffra*, *Cassia abbreviata*, *Cassia occidentalis*, *Combretum hereroense*, *Combretum imberbe*, *Dichrostachys cinerea*, *Boscia albitrunca*, *Momordica balsamina,* and *Peltophorum africanum* [18].

Ziziphus mauritiana, also known as Masau in Nyanja, is a wild fruit plant very rich in vitamin C. It contains 20 to 30% sugar, up to 2.5% protein, and 12.8% carbohydrates. The plant is a remedy for STIs because aqueous extracts and powders have broad-spectrum antibacterial activity. Its extracts are also used as a dressing to prevent bacterial infections and to aid in wound healing during male circumcision among the Lunda and Luvale people of Zambia [50]. The anti-HIV plant *Ximenia americana* contains oleic, hexacos-17-enoic (ximenic), linoleic, linolenic, and stearic acids. Its oil consists of very long chain fatty acids with up to 40 carbon atoms. *X. americana* is also used to manage STIs including gonorrhoea in Western Province, Zambia [18].

Euclea divinorum, a treatment for gonorrhoea in Livingstone, had antibacterial action with minimum inhibitory concentration values ranging from 25.0 mg/mL to 0.8 mg/mL and moderate cytotoxicity [51]. *Ximenia americana* and *Croton megalobotrys*, known as Mtswanza and Muchape (resp.) among the Kore-kore people of Chiawa District in Zambia, are also prepared as formulations for gonorrhoea [52].

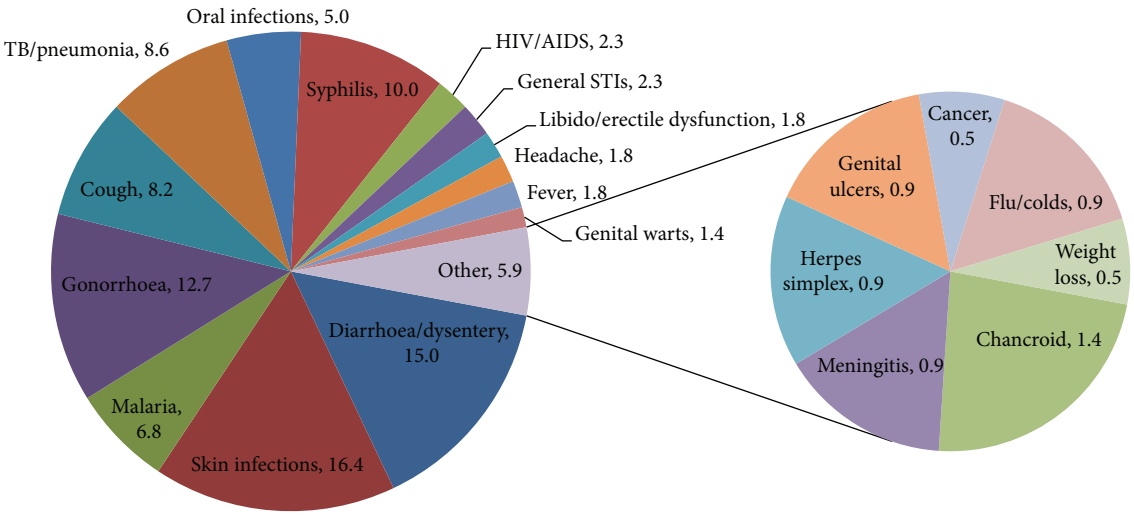

Figure 4: Proportions of plants used to treat different disease conditions.

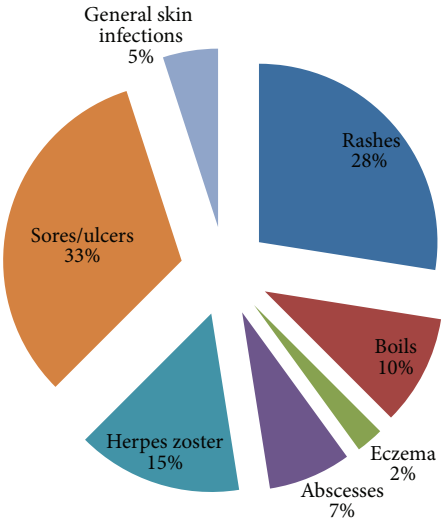

Figure 5: Percentage distribution of plants used to treat various skin conditions.

Many species of *Acacia* are used to treat TB and pneumonia, owing to their antibacterial and anti-HIV activities [53, 54]. *Acacia nilotica* leaf, bark and root ethanol, or ethyl acetate extracts were active against *Mycobacterium aurum*, MIC = 0.195–1.56 mg/mL [55]. *Combretum imberbe* contains pentacyclic triterpenes, with MIC = 1.56–25 μg/mL against *Mycobacterium fortuitum* [55]. *Maytenus senegalensis* is a known anti-HIV and antimycobacterial treatment in Uganda and Tanzania [56, 57]. A *Cleome* species was used to treat TB in Livingstone. In South Africa, Hurinanthan [58] found that *Cleome monophylla* leaf extract had anti-HIV-1 reverse transcriptase activity. *Cleome gynandra* is a treatment for chancroid in Sesheke and a remedy for malaria in other parts of Zambia [17, 18].

Studies show that HIV/AIDS is associated with low libido in men, sometimes because of depression and poor moods [59, 60]. Men on ART were also associated with sexual dysfunction [61]. Unsurprisingly, loss of libido and erectile dysfunction in men were commonly associated with HIV infection in Livingstone. HIV-infected men suffering from loss of libido and erectile dysfunction often used herbs to restore their sexual prowess. *Mucuna pruriens*, a plant with antibacterial activity [62], is also known to improve fertility, sexual behaviour, and erectile function in animals [63–65]. Extracts of the plant *Hoslundia opposita* corrected erectile dysfunction in Livingstone men living with HIV infection and were also commonly used to manage noninsulin dependent diabetes mellitus in Tanzania [66]. Erectile dysfunction and loss of libido are common in men with diabetes [67].

5. Conclusions

In Livingstone, Southern Province, Zambia, traditional healers and other knowledge holders use 94 medicinal plant species to manage HIV/AIDS-related diseases mainly skin infections, diarrhoea, STIs, TB, cough, malaria, and oral infections. Majority of the plants belonged to the families Fabaceae and Combretaceae. Most plant leaves and roots were utilized to treat two or more disease conditions related to HIV infection. These multiuse medicinal plants probably contain broad-spectrum antimicrobial agents but may also face the threats of overharvesting, thus requiring special regulations for their genetic conservation.

The indigenous knowledge of medicinal plants is quite consistent especially for managing common HIV/AIDS-related conditions such as malaria, oral infections, fever, flu, colds, and headache. Although the results of this study are consistent with ethnobotanical and antimicrobial data from many reports in the literature, further studies are needed to confirm the antimicrobial efficacies, pharmacological, cytotoxicity, and active chemical ingredients of the plants.

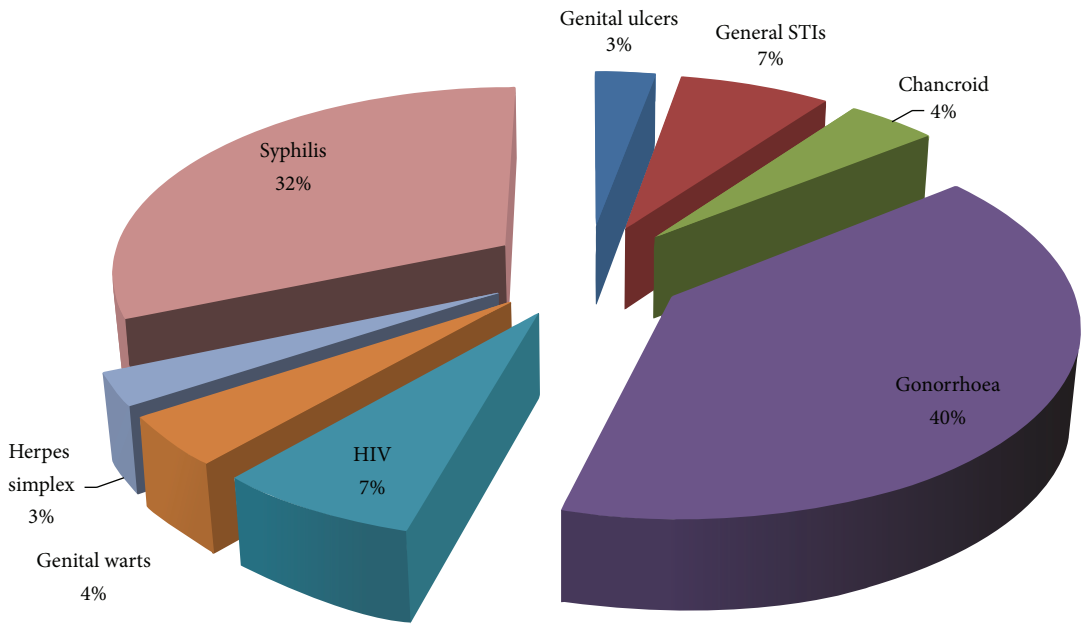

Figure 6: Percentage distribution of plants used to treat various STIs.

Competing Interests

The author has no conflict of interests or competing interests to declare. Professor Chinsembu is the chair of the steering committee on the scientific validation of plants for HIV/AIDS treatment in Namibia.

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Research Article

The Status of Ethnobotanical Knowledge of Medicinal Plants and the Impacts of Resettlement in Delanta, Northwestern Wello, Northern Ethiopia

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The present study was conducted in Delanta (Ethiopia) to examine the use of medicinal plants and investigate the impacts of the 1984/85 resettlement program on the local people's knowledge on herbal medicine and its uses. The research was conducted with 72 informants in six study sites through semistructured interviews, group discussion, and market survey. In this study, 133 species belonging to 116 genera and 57 families were documented. These plants were mentioned for uses in the treatment of about 76 human and livestock ailments. The family Asteraceae was represented by the highest number with 14 species. Herbs accounted for 52.6% of the total species and leaves (32.6%) were the most frequently used parts. The analysis showed that the resettlement program has both positive and negative impacts on nature rehabilitation and local knowledge along with many human induced threats. Most of the plant knowledge is held by traditional healers and permanent residents. The people's preference for some medicinal plants gave indications of continuity of the ethnomedicinal information among the inhabitants. The findings inform that efforts need to be directed to in situ conservation in two of the plant community types which could protect a good proportion (about 50%) of the medicinal plant species.

1. Introduction

The concept of ethnobotanical knowledge has originated from local people, which has the potential to redress some of the shortcomings of contemporary Western knowledge [1, 2]. It is passed down from generation to generation and closely interwoven with people's cultural values [3]. Traditional societies throughout the world hold a wealth of such knowledge which they have built up during prolonged interactions with the natural world and which remains fundamental to their physical, spiritual, and social interests [4, 5]. While plants can provide multiple uses, the traditional curative practice of health problem is among the most important ones for peoples' lives [6–8] and it is also one of the sources of modern health treatment [9]. Since time immemorial, traditional medicinal plants (TMPs) have been used in virtually all cultures for treatment of most human and livestock ailments [3, 10]. The uses of plant species as TMPs represent by far the biggest human use in terms of number of species of the natural world [11]. It is estimated that 70–80% of people worldwide [12] and 80% of the people of Ethiopia rely chiefly on traditional herbal medicines to meet their primary healthcare needs. The ways to combat diseases through TMPs are also as diverse as the different cultures [13–15]. The natural plant world is thus full of potential for new drug discovery. There are undoubtedly many more secrets still hidden in the world of plants [16]. These resources are found in locally available plants and they benefit from local knowledge (LK) that is simple to use and affordable. Reasonable support for TMPs will not only help bridge some of the gaps between the demand for and supply of modern pharmaceuticals, but also widen healthcare alternatives for posterity [4, 15].

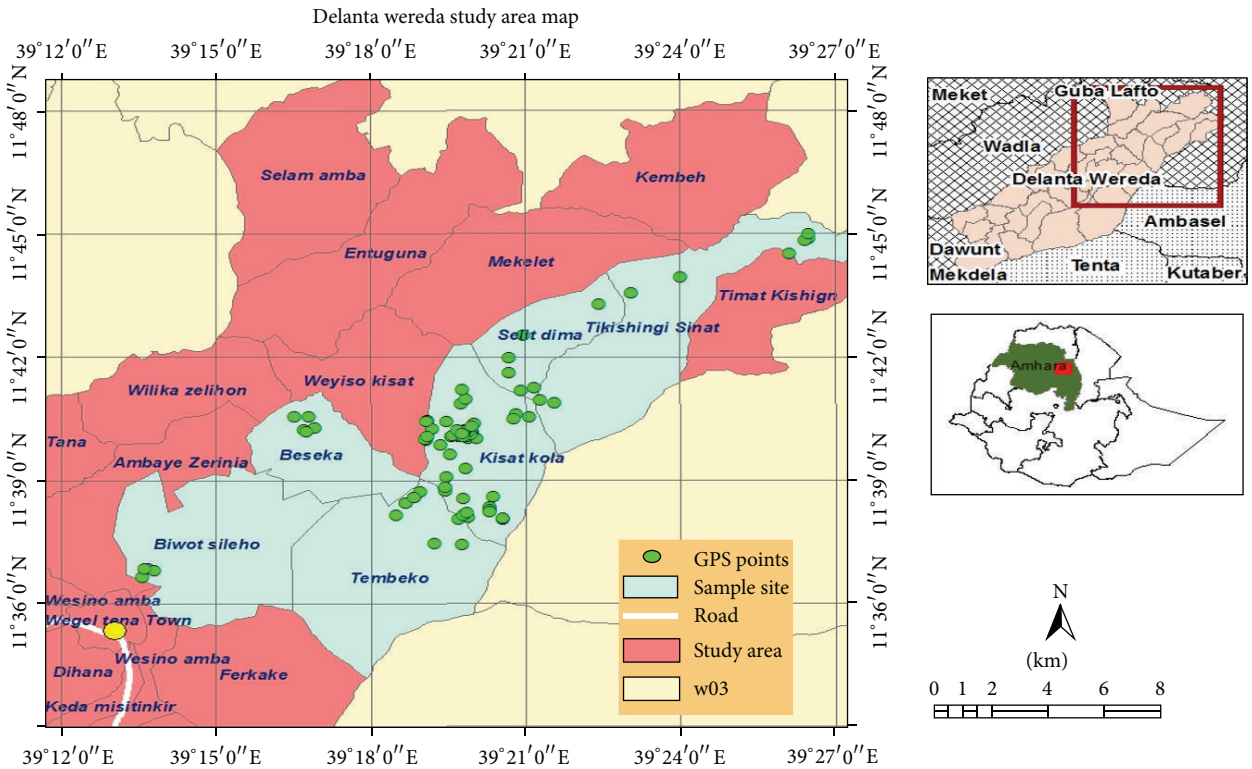

Figure 1: Map of Ethiopia showing the location of Delanta district and the study sites.

The most serious dilemma facing Delanta was recurrent droughts [17, 18] that often led to famines being usually accompanied by epidemics of different diseases [19]. Resettlement of local communities, acculturation, and inadequate understanding of the potentials of TMPs and the associated LK to the present and future generations have led to a decline in sustainable use of these biological resources [14, 20]. Natural and anthropogenic causes of wild vegetation loss and transformation of cultures further exacerbate the situation in most parts of Ethiopia including Delanta. Hence, promoting the cultures and the LK are vital for halting the loss, shaping and conserving the floristic diversity. Notably, attempts to respond to healthcare issues lead to ethnobotanical documentation on TMPs [9]. However, as the ethnobotanical information is not documented and remains in the memory of elderly practitioners and end users, the knowledge base continues to be threatened [21, 22]. Adequate information on the TMPs of Ethiopia could only be obtained when studies are undertaken in the various parts of the country where no ethnobotanical explorations have been made [23].

Maintenance of the balance between conservation and human needs has always been a complex matter. Environmental resettlement programs have been positive with respect to alleviating the problems of recurrent food insecurity and enhance restoration of useful wild plants in the original places from where people were moved out. This, however, requires careful evaluation of people's attitudes and perceptions of LK [24, 25]. Resettlement took place in Delanta at different times [17, 18, 26, 27] and the resettlement is still continuing. More floras, however, were lost by drought, further aggravated by returning of thousands of resettlers; recovery efforts have been even more difficult [19]. As a plant species is lost from a locality, the information contained in it will also be slowly blurred and finally become lost forever. Urgent ethnobotanical studies and subsequent conservation measures are, therefore, required to salvage these resources from further loss. Thus, the main objective of this study was to document the availability of plants which have been used as remedy to combat ailments and the LK on use of these resources together with the impacts of the 1984/85 resettlement as so far no such study has been conducted in Delanta.

2. Material and Methods

2.1. Description of the Study Area and Climate. Delanta district is located at 530 km north of Addis Ababa in northwestern Wello. The major town, Wegeltena, is situated at 11°35′N latitude and 39°13′E longitude with an elevation of 2555 m (Figure 1). The district has an estimated total population of 128, 416 with an area of 1060.17 square kilometers. The largest ethnic group is the Amhara (99.96%) and Amharic is spoken as a first language [[28], Delanta District Agricultural and Rural Development Office, unpublished annual report of 2011]. The Health Centre of Delanta District has eight health stations and 32 health posts. However, access to modern health services is very limited in both personnel and equipment availability. Therefore, as Abebe et al. [13] indicated, a better alternative for the majority is to use the traditional

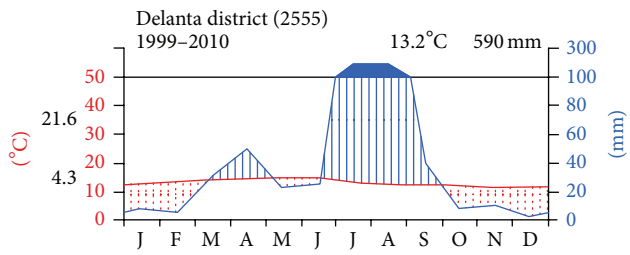

Figure 2: Climadiagram of the study area from the years 1999 to 2010 (data source: National Meteorological Service Agency of Ethiopia, Kombolicha Branch Directorate, 2011).

herbal remedies. The statistical data of the Delanta District Health Centre [unpublished annual report of 2011] shows that, among 20 types of diseases, the most frequently occurring are helminthiasis, lower and upper respiratory tract infections (LRTI and URTI), and arthritis.

The study area receives bimodal rainfall. The major peak is important for crop production when the annual rainfall is received from mid-June to the beginning of September. The climadiagram in Figure 2 illustrates that mean annual rainfall and the temperature are 590 mm and 13.2°C, respectively. As the climadiagram and some authors [14, 29] indicate, the natural vegetation of the study area largely falls under the dry ever green montane forest occurring in the altitudinal ranges of 1,500 to 3,200 m. However, the intense deforestation of the highlands led to massive loss of forest cover. One of the main contributing factors to the weakness of environmental protection and its proneness to drought and famine has been the deterioration of the natural vegetation.

The district is divided into four agroecological zones. Farmers depend on both BELG and MEHER rainfall. Although these two production systems vary in source of income along with elevation, crop production is the largest source of income, followed by livestock and off-farm sources in the district [18, 28]. With regard to geology of Delanta, very stable and excellent qualities of Ethiopian opals are found in a specific alternating layer of basalt and rhyolitic ignimbrite thick volcanic series [30]. However, fatal accidents and loss of biodiversity (wild useful plants) caused by collapsing rocks or falls from cliffs have been reported and observed. Thus, the important solution to balance the mining benefits and the loss of biodiversity could be replantation of trees in the mine areas.

2.2. Ethnobotanical Methodology. Ethnobotanical information on the traditional use and management of TMPs was collected through participatory rural appraisal involving semistructured interviews and focus group discussions. All the discussions and interviews were conducted in Amharic language. The impact of the 1984/85 resettlement on vegetation rehabilitation was examined with guided field walk. Market survey was integral part of this research. Purposive sampling method was used and six representative sites (*kebeles*) (kebeles are the smallest administrative division next to district) consisting of 38 villages were selected within the district. Most of the sites were in the middle agroecological zones of the eastern part where the 1984/85 famine was especially worse [18, 26]. In total, 72 informants aged 20 to 88 years (55 males and 17 females) were selected, of whom 18 key informants were selected by purposive sampling based on recommendations of elders and local authorities from every six study sites with equal numbers.

Plant specimens were collected with local names and GPS data, pressed, dried, and brought to the National Herbarium (ETH), Addis Ababa University, for final determinations and confirmation using taxonomic keys in the flora of Ethiopia and Eritrea, comparison with authentic specimens, and expert assistance. The voucher specimens with labels were then deposited at the ETH. The vegetation of the study area was described using both the emic and etic categorization methods. The dominant or associated codominant species gave etic plant community types and the emic categories followed Martin's system of emic vegetation classification [1], which relied on the way the people perceived plants and gave names in Amharic language.

The collected data were analyzed using descriptive statistics to evaluate the percentage and frequency of different aspects of TMPs. Preference ranking was conducted by using nine randomly selected key informants to rank five TMPs' use against febrile illness and the degree of scarcity of other five TMP species. In paired comparison, nine key informants were asked to choose the top five TMPs used to treat stomachache based on their medicinal values. The number of pairs for each was calculated by applying the formula: Number of pairs $= n(n-1)/2$, where n is the number of items. The total number of items was obtained by summing up the total scores obtained. Direct matrix ranking was used to compare seven multipurpose TMPs and six principal threats using nine key informants following Cotton [5]. Informant consensus factors were calculated for each ailment category to identify the agreements of informants on the reported cures using the following relationship: ICF = (Nur − Nt)/(Nur − 1), where Nur is the number of use reports from informants for a particular plant use category and Nt is the number of taxa that are used for plant use category for all informants. Eight categories were identified based on local explanation. ICF value ranges from 0 to 1. Abundance score was conducted to reflect the trends of indicator species in homegardens and wild vegetation during periods from the 1970s to 2011. The informants were asked to score the availability of an indicator species of TMPs. The scores ranged

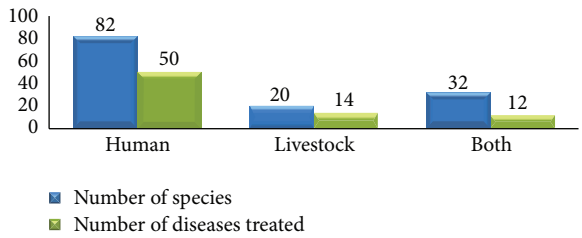

Figure 3: Proportion of treatment of human, livestock, and both human and livestock ailments.

from 0 to 2 where 0 reflected none or nearly none; 1 a few or some; 2 many or readily available following Nanyunja [20].

3. Results

3.1. Diversity and Growth Habits of TMPs. In total, 133 TMP species (including two vascular seedless plant taxa) distributed into 116 genera and 57 families were documented. Those species were collected from different habitats, notably wild vegetation that could be enclosed and unenclosed, farmlands, and homegardens. The growth form analysis of total TMPs indicated that the most widely used plant remedies are obtained from herbs (71 species, 53.4%), followed by shrubs (48 species, 36.1%) and trees (14 species, 10.5%). Of the total 57 families, Asteraceae and Lamiaceae were found to be represented by the highest number of species (14 and 12, resp.), followed by Euphorbiaceae and Solanaceae with eight species each. Three families had four to five species, another five families had three species each, 16 families had two species each, and the remaining 29 families were represented by one species each (see Appendix 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/5060247).

TMPs Used for Treating Human and Livestock Ailments. Of the reported 133 TMP species, 82 (61.7%) species representing 72 genera and 44 families were reported as medicine for treating human ailments (Figure 3). Generally, all the TMPs were used for the treatment of about 76 ailments of human and livestock (Table 1).

With regard to plant parts, leaves were the most frequently used parts (32.6%) used to treat various ailments, followed by roots (21.7%), and further details are given in Table 2. Most frequently used preparation methods of TMPs in the area are chopping, crushing, and pounding, which account for the highest proportion (38.3%), followed by grinding in powder form (20.0%); roasting, boiling, and cooking (11.3%); rubbing and unprocessing (11.3%); squeezing (10.8%); and chewing and absorbing (8.3%). Once herbal medicaments were prepared, dosages were estimated using different locally available materials. Doses of liquid remedies were estimated by using plastic cups or glasses (could be coffee-cup, tea-cup, or water-cup) or gourd utensils, lid of rubber, or number of drops. Spoons (teaspoon) for powders, counting numbers for seeds and fruits, and index finger size for roots are some of the traditional tools of estimation.

After estimating the doses, different routes of administration were used. Oral route (43.9%) was being the most common route of administration, followed by dermal route (28.7%) as shown in Table 2.

In Delanta, no side effects were reported by informants except some species like *Calotropis procera*, *Euphorbia* spp., and *Lobelia rhynchopetalum*. The white latex of these species was used for treating different ailments, which was reported as noxious for humans if not properly handled. *Hagenia abyssinica* and *Silene macrosolen* were said to result in vomiting and diarrhea and if the risk is diagnosed, patients were given SHIRO WOT (sauce made of pulse grains) and powder of *Linum usitatissimum* infusion in water to reduce the pain. *Cucumis ficifolius* and *Phytolacca dodecandra* were said to have similar counter indications unless proper care is taken in dosage determination. Milk products, salt, honey, coffee, traditional ale, food, and water are some additives used by healers when preparing remedies to improve the taste and ointments of remedies. Most of the plant remedies were employed in fresh forms (54.4%) followed by dried (26.5%) and both fresh and dried forms (19.1%). The local people stored dried remedies for future use only for some diseases. The majority of prepared remedies were applied through eating, sucking, decanting, and inhaling (internal application, 67%), followed by creaming and tying (external application, 33%).

Consensus on Use, Popularity, and Importance of TMPs. The top ten TMPs were known by more than one-fourth of the informants. *Ocimum lamiifolium* is the most popular, cited by 66 informants (91.7%) for its medicinal value, followed by *Zehneria scabra* with 61 (84.7%), and others are given in Table 3.

Febrile illness was the third common disease of both human and livestock in the district health office. Preference ranking of five TMPs was reported as effective for treating febrile illness. Table 4 shows that *Ocimum lamiifolium* and *Zehneria scabra* came in the first and second ranks.

Paired comparison was made among five TMPs that were identified by the informants to be used in treating stomachache (Table 5), which was the 2nd common human disease according to data from the district health office. The results showed that *Schinus molle* and *Verbena officinalis* ranked first and second.

Informant Consensus Factor (ICF). Diseases that were found to be prevalent in the area were treated by a variety of TMPs. The highest ICF value (0.91) has been shown from the categories of respiratory disease, febrile illness, and throat

Table 1: TMPs used against human and livestock ailments and detailed information on methods of preparation. Description of data: Uf.: used for (L: livestock; H: human; Hl.: both), Cp.: condition of preparation (F: fresh; D: dried; and Fd.: fresh & dried), Ra.: route of administration (D: dermal, Op.: optical, N: nasal, O: oral, A: anal, and V: vaginal), and Pu.: plant parts used (R: root, S: stem, Sb.: stem bark, Rh.: rhizome, L: leaf, F: fruit, Fl.: flower, Se.: seed, B: bulbs, La.: latex, W: whole parts, and Ag.: abovegroufnd part).

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Achyranthes aspera	L	H	Conjunctivitis	Op.	F	Pounded and squeezed leaf juice is filtered with cotton cloth and dropped into the eye
	L	H	Impetigo (*Kunchir*)	D	F	Crushed leaf is bandaged on the wound
	R	H	Hemorrhage at birth	O	F	Pounded and squeezed leaf is filtered with water to be drunk in a half size of water cup
	R	Hl.	Bleeding of any part	D	F	It is crushed and then dressed on the bleeding part of livestock and human
Aeonium leucoblepharum	L	H	Emergency (trauma)	D	F	Crushed leaf juice is creamed on head and face or any parts that felt sickness
Ajuga integrifolia	L	H	Tonsillitis	O	F	Pounded and squeezed leaf juice is drunk with a coffee glass
Allium cepa	B	L	Leech infestation	N	Fd.	It is crushed and mixed with water and poured into the nose in both openings
	B	H	Leech infestation	O	Fd.	It is crushed and mixed with water and then poured orally
Allium sativum	B	H	Influenza virus	N	F	The bulb is peeled and the aroma sniffed
	B	H	Dry cough	O	Fd.	The bulb is chopped and mixed with ground seed of *Guizotia abyssinica* and *Nigella sativa* powder form of *Thymus schimperi* and crushed *Zingiber officinale* is drunk continuously
	B	H	Evil eye	D/N	F	Its bulb with the roots of *Withania somnifera, Lobelia giberroa, Sida schimperiana, Carissa spinarum, Dodonaea angustifolia, Verbena officinalis, Capparis tomentosa, Croton macrostachyus, Verbasicum siniaticum, Jasminum grandiflorum, Ceratostigma abyssinicum, Clerodendrum myricoides, Ferula communis, Cyphostemma adenocaule*, and *Cucumis ficifolius* and whole parts of *Artemisia afra, Ruta chalepensis*, and *Lepidium sativum* are crushed and the smoke on the burning firewood is sniffed; the powder form is tied on the neck
	B	H	Heartburn (*Tkusat*)	O	F	Chopped bulb is boiled in water and then the decoction is drunk in a tea glass
Aloe camperi	Fl.	H	Idiopathy (*likift*)	D	F	It is cooked with chopped root of *Impatiens rothii* and flower of *Buddleja polystachya* and then the irritated feet and fingers are immersed in the cooker for three consecutive days
	L		Evil spirit at birth	N	D	It is placed on burning dung with *Otostegia integrifolia* and fumigated the house and is inhaled
Aloe pulcherrima	La.	Hl.	Wound (irritation)	D	F	It is pasted the latex on the wounded part of livestock and human
	R	L	Rh factor/disease	O	D	It is pounded with the root of *Eleusine floccifolia*, *Asparagus africanus, Verbscum sinaiticum, Trajia cinerea, Ferula communis*, bark of *Myrica salicifolia,* and seed of *Sesamum orientale* with pestle and mortar and eaten with Injera for three consecutive days at 9th pregnancy month
	R	H	Rh factor/disease	O	D	It is powdered with *Asparagus africanus* and *Sesamum orientale* and then two teaspoons are taken with the same size infusion of honey for three days at ninth month of pregnancy period
Artemisia abyssinica	L	H	Common cold	N	F	The aroma of leaf is inhaled by inserting half part of it into nose
	S	H	Trachoma	Op.	F	The infected outer part of the eye is heated with roasted portion of stem
	W	H	Evil eye	O/D	Fd.	It is kept in pocket as tooth brush; the powder is tied with others like *A. sativum*
Arundo donax	Rh.	H	Birth control	O	D	Chopped and pounded rhizome is mixed with root of *Verbena officinalis*, powder form is homogenized in water, and one coffee glass is drunk once in the morning before breakfast

Table 1: Continued.

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Asparagus africanus	Ag.	Hl.	Swelling (*Agjil*)	D	F	It is crushed and homogenized in water for washing the swelling by saying "Betin"
	R	Hl.	Rh factor/disease	O	D	It is pounded with the root of other species like *A. pulcherrima*
	Ag.	H	Circumcision	D	F	The young aboveground part is crushed and the injury is applied as cream with yolk of egg
Bersama abyssinica	L	H	Ascariasis	O	F	It is boiled in water with seed of *Vicia faba* for drinking the decoction; the cooked bean is eaten
Brassica nigra	Se.	L	Bloating	O	D	It is ground and homogenized in residue of traditional ale with *Lepidium sativum* and decanted
	Se.	H	Abortion of unborn child (*Atnt Kirit*)	O	D	It is ground and homogenized in a cup of water and then drunk by pregnant woman
Buddleja polystachya	F	H	Idiopathy	D	F	Its flowers are cooked with crushed root of *Impatiens rothii*, same as *A. camperi*
Caesalpinia decapetala	L	H	Tinea favus (*Lashign*)	D	D	Crushed and powdered leaf is mixed with butter and then the infected head part is creamed
Calotropis procera	La.	H	Hemorrhoids/tumor (swollen anal veins)	A	F	Its latex is used alone or mixed with *Euphorbia polycantha, Ficus palmata, Aloe pulcherrima, Lobelia giberroa*,and squeezed leaf of *Clematis semensis* and is creamed
	La.	H	Piercing by sharper	D	F	The latex is applied as cream on part pierced by sharpen material with the help of needle
	L	H	Boils (furuncle)	D	D	Crushed and powdered leaf is applied as cream with latex of *A. pulcherrima* on inflamed part
Calpurnia aurea	L	L	Body lice (*kijamr*)	D	F	Its leaf is crushed and stirred in water to wash cattle or calves until removal of lice
	Se.	H	Giardiasis and amoebiasis	O	D	The ground seed is mixed with honey and eaten with three-liter rubber lid size in the morning until recovery, before taking other diets
Capparis tomentosa	R/Sb.	H	Evil spirit (sickness)	N	D	The root or stem bark is chopped and placed on the fire and the smoke is inhaled
	R/Sb.	H	Epidemic	N	D	The same methods are used to treat evil spirit and hang the remainder on the roof
Capsicum annuum	F	H	Appetite loss	O	F	The pod is chopped and mixed with freshly *A. sativum* bulb in small amount of water in the bowls eaten with Injera
	F	H	Malaria (revival)	O	F	The same method is used to treat loss of appetite, prior to taking other diets in the morning
Carduus schimperi	R	H	Febrile illness	O/D	Fd.	It is pounded and squeezed by adding water and is taken by a cup of tea and the remnant is creamed
	R	L	Febrile illness	O/D	Fd.	It is pounded by adding water and is decanted by a cup of water and the remnant is pasted
	R	L	Dysentery (*Mentie*)	D	D	The room of sheep or goats is fumigated and the remnants are placed there against "Mentie"
Carissa spinarum	L	Hl.	Snake bite	O	F	The liquid is chewed and absorbed soon and for animals its crushed leaf is decanted
	L	H	Rh factor/disease	O	F	It is crushed and soaked in water for one day and drunk in one cup of coffee prior to 9th month of pregnancy period
	R	H	Evil eye	D/N	Fd.	It is the same as used in *A. sativum*
Carthamus lanatus	L	H	Sexual impotency in men	O	Fd.	It is crushed and pounded with the whole parts of *Trajia cinerea* and root of *Hibiscus eriospermus,* then stirred in local beer, and drunk in one cup of coffee until recovery
Catha edulis	L	H	Diuretic	O	F	It is boiled in the pot with water at night and the decoction is drunk in the morning
Ceratostigma abyssinicum	R	H	Evil eye	D/N	D	It is the same as used in *A. sativum*; the powder is tied in the neck with Abesha cloth and the remnant is inhaled through nose
Chenopodium schraderianum	Ag.	L	Coccoides (chicken lice)	D/N	D	Aboveground parts are chopped and the room of chickens is fumigated and the chickens themselves sniffed smokes nasally

Table 1: Continued.

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Cistanche tubulosa	W	H	Fire accident	D	D	The whole parts are crushed and powdered and then the burnt part is creamed with butter
Citrus aurantifolia (Christm.) Swingle	F	H	Dandruff	D	F	The acetic fruit is pierced and squeezed and the head is creamed with yolk of egg
	F	H	Ringworm (*Agogot*)	D	F	It is pierced and squeezed; then the affected parts are creamed alone specially faces
Clematis simensis	L	H	Impetigo (*Kunchir*)	D	F	The leaf is crushed and squeezed and the affected part is creamed with powder of *Triticum* spp.
Clerodendrum myricoides	R	H	Evil eye	D/N	D	It is the same as used in *A. sativum*; the powder is tied in the neck with Abesha cloth and the remnant is inhaled through nose
Clutia abyssinica	L	L	Leech infestation	N	F	The crushed leaf is soaked in water and decanted into cattle through their nose
Colutea abyssinica	S	H	Toothache	D	F	Affected tooth is heated by chopped and roasted stem without contact with other parts
	R	H	Emergency (*Berar*)	O	Fd.	It is crushed and pounded with the root of *Inula confertiflora* and the concoction is drunk with coffee; the roots are dug by hoes with stick of *Olea europaea* ssp. *cuspidata* on Friday
Combretum molle	L	H	Malaria	O	F	Fresh leaf is boiled in water and the decoction is drunk in a cup of tea
Conyza hypoleuca	R	L	Emaciation	O	Fd.	Its root is crushed together with root of *Inula confertiflora, Echnops hispidus*, and the leaf of *Osyris quadripartita*, *Solanecio gigas*, and whole *Leucas martinicensis* in new *Lagenaria siceraria* as a container with water for two to three days and the concoction is decanted
Coriandrum sativum	Se.	H	Stomachache (*Alsha*)	O	D	The raw seed is eaten with small amount of ground salt by the patient
Croton macrostachyus	Sb.	H	Ascariasis	O	F	It is chopped and boiled in water with seed of *Vicia faba* and the decoction is drunk and the boiled beans are eaten
	R	H	Evil spirit (Sickness)	N	D	It is chopped and placed on the fire with *C. tomentosa* and *Withania somnifera* and inhaled
	R	H	Evil eye	D	D	It is the same as used in *A. sativum*; crushed and powdered root is tied on the neck
	L	L	Poison insects on Sorghum (*Akara*)	O	F	It is crushed alone or with root of *Cyphostemma adenocaula* and *String hermonthica* is soaked in ale and decanted when cattle ate the leaf of *Sorghum* that contained poisoned insects
Cucumis ficifolia	R	H	Removal of retained placenta (*Sing*)	O	F	Freshly washed root is chewed and absorbed by woman
	L	Hl.	Eye injury (inserted materials or hit)	Op.	F	It is crushed and squeezed with the leaf of *Jasminum grandiflorum* by adding small amount of water and dropped to the affected eye and then applied as cream butter for 3 days at night and washed with water in the morning prior to exposure to sunlight
	R	H	Birth control	O	F	It is chewed and absorbed with ripe fruit of *Solanum anguivi* before sexual intercourse at the end of menstrual cycle
	R	H	Gonorrhea (*Chebt*)	O	D	In powder form, one teaspoon is infused into doro wot and then eaten by Injera
	R	Hl.	Rabies virus	O	D	Powdered root is mixed with cheese in one cup of tea and then drunk
	R	H	Jaundice (*Yewuf*)	O	D	Powdered root is mixed with cheese in one tea glass and then drunk
Cucurbita pepo	F	H	Gastritis (*Cheguara*)	O	F	Peeled fruit is boiled at night and eaten in the morning before taking other diets
	L	H	Dandruff (*Forefor*)	D	F	The head is rubbed and creamed alone or with the leaf of *Datura stramonium*
	F	H	Stomachache (*kurtet*)	O	F	Peeled and boiled fruit is eaten before taking other diets soon by mother at birth

Table 1: Continued.

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Cyathula uncinulata	L	L	Leech infestation	N	F	Crushed leaf is mixed with small amount of water is poured to the cattle via their nose
	R	H	Tapeworm (*Kosso*)	O	Fd.	Crushed and pounded root is eaten with ale/beer of *Hordeum vulgare* before filtration with water, one cup of coffee, for three consecutive days
Cynoglossum coeruleum	W	H	Febrile illness (*Mich*)	D	F	The whole parts are crushed and infused in small amount of water and then the parts that felt illness are washed except chest
Cyphostemma adenocaula	R	L	Poison insects on *Sorghum* leaf	O	F	It is the same method of preparation and ingredients as used in *C. macrostachyus*
Datura stramonium	Se.	H	Toothache	D	D	Seeds mixed to butter are roasted on plate and inhaled the vapor air through opening their mouth
	L	H	Ringworm, dandruff	D	F	The fresh leaf is rubbed and applied as cream on the head like *C. pepo*
Debregeasia saeneb	L	H	Febrile illness	O/N	F	It is boiled in water and its decoction is drunk and then the vapor air is inhaled
Dodonaea angustifolia	S & L	Hl.	Bone fracture	D	Fd.	Powdered leaf is glued with its cracked stem on the broken bone with sap of *A. pulcherrima*
	R	H	Evil eye	D/N	D	It is crushed and powdered and tied on the neck and roots are inhaled with others same as *A. sativum*
Dyschoriste radicans	W	H	Scabies, itching, and injury of skin	D	Fd.	The whole parts are roasted with *Kalanchoe petitiana* and powdered and then the inflamed parts are creamed with butter until recovery but for itching with the leaf of *Rhus retinorrhoea*
	W	H	Wound (*Sheft*)	D	Fd.	It is crushed and pounded with leaf of *Kalanchoe petitiana* and pasted on the injured parts
Echinops hispidus	R & S	H	Febrile illness	O/D	Fd.	It is pounded and squeezed and soaked in water and is drunk by a cup of tea and all parts are creamed
	R & S	L	Sunstroke (*Shiwuta*)	O/D	Fd.	It is pounded and soaked in water by a cup of water and is decanted and the livestock is creamed
	R & S	L	Struck cattle	O/N	Fd.	It is pounded and infused in one cup of water and decanted, and fumigated the smoke for cattle
	R & S	H	Epidemic; evil eye	N	D	It is crushed and placed on the hot fire and inhaled by all householders
Ehretia cymosa	L	L	Leech infestation	N	F	Crushed leaf is soaked in water and the concoction decanted into the nose of cattle
Ekebergia capensis	R & Sb.	H	Gastritis, cough	O	D	In powder form, two teaspoons infusions into one cup of water are taken before meals
Eleusine floccifolia	R	L	Rh factor/disease	O	D	It is the same ingredient and preparation method of *A. pulcherrima*
Eucalyptus camaldulensis	L	H	Emergency illness	N	F	The leaf is roasted with the seed of *Lepidium sativum* on the heated tool and the smoke is inhaled by adding cool water on the roasted leaf and at the same time the eye is closed
Eucalyptus globulus	L	H	Headache	N	F	It is rubbed and its aroma inhaled
	L	H	Common cold	N	F	It is rubbed and its aroma inhaled without calling its name "Nech-Bahirzaf"
Euphorbia abyssinica	S	L	Cough (*Kuro*)	N	D	The stem is placed on burning dung and the smoke sniffed to treat Donkey's cough
	La.	H	Ascariasis	O	F	3–5 drops of its latex are homogenized in water with the powder of *Eragrostis tef* and Injera is made that is to be eaten by infected patient
	Fl.	H	Leprosy (*sgadewie*)	D	Fd.	Pounded flower is infused in honey and applied as cream on wound
Euphorbia platyphyllos	W	H	Ascariasis	O	D	In powder form, two teaspoons are roasted with equal size of roasted *Hordeum vulgare* in the form of Injera and eaten for three consecutive days
	La.	H	Skin infection (*barle*)	D	F	The latexes are applied as cream on the affected parts only

Table 1: Continued.

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Euphorbia polyacantha	La.	H	Impetigo (*Kunchir*)	D	F	The wound is bled first and then its latex with latex of *Euphorbia tirucalli* is applied as cream
	La.	H	Boils (*Bigungi*)	D	F	Its latex is applied as cream with latex of *C. procera* on the wound part only
	La.	H	Skin cancer (tumor)	D	F	It is the same method of preparation as used in *C. procera*
Euphorbia tirucalli	La.	L	Donkey's wart	D	F	The milky latex is pasted alone or with the latex of *E. polycantha* and squeezed leaf of *C. simensis*, after bleeding the wound with blade until recovery
Euryops pinifolius	L	H	Stomachache (*kurba*)	O	F	The leaf is chewed and absorbed during feeling pain
Ferula communis	R	L	Sterile cow/be fertile	O	D	Roots are dug at seven distinct places; pounded root is eaten with Injera
	R	H	Intestine pain	O	D	Two teaspoonful powders was infused in to glass of water (one cup of tea amount) and drunk
Ficus palmata	La.	H	Impetigo (*Kunchir*)	D	F	The wound is bled first and then its latex is applied as cream alone until recovery
	La.	H	Hemorrhoids	D	F	It is the same method and ingredient used in *C. procera*
Ficus vasta	L	H	Febrile illness	Op.	F	It is boiled with the leaf of *C. spinarum* and *Zehneria scabra* and then the vapor is inhaled
Foeniculum vulgare	L	H	Diuretic (*Shntemat*)	O	D	It is boiled and then the decoction is drunk by adding sugar like tea
Gossypium hirsutum	Se.	H	Malaria	O	D	Ground seed is soaked in water with small amount of salt and drunk by a cup of tea
Grewia ferruginea	Sb.	L	Leech infestation	N	F	Chopped and pounded stem bark is mixed with water and poured into the nose of cattle
	L	L	Removal of placenta	O	F	It is crushed and pounded with the root of *Verbscum sinaiticum* and decanted for the cattle
	L	H	Eye injury	Op.	F	It is squeezed alone or with *Rumex nervosus* and dropped into eye injured by *Euphorbia* latex
Guizotia abyssinica	Se.	H	Dry cough	O	D	It is pounded with seed of *Nigella sativum, A. sativum,* flower of *Thymus schimperi*, and rhizome of *Zingiber officinale* by pestle and mortar and then the concoction is drunk until recovery
	Se.	L	Swelling bull neck	D	D	The seed is chewed and the chewed seed is pitted on the wounded neck of bull
Hagenia abyssinica	Sb.	H	Malaria	O	D	It is chopped and powdered with the root of *Silene macrosolen, Phytolacca dodecandra*, and *C. ficifolus* and the leaf of *C. myricoides* and is drunk in a half size cup of coffee
	Se.	H	Tapeworm	O	D	The seed is pounded by pestle and mortar and mixed in ale (before filtration) and then a small amount is eaten in a separate house with one boy; if the risk comes, the "Shiro" stew is taken
Hibiscus eriospermus	R	H	Impotency in men	O	Fd.	It is the same method and ingredient used in *C. lanatus*
Hypoestes forskaolii	R	H	Stomachache	O	F	The cleaned root is chewed and the liquid absorbed
	L	Hl.	Leech infestation	O/N	F	Pounded leaf is soaked in water and drunk orally for human and decanted nasally for cattle
Impatiens rothii	Tu.	H	Idiopathy (*likift*)	D	F	It is the same method as used in *A. camperi* and *B. polystachya*
	Tu.	H	Fire accident	D	Fd.	Chopped and pounded tubular root is pasted on the injured body
Inula confertiflora	L	L	Cataract (*mora*)	Op.	F	The young leaf is chewed with the leaf of *A. aspera* and spitted to the affected cattle eye
	L	L	Leech infestation	N	F	It is the same as *E. cymosa*
	R	L	Emaciation	O	Fd.	It is the same method of preparation and ingredient of *C. hypoleuca*
Jasminum grandiflorum	Sh.	H	Hemorrhage at birth	V	F	It is crushed with the leaf of *Solanum incanum* and *C. pepo* and applied as cream on the vagina
	L	Hl.	Eye injury	Op.	F	It is the same method of preparation and ingredient of *C. ficifolius*
	L	H	Trachoma (*Aynemaz*)	Op.	F	Its young leaf and leaf of *C. myricoides* and *I. confertiflora* are crushed and dried under sunlight and then powdered to cream the infected part with "cul"

Table 1: Continued.

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Juniperus procera	L	L	Febrile illness	O	F	Crushed and pounded leaf is stirred in water and then decanted for all pack animals
	L	L	Rh factor/disease	O	F	Crushed and pounded leaf is stirred in water and then decanted for cow
	L	L	Leech infestation	N	F	Crushed and pounded leaf is stirred in water and then decanted for cattle
Justicia schimperiana	L	L	Body lice	D	F	Its leaf is crushed and pounded and then immersed in water to wash the body of cattle
	L	L	Dysentery	O	F	It is crushed with *Salvia schimperi* and soaked in water with ash for decanting for sheep and goat
Kalanchoe petitiana	S	L	Swelling bull neck	D	F	Once bled with blade, chopped and peeled stem twig is tied on injured neck of bull
	Ag.	H	Scabies (*Lifie*)	D	Fd.	It is the same method and ingredient as *D. radicans*
Lagenaria siceraria	F	L	Swelling bull neck	D	D	The fruit is roasted, used as container of butter on flame, and the injured neck of bull is rubbed
	L	H	Ringworm	D	F	It is crushed and applied as cream on infected head and other skin parts until recovery
Laggera tomentosa	L	H	Stomachache	O	F	The leaf is chewed and the liquid absorbed
	L	Hl.	Leech infestation	O/N	F	It is the same as *H. forskaolii* preparation
Launaea intybacea	R	H	Stomachache	O	F	The root is chewed and the liquid absorbed
Leonotis ocymifolia	L	L	Bloating	O	F	Crushed and pounded leaf is stirred in water and then decanted for cattle
	L	L	Struck cattle	O	F	Crushed and pounded leaf is stirred in water and then decanted for cattle
Lepidium sativum	Se.	L	Bloating (*Nifat*)	O	D	It is the same method and ingredient of *B. nigra*
Leucas martinicensis	W	L	Emaciation	O	Fd.	It is the same method of preparation and ingredient of *C.hypoleuca*
Lippia adoensis	L	H	Ringworm (*Aguagot*)	D	F	Crushed and pounded leaf is applied as cream with the milk of pregnant cow on the injured part
Lobelia rhynchopetalum	R & S	H	Evil eye	O/D	D	It is the same method and ingredient of *A. sativum*
	La.	H	Hemorrhoids/tumor	D	F	It is the same method and ingredient used in *C. procera*
Malva verticillata	R	H	Dandruff	D	F	Its root is crushed in water until formation of bubble and then the head is washed
	Sb.	Hl.	Wound (any type)	D	F	Chopped and pounded stem bark is pasted with powdered *Usea* sp. on wound
Mentha longifolia	L	L	Leech infestation	N	F	It is pounded and soaked in water and then the concoction decanted in nose of cattle
Momordica foetida	W	H	Psychiatric disorder	D	F	The whole parts are pounded and immersed in pot water for three days and body is washed
Myrica salicifolia	Sb.	L	Rh factor/disease	O	Fd.	It is the same ingredient and preparation method of *A. pulcherrima*
Myrsine africana	F	H	Ascariasis	O	F	Ripe fruit is eaten and traditional beer/ale is drunk in the morning before any meals
	L	H	Tapeworm	O	F	The leaf is pounded and squeezed by adding water and then drinking half a cup of water
Myrtus communis	L	H	Itching (*Ekek*)	D	D	The leaf is crushed and powdered with the leaf of *Rhus retinorrhoea* and butter; then the inflamed body is creamed and stayed under sunlight for a few minutes
	Ag.	H	Epidemic	N	D	The crushed aboveground parts are inhaled on hot fire to reduce transmission of disease
Nicotiana rustica	L	L	Leech infestation	N	F	The leaf is pounded and soaked in water and then the concoction is decanted into nose of cattle
Nigella sativa	Se.	H	Stomachache (*Alsha*)	O	D	The ground seed is boiled with oil and drunk by tea glass
	Se.	H	Dry cough	O	D	It is the same method and ingredient of *G. abyssinica*
Ocimum lamiifolium	L	H	Headache; febrile	O/D	F	One cup of squeezed leaf is drunk with coffee orally and the remainder is applied as cream to injured parts

Table 1: Continued.

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Olea europaea ssp. *cuspidata*	S	H	Toothache	D	F	It is similar method to that used in *C. abyssinica*
	S	H	Human's wart	D	F	The bleeding wart is heated with chopped and roasted stem with the sap of *Rumex nervosus*
Orobanche ramosa	W	H	Fire accident	D	D	Crushed and roasted parts are powdered and then are applied as cream with butter on injured parts
Osyris quadripartita	L	L	Emaciation	O	Fd.	It is the same method of preparation and ingredient of *C. hypoleuca*
Otostegia fruticosa	L	H	Diarrhea	O	F	One-half of a cup of coffee of squeezed leaf is drunk, specially for babies
Otostegia integrifolia	L	H	Vomiting, nausea, & diarrhea	O	F	The leaf is crushed and squeezed and mixed to root of *Verbena officinalis* in a cup of coffee and then the concoction is drunk
	L	L	Dysentery	O	F	It is crushed and pounded with leaf of *C. hypoleuca* and immersed in water under a new container of *L. siceraria* and then the concoction is decanted for the cattle, specially ox and cow
	Ag.	H	Evil spirit	N	Fd.	The aboveground parts are fumigated on hot fire, specially for the new birth time
Otostegia tomentosa	L	H	Ascariasis, diarrhea	O	F	The leaf is pounded and squeezed and then drunk in a cup of tea in the morning before any diet
Phytolacca dodecandra	R & L	Hl	Rabies virus	O	D	It is chopped and pounded with the root of *C. macrostachyus* and *Z. scabra* and the powder is mixed with the milk of identical color cow and calf and drunk in half tea glass
Plectranthus cylindraceus	L	H	Vomiting & diarrhea	O	F	Crushed and squeezed leaf is mixed in boiled cup of coffee and then the decoction is drunk
	L	H	Emergency (*Wereza*)	O/D	F	It is squeezed and mixed in boiled cup of coffee and then the decoction is drunk and the face is creamed
Polygala abyssinica	R	H	Against snake bite	O	F	It is chewed and absorbed before any diet during the starting new year of Ethiopia and did not take any sour taste fruits from the time on wards
	R	H	Evil spirit (*Ayinetila*)	O	Fd.	The cleaned root is chewed and absorbed
Primula verticillata	L	Hl.	Cataract (*Mora*)	Op.	F	Leaf is pounded and squeezed by using clean cloth and added to injured or infected eye
Pteris dentate	W	H	Evil spirit (*Ejeseb*)	D	Fd.	The powder is tied on the neck and held in the pocket wherever they moved
Pteris pteridioides	W	H	Evil spirit (*Ejeseb*)	D	Fd.	It is similar methods as those used in *P. dentata*
Pulicaria schimperi	L	H	Wound (infection)	D	F	Pounded leaf is pasted on wounded part
Rhamnus prinoides	L	H	Tonsillitis	O/D	Fd.	Pounded and squeezed leaf is taken in a half index finger size of cup and is applied as cream with malt on the center of head by saying "sikel, sikel"
Rhus glutinosa ssp. neoglutinosa	L	L	Leech infestation	N	F	The leaf is pounded and stirred in water that contained salt and then the concoction is decanted
Rhus retinorrhoea	L	H	Itching (*Ekek*)	D	D	It is the same method and ingredient of *M. communis*
Rhynchosia minima	R	L	Weakling bull (*Abayanet*)	O	D	It is chopped and pounded with the root of *Stephania abyssinica* and *Verbena officinalis* which are immersed in a new container of *L. siceraria* for three days and then the concoction is decanted for the bull which is falling during plowing up the farmland
Ricinus communis	Sh.	H	Eczema (*Chifie*)	D	Fd.	It is roasted and powdered with the leaf of *C. procera* and mixed with butter and then applied as cream
Rubus apetalus	R	H	Evil eye	N	D	Crushed and powdered root is fumigated and the aroma of the smoke at night is smelt
Rumex abyssinicus	R	H	Itching (*Ekek*)	D	Fd.	It is crushed and roasted and then powdered root is applied as cream with butter on wound
Rumex nepalensis	R	H	Stomachache (*Alsha*)	O	F	The cleaned root is chewed and absorbed alone or with the root of *Solanum incanum*

Table 1: Continued.

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Rumex nervosus	L	H	Eye pain	Op.	F	It is the same as *G. ferruginea*
	S	H	Ringworm (*Agogot*)	D	F	The stem is roasted on hot fire and the bubbles arisen from stem are applied as cream on wound
	S	H	Human's wart	D	F	It is the same as *O. Europaea* ssp. *cuspidata*
Ruta chalepensis	F	H	Stomachache (*kurba*)	O	Fd.	The fruit/seed is eaten alone or with the rhizome of *Zingiber officinale* and salt
	F	H	Evil eye (*lebuda*)	D	Fd.	The fruit is chewed and held with the bulb of *A. sativum* and stem of *A. afra*
Salvia schimperi	L	L	Dysentery	O	F	It is the same method and ingredient of *J. schimperiana*
Schinus molle	F	H	Stomachache (*kurba*)	O	Fd.	The ripe fruit/seed is eaten alone for both prevention and treatment of disease
Sesamum orientale	Se.	H	Rh factor/disease	O	D	It is the same method and ingredient used in *A. pulcherrima*
Sida schimperiana	R	H	Evil eye	O/D	D	It is the same method and ingredients used in *A. sativum*
Silene macrosolen	R	H	Tapeworm	O	Fd.	Crushed and pounded root in half index finger size is drunk by tea glass, and if the risk comes, powder of *Linum usitatissimum* infusion in water is taken as reducing pain
Solanecio gigas	L	L	Emaciation	O	F	It is the same method of preparation and ingredient of *C. hypoleuca*
Solanum anguivi	F	H	Birth control	O	F	It is the same method of preparation and ingredient used in *C. ficifolus*
	F	H	Itching (*Ekek*)	D	F	The fruit is pounded with pestle and mortar and then applied as cream with butter on wound
Solanum marginatum	F & Se.	L	Cough (*Chifra*)	N	F	Its fruit is pierced and its fluid and seed are collected in a cup to be decanted nasally
	F & Se.	H	Rabies virus	O	F	Its fluid is collected with squeezed leaf of *P. dodecandra* and then drunk with cup of tea
	F	L	Evil eye	O	F	Roasted and pierced ripe fruit can be eaten with straw or hay fodders
Solanum incanum	L	Hl.	Bleeding (epistaxis)	D	F	Its leaf is crushed and tied to bleeding nose alone or with the leaf of *A. aspera* and *R. nervosus*
	R	H	Stomachache	O	F	It is the same method and ingredient used in *R. nepalensis*
Solanum nigrum	L	H	Gastritis	O	F	The washed or cleaned raw leaf before any meals is chewed
Stephania abyssinica	R	H	Against snake bite	O	F	It is the same as *P. abyssinica* except that there is no restriction on taking any sour taste fruits
Striga hermonthica	W	L	Poisoned insects	O	Fd.	It is the same method and ingredients used in *C. macrostachyus*
Thymus schimperi	F	H	Dry cough	O	D	It is the same method and ingredients used in *G. abyssinica*
	L	H	Hypertension	O	Fd.	The crushed or the normal leaf is boiled and then taken like a tea with a tea glass
Tragia brevipes	W	H	Impotency (in men)	O	Fd.	It is the same method and ingredient used in *C. lanatus*
Urtica simensis	L	H	Night blindness	D/N	F	The leaf is boiled in hot water and its aroma fumigated three times by opening the lid
	Rh.	H	Abortion	V	F	It is peeled with blade; three half little finger size rhizomes are inserted in the vagina
	L	H	Eye injury	Op	F	Pounded and squeezed leaf is dropped on the injured eye by insertion of bad materials

Table 1: Continued.

Scientific name	Pu.	Uf.	Disease treated	Ra.	Cp.	Method of preparation and application of TMPs
Verbena officinalis	R	H	Stomachache/nausea	O	F	The liquid is chewed and sucked and the residue is spitted
	R	L	Weakling bull	O	D	It is the same method and ingredient used in *R. minima*
Verbscum sinaiticum	R	L	Struck cattle (*Betir*)	O	Fd.	One water cup of pounded root in the water is decanted alone or with *E. hispidus*
	R	L	Febrile illness	O	Fd.	Crushed and pounded root is soaked in water and poured orally for all livestock
	R	H	Evil eye	O/N	D	It is the same method and ingredient used in *A. sativum*
	R	H	Psychiatric disorder	O	Fd.	It is crushed alone or with *C. ficifolus* and then half index finger size by a tea glass is drunk
Vernonia leopoldii	L	H	Wound, bleeding	D	F	The leaf is crushed and pounded and then tied on the injured part
Vernonia schimperi	R	H	Febrile illness	D/N	Fd.	Crushed and pounded leaf is stirred in water until bubbles are formed and then all parts are creamed except chest and the dried root is fumigated on hot fire and sniffed
Vicia faba	Se.	H	Boils	D	D	It is ground and soaked in water and pasted on wound and then heated by dung of jackass
	Se.	H	Ascariasis	O	D	It is the same method and ingredient used in either *B. abyssinica* or *C. macrostachyus*
Withania somnifera	R	H	Evil eye/spirit	D/N	D	It is the same method and ingredient used in *A. sativum*
Zehneria scabra	L	H	Jaundice	O	F	The leaf is pounded and squeezed and then drunk in half a cup of tea
	R	Hl.	Rabies virus	O	D	It is the same method and ingredient used in *P. dodecandra*
	Ag.	H	Febrile illness	N	F	It is the same method and ingredient used in *F. vasta*
Zingiber officinale	Rh.	H	Stomachache	O	Fd.	It is the same method and ingredient used in *R. chalepensis*

For authorities to scientific names of each species, see Appendix 1.

Table 2: Frequency of plant parts used and route of administration of remedy from TMPs.

Plants parts	Frequency	Percentage	Route of administration	Frequency	Percentage
Leaf only	75	32.6	Oral	101	43.9
Root only	50	21.7	Dermal	66	28.7
Fruit only	16	6.9	Nasal	26	11.3
Seed only	15	6.5	Optical	11	4.8
Whole plant	15	6.5	Oral and dermal	10	4.3
Latex only	12	5.2	Dermal and nasal	8	3.5
Stem only	8	3.5	Oral and nasal	5	2.2
Aboveground parts	7	3.0	Vaginal	2	0.9
Bulb	6	2.6	Anal	1	0.4
Root and stem	5	2.2	Total	**230**	**100.0**
Stem bark only	5	2.2			
Rhizome only	3	1.3			
Root and stem bark	3	1.3			
Flower only	2	0.9			
Fruit and seed	2	0.9			
Shoot only	2	0.9			
Tuber	2	0.9			
Leaf and stem	1	0.4			
Root and leaf	1	0.4			
Total	**230**	**100.0**			

Table 3: Informant consensus on commonly known TMP species.

Scientific name	Total informants' agreement	Percentage	Rank
Ocimum lamiifolium	66	91.7	1st
Zehneria scabra	61	84.7	2nd
Carduus schimperi	56	77.8	3rd
Achyranthes aspera	42	58.3	4th
Schinus molle	37	51.4	5th
Allium sativum	35	48.6	6th
Echinops hispidus	30	41.7	7th
Cucumis ficifolius	25	34.7	8th
Aloe pulcherrima	21	29.2	9th
Withania somnifera	20	27.8	10th

infection with relatively smaller number of species (19) used by a large proportion of the healers, followed by disease related to internal parasites and gastrointestinal disorders with ICF value of 0.82, represented by the highest number of both species (46) and informants (248). On the other hand, the category of genitourinary problems was only treated by healers, which had the lowest ICF value of 0.43 with five species (Table 6).

Marketable TMPs in Delanta District. Market survey was conducted at Tirtria and Adagua in the major town. Some herbal medicinal plants were recorded based on direct observation and interviewing traders, vendors, and consumers. They reported that most of the herbal medicines were sold and bought for various use values such as spices, condiments, foods and beverages, fumigants, and cultural and spiritual aspects. These species included *Allium cepa*, *A. sativum*, *Capsicum annuum*, *Myrtus communis*, *Rhamnus prinoides*, *Cucurbita pepo*, *Olea europaea* ssp. *cuspidata*, *Otostegia integrifolia*, *Ruta chalepensis*, *Vicia faba*, and *Zingiber officinale*.

Multiple Uses and Ranking of Ethnomedicinal Plant Species. Some of the TMPs in the study area were used for other purposes. Multiple use analysis showed that firewood and charcoal are the most frequently mentioned uses (27.3%) while the wild (normal and famine) food category was the least reported (10.0%) (Figure 4).

Use diversity analysis shows that, among the six TMPs, *Carissa spinarum* is ranked 1st, followed by *Rhus vulgaris* ssp. *neoglutinosa*, and the others are given in Table 7.

Threats to and Conservation Status of TMPs. The threats resulted mainly from human activities and they varied from site to site. Among these activities, agricultural land expansion is ranked 1st, followed by fuel wood and construction material collection, overgrazing, mining opal, and drought (Table 8).

Further analysis showed that *Withania somnifera* came out in the first rank of most threatened TMP (Table 9).

Trends in Abundance of TMP Species. The degree of abundance of TMP species considering their current status was reported as 50 common species, followed by rare (45 species) and sparsely distributed species (40), based on informants' perception and direct field observation in the wild state.

Table 4: Preference ranking of TMPs against febrile illness.

Plant species that treat febrile illness	Key informants (coded K_1 to K_9) with the ranks they gave										
	K_1	K_2	K_3	K_4	K_5	K_6	K_7	K_8	K_9	Total	Rank
Carduus schimperi	4	1	2	5	4	3	5	3	1	28	3rd
Echinops hispidus	5	4	5	1	2	2	3	2	3	27	4th
Ocimum lamiifolium	3	5	4	3	5	4	2	4	5	35	1st
Vernonia schimperi	1	3	1	2	1	1	4	1	4	18	5th
Zehneria scabra	2	2	3	4	3	5	1	5	5	30	2nd

Table 5: Results of paired comparison for five TMPs used for treating stomachache.

Plant species that treat stomachache	Key informants (Coded K_1 to K_9) with the ranks they gave										
	K_1	K_2	K_3	K_4	K_5	K_6	K_7	K_8	K_9	Score	Rank
Rumex nepalensis	0	2	1	0	3	1	4	0	0	11	4th
Ruta chalepensis	2	1	3	2	4	0	0	2	1	15	3rd
Schinus molle	4	3	2	4	2	3	3	3	4	28	1st
Solanum incanum	1	0	0	1	0	2	2	1	2	9	5th
Verbena officinalis	3	4	4	3	1	4	1	4	3	27	2nd

Table 6: Informant consensus factor for the given disease category.

Category	N. spp.	Nur.	ICF
Respiratory, febrile illness, and throat infection (common cold, influenza, dry cough, tonsillitis, and sunstroke)	19	203	0.91
Internal parasites and gastrointestinal disorder (rabies virus, jaundice, stomachache, bloating, vomiting and nausea, appetite loss, malaria, diarrhea and dysentery, tapeworm, ascariasis, giardiasis, and amoebiasis, leech infestation, and heartburn)	46	248	0.82
Dermatological disorders/infections (swelling, wound, dandruff, ring worm, leprosy, eczema, itching, wart, boils, impetigo, and idiopathy)	34	178	0.81
Psychiatric disorder and birth problems (headache, toothache, blood pressure, abortion, Rh factor, retained placenta, hemorrhage at birth, and infertility)	19	68	0.73
Emaciation and weakling for livestock and epidemic for humans	9	30	0.72
Evil eye, evil spirit, and emergency (trauma)	24	59	0.60
External injury and poisoning parasites (eye problems, cataract, bone fracture, circumcision, bleeding, snake bite, struck, fire accident, and body lice)	25	48	0.49
Genitourinary problems (gonorrhea and diuretic and sexual impotency)	5	8	0.43

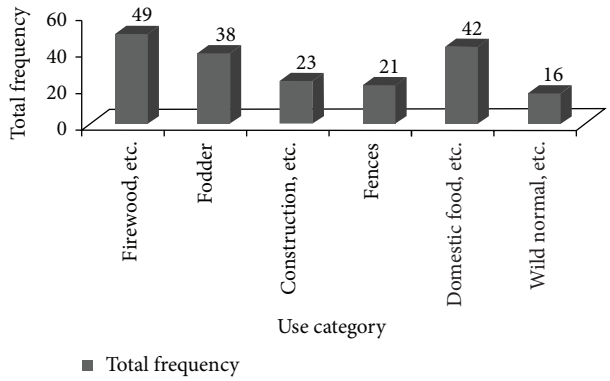

Figure 4: Proportion of other uses of the ethnomedicinal plant species.

Table 7: Results of direct matrix ranking of multipurpose TMPs.

Main uses	*Carissa spinarum*	*Olea europaea* ssp. *cuspidata*	*Rhus glutinosa* ssp. *neoglutinosa*	*Ficus palmata*	*Ficus vasta*	*Rubus apetalus*
Firewood	5	5	5	5	5	5
Medicine	5	5	3	5	3	3
Construction	4	5	5	4	5	1
Fodder	4	5	5	0	0	3
Edible fruit	5	0	3	5	5	5
Total	23	20	21	19	18	17
Rank	1st	3rd	2nd	4th	5th	6th

Table 8: Results of direct matrix ranking of factors threatening to TMPs.

Threats	Key informants (coded K_1 to K_9) with the total scores and rank										
	K_1	K_2	K_3	K_4	K_5	K_6	K_7	K_8	K_9	Total	Rank
Agricultural expansion	6	6	5	5	6	5	4	6	6	49	1st
Mining of opals	4	3	5	3	5	4	5	4	3	33	5th
Overgrazing	4	5	4	4	6	3	3	5	4	38	4th
Farm tools and construction	6	6	4	3	3	6	4	3	6	41	3rd
Drought	3	4	3	4	2	5	3	2	5	31	6th
Firewood and charcoal	5	5	6	5	5	6	6	5	4	47	2nd

Table 9: Priority ranking values (based on their degree of scarcity in the wild) for five selected TMPs.

Threatened medicinal plant species	Key informants (coded K_1 to K_6) with the total scores and rank										
	K_1	K_2	K_3	K_4	K_5	K_6	K_7	K_8	K_9	Total	Rank
Olea europaea ssp. *cuspidata*	4	3	4	5	4	3	1	5	2	31	2nd
Croton macrostachyus	2	1	1	2	1	5	4	3	4	23	4th
Juniperus procera	3	5	3	3	5	1	5	2	1	28	3rd
Aloe pulcherrima	1	2	2	1	3	4	2	1	5	21	5th
Withania somnifera	5	4	5	4	2	2	3	4	3	32	1st

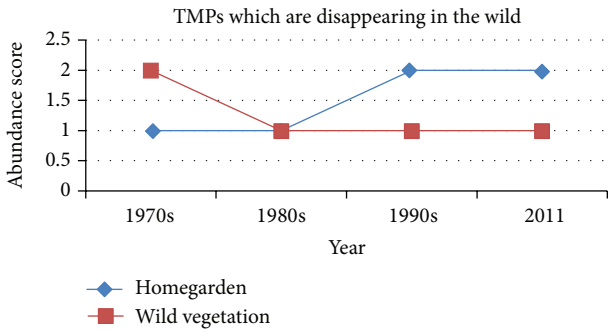

Figure 5: Trends of ethnomedicinal plant species in homegarden and wild vegetation.

Figure 5 shows that the homegarden is maintained in an increasing state of some of the indicator TMP species, particularly those said to have been lost from the wild environment in the 1970s, and then went on decreasing.

Local Ecological Knowledge of People. In the study area, people have knowledge about their environment (landscapes, vegetation, and soil types). Based on topography of the land forms and the color and fertility of soils, they classified the ecological units into six categories in Amharic language based on their lifelong LK. This is presented along with the etic categories in Table 10.

The LK of People on Plant Community Type and Distribution of TMPs. Although natural forests have disappeared in many places due to many reasons, the vegetation of the study area still keeps relatively high number of species of TMPs. However, among 19, six endemic plant taxa in the floristic region of Ethiopia and Eritrea were not recorded as they are found in the floristic region of Wello (WU). These are *Urtica simensis*, *Impatiens rothii*, *Aloe pulcherrima*, *Solanum marginatum*, *Lobelia rhynchopetalum*, and *Primula verticillata* ssp. *simensis*. The plant community types were identified through categorization of the local people (emic classification approach) and observation of the researcher (using etic classification) based on the dominant plant species as visually inspected. Eight major community types were recognized. All types of plant community types are found in six study sites except *Lobelia rhynchopetalum* and *Euryops pinifolius*

Table 10: LK of people on landscapes and soil types.

Landscape classification		Soil classification	
Emic classification	Etic classification	Emic classification	Etic classification
WOTAGEBA	Undulated land	KEYATIE	Red soil
TERRAR	Mountain	WALKA	Black soil
MEDA/REGATA	Plain	BUNAMA	Dark brown
GODGUADAMESK	Valley	ASE	Mixed soil
GEFET/GEDEL	Outcrop land	NECHATE	Silt/ashy soil
TEDAFAT/KONTER	Hilly/steep slope	KOSIE	Dung wastes

Table 11: Plant community types and characteristic plant species.

Number	Plant community type and elevation	Characteristic species	Taxa known to be endemic or near endemic to Ethiopia	Spp.
I	*Becium grandiflorum* and *Rumex nervosus* (2502 and 2738 m); ASHA (restored vegetation)	*B. grandiflorum*$^{+}$, *R. nervosus*	*B. grandiflorum*$^{++}$, *Laggera tomentosa*$^{++}$	35
II	Riverine vegetation (altitude varies); WENZ DAR	*Ehretia cymosa, Cordia africana*, and *Ficus* spp.	*Urtica simensis*$^{+}$, *Impatiens rothii*$^{++}$	5
III	*Otostegia integrifolia* and *Dodonaea angustifolia* (1802–2500 m); QUTQUATO (shrub)	*O. integrifolia, D. angustifolia*	*Vernonia leopoldii*$^{+}$	32
IV	Farmland and monastery plant community type (altitude varies); YERSHAGOT and GEDAM	*Olea europaea* ssp. *cuspidata*, *Juniperusprocera,*	*Erucastrum abyssinicum*$^{++}$, *Rhus glutinosa* ssp. *neoglutinosa*$^{+}$	20
V	Homegarden plant community type (altitude varies); YEGUARO ATIKLT	*Rhamnus prinoides, Calpurnia aurea,* and *Hagenia abyssinica*	*Aloe pulcherrima*$^{+}$, *Solanecio gigas*$^{+}$	24
VI	*Conyza hypoleuca* and *Clutia abyssinica* dominated community type (2814–3253 m); MUSH (bush)	*C. hypoleuca, C. abyssinica*	*Inula confertiflora*$^{+}$, *Solanum marginatum*$^{++}$, *Otostegia tomentosa* ssp. steudneri^{+}, *Lippia adoensis*$^{++}$, and *Kalanchoe petitiana*$^{++}$	9
VII	*Eucalyptus* plantation dominated community type (3200–3253 m); DEN/KILKIL (jungle)	*E. globulus, E. camaldulensis,* and *Carduus schimperi*	*Thymus schimperi*$^{++}$	4
VIII	*Lobelia rhynchopetalum* and *Euryops pinifolius* dominated community type (3539–3702 m); CHEKA (forest)	*L. rhynchopetalum*$^{+}$, *E. pinifolius*$^{+}$	*L. rhynchopetalum*$^{+}$, *E. pinifolius*$^{+}$, *Sedum mooneyi*$^{+}$, and *Primula verticillata* ssp. *simensis*$^{++}$	4
Total				**133**

$^{+}$Taxa which are endemic to Ethiopia while $^{++}$near endemic taxa are those shared with Eritrea.

dominated community type, which is restricted in one of the study sites, namely, Tikshign Sinat kebele between 3539 to 3702 m a.s.l. Some of the following species are not included in the present paper, but they are collected and preserved in ETH by the authors for other purposes (Table 11).

The present study noted that well-organized emic classification of local vegetation was not shown in some ecological settings (largely DEGA zones) where natural vegetation has been almost completely changed into agricultural lands. Regarding habitat, most of the TMPs are distributed in

Table 12: Mode of TMP knowledge transfer from traditional healers to others.

Means of knowledge transfer	Number of traditional healers	%
Selected family members (verbal and/or observation)		
Males only	9	50.0
For both females and males	6	33.4
Other members of societies (verbal and/or observation)		
Best relatives/peers	2	11.1
Anybody who seeks to learn the knowledge of healers	1	5.5
Total	**18**	**100.0**

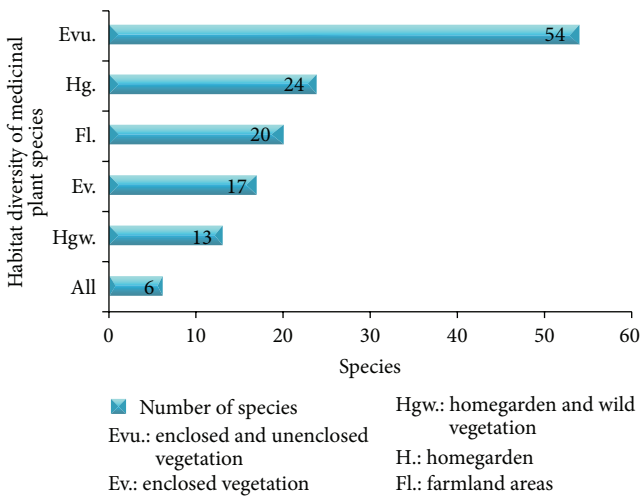

Figure 6: Proportion of habitat diversity of TMPs.

different habitats though their availability varies from place to place among species. The majority were harvested from the wild (71 species), followed by homegarden (24 species), and cultivated in farmlands (Figure 6). The wild vegetation could be either enclosed (protected by government or private sectors) or unenclosed, which is open to all people.

3.2. Comparisons of LK among Age Groups and Its Transfer System. The LK of the three age groups was compared with respect to the names and the respective uses of TMPs. The sample sizes of each age group were 14, 28, and 30 persons from 20 to 88 with 22 years' gap. The results show that the age groups within the ranges of 66–88 years reported the highest proportion of TMP names and uses. The total value is more than 100% because sometimes same plant species were mentioned by all groups (Figure 7).

The majority of traditional healers (84.4%) kept the knowledge with them and selected family members for the sake of confidentiality while others (16.6%) transferred their LK to other persons (Table 12).

Consequences of Resettlement on Environmental Component and LK of TMPs. The study reported that half of the interviewed informants were those that returned from the destination area due to the 1984/85 resettlement. This indicated that the 1984/85 drought was one of the worst disasters in the area. In case of returnees, 17 plant species have no names and some of the names given also were more general (e.g., HAREG refers to any climber plant). However, this is not seen in traditional healers who have used "medicoreligious manuscripts." In case of resident people, however, some of the TMPs are named by using the disease treated adding YE at the beginning followed by MEDANIT. For example, three species (*Cistanche tubulosa*, *Polygala abyssinica*, and *Vernonia schimperi*) are named as YESATMEDANIT, YEBABMEDANIT, and YEMICHMEDANIT, to say medicine of burns, snake bite, and febrile, respectively. It is noted in Table 13 that permanent inhabitants have complete names for each except two species (*Ekebergia capensis* and *Pulicaria schimperi*), which is not the case for the remaining groups of informants.

Of 36 returnees including some key informants, only two persons were born in the destination of resettlement areas and the rest were primarily born in the original area and then left from their original area during the 1984/85 famine. However, after staying away at different times, they came back to their original location (Delanta district). The sample sizes of the three groups of informants were 18 key informants, 25 permanent residents, and 29 returnees. The results showed that key informants are more knowledgeable about TMP species (92.6%), followed by permanent residents (81.5%). However, concerning returnees, the least results were recorded in all the three aspects (Figure 8).

The findings on the impacts of resettlement depict that the 1984/85 resettlement had both positive and negative results in the area of ethnobotanical knowledge and social and cultural activities. From negative perspective, for instance, majority of the returnees had lost specified local names and detailed preparation of TMPs, and youngsters were not willing to respect the LK and the associated taboos. For the positive perspectives, on the other hand, the former farmlands were converted into noncultivated vegetation. Such promoting ecological rehabilitation in turn provided restoration of wild useful plants and reduced human pressure on plant resources.

4. Discussion

Relatively high number of TMP species is encouraging and a good indication that the area has reasonable number of useful plant species. This is partly the result of the action to move out drought-affected people from the area and the fact that the permanent residents went on using and protecting the plants. The results agree very well with the findings in

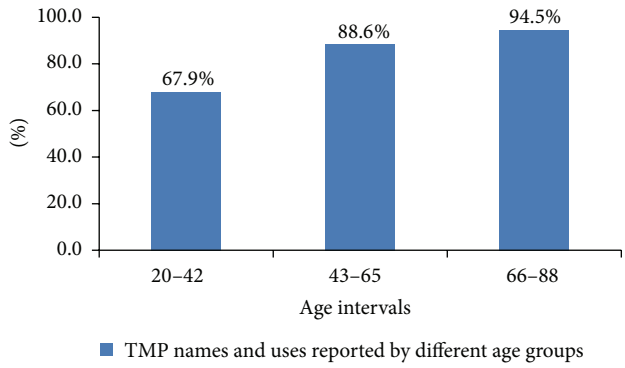

Figure 7: Variations of LK in TMPs among the three age groups of informants.

Table 13: Variation of LK on some TMP names.

Number	Scientific name	Different local names among three groups of informants		
		Key informants	Permanent residents	Returnees
1	*Aeonium leucoblepharum*	Gimdo	Yetota-Kita	—
2	*Asparagus africanus*	Yeset-kest	Kestencha	Betin
3	*Carduus schimperi*	Dendero	Yemidir Koshele	Yedega Koshele
4	*Carthamus lanatus*	Yesetaf	Koshele	—
5	*Ceratostigma abyssinicum*	Key-telenji	Key-telenji	—
6	*Chenopodium schraderianum*	Sinin	Sinign	—
7	*Cistanche tubulosa*	Dechmerch	Yesatmedanit	—
8	*Croton macrostachyus*	Bisana	Bisana/Mekenisa	Mekenisa
9	*Cyathula uncinulata*	Kuno	Kugno	—
10	*Cynoglossum coeruleum*	Yemogn-fikir	Hulu-zemede	Chigogot
11	*Cyphostemma adenocaula*	Milas-golgul	Aserkush	Hareg
12	*Datura stramonium*	Astenagr	Banjie	—
13	*Dyschoriste radicans*	Yesheftmedanit	Yesheftmedanit	—
14	*Ekebergia capensis*	—	—	—
15	*Euphorbia polyacantha*	Sete-qulqual	Yeberha-qulqual	Qulqual
16	*Justicia schimperiana*	Simiza/Sensel	Sensel	Sensel
17	*Kalanchoe petitiana*	Endehulla	Endehulla/Fitfita	—
18	*Laggera tomentosa*	Keskessie	Alashume	Alashume
19	*Launaea intybacea*	Demastefi	Yewushamilas	Yewushamilas
20	*Lippia adoensis*	Kessie	Ayib-kessie	—
21	*Myrsine africana*	Kechemo	Kerchemo	—
22	*Nicotiana rustica*	Yabesh-Tinbaho	Tinbaho/Atiya	Tinbaho
23	*Orobanche ramosa*	—	Yesatmedanit	—
24	*Plectranthus cylindraceus*	—	Yewerezamedanit	—
25	*Polygala abyssinica*	Etse-libona	Yebabmedanit/Kibegolgu	—
26	*Pulicaria schimperi*	—	—	—
27	*Ricinus communis*	Gullo	Agullo	Agullo
28	*Rumex abyssinicus*	Mekmeko	Embari-kolla	Embari-kolla
29	*Solanum incanum*	Yedi	Embuay	Embuay
30	*Solanum nigrum*	Tikur-awut	Tikur-awut	Awut
31	*Stephania abyssinica*	Etse-eyesus	Yeayit-hareg	Hareg
32	*Pteris pteridioides*	Etse-anbessa	Ems-anketkit	—
33	*Verbscum sinaiticum*	Etse-debtera	Ketetina	Ketetina
34	*Vernonia schimperi*	—	Yemichmedanit	—
35	*Zehneria scabra*	Etse-sabek	Aregresa	Hareg

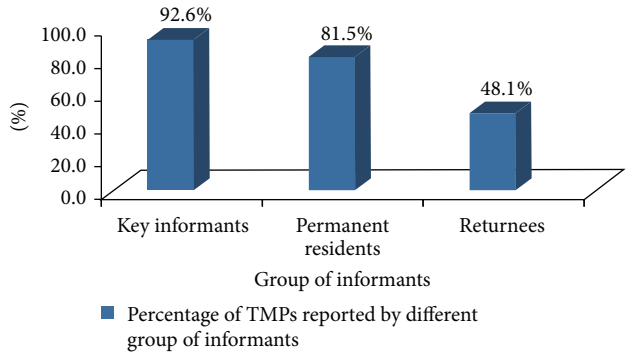

Figure 8: Variations of LK of TMPs among three groups of informants.

the Cheffa plain of southern Wello where 83 TMPs species were recorded [31]. Although there is continued deforestation and degradation in the area, the remaining species and the taxonomic diversity (59 families) promise success in conservation. As in other studies made in southwestern Ethiopia [22] and elsewhere [8], the family Asteraceae came up with high number of species. Asteraceae is also the second (next to Fabaceae) largest represented dicotyledonous family in the flora of Ethiopia and Eritrea with 440 spp. The results of this study prove that people tend to use preferably the plants that are easily available to them excluding, of course, those that are toxic or lethal. As was affirmed by other studies [8], the more common the plant species is in an area, the greater the probability of its popular use is. The study showed that the wild vegetation is the major habitat where a significant proportion of TMPs are harvested. The distributions of all, but two (IV and V), of the plant community types also confirmed this habitat. This result is in agreement with different studies carried out elsewhere in Ethiopia [7, 23] and other parts of the world [11].

The people of Delanta rely largely on herbs, which are replacing the forest resources and are relatively common in the areas where extensive degradation has taken place. This finding agrees with findings of many authors [12, 21, 32] but disagrees with other studies [31, 33]. This could be related to the level of agricultural expansion and perhaps due to the presence of two rain-fed seasons. In most cases, there are several plant species used in combination to treat specific ailments, which was considered important to increase the strength and effectiveness of the remedies. Such practice was also common in other countries [34]. According to Abebe and Ayehu [6], the use of several species for the treatment of a particular ailment could indicate the prevalence of the species. The common use of leaf for preparation of remedies could partly be due to the relative ease of finding and simplicity of preparation. Leaves were shown to be the most commonly utilized parts in other findings [12, 22, 31]. Contrary to this finding, Hunde et al. [33] reported that roots were the most widely used parts, and this may be related to the medical culture of the people and environmental condition of the area. Moreover, collecting leaf parts for medicinal purpose is usually not a threat to the survival of plants as compared to the use of whole parts, roots, and stem barks [6, 35].

Among several preparation methods, the most frequent use of chopping, crushing, and pounding could be because of ease of use of local tools. Similar finding was reported by Tamene et al. [31] but deviates from other findings [12]. Informants underlined that attention is given in determination of dosage and depends on experience of traditional healers, age of patients, and the power of TMPs. They also had relatively better measurements for humans than livestock. However, lack of precision and standardization in measurement is considered as the general weakness of traditional healthcare system [4, 32]. The findings showed that most remedies in Delanta were administered orally in agreement with other reports from northern Ethiopia [6, 31, 32]. The fact is that modern health system also reported that there was high prevalence of internal problems and gastrointestine disorder in the study area. This also emerged from the calculations of ICF values. However, Giday et al. [22] indicated that skin (dermal) was the predominant route of administration. Most remedies were prepared and used immediately after harvest. As traditional healers pointed out, the use of either fresh or dry form is based on the type of applications and the availability of TMPs. Herbal drugs were mostly applied internally (58.3%) and the plants were found around homegardens. Fresh forms were favored since this form is considered to be strong and healthy. The wider use of fresh conditions was also reported from elsewhere [22, 35].

In the community, some of the TMP species were more popular and recognized as more effective and popular remedies than others. From both rankings and comparisons, it could be understood that the most favored species are usually the most effective ones for being used against a particular ailment. Thus, the preferences of some TMPs more than others prove the reliability and continuity of the ethnomedicinal information obtained from the local people. Among eight identified disease categories in the area, respiratory diseases, febrile illness and throat problems, and internal parasites and gastrointestine disorders were the most frequently encountered with high value of ICF. This may indicate high incidence of these types of disease in the area, possibly due to the poor socioeconomic and sanitary conditions of the people perhaps related to drinking stream water and food preparation. This finding is in line with another study conducted in northwestern Ethiopia [32]. Heinrich [36] also indicated that TMPs that are believed to be effective in treating a certain disease have higher ICF values whereas a low value implies that the informants disagree on the taxa. All these signified that people rely chiefly on TMPs to meet their primary healthcare needs and this holds true in other previous studies [6, 12]. Some of the species discussed herein (Table 1) were also incorporated in modern pharmacological remedies with the same treatment and parts used by other researchers [37–39]. These are good indicators that the present study will serve as an input for further works in modern therapeutics activity.

With this study, informants noted that resettlement program contributed to biodiversity conservation in the original place as evidence of the TMPs that are regenerating in the wild vegetation and former farmlands since the local people left Delanta. This was mainly seen in the lowlands (1700–2600 m a.s.l.) of the study area that was hard hit by the 1984/85

drought as described by Rahmato [26]. Inappropriateness for farming is also served as reinforcement for the areas that have been shifted to restoration and unsettled lands. Other findings were reported by different authors elsewhere in Ethiopia [25, 40, 41] also affirmed that the basic rationale behind resettlement was to facilitate resource rehabilitation and to provide poor households with a better livelihood. According to Dhakal et al. [24], however, it needs careful evaluation of peoples' perceptions on volunteerism to handle with proper management of biodiversity.

Although many positive outcomes were seen in the recovery of habitats, there was some loss of LK and culture of resettled people after resettlement. Such mixed results were also shown in another study [24]. Informants reported that before the 1984/85 resettlement people's judgments on their conservation outcomes of vegetation which foster TMPs were very positive. With regard to returnees, however, this habit has been reducing so that resettlement (staying away from the original site) could be one of the reasons. Traditional healers who might not have gotten opportunities to come back to their original place may have lost some of the LK to treat ailments such as asthma and tuberculosis (lung problem) in the local people was mentioned. This was again another barrier in transfer of knowledge to the young generation. This resulted in the loss of specified local names of some plants and their uses. According to some key informants, some TMP species were lost by improper exploitation of root parts which was aggravated by drought: HAKENUR, WEYILO, SIREBIZU, SHEBOTETYA, TIFRENA, and CHOCHO (in Amharic). Similarly, another study in the same area [19] pointed out that recovery efforts of the lost floras have been even more difficult. Some of the returnees are encroaching and clearing both rehabilitated and natural vegetation which harbors TMPs unlawfully. Another influence of resettlement was that economic crisis of returnees—those who were totally dependent on safety net program.

Out of total 133 collected species, 17 (13%) species are endemic TMPs in the flora of Ethiopia and Eritrea. As it holds true for the total species, Asteraceae is also the leading family with five species, followed by Lamiaceae with three endemic species. These were cross-checked with different volumes of flora of Ethiopia and Eritrea and Red List book [42]. Eight of these endemic and a few other nonendemic TMP species are not described as they have been distributed in Wello floristic region (WU) in the flora. IBC [14] and Friis et al. [29] mentioned that the dry evergreen montane and grassland complex ecosystems cover the major part of the study area. Additionally, the present study revealed that the Afro-Alpine and sub-Afro-Alpine ecosystems are also found in some mountain areas and characterized by the most conspicuous endemic giant herb (*Lobelia rhynchopetalum*) and shrubs including *Euryops pinifolius*, the evergreen tree heather (*Erica arborea*), and perennial herb species in the study area. Hence, there is a need to strengthen conservation actions in these ecosystems to stop further threats of endemic species.

Based on the reports of informants, the trends of indicator species revealed that the last 40 decades were much detrimental to natural vegetation in the area. Furthermore, wild plants used in TMPs are being lost more quickly in wild vegetation than homegardens as illustrated in Figure 5. All the indicator TMP species which are threatened by different factors (Table 8) were reported to be greatly harvested for multiple uses they provide. The root parts used as medicine could be posing another threat. There are 113 multipurpose plants which have diverse services in terms of economical, ecological, and cultural aspects other than their medicinal values (Figure 4). The results of data matrix ranking of TMPs on five main uses showed that all the six species were the most favored for firewood although they are overexploited for other multiple uses. This goes in line with other findings [5, 6, 10, 11]. Thus, conservation strategy is needed in the area to save these species from further reduction or total extinction due to unsustainable use and overexploitation.

In view of long human settlement history of the area, the natural vegetation has enormously been altered. It came out clearly from the study that the threats facing TMPs are both anthropogenic (e.g., agricultural expansion, which is ranked 1st) and natural factors (e.g., drought), which are having detrimental effects on wild medicinal resources. This finding is in agreement with other studies conducted elsewhere in Ethiopia [7, 43]. The previous studies conducted elsewhere also confirmed that LK of wild plants in Ethiopia is in danger of being lost, as habits, value systems, and the natural environment change [6, 7].

When the pressures of threats increased and some multiuse plants became rare in the wild vegetation, most traditional healers and some local people started to conserve them by growing such species intentionally in their homegardens and farmland margins. This report is consistent with that of Asfaw and Tadesse [7], as they explained that homegarden is a strategic farming system for conservation and enhancement of TMPs. The other ways by which conservation of useful plants is effected relate to the culture of people themselves and some taboos. For instance, *Phytolacca dodecandra* and some *Solanum* spp. were not used for firewood, because the local people believe that those who used these species for firewood become poor and divorced from spouses. Furthermore, cutting of big trees with large umbrella branches was culturally not accepted because of the belief that one who cuts them will have a shortened life and may soon pass away. This is widely seen in plants like *Ficus* spp. (YETINCHAW LEMLEM), *Euphorbia abyssinica*, and *Hagenia abyssinica* (ADBAR-ZAF) related to spiritual issue. It also holds true for sacred forests which are found around monastery and church yards. Such habit of conserving useful plants is still alive in the study sites but is ageing in the majority of present generations. Likewise, Tamene et al. [31] reported about the preservation of forest islands by the community's sociocultural factors as rituals. Therefore, incorporation of local communities' own vision and LK concerning conservation and sustainable use of TMPs has to be given priority. It is likely that the local people closely watch and know how the resources are consumed and conserved [20, 44].

It is obvious that overgrazing and deforestation are very serious in northern highlands of Ethiopia. Presently, however, the local people have practiced check dam construction and tree plantation with enclosed vegetation of such highlands to reduce erosive rain storms and to preserve useful plants as

well. The significance of TMPs to people can be sufficiently great that arrangements made for the conservation and sustainable use of TMPs have now grown to be a timely issue in Ethiopia. In general, biodiversity has to be protected for its multidirectional values [45, 46].

Though the local people are exposed to high cultural and habit change due to the repeated drought and resettlement conditions, the ethnobotanical knowledge on classification of their ecology and diverse use of TMPs were transmitted orally through generations. Different studies affirmed that the local people are knowledgeable about their environment in general and plants in particular [1, 33]. The findings of Giday et al. [22] indicated that boys were usually favored. Likewise, 50% of free transfers of knowledge particularly took place from parents to sons in this study. Similarly, several researchers reported that the distribution of LK is hierarchically placed and the services are obtained from the family, the village, or beyond. The secrecy of LK on medicinal practices could be one of the reasons for the uneven distribution of LK of TMPs in Ethiopia among community members [7, 21].

Comparison of LK on TMPs among age groups proves that knowledge on TMPs is wider among elderly persons while the youngsters are comparatively less knowledgeable. Since majority of young age groups are educated, modern education might have made the young generation underestimate the traditional practice. This is in line with the study of TMP in Kafficho and Butajira people [23, 47], which showed that illiterates and older residents are more likely to use traditional medicine than the educated and youngsters. Thus, age and education are main factors that appeared to influence the use of LK. On the other hand, the LK on the use of three groups of plants also varies among three groups of inhabitants. In fact, the knowledge of key informants on utilization of TMPs is proportionally high (92.6%). It is noted that the knowledge of TMPs is a means of income generation for the healers in the study sites. The folk naming of the plants by permanent residents, usually associated with their function and traits, is consistent with another study conducted by Awas and Demissew [23], which indicated that some names are attached to the disease treated and some are attributed to domestic or wild animals. However, returnees are the least knowledgeable and the names they gave to the plants were not specified. This could be due to the absence of returnees at the time of famine (1984/85). Furthermore, Martin [1] described that the knowledge on plant uses changes with time and space and with change of resources and culture. Therefore, the expansion of modern education and resettlement of people (to elsewhere) have resulted in the deterioration of LK and practices with the dislocated members of the community.

5. Conclusions

The present study indicated that the local people of Delanta are custodians of large number of TMP species (133) that they named and explained for the treatment of various human and livestock ailments (76). Notably, the highest proportion of TMPs (65.8%) were cited for human ailments. Various ethnobotanical analytical tools showed that the local people preferred some species over the others in treating ailments and other uses. About 85% of these resources also provide multiple uses. While most of the TMPs are harvested from the wild vegetation, the area is exposed to many threats such as agricultural expansion and other human induced pressures. As a result of these factors, some of the plants and the associated LK are under threat and declining. The ethnobotanical knowledge on TMP species varied among key informants, permanent residents, and returnees. Returnees were the least knowledgeable and this is one of the negative consequences of resettlement. The findings in general indicate that resettlement provided an opportunity to improve the restoration of useful plants and proved to be advantageous to biodiversity conservation in the original location (Delanta) and people should be resettled within their close community in order to avoid any disruption of their LK.

Homegardens and farmland margins have contributed to serving as preserving places of species presently in short supply and this is in need of further enhancing. Thus, strengthening the conservation of TMPs in such places is very important. For better diversity of species in general and TMPs in particular, in situ conservation measures need to be particularly directed to plant community types which could allow conservation of 50% of TMPs (*Becium grandiflorum* and *Rumex nervosus*; *Otostegia integrifolia* and *Dodonaea angustifolia* dominated community types). These potential TMP species may even give an insight for further pharmacological and therapeutic development in Ethiopia.

Conflict of Interests

The authors declared that they have no competing interests.

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Research Article

Ethnomedical Knowledge of Plants Used for the Treatment of Tuberculosis in Johor, Malaysia

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This study documented ethnomedical knowledge of plants used for the treatment of tuberculosis (TB) and its related symptoms as practiced by the Jakun community of Kampung Peta, situated in Endau Rompin Johor National Park, Johor, Malaysia. Eight key informants were selected by snowball sampling technique and data about medicinal plants were collected by semistructured interviews, participatory observations, and focus group. Qualitative analysis was undertaken using thematic analysis. There were 23 species of plants (22 genera, 20 families) documented and herbarium specimens were deposited at the UTHM Herbarium. *Dipterocarpus sublamellatus* was recorded for the first time with ethnomedical uses while other species were previously reported. The qualitative approach employed in this study demonstrates the emic perspective in terms of perceptions on traditional herbal medicine, transfer of knowledge, significant taboos related with medicinal plants, and their conservation efforts. Local and biomedical terminology in treatment of TB showed substantial correspondence. The outcomes obtained in the study are worth being further investigated for conservation strategies and are worthy of verifying their ethnomedical claims scientifically.

1. Introduction

Tuberculosis (TB) is a key global health problem [1]. This ancient, airborne infectious disease is caused by *Mycobacterium tuberculosis* bacterium. In 2012, it is estimated that 8.6 million people developed TB and 1.3 million died from it [1]. Its epidemiology shows that this disease can affect a whole community by causing significant mortality and morbidity to human being [2]. Additionally, occurrence of drug-resistant strains of TB poses serious threat to the current situation and the typical anti-TB drugs have caused ruthless side and adverse effects to the patients [3]. An ideal anti-TB regimen is not yet available to combat the resistant strains of TB and the recommended treatment regimens are problematic [4]. Since Johor, having the second highest prevalence of tuberculosis (TB) cases in Peninsular Malaysia, is also home to the Jakun, perhaps documenting the existing ethnomedical knowledge of the Jakun of the treatment for TB and its symptoms could be a leading way towards future discovery of medication for TB [5]. Therefore, the search for at least one potentially new drug derived from nature should be initiated [6]. In this case, ethnomedical knowledge of the Jakun community could provide a lead in primary screening of potential anti-TB agents.

Malaysia is ranked as the twelfth megadiverse country in the world due to its richness and endemism of flora and fauna [7]. Peninsular Malaysia has been estimated to have more than 2,000 species of medicinal plants and there are about 200 species being used by different ethnic groups all around the country [8]. Endau Rompin forest (2°25′12.94″N, 103°15′40.94″E) is one of the few remaining areas of virgin lowland rainforest in the southern part of Peninsular Malaysia. Geographically, it is the mainland Asian's southernmost stretch of tropical rainforest. In 1993, 48,905 hectares of the Endau Rompin forest was gazetted as a national park by the state government of Johor [9]. Kampung Peta is a village located outside the boundaries of the park (Figure 1). It has become the main entrance to Endau Rompin Johor National Park in the municipality of Mersing, Johor. Within the rich lowland mixed dipterocarp forest of the park lie various species of plants that provide substantial sources for

Figure 1: Location of Endau Rompin Johor National Park and Kampung Peta [11].

food, medicines, shelters, timber products, and many more to the nearby civilization [10].

Orang Asli is a local term for indigenous people in Peninsular Malaysia. A total of 18 tribes of *Orang Asli* are estimated to be 150,000 people, covering 0.5% of the whole Malaysian population [12]. They are clustered into three major groups: Negrito (northern region), Senoi (middle region), and Proto-Malay (southern region). A tribe called the *Jakun* is a subgroup of Proto-Malay and is the most dominant *Orang Asli* tribe in Johor [13]. The Jakun community of Kampung Peta are descendants of the first inhabitants of Endau River valley [14]. The population of Jakun community in Kampung Peta is about 220 people with 67 households, which represents 2% of the whole *Orang Asli* in Johor [15]. They are still practicing traditional lifestyle amidst modern facilities and strongly adhere to their ancestors beliefs as their way of life. They speak the Jakun dialect which is a subdialect of the Malay language. Their livelihood relies heavily on natural resources around them, which includes combination of fishing, hunting, farming, and trading forest products. Recent years have shown tremendous changes in their lifestyle. Due to socioeconomic improvement, the younger generation of Jakun are able to get higher education and many have migrated to other places [16].

The first documentation work about medicinal plants used by the Jakun community in Kampung Peta recorded 52 plants species used for minor common ailments [17]. Additionally, 118 species of plants from Endau Rompin Johor National Park were also surveyed against their alkaloid, saponin, triterpene, and steroid contents [18]. Recent inventory data taken from 2005 to 2008 showed that approximately 54 nontimber plant families are used for various ethnobotanical uses that include Rubiaceae (16 species), Arecaceae (12 species), Annonaceae (9 species), Melastomataceae (7 species), Euphorbiaceae (5 species), Leguminosae and Zingiberaceae (4 species), and Connaraceae, Liliaceae, Myrtaceae, Rhizophoraceae, and Piperaceae (3 species) [19–21]. This information demonstrates the prominence and dependency of the Jakun community on such plants that may have potential value as sources of active medicinal principles. Although the Jakun community in Kampung Peta still depend on their ethnomedical knowledge for primary healthcare, it is easy to lose this attribute as the world progresses towards modernization. Therefore, by conducting proper documentation, existing ethnomedical knowledge could be maintained and not easily manipulated.

This paper aims to document plants used for the treatment of TB and its related symptoms guided by ethnomedical knowledge of the Jakun community in Kampung Peta, Johor, south of Peninsular Malaysia. To the best of our knowledge, no previous ethnomedical study had been conducted specifically for treatment of TB from this community. The new information gained from this study might initiate further studies to aim at exploring the anti-TB potentials of the plants, supporting the sustainability of traditional herbal medicine in local community, and conserving plants diversity.

2. Methodology

2.1. Ethical Authorizations. Following ethical guidelines from [22], approval from Department of Orang Asli Development (JAKOA) under the Malaysia Ministry of Rural and Regional Development was acquired. Plants were collected under a permit approved by Johor National Parks Corporation (JNPC). Written Prior Informed Consent (PIC) was obtained and Access and Benefit Sharing (ABS) was explained during data collection. The inclusion of the headman (*tok batin*) of the Jakun of Kampung Peta indicated our commitment in fulfilling ABS mechanism at this juncture of time. Participation of informants was dependent on their self-willingness and acceptance of the terms in PIC and ABS, which were specially developed for this research program.

2.2. Data Collection. Fieldworks were conducted between April 2013 and April 2014. Key informants were recruited using snowball sampling method [23–25]. Initially, a courtesy call was made to the village headman. The purpose of the research was briefed to him and he then assigned his people to participate in the interviews. During the discussion with the first informant (R1), she then referred to other informants. Criteria of selection were also based on (i) the recognition that they are local practitioners by the Jakun community, (ii) their ability to identify plants and explain the uses, and (iii) the recommendation by park officers for their involvement in traditional herbal medicine. Eight key informants of Jakun community were selected from Kampung Peta as shown in Table 1. Each informant has vast experience in the areas of traditional practices, herbal formulations, field identification,

Table 1: Characteristics of the selected key informants in Kampung Peta.

Code	Gender	Age	Marital status	Belief	Knowledge gained from	Duration of practice	Education level	Occupation
R1	F	66	Widowed	A	S, O, P, and H	Since small	Primary school	Retired park staff, farmer
R2	F	57	Married	A	S, O, P, and H	Since small	No formal education	Retired park staff, farmer
R3	M	58	Married	A	S, O, and P	Since young	No formal education	Handicraft, herbal, and forest products entrepreneur
R4	F	55	Married	A	S, O, P, and H	After being married	No formal education	Park staff, farmer
R5	F	40	Single	A	S, O, P, and H	Since young	No formal education	Park staff
R6	F	44	Married	I	S, O, P, and H	Since small	Primary school	Park staff, trade forest products
R7	M	45	Single	A	S, O, and P	Since small	No formal education	Park staff
R8	F	55	Married	A	S, O, and G	3 months	Primary school	Park staff

Codes R1–R8 refers to informant's name. R1: Dido Lanau, R2: Lindan Jala, R3: Awang Kudi, R4: Kikai Akar, R5: Resnah Jala, R6: Azizah Hussien, R7: Salam Liman, R8: Kechek Chuka, F: female, M: male, A: animism, I: Islam, S: self-experienced, O: observation, P: parents, H: herbalist, and G: God or spirit.

and collection of medicinal plants. One of the informants (R8) was selected for her experience in preparing herbal remedies to treat her son who claimed to have active TB and now recovered from it.

In-depth, semistructured interviews were carried out as guided [26, 27]. The interviews were comprised of three parts: (i) demographic profile of the informants such as name, gender, age, marital status, religious belief, how they gained the knowledge, duration of practice, education level, and occupation; (ii) information about medicinal plants consumed by the Jakun related to signs and symptoms of TB (cough, cough with blood, cough with sputum, fever, night fever, loss of weight or appetite, asthma, rheumatism, and fatigue), including the local names, parts used, method of preparation, dosage, and administration; and (iii) significant aspects of Jakun's ethnomedical knowledge such as beliefs or taboos related to the plants. Each interview session lasted an average of two hours, ranging from 30 minutes to three hours.

Participatory observations were also done during casual or social meetings for any occurrences of what related to ethnomedical knowledge of plants among the Jakun community. This also created a unique opportunity for the main researcher to get closer, to build up rapport and trust, and to minimize the cultural gap between the main researcher and the informants.

A 2-day training course on "*Documentation of Ethnobotanical Knowledge of Indigenous People*" was organized by Universiti Tun Hussein Onn Malaysia (UTHM) to form a focus group. The objectives for this course were to provide training on ethnobotanical documentation and to establish an open-ended discussion among researchers, state agencies, and four representatives from the Jakun community. The discussion session was directed to encourage the representatives to share and discuss their knowledge in greater depth. Questions like "How do you feel about your mom using herbs? Do you think it is ancient or out-dated?" were asked. In this way, the representatives were able to provide in-depth answers as individuals.

2.3. Plants Identification. Plant samples were collected following the standard guidelines with consideration to the conservation of the species [28]. Triplicates of each Herbarium specimens were pressed, oven-dried at 40°C for two weeks, and mounted on Herbarium sheets, which were then deposited at Universiti Tun Hussein Onn Malaysia (UTHM) Herbarium Collection for future references. Other standard data such as location, vegetation, habitat description, other medicinal plants present, and local plant name were recorded at each field site on preprepared forms. Digital photographs showing morphological features were also taken. The prepared specimens were compared to previously identified specimens from Kepong National Herbarium (KEP). The authentication was done by Kamarudin Saleh from Forest Research Institute Malaysia (FRIM).

2.4. Data Analysis. Tables and graphs were generated in standard software, namely, Microsoft Excel 2013 [29]. Data from the transcribed interviews were analysed qualitatively following the emic approach [30]. Thematic analysis, which was derived from informants' own concepts, was applied to conceptualize the data, identify themes, and assign concept codes [31]. Reported uses of various medicinal plants were

compared with previously published ethnomedical literatures about medicinal plants in Endau Rompin Johor National Park to cross-check and identify new medicinal uses [32] and any loss of knowledge [33].

3. Results and Discussion

3.1. Demographics. The eight key informants were two males and six females, with ages ranging between 40 and 66 years. In common, they were individuals who gained knowledge of medicinal uses of plants from self-experiences and observations and through their parents as detailed in Table 1. Additionally, some of them were formally trained by a local herbalist due to their occupational requirement as park staffs. The God, forest spirits, or deceased ancestors revealed the knowledge through dreams, as experienced by one of the informants' son. This showed that belief and ethnomedical knowledge were integrated in this study. Although they were not regarded as the local experts or herbalists, they were the traditional herbal medicine practitioners that would genuinely describe the plants they were very familiar with to the researcher. Additionally, the main advantage of employing the snowball sampling method was that the subsequent key informants were introduced to the researcher based on acknowledgement by their own tribe. Thus, in this study, characteristics such as age, gender, marital status, belief, and education level did not influence the acquisition of their ethnomedical knowledge of plants.

3.2. Ethnomedical Knowledge of Plants

3.2.1. Plant Families, Habitat. The ethnomedical knowledge about the plants was summarized in Table 2. A total of 23 species of medicinal plants were documented in this study. From Table 2, 22 genera and 20 botanical families were presented, indicating that the medicinal plants were much diversified taxonomically. The top most represented families were Arecaceae, Aristolochiaceae, and Rubiaceae with two species each of the total distribution. Others were the remaining 17 families (Loganiaceae, Musaceae, Cucurbitaceae, Sterculiaceae, Annonaceae, Dipterocarpaceae, Dilleniaceae, Hypoxidaceae, Myrtaceae, Nepenthaceae, Urticaceae, Simaroubaceae, Euphorbiaceae, Poaceae, Anacardiaceae, Ebenaceae, and Connaraceae), which represented only one species each.

The plant families consist of various habitats such as trees (7 species), climbers (7 species), shrubs (4 species), herbs (3 species), and hemiepiphyte (1 species). In this study, the significant uses of the climbers in the Jakun ethnomedical knowledge showed a substantial relationship between traditional knowledge and plant conservation. As examples, the climbers are greatly dependent on large trees to grow and survive and vice versa [34, 35]. At the same time, the climbers play an essential role as remedial resource to the local community. Uncontrolled logging and deforestation could cause threats to the species of climbers and eventually erode local knowledge about medicinal plants [36]. Therefore, not only is documenting ethnomedical knowledge of plants an inventory *per se*, but it also contributes to the issue of biodiversity conservation threats such as deforestation, habitat modification, and unsustainable overexploitation.

3.2.2. Symptoms of TB. The 23 medicinal plants species recorded in this study were used to treat an active TB disease (claimed by the Jakun community) and nine of TB-related symptoms. The most frequently cited medicinal plants were used for fever (30%) as it is a common ailment even in other communities. Following that is cough (22%), fatigue (17%), and asthma (13%). 9% of the species were used to treat cough with blood, night fever, cough with sputum, and rheumatism, whereas 4% were documented to treat active TB and loss of appetite.

3.2.3. Parts Used. In this study, various plant parts were used for the herbal preparation. Commonly, roots and stems were used and this applied to 39% of all plants listed. This is followed by shoots involving 9%. The least used parts were flowers, fruits, seeds, and stem barks, for 4% of listed plants. According to informants, the root is the main plant part used in the Jakun traditional medicine. This may arise from the fact that the roots act as reservoirs for water and mineral uptakes, which is rich with variety of secondary metabolites such as steroids, alkaloids, terpenes, and volatile organic compounds [37]. 83% of the documented species were used individually, while the remaining 17% were recommended to be used in mixtures.

3.2.4. Preparation and Administration. In the Jakun community, herbal remedies are usually prepared fresh. If this is not the case, they will dry the plant parts (usually the roots) and keep them in a proper storage before use. The most common method of preparation was decoction in water (43% of listed plants) followed by collection of sap (35%) and being eaten raw (13%). The less common methods include infusion in water and being cooked as food (9% each) and maceration in water and decoction in oil (4% each). Decoction in water is equivalent to aqueous extraction and it appears to be much favoured because it is easier to prepare. Additionally, water is the best solvent to dissolve hydrophilic compounds that are responsible for various antimicrobial activities [38]. In this study, the most typical way of administration was taken in a form of drink (83%) followed by taken as food (17%) and applied on tongue (9%). The least typical ways of administration were as massage oil and cold press and for bathing (4% each). These elements might explain the relatively good association between preparation and administration of herbal remedies, and more than three-quarters of the listed plant species (87%) were taken orally as compared to those taken for topical applications (4%).

3.2.5. Conservation Status. 22 species of the plants documented in Table 2 are taken from the wild, whereas only one species (*Gardenia* sp.) is cultivated. Medicinal plants are generally harvested from nearby forest areas by the local people. These results corroborate the ideas of Ceuterick et al. [31], who suggested that local people use herbal remedies that are readily available and easily accessible in the natural vegetation around their settlement. However,

Table 2: List of medicinal plants recorded in this study.

Botanical information	Symptoms	Parts used	Methods of preparation	Ways of administration	Frequency of citation	Source of plants
Strychnos ignatii Berg. Akar Ipoh Loganiaceae Climber SUNR(P)001	Fever, rheumatism	Stem	Decoction in water, infusion in water	Oral: drink	6	The wild
Calamus sp. Rotan sepetang Arecaceae Climber SUNR035	Fever	Stem	Sap collected	Oral: drink	6	The wild
Calamus scipionum Lour. Rotan semambu Arecaceae Climber SUNR040	Fever	Stem	Sap collected	Oral: drink	5	The wild
Musa gracilis Holttum Pisang sum Musaceae Herb SUNR003	Cough	Stem, flower	Sap collected	Oral: drink, applied on tongue	5	The wild
Thottea praetermissa T.L. Yao Perut keletong Aristolochiaceae Shrub SUNR034	Cough, cough with sputum	Root	Decoction in water, raw	Oral: drink, eaten raw	5	The wild
Hodgsonia macrocarpa (Blume) Cogn. Teruak Cucurbitaceae Climber SUNR001	Fever	Stem	Sap collected	Oral: drink	4	The wild
Scaphium macropodum (Miq.) Beumée ex. Heyne Kembang semangkok Sterculiaceae Tree SUNR021	Fever (high)	Seed	Infusion in water	Oral: drink, mucilage eaten	4	The wild
Polyalthia bullata King Tungkat Ali Hitam Annonaceae Shrub SUNR030	Fatigue	Root	Decoction in water	Oral: drink	4	The wild
Dipterocarpus sublamellatus Foxw. Keruing air Dipterocarpaceae Tree SUNR037	TB	Stem bark	Decoction in water, decoction in oil	Oral: drink. Topical: massage oil, for bathing	4	The wild
Tetracera macrophylla Wall. ex. Hook.f. & Thomson Empelas Dilleniaceae Climber SUNR002	Night fever	Stem	Sap collected	Oral: drink	3	The wild

Table 2: Continued.

Botanical information	Symptoms	Parts used	Methods of preparation	Ways of administration	Frequency of citation	Source of plants
Molineria latifolia (Dryand.) Herb. ex. Kurz var. *latifolia* Lembak Hypoxidaceae Herb SUNR014	Loss of appetite	Fruit	Raw	Oral: eaten raw	3	The wild
Rhodamnia cinerea Jack Pelonggot Myrtaceae Tree SUNR019	Fever, fatigue	Stem	Sap collected	Oral: drink	3	The wild
Nepenthes ampullaria Jack Sentoyot Nepenthaceae Climber SUNR024	Asthma, rheumatism	Root	Decoction in water	Oral: drink	3	The wild
Poikilospermum suaveolens (Blume) Merr. Demom malam Urticaceae Hemi-epiphyte SUNR026	Night fever	Stem	Sap collected	Oral: drink	3	The wild
Eurycoma longifolia Jack Tungkat Ali Putih Simaroubaceae Tree SUNR029	Fatigue	Root	Decoction in water. In combination with *Rennellia elliptica*, *Polyalthia bullata*, and others	Oral: drink	3	The wild
Gardenia sp. Bunga cina Rubiaceae Shrub SUNR020	Fever	Shoot, leaf	Maceration in water	Topical: cold press	3	Cultivated
Macaranga gigantea (Rchb.f & Zoll.) M.A. Tudung Euphorbiaceae Herb SUNR005	Cough	Stem	Sap collected	Oral: applied on tongue	2	The wild
Leptaspis urceolata (Roxb.) R.Br. Lapun puyuh Poaceae Herb SUNR012	Asthma, cough with sputum	Root	Decoction in water	Oral: drink	2	The wild
Thottea grandiflora Rottb. Hempeduk beruang; Telingok kelawar Aristolochiaceae Shrub SUNR022	Cough, asthma	Root	Decoction in water	Oral: drink	2	The wild

Table 2: Continued.

Botanical information	Symptoms	Parts used	Methods of preparation	Ways of administration	Frequency of citation	Source of plants
Campnosperma auriculatum (Blume) Hook.f. Habong Anacardiaceae Tree SUNR028	Cough with blood	Shoot, root	Decoction in water, raw, and cooked	Oral: drink, eaten raw, and cooked as food	2	The wild
Diospyros cauliflora Blume Uncertain* Ebenaceae Tree SUNR013	Cough	Uncertain*	Uncertain*	Uncertain*	1	The wild
Rourea mimosoides (Vahl.) Planch. Pengesep Connaraceae Climber SUNR033	Cough with blood	Root	Decoction in water	Oral: drink	1	The wild

*The informants were unable to provide the detailed information regarding the criteria for this plant.

forestry overexploitation for timber products [39, 40] and wide popularity of their local use lead to overharvesting [41] and perhaps put them into higher risk of extinction in the future if no conservation efforts are engaged. In this study, all informants showed an understanding about conservation practices. Their strong affection towards the forest was observed by the researcher during the fieldworks. The implementation of *ex situ* conservation through home garden and *in situ* conservation through the establishment of ethnobotanical garden in national park area was efforts made by the Jakun community and the national park authority.

> If I get medicines that are rare... highly healing... I will plant them. (R1, 2014, personal communication)

It is interesting to note that Jakun's ethnomedical knowledge reflected their thoughtful conservation efforts and respects towards nature. Apart from replanting the medicinal plants, they also practice to reuse the raw materials.

> I will not waste the materials. After using, I collect the decoction and I dry the remaining materials again to reuse them. (R6, 2014, personal communication)

Perhaps, unintentionally, these ethnomedical practices that implement sustainable method of harvesting have contributed to the conservation of medicinal plants. In addition, the awareness of loss of herbs among the Jakun community shows that the natural resources are increasingly threatened and intensifying efforts need to be implemented immediately to curb this problem. One of the informants stated that majority of the medicinal plants are easily available but certain species are also available with difficulty.

> Before this it was very easy to find. Now, it is hard. (R4, 2014, personal communication)

Pardo-de-Santayana and Macía [42] agreed that local resources particularly the plants they use as food and medicine are crucial to ensure that those communities can continue to live and benefit from their local ecosystems in a sustainable way.

3.2.6. Frequency of Citation. The plants with the highest frequency of citation by informants are *Strychnos ignatii* and *Calamus* sp. (6 citations), whereas plants with the lowest frequency of citation by the informants are *Diospyros cauliflora* and *Rourea mimosoides* (1 citation). Even though six species were cited by less than three informants ($n < 3$), their medicinal uses appear to be worthy of further investigations to verify their possible pharmacological activities especially those used to treat constitutional symptoms of TB such as night fever and cough with sputum [30]. However, being named by at least three informants ($n \geq 3$) is the most typical cut-off point used by ethnobotanists to establish agreement [43].

3.2.7. Novel Knowledge. Comparison with previous documentation works appeared to suggest that this study attained one new ethnomedical knowledge and one new claim. Majority of the species reported by the informants were already known as medicinal plants in Malaysia except for *Dipterocarpus sublamellatus*. Therefore, in this study, *D. sublamellatus* was documented for the first time with ethnomedical knowledge while the rest of the listed species were formerly reported with diverse medicinal uses from other indigenous communities. *D. sublamellatus* was specifically used to treat active TB as claimed by some of the key informants. It is interesting to note that this particular species is a member of Dipterocarpaceae family, which was reported to contain sesquiterpenes, triterpenes, coumarin derivatives, phenolics, essential oil, and isoquinoline alkaloids groups [44, 45]. The use of *Gardenia* sp. as medicinal plants for the Jakun

Table 3: Themes that emerged via the coding process.

Themes	Subthemes	Codings
Perceptions on traditional medicine	Primary source of healthcare for elder generation	Primary
	Alternative source of healthcare for younger generation	Alternative
Transfer of knowledge	Mothers have a significant influence	Mothers
	The young generation are not interested to learn traditional knowledge due to modern lifestyle	Time
Conservation of medicinal plants	Some valuable and in-demand herbs are difficult to find	Difficult to find
	The location to collect plants is far	Too far
	They use only small amount, use them when necessary, and reuse the materials	Reuse
	They plant the seedlings	Replant
Taboos	Avoid taking prohibited meals during treatment	Eating
	Nice weather is a good time	Collecting
Confusion of names	Appearance of uncertainties	Names

community, which was another new claim recorded in this study, was not previously recorded. The knowledge might be gained by cross-cultural interaction with outsiders like the Malays and Chinese, as it is typical ornamental and medicinal plant in these cultures [46].

3.3. Thematic Analysis. The thematic analysis approach was helpful to recognize the culturally valuable ethnomedical knowledge of the Jakun community. Repetition of certain words provided a cue to assign coding and identify themes. In addition, the repetition of questions was deliberated to provide a focus for analysis. For instance, the word "time" appeared frequently during the interviews in describing events of plant collection and herbal administration. Subcodings such as "collecting" or "eating" would be a reference to a theme such as "taboos." Once the themes emerged, data were fragmented to lift coded elements out of the context of each interview to list comments and information by group [24, 25]. Table 3 lists the themes that emerged from the codings.

3.3.1. Perceptions on Traditional Medicine. Medicinal plants were fairly important in the Jakun community for both the elderly and the young generations. The use of traditional medicine did not seem to conflict with the use of modern medicine. In many cases, they complemented each other. However, there were some contraries among the elder and younger generations of Jakun community in Kampung Peta. As examples consider the following:

> We never abandon our traditional practices. Just like you, the Malay; if you don't get well surely you will go to the hospital. We still carry out as what our ancestors have been practicing before and never leave it behind. (R1, 2014, personal communication)

> "If modern medicine is not effective, I have to look for forest remedies as an alternative." (Son of R1, 2014, personal communication)

The elder generation uses traditional herbal medicine as the primary source of healthcare while the younger generation uses traditional herbal medicine as the alternative source of healthcare if the modern medicine seems not effective. From the focus group discussion, Jakun's representative expressed his feelings of being the young generation of Jakun who is keeping up with the modern lifestyle and his effort to preserve their traditional knowledge. He mentioned the following:

> I do not feel ashamed to the fact that my mother is practicing traditional herbal medicine. Indeed, I feel so proud of it. I also want to learn about it and use it to my daughter. (Rudi bin Kudi, 2013, personal communication)

Although the elder generations are practicing less frequently ethnomedicine due to modernization, such declaration as above proved that the younger generations of the Jakun in Kampung Peta are still supporting the strong practices of ethnomedical knowledge of their ancestors.

3.3.2. Transfer of Knowledge. During present study, it was found that the knowledge about utilization of medicinal plant species is generally accumulated by observation and experiences and transferred orally to the next generation without any systematic process. However, it is certain that such knowledge system is at the risk of fading in the future [47]. Lack of interest from the youth is one of the main concerns among the elderly. The young generation of Jakun shows less attention and are not keen on learning their traditional knowledge from the elder generation. A likely

explanation is that because it has little scope for money. Therefore, they engage themselves in other occupations [48]. One of the informants narrated the following:

> Even so... the community... mostly the new generation could not recognize the medicinal plants. This is why I tell them, they are the local people but they do not recognize the cures from the forest. (R1, 2014, personal communication)

Commitment towards other responsibilities such as seeking formal education was given more priority compared to learning and teaching about ethnomedical knowledge. The informant explained the following:

> How can we teach our grandchildren about this knowledge while they are studying at school? (R4, 2014, personal communication)

Assimilation to modern lifestyle by the young generation most probably contributes to the huge impact on transfer of knowledge. At the time this study was conducted in 2013, the community in Kampung Peta had already gained access to modern medical treatment that was frequently used. It was in the form of a small clinic built by the government in the village to routinely monitor health status of the Jakun community. In addition, they received regular biweekly visits by the medical officers. Moreover, it takes only two hours by car or motorcycle from the village to Mersing Hospital, where doctors are available. Consequently, all of these lessen the exposure to ethnomedical knowledge as a source of remedies [49].

Despite the challenges in transferring the knowledge, having a family and being a parent lead to the awareness in learning about traditional herbal medicine.

> He (referring to her son)... now knows a little about forest remedies; after he has a daughter. A few years back before he could not tell anything at all. He definitely knew nothing. Just after his daughter was sick, he asked my opinion on which forest remedies are better. (RI, 2014, personal communication)

Medicinal plants have traditionally been used at home to treat family sickness. In this case, women have particular roles in transferring the ethnomedical knowledge in their capacities as mothers [50].

3.3.3. Taboos Associated with Medicinal Plants. In Jakun's ethnomedical knowledge practices, a few conditions must be followed during the plant collection, preparation, and treatment to ensure efficacy. For medical purposes, medicinal plants should be collected in certain settings such as during the full moon or early in the morning. Indeed, time of harvest is a possible source of variation for the bioactivity of the extracts [51]. They are particularly prohibited to collect plants during "*hujan panas*" or summer rain. They believe that summer rain brings harmful effects on the collector's health and the plants might contain toxic metabolites. Additionally, they are aware of the safety and dosage issues particularly if they take traditional medication together with modern medicine. As demonstrated in Table 2, most of the medicinal plants are prepared as water infusion. The water infusion mainly extracts bioactive compounds such as anthocyanins, tannins, saponins, and terpenoids [52]. As a result, the herbal preparation should only be taken after meal and the Jakun community would avoid any acidic or spicy food during treatment to avoid stomach pain.

3.3.4. Confusion in Names. Confusion of plant names and terminologies and the appearance of uncertainties as shown in Table 2 indicate the erosion of ethnomedical knowledge among the Jakun and this was apparent in this study. Khuankaew et al. [49] suggested that lack of experience with the ethnomedical knowledge practices, which is very vital in the transmission of knowledge, might be a possible factor. This event also suggests that certain knowledge might potentially be lost as a form of deculturation. The reason as to why the Jakun people stop using certain remedies may be due to availability of better alternatives (modern medicine). Ceuterick et al. [31] concluded that herbal remedies can function as ethnic markers. Thus, erosion of this traditional knowledge and practices may possibly weaken Jakun's sense of identity.

During the interviews, some of the informants gave information about the plants that they previously consumed themselves. On the other hand, some of the informants gave information about medicinal plants that they thought the researcher might be interested in although they have little knowledge about the plant. It is important to bear in mind the possible bias in these responses. Hence, confirmation using quantitative approach should be employed to ratify the statement and to eliminate bias of information.

3.4. Correspondence between Local and Biomedical Terminology. Following their emic perceptions, all of the key informants were able to differentiate the symptoms of TB as described by the researcher and to define their ethnomedical terms according to their understanding. Table 4 lists the symptoms of TB given by informants and their equivalent biomedical terms.

Based on Table 4, 14 local terms of TB-related symptoms were listed and each term was capable of being translated into standard biomedical terms. Terminology is one of the challenges during ethnomedical knowledge documentation [51, 52]. Mcclatchey [53] emphasized that it is critical to use terms that are meaningful within a community, even if they are obscure to scientific fields. This is because culture defines medicine while disease etiologies differ between ethnomedical systems [54]. As the one discussed here, Heinrich et al. [55] argued that translating indigenous and local diagnosis into biomedical terms is ideally essential for future clinical assessment.

In the Jakun community, TB is closely associated with black magic. "*Hasad dengki*" or jealousy was speculated as the cause for this disease. According to the informant,

> in our community, this disease is typically linked to jealousy. It is intended to destroy the person.

Table 4: Symptoms of TB given by informants and their equivalent biomedical terms.

Ailment categories	Biomedical terms	Local terms
Respiratory diseases and fever	Cough	*Se'eh, batuk, gatal-gatal tekak, sakit lidah*
	Cough with sputum	*Batuk berkahak*
	Ordinary fever	*Demam, panas dalam*
	High fever	*Demam panas*
	Asthma	*Semput*
	Chest pain	*Sakit dada*
	Night fever	*Demam malam*
Arthritis	Rheumatism	*Sakit dalam badan*
	Joint pain	*Sakit lutut, sakit sendi*
Ear, nose, throat bleedings	Nose	*Hidung dan tekak berdarah*
	Sore throat	*Sakit leher*
	Cough with blood	*Batuk berdarah*
Others	Fatigue	*Lemah badan*
	Loss of appetite	*Kurang/Tiada selera makan*

> We no longer practice such custom and I, myself prohibit it. (Sangka Chuka, 2013, personal communication)

In Kampung Peta, "*bomoh*" could also be consulted to cure less acute conditions by employing his knowledge of the chemical properties of plants. Therefore, any plants might be used as a medicinal plant with some addition of charm or "*jampi*." Some of the plants introduced by the "*bomoh*" as medicinal plants might over time be incorporated into the group of medicinal plants used by common people in the village. Additionally, the Jakun community also believed that, other than the "*bomoh*," any selected individual could receive knowledge about forest remedies through dreams revealed by the spirits of the jungle (*semangat hutan* or *dewa*) or their deceased ancestors, who sympathize with their sufferings. Additionally, the Jakun community have not entirely stopped believing in black magic and the powers of plants to impose curses to cure or neutralize curses. But as the Malays and Chinese came in they perceive all black magic as the work of demons and it should be strictly avoided. They still practice animism and believe that God has given plants their specific qualities and their power to act as remedies.

4. Conclusions

This study has contributed to the scientific documentation of medicinal plants used for the treatment of TB in Johor, Malaysia. The 23 species of medicinal plants recorded in this study demonstrate that the Jakun community in Kampung Peta are still rich in ethnomedical knowledge particularly of treatment of TB and its related symptoms. The most frequently cited species were *Strychnos ignatii* and *Calamus* sp. *Dipterocarpus sublamellatus* was recorded for the first time for its ethnomedical knowledge and traditionally claimed to treat active TB by the Jakun. While other species were formerly reported, *Gardenia* sp. was a new addition to Jakun's ethnomedical knowledge. Jakun's ethnomedical knowledge needs to be conserved as the larger percentage of the traditional practitioners is older generation and some of the knowledge was apparently eroded in this study. The qualitative approach employed in this study successfully provide the emic perspective in terms of perceptions on traditional herbal medicine, transfer of knowledge, significant taboos related with medicinal plants, and their conservation efforts. Local people and biomedical terminology in treatment of TB showed substantial correspondence. Further studies are in progress on the antituberculosis assay to validate their traditional claims.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

In Vitro Pharmacological Activities and GC-MS Analysis of Different Solvent Extracts of *Lantana camara* Leaves Collected from Tropical Region of Malaysia

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We investigated the effect of different solvents (ethyl acetate, methanol, acetone, and chloroform) on the extraction of phytoconstituents from *Lantana camara* leaves and their antioxidant and antibacterial activities. Further, GC-MS analysis was carried out to identify the bioactive chemical constituents occurring in the active extract. The results revealed the presence of various phytocompounds in the extracts. The methanol solvent recovered higher extractable compounds (14.4% of yield) and contained the highest phenolic (92.8 mg GAE/g) and flavonoid (26.5 mg RE/g) content. DPPH radical scavenging assay showed the IC_{50} value of 165, 200, 245, and 440 μg/mL for methanol, ethyl acetate, acetone, and chloroform extracts, respectively. The hydroxyl scavenging activity test showed the IC_{50} value of 110, 240, 300, and 510 μg/mL for methanol, ethyl acetate, acetone, and chloroform extracts, respectively. Gram negative bacterial pathogens (*E. coli* and *K. pneumoniae*) were more susceptible to all extracts compared to Gram positive bacteria (*M. luteus*, *B. subtilis*, and *S. aureus*). Methanol extract had the highest inhibition activity against all the tested microbes. Moreover, methanolic extract of *L. camara* contained 32 bioactive components as revealed by GC-MS study. The identified major compounds included hexadecanoic acid (5.197%), phytol (4.528%), caryophyllene oxide (4.605%), and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (3.751%).

1. Introduction

Nature has existed as a source of almost all drugs for many years and natural products were the only source of medicine for mankind ever since the ancient period. Herb based products play an important role in primary human health care as the majority (80%) of the global population rely on traditional medical practices [1, 2]. Most of the modern drugs are derived either from plant sources or from their derivatives for various medicaments and are extensively used in the pharma industry [2]. In addition to the prevailing health problems, emerging infectious diseases and disorders have seriously caused the world population to suffer with a high mortality rate. It is reported that about 50% of all fatality occurring in tropic countries is mainly due to the current infectious diseases [3]. Also, increase of antimicrobial resistance among the pathogens is a rising problem which is challenging the scientific advancement of the medical world [4]. This situation has prompted researchers to develop efficient new antimicrobial agents. Therefore, exploration of natural products as leads to discover new drug molecules is continuously made to understand their therapeutic potential with special reference to biological activities, efficiency, and safety aspects. Exploration of medicinal plants for curative purposes is mainly based on the available traditional information from the experts and local population [5, 6].

Lantana camara L. is a medicinal aromatic plant that belongs to the family Verbenaceae and occurs in most parts of the world as an evergreen notorious weed species. It is also considered as an ornamental garden plant. It is widely used

in different traditional medical practices for treating various health problems. Different parts of the plant are used in treating various human ailments such as measles, chicken pox, tetanus, malaria, cancers, asthma, ulcers, fevers, eczema, skin rashes, cardiac disorders, and rheumatism [7, 8]. Also leaf extracts and essential oil of *L. camara* leaves possess larvicidal activities, antioxidant, anti-inflammatory, analgesic, antidiabetic, hypolipidemic, anthelmintic, wound healing, and antipyretic properties [9, 10]. The therapeutic potential of the plant is due to the occurrence of many bioactive phytocompounds such as terpenoids, alkaloids, flavonoids, phenolics, glycosides, and steroids as major phytoconstituents [11]. Some of the important bioactive compounds include quercetin, isorhamnetin, oleanolic acid, lantadene A, β-sitosterol pomonic acid, camaric acid, verbacosides, lantanoside, linaroside, octadecanoic acid, palmitic acid, and docosanoic acid. The essential oil from the leaves is rich in monoterpenes and sesquiterpenes [11–13]. Various factors including genetic, geographical location, plant parts, and environmental factors have been shown to influence the accumulation of phytochemical contents in different parts of *L. camara* and its essential oil composition [14–16]. Also, occurrence of varietal differences in phytoconstituents of *L. camara* has been documented by Sharma et al. [17]. More recently, in vitro study indicated the existence of chemical differences in methanol leaf extract of four varieties of *L. camara* collected from India and their antioxidant property was found to differ [8]. Certainly, more research efforts should be carried out to explore the potential benefits of *L. camara* for treating various health problems. Therefore, the present investigation was aimed at determining the phytochemical constituents and antioxidant and antimicrobial activities of different solvent extracts of *L. camara* leaves collected from the tropical region of Malaysia. Moreover, the bioactive components of the extracts were also identified using GC-MS analysis.

2. Materials and Methods

2.1. Plant Collection. *L. camara* plant material was collected from the forest area near Universiti Putra Malaysia, Serdang, Selangor, Malaysia, during the month of May 2015. Plant material was authenticated by N. A. P. Abdullah, Department of Crop Science, Universiti Putra Malaysia, Malaysia, and the voucher specimen (LC-102015) was deposited at the department. The leaves were detached from the collected materials, washed with water and dried under shade for 1 week, and finely powdered using electric blender. The powdered material was kept at room temperature for future use.

2.2. Preparation of Extracts. Five grams of powdered leaves was kept in a beaker to which 100 mL of various organic solvents (ethyl acetate, methanol, acetone, and chloroform) was added and thoroughly shaken. Later the mixture was placed at room temperature for 48 hrs and stirred 2-3 times a day. After filtering the mixture, the filtrate was evaporated to dryness using Rotavapor. The final extracts were weighed to determine the yield (%) and the dried extracts were stored at 4°C in a refrigerator for further studies.

2.3. Phytochemical Screening. The presence of various phytochemical constituents such as alkaloids, saponins, flavonoids, phenolics, anthraquinones, tannins, cardiac glycosides, steroids, and terpenoids was screened qualitatively by using standard procedures [18–20].

2.4. Determination of Total Phenolic and Flavonoid Contents. Total phenolic contents present in different solvent extracts were determined by using FC (Folin-Ciocalteu) colorimetric method as detailed by Salar and Seasotiya [21]. About 0.1 g of dried extract was suspended in 1 mL of the respective solvents and 0.1 mL of this solution was mixed thoroughly with 1 mL of sodium carbonate (20%) solution and 0.5 mL of 50% FC reagent. Thereafter, the solution was allowed to remain at room temperature for about 20 min to observe the color change. The absorbance was taken against the blank (water) at 730 nm. Using different concentrations of gallic acid, a standard calibration curve was generated. Total phenolic content was represented as mg of gallic acid equivalents (GAE) per gram of dried extract.

Total flavonoids content present in different solvent extracts was verified by the modified procedure of Zhishen et al. [22] using colorimeter. Briefly, 2 mL of distilled water was mixed well with 0.5 mL of solvent extract. Thereafter, 150 μL of $NaNO_2$ solution (5%) was added and kept for 5 min at room temperature. It was followed by adding 600 μL of $AlCl_3$ (10%) and 2 mL of NaOH (4%). After mixing thoroughly, the solution was made up to 5 mL with distilled water and set aside for 15 min at room temperature. By using water as blank, absorbance was measured at 510 nm. A standard calibration curve was generated using different concentrations of rutin. Total flavonoid content was articulated as mg of rutin equivalent (RE) per gram of dried extract.

2.5. Antioxidant Activity

2.5.1. Free Radical Scavenging Activity. The antioxidant activity of each extract was assessed using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay as explained by Mohanty et al. [23] with little modifications. In short, 0.3 mL of different extract at varied concentrations (100–1000 μg/mL) was mixed with 2 mL of 0.1 mM DPPH solution and incubated for 30 min under dark conditions at room temperature. Afterwards, using UV-visible spectrophotometer, the absorbance was taken at 517 nm against methanol (blank) while ascorbic acid served as a standard sample. The reaction mixture showing lower absorbance is an indication of higher activity of radical scavenging. The inhibition percentage of free radical scavenging was calculated based on the following formula:

$$\text{DPPH scavenging activity } (\%) = \frac{A\text{control} - A\text{sample}}{A\text{control}} \times 100. \quad (1)$$

For each extract, IC_{50} values (the minimum quantity of extract necessary for scavenging free radicals up to 50%) were calculated from the standard plot.

Table 1: Qualitative screening of phytochemicals present in different solvent extracts of *L. camara* leaves.

Phytochemicals	Ethyl acetate	Methanol	Chloroform	Acetone
Alkaloids	−	+	−	+
Flavonoids	+	+	+	+
Saponins	+	+	−	+
Phenolics	+	+	+	+
Tannins	−	−	+	−
Anthraquinones	+	−	−	+
Cardiac glycosides	+	+	+	+
Terpenoids	−	+	+	−
Steroids	−	+	−	−

Note: + = present, − = absent.

2.5.2. Hydrogen Peroxide (H_2O_2) Scavenging Activity. The potential capacity of different solvent extracts to scavenge H_2O_2 was carried out by the method outlined by Mohanty et al. [23]. In brief, various quantities of plant extract (100–1000 μg/mL) were prepared as detailed in previous experiment. By using phosphate buffer (pH 7.4), H_2O_2 solution (4 mM/L) was prepared. Varied amounts of plant extracts were mixed with 0.6 mL of H_2O_2 solution and kept for 30 min incubation at room temperature. The activity of H_2O_2 was analyzed by taking absorbance at 230 nm using phosphate buffer without H_2O_2 as blank solution. H_2O_2 scavenging activity (%) was calculated by using the following equation:

$$H_2O_2 \text{ scavenging activity } (\%) = \frac{A\text{control} - A\text{sample}}{A\text{control}} \times 100. \quad (2)$$

For each extract, IC_{50} value (the minimum quantity of extract necessary for scavenging free radicals up to 50%) was calculated from the standard plot.

2.5.3. Determination of Antibacterial Activity. The antibacterial activities for the extracts were evaluated by using disc diffusion method. The experiment included both Gram positive (*Micrococcus luteus*, *Bacillus subtilis*, and *Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) pathogenic strains of bacteria. One mg of each extract was dissolved in 1 mL of DMSO (dimethyl sulfoxide) and about 2.5, 5.0, and 10 μL of this solution were impregnated on sterilized filter paper discs (6 mm size). The discs were kept on the nutrient agar medium preinoculated uniformly with the known bacterial culture. The discs soaked with chloramphenicol (50 μL of 50 μg/mL) served as positive control while discs soaked with 50 μL DMSO served as negative control. All culture plates were kept in an incubator at 37°C for 24 h and the bacterial inhibition zone was recorded and expressed in mm. For each bacterial strain, the test was repeated 3 times.

2.6. GC-MS Analysis. GC-MS analysis of the active methanol extract of *L. camara* was carried out by using the GC-MS instrument (Model GCMS-QP2010 Ultra, Shimadzu Co., Japan) equipped with a capillary column DB-1 (0.25 μm film × 0.25 mm i.d. × 30 m length). The instrument was operated in electron impact mode at ionization voltage (70 eV), injector temperature (230°C), and detector temperature (280°C). The carrier gas used was helium (99.9% purity) at a flow rate of 1 mL/min and about 1 μL of the sample was injected. The oven temperature was initially programmed at 80°C (isothermal for 5 min.) and then increased to 200°C at 5°C/min and finally to 280°C at 5°C/min (isothermal for 16 min). The identification of compounds from the spectral data was based on the available mass spectral records (NIST and WILEY libraries).

2.7. Statistical Analysis. All the data measured in each experiment included 3 replications ($n = 3$) and the results were represented as mean ± SD. The one-way analysis of variance (ANOVA) was performed to compare the data and Tukey's test was used to find out the statistically significant differences at $p < 0.05$ using statistical software, GraphPad Prism version 5.0.

3. Results and Discussion

The results of preliminary investigation on the phytochemicals present in different solvent extracts of *L. camara* are presented in Table 1. Different phytocompounds such as alkaloids, saponins, flavonoids, phenolics, anthraquinones, tannins, cardiac glycosides, steroids, and terpenoids were detected in the crude extracts. Flavonoids, phenolics, and cardiac glycosides were noticed in all solvent extracts used. Methanol extracts with 7 phytoconstituents were the best among the organic solvents evaluated in our study. Acetone extract showed the presence of 6 phytoconstituents while ethyl acetate and chloroform extracts contained 5 phytocompounds. All these identified phytochemicals are known to have a wide range of biological activities including antibacterial, antifungal, antiviral, antioxidant, and cytotoxic properties [19]. Understanding the occurrence of phytochemicals in medicinal plants is advantageous and presently, the discovery of new drug compounds or lead molecules from plants is mainly based on the systematic examination of different plant extracts or plant based products. Also, this preliminary knowledge can decipher a new source for economically valued chemical compounds [1, 23].

Table 2: Dry weight and total yield of different solvent extracts of *L. camara* leaves.

Solvent extracts	Weight of the extract (μg ± SD)	Yield (%)
Ethyl acetate	501.3 ± 3.5^{b}	10.0
Methanol	721.3 ± 1.5^{a}	14.4
Acetone	260.6 ± 4.0^{c}	5.2
Chloroform	141.6 ± 2.5^{d}	2.8

Note: each value is expressed as mean ± standard deviation (SD) ($n = 3$). Values in the column followed by a different letter superscript are significantly different ($p < 0.05$).

Table 3: Total phenolics and flavonoids content of different solvent extracts of *L. camara* leaves.

Solvent extracts	Total phenolic content (mg GAE/g) ± SD	Flavonoid content (mg RE/g) ± SD
Ethyl acetate	75.6 ± 0.9^{b}	16.7 ± 0.6^{c}
Methanol	92.8 ± 1.7^{a}	26.5 ± 0.5^{a}
Acetone	62.9 ± 1.7^{c}	20.6 ± 0.3^{b}
Chloroform	33.7 ± 0.5^{d}	21.2 ± 0.8^{b}

Note: each value is expressed as mean ± standard deviation (SD) ($n = 3$). Values in the column followed by a different letter superscript are significantly different ($p < 0.05$) and values having the same letters are not statistically significant ($p < 0.05$). GAE: gallic acid equivalent, RE: rutin equivalent.

The weight of the leaf extracts and their yield obtained from different solvent extracts of *L. camara* are presented in Table 2. Different solvents showed a significant influence on the total dry weight and yield of the extracts. Relatively, the extract from methanol resulted in superior extraction yield (14.4%) with 721.3 ± 1.5 μg of dry weight. The recovery of extractable constituents from different extracts remained in the following order of methanol > ethyl acetate > acetone > chloroform. Our results are in conformity with previous studies supporting the use of methanol as the best solvent to recover higher extractable compounds from various medicinal plants [23, 24]. Similarly, Anwar et al. [16] have stated that methanol as the best solvent for extraction from *L. camara*. Also, methanol was commonly employed by other researchers in *L. camara* for various biological studies [8, 25–27]. However, literature study shows that there are no available reports on the comparative yield analysis obtained from different solvent extracts till date. The existence of significant differences of dry weight of the extracts and yields between various organic solvents can be because of different polar nature of the solvents tested [24].

Polyphenols and flavonoids are the plant secondary metabolites occurring in several medicinal plants known to possess antimicrobial, antioxidant, antispasmodic, antidepressant, antitumor, antimutagenic, anti-inflammatory, and many other biological activities [27, 28]. In plants, these phenolic compounds provide defense against various pathogens, regulate cell division and growth, and help in pigmentation and many other metabolic pathways [29]. Therefore, we investigated the occurrence of total phenolic and flavonoid content in different organic solvent extracts (Table 3). The results clearly indicated the existence of statistically significant differences ($p < 0.05$) among the different extracts. The highest phenolic content (92.8±1.7 mg GAE/g) and flavonoid content (26.5 ± 0.5 mg RE/g) were observed in the methanol leaf extract of *L. camara*. On the other hand, ethyl acetate, acetone, and chloroform extract contained phenolic content of 75.6 ± 0.9, 62.9 ± 1.7, and 33.7 ± 0.5 mg GAE/g, respectively. Total flavonoid content in ethyl acetate, acetone, and chloroform extract was found to be 16.7 ± 0.6, 20.6 ± 0.3, and 21.2 ± 0.8 mg RE/g, respectively. Similarly, previous studies have shown the occurrence of rosmarinic and caffeic acid as the major phenolic compounds and few flavonoids such as 3,7-dimethoxy-, 3-methoxy-, and 3,7,4′-trimethoxy-quercetin, hispidulin, 3, pectolinarigenin 7-O-β-D-glucoside, and camaraside glycoside were evident in the plant extract of *L. camara* [7, 11, 26]. Due to high polarity, methanol was found to exhibit better efficiency in extracting various polar phytocompounds (phenolics and flavonoids) from the leaves of *L. camara*. Both total phenolic and flavonoid contents obtained in our study were relatively much higher than the quantity obtained by earlier researchers in the same species but collected from geographically distant places [8, 16, 26]. However, total phenolic content was much lesser than the quantity (245.5 ± 3.5 mg gallic acid/g) as reported by Mahdi-Pour et al. [15] from methanol leaf extract of *L. camara* located in Kedah, Malaysia, and this difference can be attributed the influence of environmental factors and geographical location.

The formation of increased free radicals in human body may cause cell damage andinduces various disorders such as cancer, myocardial infarction, atherosclerosis, and neurodegenerative disorders. However, antioxidant compounds derived from natural source or plants can repair these free radicals formed in cells and thereby, antioxidants are very useful in preventing various disorders [2, 15, 23]. The antioxidant capacities of either natural products or crude plant extracts are usually evaluated by making use of DPPH radical scavenging test [23]. In principal, the test depends on the capacity of DPPH free radicals reacting with plant metabolites such as phenolic and flavonoid compounds (H^+ donors) occurring in the sample. After the reaction, DPPH solution turns from purple to yellow color due to acquiring of a proton from the donor species. The intensity of color change directly relates to the scavenging ability of the biological sample [27]. Figure 1 shows the DPPH scavenging activity of *L. camara* leaf extracts in comparison to positive control (ascorbic acid). In all the solvent extracts, radical scavenging activity was found to be concentration dependent. The highest percentage of scavenging activity (86.4 ± 0.2 μg/mL) was observed in methanolic leaf extracts at 500 μg/mL concentration. The next best solvent extract was found to be acetone (80.5 ± 0.3 μg/mL) followed by ethyl acetate (72.4 ± 0.3 μg/mL) and chloroform (526.1 ± 0.3 μg/mL). However, the activities of all extracts were inferior to that of the ascorbic acid standard. The IC_{50} values of DPPH free radical scavenging activity were found to be in the following order: ascorbic acid (80 μg/mL) > methanol extract (165 μg/mL) > ethyl acetate (200 μg/mL) > acetone (245 μg/mL) > chloroform (440 μg/mL). These results are on a par with or even superior to that of reports by other researchers on several other crude extracts of the plant

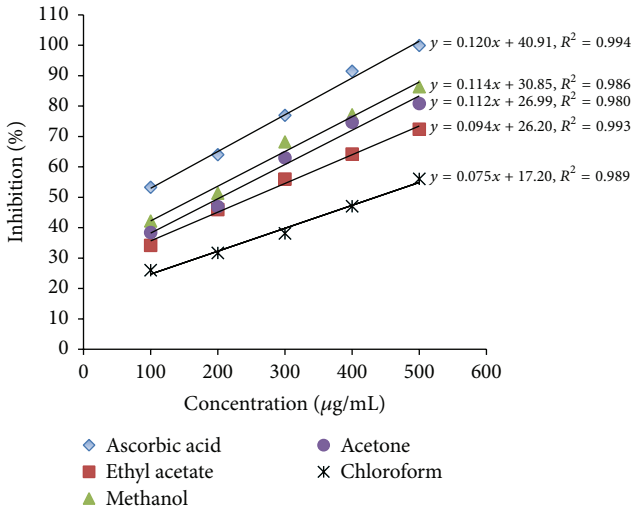

Figure 1: DPPH scavenging activities of various solvent extracts of *L. camara*.

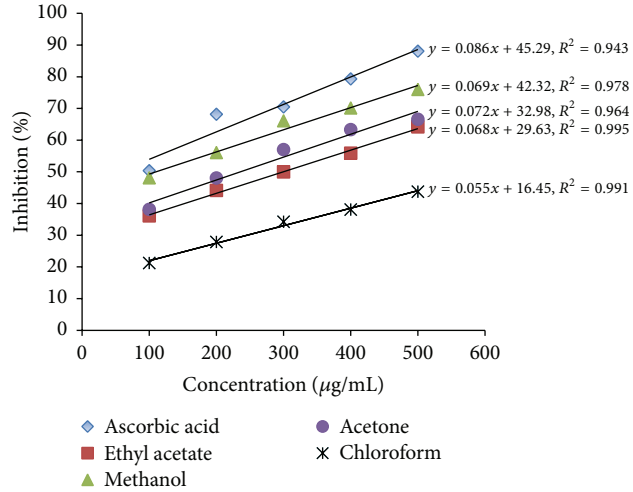

Figure 2: H_2O_2 scavenging activities of various solvent extracts of *L. camara*.

evaluated under the same conditions using DPPH assay [27]. The higher antioxidant potential of plant extracts is correlated to the occurrence of many antioxidant compounds especially polyphenols [23]. Although the antioxidant activity of *L. camara* leaf extracts by using DPPH assay was reported by earlier researchers [15, 16, 26], none of them compared the effects of different solvents on antioxidant potential. In our study, due to higher solubility of antioxidant compounds, the methanol extract exhibited increased radical scavenging activity compared to other solvent extracts. Similarly, other researchers have stated that free radical scavenging potential of plant extracts depends mainly on the occurrence of bioactive compounds, particularly polyphenols [8, 30].

In biological system, a large quantity of hydroxyl radicals is formed due to activation of immune cells which are known to be highly toxic radicals and causes extreme damage to all molecules occurring in live cells. These radicals can trigger cell toxicity and mutagenesis by damaging DNA nucleotides [8, 31]. Therefore, measuring hydroxyl radical scavenging activity can provide good information on the antioxidant potential of *L. camara* leaf extracts obtained by different solvents. The results of hydroxyl radicals inhibition obtained from our study are depicted in Figure 2. It is evident that, irrespective of the organic solvents used for extraction, the percent inhibition of hydroxyl radicals increased with the concentration of the extracts. At higher concentration of 500 μg/mL, the percentage of hydroxyl scavenging activity for ascorbic acid, ethyl acetate, methanol, acetone, and chloroform extracts was found to be 88.1 ± 0.4, 64.2 ± 1.0, 76 ± 0.3, 66.6 ± 0.5, and 43.7 ± 0.2%, respectively. The IC_{50} value was found to be in the following order of ascorbic acid (60 μg/mL) > methanol (110 μg/mL) > ethyl acetate (240 μg/mL) > acetone (300 μg/mL) > chloroform (510 μg/mL). The lower the IC_{50} value, the higher the scavenging activity and hence, methanol extract was found to possess superior antioxidant potential compared to other extracts tested. However, their effect was considerably lesser than the standard, ascorbic acid. These results completely corroborate the earlier reports that have proved the existence of correlation between the antioxidant property and the concentration and composition of different plant metabolites occurring in the extracts [23, 32]. In our study, methanol extracts contained significantly higher quantity of phenolics (92.8 ± 1.7 mg GAE/g) and flavonoids (26.5 ± 0.5 mg RE/g) and hence possessed superior antioxidant potential compared to other solvent extracts.

At present, the increased prevalence of deadly diseases and microbes adapting to antibiotic resistance is a great concern in the medical world [33]. Hence, more research interest is shown by medical community towards the development or discovery of novel antimicrobial agents. Due to the severe side effects of several synthetic antibiotics, research preference is mainly focused on discovering plant based natural drugs [5, 34]. Since *L. camara* possessed numerous secondary metabolites, we evaluated the effects of their different solvent extracts against some common human pathogenic bacterial strains. The results of our study revealed that all solvent extracts of *L. camara* were effective against both Gram positive and Gram negative bacterial strains tested, but their efficacy varied (Table 4). With the increase in the concentration, there was an enhanced antibacterial activity irrespective of the solvent used for extraction. Gram negative bacteria were more susceptible to all extracts compared to Gram positive bacteria. In general, Gram negative bacteria are known to exhibit high resistance towards wide range of chemical agents and antibiotics compared to Gram positive bacteria. Besides, Gram negative bacteria are reported to be the most prevailing pathogens causing a large number of deaths [34, 35]. Thus, *L. camara* leaf extracts can be more beneficial in treating most of these Gram negative disease causing pathogens. Methanol extract had the highest inhibition activity against all the tested microbes when compared to any other solvent extracts. The methanol leaf extract exhibited the highest activity against *E. coli* (24.1 ± 0.4 mm) followed by *K. pneumoniae* (18.1 ± 0.4 mm) at higher concentration (10 μL/disc). *S. aureus* was also more vulnerable to methanol extract with 18.0 ± 0.4 mm

Table 4: Antibacterial activity of different solvent extracts of *L. camara* at different concentrations.

Solvent extracts (μL/disc)	Zone of inhibition (mm)				
	Escherichia coli	*Klebsiella pneumoniae*	*Micrococcus luteus*	*Bacillus subtilis*	*Staphylococcus aureus*
Ethyl acetate					
2.5	08.2 ± 0.8	06.9 ± 0.5	06.1 ± 0.7	10.0 ± 0.5	—
5.0	13.0 ± 0.9	08.3 ± 0.5	07.1 ± 0.3	13.8 ± 0.5	06.1 ± 0.1
10.0	12.0 ± 0.5	10.5 ± 0.8	07.0 ± 0.5	13.9 ± 0.4	10.6 ± 0.4
Methanol					
2.5	14.6 ± 0.5	08.2 ± 0.3	08.1 ± 0.7	10.0 ± 0.3	14.1 ± 0.2
5.0	18.2 ± 0.2	14.5 ± 0.5	12.2 ± 0.3	14.0 ± 0.6	16.1 ± 0.6
10.0	24.1 ± 0.4	18.1 ± 0.4	18.0 ± 0.5	16.1 ± 0.2	18.0 ± 0.4
Acetone					
2.5	16.1 ± 0.4	06.1 ± 0.6	07.9 ± 0.2	14.2 ± 0.3	05.8 ± 0.6
5.0	24.0 ± 0.4	10.1 ± 0.6	12.3 ± 0.2	15.9 ± 0.4	10.2 ± 0.2
10.0	28.2 ± 0.6	16.2 ± 0.2	12.2 ± 0.2	16.3 ± 0.4	12.2 ± 0.4
Chloroform					
2.5	10.6 ± 0.6	—	—	10.5 ± 0.9	—
5.0	12.9 ± 0.6	09.2 ± 0.2	06.8 ± 0.7	12.1 ± 0.7	07.2 ± 0.2
10.0	14.8 ± 0.3	11.4 ± 0.8	08.2 ± 0.2	12.4 ± 0.4	07.9 ± 0.2

Note: the negative control discs were soaked with 50 μL DMSO and the positive control discs with 50 μL (50 μg/mL) *chloramphenicol*. Each value represents the mean ± standard deviation (SD) of 3 replicates per treatment in 3 repeated experiments. "—" represents no activity.

of inhibition zone at 10 μL/disc concentration. Among the tested bacterial strains, *E. coli* and *B. subtilis* showed increased zone of inhibition to all the solvent extracts and were the most susceptible strains. Ethyl extract was also most effective against *B. subtilis* while other bacterial strains were moderately inhibited. Likewise, acetone and chloroform extracts were also effective in inhibiting *E. coli* and *B. subtilis*. However, at lower concentrations, ethyl acetate extract showed no inhibition against *S. aureus*. In contrast, chloroform extract was less effective to all bacteria and at lower concentration, it failed to show antibacterial activity. *L. camara* finds its application in many parts of the world to cure various human ailments [7]. Previously, researchers have reported the varied antimicrobial potential of this plant and thus our reports support these findings [13, 14]. These results substantiate the findings of Naz and Bano [26] where methanol extract of *L. camara* leaves showed the highest antimicrobial activity. However, this is the first ever attempt which emphasizes the influence of different solvent extracts on human pathogenic bacterial strains. Our study clearly indicated the existence of considerable differences in the antibacterial activity among the various solvent extracts evaluated. This could be due to varied phytochemical constituents present in different solvent extracts.

Further, we used GC-MS analysis to identify the bioactive compounds occurring in the most competent solvent extract of *L. camara*. GC-MS profiling was performed only for methanolic leaf extract due to the fact that it contained many phytochemicals and exhibited superior biological activities. The distinctive chromatogram of the methanolic leaf extract of *L. camara* is shown in Figure 3. The analysis separated and identified a total of 32 known compounds belonging to different chemical classes (Table 5). The major compounds included hexadecanoic acid (5.197%), phytol (4.528%), caryophyllene oxide (4.605%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (3.751%), 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (2.954%), α-D-galactopyranoside, methyl (2.790%), coumaran (2.288%), germacrene-D (2.185%), bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl (2.065%), spathulenol (1.888%), 1,2,3-propanetriol, 1-acetate (1.689%), propane-1,2,3-triol (1.1615), and 2,4(1H,3H)-pyrimidinedione, 5-methyl- (1.180%). Few other compounds identified in the extract are α-elemol, myristic acid, neophytadiene, furfuryl alcohol, propargyl alcohol, and acetic acid, fluoro-, ethyl ester. Many of these identified constituents are known to possess several pharmacological activities. Hexadecanoic acid, a major phytoconstituent of *L. camara* methanolic leaf extract, is known to possess strong antimicrobial activity [36]. The diterpene, phytol, is an important compound reported with antioxidant, cytotoxic, and antimicrobial properties [37]. Similarly a conjugated saponin, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one, is reported to possess strong antioxidant, anticancer, and anti-inflammatory properties [38, 39]. Caryophyllene oxide, spathulenol, and germacrene-D are known to possess anticarcinogenic, anti-inflammatory, and antibacterial properties [2, 40]. As a biofumigant, coumaran is reported to act against insect pests found in stored food grains [41]. The compound 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one has been reported in plant extracts exhibiting antioxidant, antiproliferative, and anti-inflammatory properties [40]. More recently, anti-inflammatory and cytotoxicity activities of hexadecanoic acid, methyl ester have been reported by Othman et al. [42]. However, pharmacological activities of other compounds of *L. camara* methanolic leaf extract are yet to be determined. Therefore, we assume that the strong bioactivities exhibited

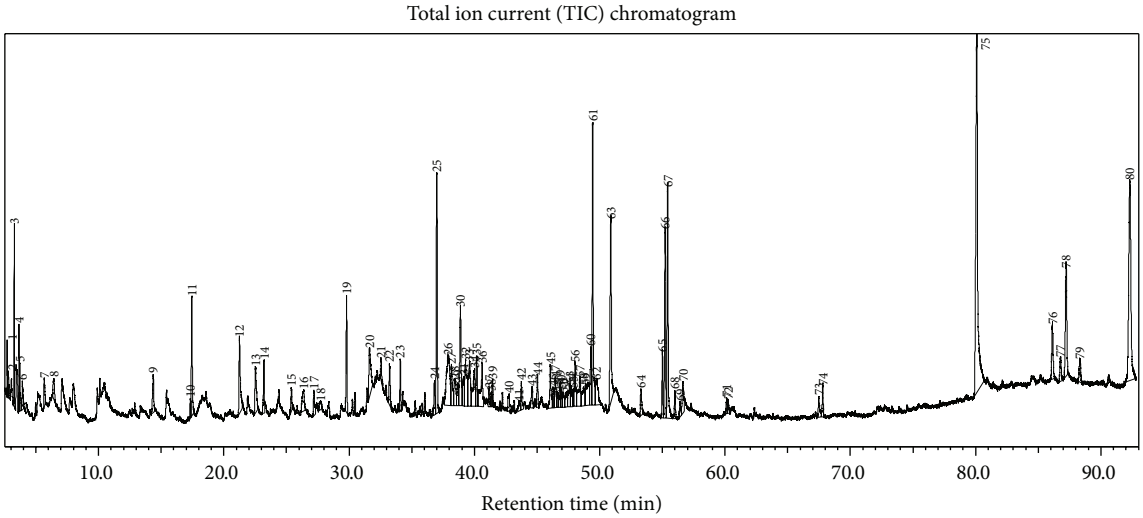

Figure 3: GC-MS chromatograph of methanolic leaf extract of *L. camara*.

Table 5: The major phytocompounds detected in the methanolic leaf extract of *L. camara* by GC-MS analysis.

S. number	Name of the compound	Peak number*	Retention time (min)	Area (%)
1	2-Propanone, 1-hydroxy-	1	2.703	0.481
2	Propane-1,2,3-triol	3	3.275	1.161
3	Propargyl alcohol	4	3.653	0.877
4	Acetic acid, fluoro-, ethyl ester	5	3.719	0.737
5	Furfuryl alcohol	7	5.672	0.750
6	2,4(1H,3H)-Pyrimidinedione, 5-methyl-	9	14.366	1.180
7	2,3-Dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one	11	17.443	2.954
8	Coumaran	12	21.251	2.288
9	1,2,3-Propanetriol, 1-acetate	13	22.529	1.689
10	Cyclohexasiloxane, dodecamethyl-	14	23.200	0.978
11	4-Vinylguaiacol	15	25.393	0.809
12	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl	19	29.798	2.065
13	Germacrene-D	21	32.547	2.185
14	Longifolene	22	33.22	0.889
15	4-Epi-cubedol	23	34.084	0.817
16	Caryophyllene oxide	25	36.995	4.605
17	α-D-Galactopyranoside, methyl	26	37.909	2.790
18	Humulene epoxide II	27	38.173	0.696
19	Spathulenol	30	38.886	1.888
20	α-Elemol	34	39.997	0.507
21	Myristic acid	42	43.751	0.652
22	Neophytadiene	45	46.059	0.820
23	3-Eicosyne	53	47.594	0.211
24	9-Octadecenoic acid (Z)-, methyl ester	60	49.296	0.093
25	Hexadecanoic acid, methyl ester	61	49.438	0.696
26	Hexadecanoic acid	63	50.888	5.197
27	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	65	55.007	1.037
28	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	66	55.225	3.751
29	Phytol	67	55.426	4.528
30	Octadecanoic acid, methyl ester	68	56.428	0.330
31	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	70	56.640	1.019
32	Phthalic acid, di(2-propylpentyl) ester	74	67.804	0.688

*Peak number is represented in Figure 3.

by *L. camara* in this study are correlated to the occurrence of these bioactive compounds in the methanol solvent extract. However, further studies on the isolation, characterization, and biological evaluation of these identified compounds are necessary to confirm their potential benefits.

4. Conclusion

In conclusion, the present investigation clearly revealed that phytochemical composition of *L. camara* leaf extract varied with respect to different solvents. Total phenolic and flavonoid content significantly varied among the different solvent extracts. Methanol solvent extract of *L. camara* leaves contained more extractable metabolites compared to any other solvents. Moreover, all solvent extracts of *L. camara* showed considerable antioxidant and antimicrobial activity with varying differences due to differences in their phytochemical composition. Thus, our study suggests that methanol leaf extract of *L. camara* containing many bioactive compounds may possibly be utilized as a therapeutical source for developing beneficial drugs to manage various human diseases and disorders.

Disclosure

Mohd. Sayeed Akhtar is a coauthor.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Research Article

The Antioxidant and Starch Hydrolase Inhibitory Activity of Ten Spices in an *In Vitro* Model of Digestion: Bioaccessibility of Anthocyanins and Carotenoids

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The antioxidant and starch hydrolase inhibitory activities of cardamom, cloves, coriander, cumin seeds, curry leaves, fenugreek, mustard seeds, nutmeg, sweet cumin, and star anise extracts were investigated in an *in vitro* model of digestion mimicking the gastric and duodenal conditions. The total phenolic contents in all spice extracts had statistically significantly ($P < 0.05$) increased following both gastric and duodenal digestion. This was also in correlation with the antioxidant assays quantifying the water-soluble antioxidant capacity of the extracts. The lipophilic Oxygen Radical Absorbance Capacity assay did not indicate a statistically significant change in the values during any of the digestion phases. Statistically significant ($P < 0.05$) reductions in the anthocyanin contents were observed during the digestion phases in contrast to the carotenoid contents. With the exception of the cumin seed extract, none of the spice extracts showed statistically significant changes in the initial starch hydrolase enzyme inhibitory values prior to gastric and duodenal digestion. In conclusion, this study was able to prove that the 10 spices were a significant source of total phenolics, antioxidant, and starch hydrolase inhibitory activities.

1. Introduction

Antioxidant and starch hydrolase inhibitory activities are two of the most coveted mechanisms to which prevention of noncommunicable diseases such as cardiovascular disease (CVD), diabetes, and cancer is currently attributed. While antioxidants provide protection from cellular damage due to free radicals, starch hydrolase inhibitory activity is known to prevent the sudden release of glucose into the physiological system, thereby preventing the biochemical pathways which trigger the production of free radicals inside the mitochondria [1, 2]. Carotenoids and phenolic compounds have been identified as two major dietary antioxidants with both categories containing over hundred member compounds. Carotenoids are fat-soluble pigments, while phenolic compounds commonly exist as free, esterified, etherified, and insoluble-bound forms. The most abundant form of phenolic compounds is flavonoids, which are abundantly found in edible fruits, leafy vegetables, roots, tubers, bulbs, herbs, spices, and legumes [3, 4]. Other than antioxidant activity, certain flavonoids are known to possess the ability to modulate cellular enzyme activities—a trait which is responsible for the inhibition of starch hydrolases such as α-amylase and α-glucosidase [5]. Measurement of antioxidant and starch hydrolase inhibitory activities is well documented. Several chemical and biochemical assays have been utilized for the quantification of the total antioxidant capacity (TAC) of plant products [6]. In addition, with reference to phenolic compounds, a number of studies present a measure of the total polyphenol content of food products in order to draw comparisons with other similar products and to provide more detailed information about this subgroup of antioxidants [7, 8]. However, in this respect, a major obstacle in evaluating the role of individual food components in modifying disease risk

is the scarcity of information on factors that influence their bioavailability and bioaccessibility [9].

Bioavailability is the degree to which a drug, nutrient, dietary supplement, or nutraceutical is available to the body. On the other hand, bioaccessibility is defined as the amount of a food constituent that is present in the gut, as a consequence of the release of this constituent from the solid food matrix, and may be able to pass through the intestinal barrier [10]. Several *in vitro* methods have been used to determine the bioaccessibility and bioavailability of individual antioxidant compounds in order to isolate those which remain stable and active throughout the digestion and absorption processes [5, 8, 9]. Only bioactive compounds released from the food matrix by the action of digestive enzymes present in pancreatic and duodenal digestion and bacterial microflora (large intestine) are bioaccessible in the gut and therefore potentially bioavailable. From this perspective, the amount of bioaccessible food antioxidants and other therapeutic compounds of interest may differ quantitatively and qualitatively from polyphenols included in food databases [11]. Given these conditions, the aim of this research was to quantify polyphenol contents, TAC, and starch hydrolase inhibitory activity of the acetone/water extracts of 10 commonly consumed spices and assess the stability of these parameters after the gastric and duodenal digestion phases in an *in vitro* model. Besides flavoring purposes, spices and herbs are known for their medical or antiseptic properties—characteristics which have been owed to the presence of immense amounts of antioxidant compounds [12]. In fact, the preservative effect of many spices and herbs suggests the presence of antioxidant and antimicrobial constituents [12, 13]. Special attention was rendered towards the analysis of anthocyanins which are abundantly present in the selected spices. Although flavonoids and phenolic acids are relatively more stable under the duodenal digestion conditions, anthocyanins are known to undergo ring fission during physiological digestion due to the varied pH conditions [14]. Selected carotenoids compounds are also quantified in order to provide a more holistic view on the effect of the digestion procedure on various types of antioxidant compounds.

2. Materials and Methods

All reagents, chemicals, and HPLC standards used for this study were purchased from Sigma Chemicals (St. Louis, MO, USA).

2.1. Preparation of Spice Powders and In Vitro Digestion Procedure. The following spices were chosen for the study based on their therapeutic properties, previous studies on antioxidant and starch hydrolase inhibitory properties as documented in the published literature [15–17]: cardamom (*Elettaria cardamomum*), cloves (*Syzygium aromaticum*), coriander (*Coriandrum sativum*), cumin seeds (*Cuminum cyminum*), curry leaves (*Murraya koenigii*), fenugreek (*Trigonella foenum*), mustard (*Brassica nigra*), nutmeg (*Myristica fragrans*), sweet cumin (*Pimpinella anisum*), and star anise (*Illicium verum*). Dried powders of the spices were obtained from the Ayurvedic Medicinal Hall in Kandy, Sri Lanka. The methodology by Wu et al. [18] was followed for the preparation of the herbal extracts using acetone/water/acetic acid (70 : 29.5 : 0.5). The *in vitro* digestion model was adapted from Ryan et al. [12]. In brief, the extracts of samples were transferred to clean amber bottles and mixed with saline (balanced salt solution) to create a final volume of 20 mL. The samples were acidified to pH 2.0 with 1 mL of a porcine pepsin preparation (0.04 g pepsin in 1 mL 0.1 M HCl) and incubated at 37°C in a shaking water bath at 3000 g for 1 h. After gastric digestion, 500 μL of each sample was extracted and stored at −20°C. The pH was then increased to 5.3 with 0.9 M sodium bicarbonate followed by the addition of 200 μL of bile salts glycodeoxycholate (0.04 g in 1 mL saline), taurodeoxycholate (0.025 g in 1 mL saline), and taurocholate (0.04 g in 1 mL saline) and 100 μL of pancreatin (0.04 g in 500 μL saline). The pH of each sample was increased to 7.4 with 1 M NaOH. Samples were incubated in a shaking water bath at 95 rpm at 37°C for 2 h to complete the intestinal phase of the *in vitro* digestion process. After the intestinal phase, 500 μL of each sample was extracted and stored at −20°C. Digested samples were analyzed within 2 weeks.

2.2. Total Phenolic Content and Antioxidant Activity Assays. The total phenolic contents were determined according to Singleton et al. [17]. The values were expressed as μg gallic acid equivalents per gram fresh weight (μg GAE/g) of sample. Quantification of the water-soluble Oxygen Radical Absorbance Capacity ($ORAC_{FL}$) was carried out according to the method by Prior et al. [19], while the lipophilic Oxygen Radical Absorbance Capacity ($ORAC_{oil}$) was carried out according to the method by Hay et al. [20]. The DPPH method was conducted using the method by Brand-Williams et al. [21]. The FRAP assay was carried out as described by Benzie and Strain [22]. In addition, the antioxidant activities of the samples were analyzed by investigating their ability to scavenge the ABTS•+ using a methodology previously reported by Ozgen et al. [23]. All assays were carried out in 96-well plate format using the Synergy HTX multimode microplate reader and Gen5 software (Biotek, Winooski, VT, USA).

2.3. Assays of α-Amylase and α-Glucosidase Inhibitory Activities. The α-amylase inhibitory activity of the extracts was carried out according to the method by Liu et al. [24], while the α-glucosidase inhibitory activity was carried out according to the method by Koh et al. [25]. Both assays were carried out in 96-well plate format using the Synergy HTX multimode microplate reader and Gen5 software (Biotek, Winooski, VT, USA). Data were expressed as IC_{50} (mg/mL).

2.4. Total Monomeric Anthocyanin Pigment Content and High Performance Liquid Chromatography (HPLC) Determination of the Individual Anthocyanin Compounds. The AOAC official method 2005.02 [26] was carried out to quantify the total monomeric anthocyanin pigment content. The values were expressed as mg cyanidin-3-glucoside equivalents per gram wet weight of spice. The quantities of the individual anthocyanin compounds cyanidin-3-O-galactoside (C3Ga), cyanidin-3-O-glucoside (C3Gl),

cyanidin-3-O-arabinoside (C3Ar), delphinidin-3-glucoside (D3Gl), peonidin-3-O-galactoside (P3Ga), and peonidin-3-O-arabinoside (P3Ar) were measured according to the method by Brown and Shipley [27]. Ten milliliters of solvent was centrifuged at 3000 g for 10 min. A volume of 1000 μL of the extracts were diluted with 500 μL of the extraction solvent (acetone/water/acetic acid, 70 : 29.5 : 0.5). Approximately 1 mL of solution was filtered through a 0.45 μm nylon filter into an amber HPLC vial. The quantities of the individual anthocyanin compounds were quantified in the extracts using standards. A Shimadzu (Kyoto, Japan) HPLC system equipped with a diode array detector (SPDM10Avp) and a Phenomenex Luna C-18(2) column (4.6 mm i.d. × 25 cm, 5 μm) was used for the quantification.

2.5. HPLC Determination of Carotenoids. Sample extraction and HPLC analysis for carotenoids (neoxanthin, violaxanthin, lutein, zeaxanthin, α-carotene, and β-carotene) were carried out according to the internal standard (IS) method by Lee et al. [28]. The Shimadzu (Kyoto, Japan) HPLC system equipped with a Phenomenex Luna C-18(2) column (4.6 mm i.d. × 25 cm, 5 μm) was used for the quantification having cross-linked end-capping with diode array (SPDM10Avp) detection. At least triplicate extractions were performed for each sample. IS solution was weekly prepared according to the method by Lee et al. [28]. For calibration, 100 μL of the IS solution was mixed with 100 μL of standard mixtures of various concentrations. Stock solutions of each standard were prepared individually with relevant solvents as described by Lee et al. [29].

2.6. Statistical Analysis. All results are presented as mean ± standard error mean (SEM). For comparisons, data was analyzed by ANOVA and Tukey's multiple comparison test (SPSS, version 17). A probability of 5% or less was accepted as statistically significant.

3. Results and Discussion

3.1. Total Phenolic Contents, Antioxidant Activities, Anthocyanin, and Carotenoid Contents. The total phenolic contents are shown in Table 1. Cloves had the highest total phenolic content (22.83 ± 0.20 mg GAE/g) followed by curry leaves (21.94 ± 0.19 μg GAE/g). Nutmeg had the lowest total phenolic content (0.80 ± 0.03 μg GAE/g). Some of these herbs were also evaluated for the total phenolic content in a study by Przygodzka et al. [30], namely, cardamom, cloves, coriander, nutmeg, and star anise. However, the values which were obtained in this study were much higher, most likely owing to the method of extraction as well as the source from which they were obtained. In the instance of cumin, the reported total phenolic contents differed from the study by Vallverdú-Queralt et al. [31], in which the value was higher. Vallverdú-Queralt et al. [31] had also used an extraction method which differed from the present study. Overall, the extraction method and the source from where the spices were obtained have a significant effect on the final reported value as highlighted by Zheng and Wang [32]. Following the gastric phase of digestion, all spice extracts had statistically significant increases ($P < 0.05$) in the total phenolic content. The trend continued onto the duodenal phase of digestion as well ($P < 0.05$). The antioxidant activity values are shown in Tables 2 and 3. Despite the increases in the total phenolic contents in both pancreatic and duodenal digestion phases, the $ORAC_{FL}$, ABTS, DPPH, and FRAP results did not indicate similar trends with a few exceptions. Overall, the antioxidant activity values coming from these assays were observed to have either been maintained during the digestion phases or statistically significantly increased ($P < 0.05$). The $ORAC_{oil}$ values did not display any statistically significant increases or decreases as compared with the values prior to the gastric and duodenal digestion phases as well, with the only exception being cardamom, where a statistically significant increase ($P < 0.05$) was observed in the duodenal digestion phase. Despite the ABTS, DPPH, and FRAP assay values following an almost similar trend as the $ORAC_{FL}$ values, their correlation with the total phenolic contents was comparatively less than the $ORAC_{FL}$ values. A clear correlation between the TAC values was also not observed. The $ORAC_{FL}$ assay has been applied extensively to evaluate the antioxidant capacity of a large variety of food products and many supplement and functional food companies compare their products, including juices, favorably to fruits and vegetables using the $ORAC_{FL}$ results from those studies [15]. The $ORAC_{FL}$ method is the currently most widely recognized assay used by food manufacturers despite its significant internal variability. From this assay, the antioxidant activity is determined using the area under the curve of a measurement of the protection from oxidation by free radicals generated in a temperature-dependent reaction. On the basis of technical issues related to temperature gradients across the plate in commonly used plate readers, this assay can have significant internal variability [30]. Although this technical issue does not pertain to end point determinations such as ABTS, FRAP, and DPPH assays, the $ORAC_{FL}$ assay data was still included in this study for the overall determination of the

Table 1: Total phenolic content of the spices prior to digestion as well as after gastric and duodenal digestion. $^*P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

Spice	Prior (μg GAE/g)	Gastric (μg GAE/g)	Duodenal (μg GAE/g)
Cloves	22.83 ± 0.20	23.20 ± 0.03*	23.25 ± 0.02*
Curry leaves	21.94 ± 0.19	23.87 ± 0.02*	23.89 ± 0.01*
Sweet cumin	4.99 ± 0.19	5.301 ± 0.14*	5.312 ± 0.03*
Cumin seeds	4.92 ± 0.07	5.29 ± 0.17*	5.48 ± 0.03*
Coriander	4.23 ± 0.12	5.16 ± 0.02*	5.24 ± 0.04*
Mustard	3.76 ± 0.04	4.092 ± 0.16*	4.47 ± 0.20*
Fenugreek	3.56 ± 0.03	4.66 ± 0.24*	4.82 ± 0.03*
Star anise	3.25 ± 0.13	4.13 ± 0.06*	4.25 ± 0.04*
Cardamom	1.18 ± 0.06	1.86 ± 0.08*	1.87 ± 0.04*
Nutmeg	0.80 ± 0.03	0.90 ± 0.06*	0.92 ± 0.04*

Table 2: Changes to the $ORAC_{FL}$ and $ORAC_{oil}$ values in μmol Trolox Equivalents per gram (μmol TE/g) of the spices prior to digestion as well as after gastric and duodenal digestion. $^{*}P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

Spice	$ORAC_{FL}$ (μmol TE/g)			$ORAC_{oil}$ (μmol TE/g)		
	Prior	Gastric	Duodenal	Prior	Gastric	Duodenal
Cloves	782.1 ± 6.5	785.6 ± 2.5	724.9 ± 1.1	658.2 ± 4.7	649.8 ± 3.9	658.9 ± 3.8
Curry leaves	752.6 ± 4.8	755.9 ± 2.1*	758.9 ± 1.6	642.9 ± 3.8	641.9 ± 3.7	647.9 ± 2.5
Sweet cumin	238.9 ± 5.8	231.2 ± 1.9	237.8 ± 2.1*	542.1 ± 2.9	539.8 ± 2.7	548.1 ± 3.1
Cumin seeds	210.3 ± 3.5	231.2 ± 1.9*	229.8 ± 2.1*	489.6 ± 6.5	479.6 ± 5.8	487.1 ± 2.4
Coriander	197.5 ± 3.2	185.9 ± 1.3*	189.7 ± 1.4*	471.8 ± 3.9	467.8 ± 3.8	467.5 ± 2.1
Mustard	177.5 ± 2.3	180.3 ± 1.6	185.9 ± 2.3	408.9 ± 5.4	410.8 ± 2.7	411.2 ± 3.7
Fenugreek	165.2 ± 3.4	165.9 ± 1.4	167.5 ± 1.6	359.8 ± 2.8	361.7 ± 2.4	357.6 ± 4.7
Star anise	158.4 ± 2.6	159.7 ± 1.5	159.3 ± 1.2	347.2 ± 3.9	359.2 ± 3.4	359.2 ± 3.7
Cardamom	92.5 ± 3.5	89.7 ± 2.0*	91.2 ± 1.3*	318.7 ± 2.6	320.6 ± 2.5	342.0 ± 1.9*
Nutmeg	65.8 ± 1.2	63.8 ± 1.8*	75.4 ± 1.8*	304.9 ± 2.1	305.1 ± 2.8	306.4 ± 2.9

Table 3: Changes to the ABTS, DPPH, and FRAP assay values of the spices prior to digestion as well as after gastric and duodenal digestion. $^{*}P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

Spice	ABTS (μmol TE/g)			DPPH (EC_{50}, mg/kg)			FRAP (μmol TE/g)		
	Prior	Gastric	Duodenal	Prior	Gastric	Duodenal	Prior	Gastric	Duodenal
Cloves	198.7 ± 1.9	195.9 ± 2.4	197.2 ± 1.7	65.8 ± 2.5	67.8 ± 1.9	67.8 ± 2.7	2698 ± 10	2254 ± 19*	2257 ± 11*
Curry leaves	182.4 ± 3.6	182.4 ± 1.9	185.8 ± 1.4	69.8 ± 1.8	64.8 ± 1.6	69.8 ± 2.4	2158 ± 11	2125 ± 14	2124 ± 16
Sweet cumin	163.5 ± 2.4	168.9 ± 1.7	162.4 ± 1.4	75.4 ± 1.9	78.7 ± 4.5	74.9 ± 4.7	1759 ± 21	1758 ± 21	1747 ± 14
Cumin seeds	152.4 ± 1.9	152.0 ± 3.1	155.9 ± 1.5	84.1 ± 1.7	88.5 ± 1.4*	89.6 ± 1.7*	1542 ± 14	1525 ± 20	1547 ± 19
Coriander	144.2 ± 3.1	148.9 ± 1.7	149.5 ± 1.8	91.4 ± 2.5	97.8 ± 2.5*	99.6 ± 2.8*	1420 ± 13	1458 ± 14	1447 ± 21
Mustard	124.7 ± 1.8	125.4 ± 1.1	122.5 ± 1.5	110.2 ± 3.4	115.9 ± 2.4	112.8 ± 1.5	1025 ± 17	1014 ± 10	1189 ± 17*
Fenugreek	114.7 ± 1.9	113.2 ± 1.4	112.0 ± 1.9	127.4 ± 2.5	128.9 ± 1.7	124.2 ± 1.4	1022 ± 12	932 ± 10*	1055 ± 19
Star anise	92.5 ± 4.3	91.6 ± 1.8	91.8 ± 1.4	178.4 ± 2.9	177.2 ± 1.8	178.9 ± 2.4	954 ± 16	947 ± 14	957 ± 10
Cardamom	64.2 ± 1.9	65.6 ± 1.8	52.9 ± 1.1*	188.7 ± 1.8	187.4 ± 2.4	188.9 ± 2.3	847 ± 10	841 ± 11	858 ± 17
Nutmeg	52.8 ± 1.4	53.9 ± 1.8	51.4 ± 1.1	197.5 ± 4.5	199.8 ± 4.7	189.5 ± 4.8	721 ± 16	754 ± 19	759 ± 14

TAC of the plant extracts. As a whole, it is important to run multiple antioxidant assays rather than just one method alone to get a better estimate of the antioxidant capacity of a food material. As for the $ORAC_{oil}$ values, the measurement refers to the amount of antioxidant activity originating from lipid-soluble antioxidant compounds. From the absence of statistically significant fluctuations in the spice extracts prior to digestion, it may be concluded that the lipid-soluble antioxidant compounds are relatively stable against the chemical and enzymatic reactions taking place in the gastric and duodenal digestion phases as compared with the water-soluble antioxidant compounds.

Changes to the total monomeric anthocyanin content and the individual anthocyanin compounds are shown in Tables 4 and 5, respectively. Changes to the carotenoid contents are shown in Table 6. While the anthocyanin contents had statistically significant decreases ($P > 0.05$) for all the compounds analyzed, this was in contrast to the carotenoid contents where they were not observed to have statistically significant changes following gastric and duodenal digestion. It could be highlighted in this instance that the statistically significant decrease ($P > 0.05$) observed in the anthocyanin contents does not necessarily indicate a reduction in the amount of compounds. Structural transformation of the anthocyanins, especially under the varied pH conditions of the digestion model, would render them undetectable by the total monomeric and HPLC-based methods employed for the analysis. This conclusion was further supported by the analysis of total phenolic contents in Table 1. Assessment of the anthocyanin contents was essential given that spices and herbs are known to contain copious amounts of these compounds, resulting in the flavor and colour they are known to impart in food products. Given their therapeutic properties, assessment of the bioavailability of these compounds during physiological digestion could be deemed as vital. Nevertheless, the chemical structure of anthocyanins

Table 4: The total monomeric anthocyanin pigment contents of the spices prior to digestion as well as after gastric and duodenal digestion in mg cyaniding-3-glucoside equivalents per g wet weight of spice (mg/g). $^{*}P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

Spice	Prior (mg/g)	Gastric (mg/g)	Duodenal (mg/g)
Cloves	89.8 ± 2.5	59.2 ± 6.5*	56.8 ± 1.6*
Curry leaves	58.9 ± 1.8	29.8 ± 1.9*	31.5 ± 2.4*
Sweet cumin	48.3 ± 1.6	26.5 ± 1.2*	29.8 ± 3.5*
Cumin seeds	48.7 ± 2.0	25.6 ± 1.3*	26.8 ± 1.9*
Coriander	35.9 ± 2.5	20.8 ± 1.4*	19.8 ± 2.1*
Mustard	30.2 ± 2.0	19.6 ± 2.1*	19.2 ± 1.0*
Fenugreek	31.2 ± 1.8	18.9 ± 1.9*	18.2 ± 1.5*
Star anise	28.5 ± 1.2	19.6 ± 1.1*	18.9 ± 1.5*
Cardamom	27.5 ± 1.1	18.5 ± 1.2*	17.9 ± 1.6*
Nutmeg	26.3 ± 1.4	15.2 ± 1.1*	14.9 ± 1.8*

does deem them more vulnerable to ring fission, resulting in somewhat of a loss of their therapeutic effects [14]. The carotenoids which were selected for quantification in this study were based on previous evidence as to their therapeutic effects [33–36]. Nevertheless, carotenoids as an entire group of compounds could be deemed as being more relatively stable as compared with phenolic compounds when it comes to exposure to gastric and duodenal digestion conditions.

Overall, in terms of the antioxidant capacity and associated therapeutic compounds, the results showed the antioxidant capacity of the spice extracts was stable during gastric and duodenal digestion. In the study by Bermúdez-Soto et al. [37], the researchers reported a reduction in various polyphenols following similar two phase digestions of a variety of fruit juices, where a largely increased polyphenol content was shown after the gastric phase; however, these contents fell below the predigestion levels after the duodenal phase. In this instance as well, it may be hypothesized that this may have been due to a structural transformation in the polyphenols which render them undetectable by HPLC—a hypothesis which was brought forward in the study by Wootton-Beard et al. [7] as well. However, Bermúdez-Soto et al. [37] had not quantified the total phenolic content—an aspect which would have provided a better idea as to confirmation of the hypotheses. In addition, in comparison with the study by Bermúdez-Soto et al. [37], this research work also highlights the efficacy of multiple methods of analysis to prevent the exaggerated reporting of the antioxidant potential of plant-based food products.

3.2. Starch Hydrolase Inhibitory Activities. Inhibition of starch hydrolases could be deemed as a more novel aspect when it comes to the properties of functional food. This inhibitory activity leads to a reduced breakdown of glucose, thereby controlling the amount of calories and insulin response in a physiological system. The starch hydrolase inhibitory activities of the spice extracts are shown in Table 7. With the exception of the cumin seed extract, none of the spice extracts showed statistically significant changes to the initial enzyme inhibitory values prior to gastric and duodenal digestion. This observation was of therapeutic significance, given that the initial starch hydrolase inhibitory activities of the spice extracts were maintained despite the digestion reactions. Fenugreek had the highest α-amylase and α-glucosidase inhibitory activities, while cumin seeds had the lowest. Overall, the spices were observed to inhibit α-amylase better than α-glucosidase, given the mean inhibitory values. Inhibition of α-amylase is considered to be more important when it comes to reducing the breakdown of starch, since it triggers the production of the substrate for the subsequent action of α-glucosidase [38]. Therefore, it was noteworthy in terms of therapeutic significance that the spice extracts were able to inhibit α-amylase better than α-glucosidase. Given this requirement, even many of the commercially available antidiabetic drugs to date, such as acarbose, primarily target the inhibition of α-amylase rather than α-glucosidase. A clear correlation between the starch hydrolase inhibitory activities, total phenolic contents, and anthocyanins or carotenoid contents was not observed in this study. Thus, the starch hydrolase inhibitory potential may not have been necessarily drawn from the class of compounds which were investigated and quantified in this study.

4. Conclusions

This study was able to prove that the 10 spices were a significant source of total phenolics, antioxidant activity, and starch hydrolase inhibitory activities, despite the wide variety in the values observed from these parameters of analysis. However, the bioaccessibility and therefore the bioavailability of the compounds which impart the antioxidant properties need to be further evaluated prior to arriving at formidable conclusions as to their efficacy against disease conditions. With the design chosen, the study simply evaluated how the digestion process affects phenolic compounds and carotenoids already extracted, but during the real digestion process, these components may interact with other food constituents, affecting the overall bioaccessibility of the bioactive compounds. Nevertheless, this study was able to provide the first measurement concerning the stability of the antioxidant and starch hydrolase inhibitory potential of these particular spices following *in vitro* digestion. Although cell-based, animal-based, or clinical trial-based studies are able to provide more conclusive evidence, the *in vitro* digestion model used in this study could be used as a preliminary screening step prior to embarking on study models which require much more resources and planning. The study also emphasizes the importance of using multiple methods of analysis for the measurement of the total antioxidant capacity in the absence of any single accepted assay. The importance of this practice has been further highlighted in the study by Wootton-Beard et al. [7] as well as Wootton-Beard and Ryan [39], thus justifying the usage of multiple methods in this study as well.

Table 5: Quantities of D3Ga, C3Ga, C3Gl, C3Ar, P3Ga, and P3Ar present in the herbal extracts prior to digestion (P) as well as after gastric (G) and duodenal (D) digestion. $^{*}P < 0.05$ denotes statistically significant difference as compared with prior *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

Spice	D3Ga (mg/g)			C3Ga (mg/g)			C3GI (mg/g)			C3Ar (mg/g)			P3Ga (mg/g)			P3Ar (mg/g)		
	P	G	D	P	G	D	P	G	D	P	G	D	P	G	D	P	G	D
Cloves	1.21 ± 0.09	0.50 ± 0.08*	0.27 ± 0.01*	1.80 ± 0.08	1.00 ± 0.07*	0.98 ± 0.06*	2.10 ± 0.06	1.25 ± 0.08*	1.15 ± 0.05*	1.39 ± 0.04	0.68 ± 0.08*	0.58 ± 0.01*	1.95 ± 0.01	1.47 ± 0.02*	1.17 ± 0.04*	1.67 ± 0.02	1.24 ± 0.03*	1.15 ± 0.01*
Curry leaves	0.98 ± 0.02	0.74 ± 0.10*	0.69 ± 0.08*	1.44 ± 0.09	1.22 ± 0.04*	0.54 ± 1.47*	1.21 ± 0.08	1.22 ± 0.06*	0.88 ± 0.04*	0.66 ± 0.04	0.58 ± 0.01*	0.47 ± 0.04*	0.87 ± 0.02	0.74 ± 0.01*	0.69 ± 0.02*	1.21 ± 0.04	0.89 ± 0.10*	0.63 ± 0.05*
Sweet cumin	0.47 ± 0.01	0.23 ± 0.01*	0.19 ± 0.02*	0.49 ± 0.04	0.39 ± 0.07*	0.22 ± 0.04*	1.24 ± 0.02	1.00 ± 0.04*	0.95 ± 0.11*	0.95 ± 0.08	0.67 ± 0.05*	0.62 ± 0.02*	1.49 ± 0.09	0.25 ± 0.01*	0.31 ± 0.02*	1.22 ± 0.07	1.20 ± 0.08*	1.17 ± 0.04*
Cumin seeds	1.31 ± 0.05	1.20 ± 0.01*	1.05 ± 0.06*	1.20 ± 0.36	0.58 ± 0.01*	0.44 ± 0.03*	1.35 ± 0.02	0.94 ± 0.06*	0.84 ± 0.03*	1.24 ± 0.06	1.15 ± 0.06*	0.84 ± 0.05*	0.87 ± 0.03	0.64 ± 0.02*	0.54 ± 0.01*	0.97 ± 0.08	0.87 ± 0.01*	0.68 ± 0.02*
Coriander	1.12 ± 0.05	1.00 ± 0.03*	0.85 ± 0.02*	0.65 ± 0.02	0.51 ± 0.02*	0.48 ± 0.01*	0.75 ± 0.04	0.61 ± 0.02*	0.57 ± 0.01*	0.67 ± 0.01	0.44 ± 0.02*	0.42 ± 0.01*	0.74 ± 0.01	0.59 ± 0.01*	0.54 ± 0.02*	0.64 ± 0.02	0.29 ± 0.01*	0.22 ± 0.02*
Mustard	0.58 ± 0.02	0.25 ± 0.01*	0.20 ± 0.01*	0.67 ± 0.02	0.61 ± 0.03*	0.54 ± 0.08*	0.81 ± 0.03	0.61 ± 0.02*	0.51 ± 0.04*	0.74 ± 0.01	0.61 ± 0.03*	0.51 ± 0.02*	0.94 ± 0.01	0.88 ± 0.04*	0.77 ± 0.02*	0.47 ± 0.02	0.36 ± 0.07*	0.29 ± 0.01*
Fenugreek	0.39 ± 0.02	0.11 ± 0.01*	0.10 ± 0.01*	0.48 ± 0.03	0.32 ± 0.02*	0.25 ± 0.01*	0.55 ± 0.04	0.51 ± 0.03*	0.50 ± 0.03*	0.47 ± 0.06	0.31 ± 0.03*	0.31 ± 0.01*	0.77 ± 0.03	0.65 ± 0.04*	0.54 ± 0.01*	0.66 ± 0.02	0.60 ± 0.01*	0.54 ± 0.02*
Star anise	0.85 ± 0.02	0.65 ± 0.01*	0.60 ± 0.02*	0.80 ± 0.01	0.75 ± 0.02*	0.75 ± 0.01*	0.66 ± 0.05	0.49 ± 0.01*	0.45 ± 0.02*	0.58 ± 0.02	0.55 ± 0.05*	0.50 ± 0.03*	0.49 ± 0.01	0.39 ± 0.02*	0.28 ± 0.01*	0.33 ± 0.02	0.28 ± 0.05*	0.25 ± 0.01*
Cardamom	0.79 ± 0.01	0.52 ± 0.01*	0.49 ± 0.01*	0.38 ± 0.02	0.31 ± 0.01*	0.29 ± 0.02*	0.84 ± 0.02	0.77 ± 0.03*	0.75 ± 0.02*	0.29 ± 0.01	0.25 ± 0.02*	0.25 ± 0.01*	0.36 ± 0.04	0.32 ± 0.02*	0.31 ± 0.01*	0.68 ± 0.02	0.62 ± 0.05*	0.60 ± 0.03*
Nutmeg	0.57 ± 0.02	0.41 ± 0.01*	0.40 ± 0.04*	0.87 ± 0.05	0.81 ± 0.06*	0.74 ± 0.06*	0.91 ± 0.06	0.72 ± 0.03*	0.61 ± 0.05*	0.88 ± 0.04	0.71 ± 0.06*	0.66 ± 0.05*	0.49 ± 0.01	0.34 ± 0.02*	0.30 ± 0.02*	0.45 ± 0.02	0.26 ± 0.01*	0.26 ± 0.01*

Table 6: Quantities of neoxanthin, violaxanthin, lutein, zeaxanthin, α-carotene, and β-carotene present in the herbal extracts prior to digestion (P) as well as after gastric (G) and duodenal (D) digestion. $P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

Spice	Neoxanthin (mg/g)			Violaxanthin (mg/g)			Lutein (mg/g)			Zeaxanthin (mg/g)			α-Carotene (mg/g)			β-Carotene (mg/g)		
	P	G	D	P	G	D	P	G	D	P	G	D	P	G	D	P	G	D
Cloves	0.78 ± 0.05	0.75 ± 0.06	0.77 ± 0.02	1.10 ± 0.08	1.11 ± 0.09	1.15 ± 0.08	1.15 ± 0.09	1.12 ± 0.05	1.13 ± 0.02	0.95 ± 0.08	0.96 ± 0.08	0.97 ± 0.08	1.28 ± 0.01	1.29 ± 0.02	1.29 ± 0.02	1.28 ± 0.01	1.25 ± 0.02	1.26 ± 0.02
Curry leaves	0.62 ± 0.05	0.65 ± 0.04	0.66 ± 0.03	0.87 ± 0.02	0.88 ± 0.03	0.89 ± 0.05	0.79 ± 0.02	0.78 ± 0.03	0.79 ± 0.02	1.18 ± 0.02	1.17 ± 0.03	1.15 ± 0.06	0.84 ± 0.02	0.84 ± 0.02	0.85 ± 0.01	0.79 ± 0.05	0.80 ± 0.04	0.81 ± 0.02
Sweet cumin	0.79 ± 0.01	0.81 ± 0.02	0.80 ± 0.01	1.08 ± 0.09	1.09 ± 0.02	1.09 ± 0.02	0.88 ± 0.02	0.87 ± 0.01	0.87 ± 0.01	0.88 ± 0.06	0.87 ± 0.05	0.88 ± 0.06	1.05 ± 0.06	1.05 ± 0.03	1.04 ± 0.06	0.95 ± 0.06	0.95 ± 0.05	0.95 ± 0.04
Cumin seeds	1.10 ± 0.03	1.12 ± 0.01	1.12 ± 0.02	0.55 ± 0.01	0.56 ± 0.02	0.56 ± 0.01	0.62 ± 0.01	0.63 ± 0.01	0.63 ± 0.01	0.58 ± 0.02	0.56 ± 0.01	0.55 ± 0.02	0.49 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.64 ± 0.09	0.66 ± 0.05	0.65 ± 0.01
Coriander	0.65 ± 0.01	0.69 ± 0.04	0.69 ± 0.04	0.75 ± 0.01	0.76 ± 0.02	0.76 ± 0.01	0.85 ± 0.02	0.86 ± 0.01	0.86 ± 0.01	0.69 ± 0.01	0.70 ± 0.02	0.70 ± 0.02	0.65 ± 0.01	0.66 ± 0.02	0.66 ± 0.01	0.59 ± 0.03	0.59 ± 0.01	0.59 ± 0.01
Mustard	0.84 ± 0.03	0.85 ± 0.01	0.85 ± 0.01	0.71 ± 0.02	0.70 ± 0.02	0.70 ± 0.02	0.66 ± 0.01	0.66 ± 0.01	0.66 ± 0.01	0.59 ± 0.01	0.59 ± 0.01	0.58 ± 0.02	0.61 ± 0.02	0.61 ± 0.02	0.61 ± 0.02	0.63 ± 0.09	0.65 ± 0.01	0.65 ± 0.02
Fenugreek	0.45 ± 0.01	0.46 ± 0.01	0.46 ± 0.01	0.58 ± 0.02	0.58 ± 0.01	0.58 ± 0.01	0.62 ± 0.01	0.62 ± 0.02	0.62 ± 0.02	0.39 ± 0.02	0.40 ± 0.01	0.40 ± 0.01	0.49 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.61 ± 0.02	0.62 ± 0.02	0.62 ± 0.02
Star anise	0.39 ± 0.03	0.39 ± 0.02	0.40 ± 0.02	0.88 ± 0.03	0.85 ± 0.04	0.86 ± 0.05	0.90 ± 0.02	0.91 ± 0.01	0.90 ± 0.04	0.62 ± 0.03	0.61 ± 0.09	0.61 ± 0.08	0.69 ± 0.08	0.70 ± 0.07	0.71 ± 0.06	0.61 ± 0.03	0.61 ± 0.02	0.61 ± 0.01
Cardamom	0.35 ± 0.02	0.36 ± 0.02	0.36 ± 0.01	0.54 ± 0.02	0.55 ± 0.01	0.55 ± 0.01	0.62 ± 0.04	0.65 ± 0.02	0.69 ± 0.05	0.71 ± 0.02	0.71 ± 0.02	0.71 ± 0.02	0.69 ± 0.01	0.69 ± 0.01	0.69 ± 0.01	0.53 ± 0.04	0.53 ± 0.04	0.53 ± 0.02
Nutmeg	0.39 ± 0.01	0.41 ± 0.02	0.41 ± 0.02	0.51 ± 0.03	0.55 ± 0.01	0.55 ± 0.01	0.49 ± 0.01	0.48 ± 0.02	0.48 ± 0.01	0.66 ± 0.02	0.66 ± 0.01	0.65 ± 0.01	0.71 ± 0.02	0.71 ± 0.02	0.72 ± 0.02	0.31 ± 0.05	0.31 ± 0.05	0.30 ± 0.01

Table 7: α-Amylase and α-glucosidase inhibitory activities of the spice extracts prior and following digestion in the gastric and duodenal phases. $^{*}P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion.

Sample code	α-Amylase inhibitory activity			α-Glucosidase inhibitory activity		
	Prior (IC_{50}, μg/mL)	Gastric (IC_{50}, μg/mL)	Duodenal (IC_{50}, μg/mL)	Prior (IC_{50}, μg/mL)	Gastric (IC_{50}, μg/mL)	Duodenal (IC_{50}, μg/mL)
Fenugreek	48.9 ± 1.6	49.5 ± 3.6	48.9 ± 1.8	55.8 ± 1.9	56.7 ± 1.4	56.9 ± 1.7
Curry leaves	55.9 ± 1.4	58.7 ± 1.9	58.9 ± 1.8	59.6 ± 1.5	60.2 ± 1.6	61.2 ± 1.8
Coriander	60.3 ± 1.8	61.4 ± 1.8	61.9 ± 1.7	69.8 ± 1.4	70.8± 1.6	71.9 ± 1.4
Nutmeg	75.9 ± 1.2	75.9 ± 1.2	76.8 ± 1.9	81.2 ± 1.7	82.6 ± 1.6	83.9 ± 1.4
Star anise	83.6 ± 1.6	84.9 ± 1.5	88.7 ± 1.4	92.6 ± 1.9	95.8 ± 2.0	96.8 ± 2.1
Sweet cumin	104.9 ± 1.9	106.2 ± 1.7	109.5 ± 2.1	119.5 ± 1.1	120.3 ± 1.2	121.5 ± 1.8
Cloves	128.9 ± 3.6	139.5 ± 2.4*	140.2 ± 3.6*	149.8 ± 2.6	151.9 ± 2.4	154.3 ± 2.5
Mustard	169.8 ± 2.6	170.6 ± 2.8	171.6 ± 2.4	179.6 ± 1.5	184.6 ± 2.1	188.4 ± 2.6
Cardamom	197.9 ± 1.6	198.6 ± 1.7	198.6 ± 1.7	256.3 ± 6.4	256.9 ± 6.2	256.9 ± 6.2
Cumin seeds	254.6 ± 4.8	269.4 ± 4.8*	266.1 ± 4.7*	279.6 ± 2.6	281.6 ± 2.5	281.4 ± 2.3

Conflict of Interests

The authors do not have any conflict of interests to disclose, financial or otherwise.

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Research Article

The Ameliorating Effect of Myrrh on Scopolamine-Induced Memory Impairments in Mice

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Myrrh has been used since ancient times for the treatment of various diseases such as inflammatory diseases, gynecological diseases, and hemiplegia. In the present study, we investigated the effects of aqueous extracts of myrrh resin (AEM) on scopolamine-induced memory impairments in mice. AEM was estimated with (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal as a representative constituent by HPLC. The oral administration of AEM for 7 days significantly reversed scopolamine-induced reduction of spontaneous alternation in the Y-maze test. In the passive avoidance task, AEM also restored the decreased latency time of the retention trial by scopolamine treatment. In addition, Western blot analysis and Immunohistochemistry revealed that AEM reversed scopolamine-decreased phosphorylation of Akt and extracellular signal-regulated kinase (ERK). Our study demonstrates for the first time that AEM ameliorates the scopolamine-induced memory impairments in mice and increases the phosphorylation of Akt and ERK in the hippocampus of mice brain. These results suggest that AEM has the therapeutic potential in memory impairments.

1. Introduction

Memory impairment can be caused not only by aging or stress, but also by the neurodegenerative diseases such as Alzheimer's disease (AD). The loss of cholinergic function by cholinergic neuronal degeneration in the central nervous system significantly contributes to the cognitive decline associated with AD [1]. Accordingly, scopolamine, a competitive antagonist for muscarinic acetylcholine receptor (mAChR), induces memory impairments in rodents which parallel those in AD patients [2]. It is assumed that the scopolamine-induced amnesic animal model is very useful tool for screening the protective agents against memory impairment of AD symptoms [3, 4]. Acetylcholinesterase (AChE) is a key enzyme for hydrolysis of acetylcholine; thereby it regulates cholinergic function. Indeed, AChE inhibitors such as donepezil and rivastigmine are prescribed for ameliorating AD symptoms [5]. However, these medicines for AD treatment have side effects: hepatotoxicity, nausea, and diarrhea are concerns [4, 6]. Therefore, the interest has been drawn towards developing natural product based drugs which are generally more accessible with few or no side effects [7, 8].

One of the molecular signaling pathways associated with memory functions is extracellular signal-regulated kinase (ERK). ERK1/2, a member of mitogen-activated protein kinase superfamily, is expressed ubiquitously, conserved well, and responsible for intracellular response transmitted from extracellular signal. The activation of mAChRs in the neurons

induces the elevation of intracellular calcium level, phosphoinositol turnover that activates ERK [9]. ERK activation is necessary for the establishment of long-term potentiation (LTP), the cellular mechanism underlying synaptic plasticity and memory [10]. In addition, Akt is another signaling molecule involved in learning and memory. Akt activation is also necessary for hippocampal LTP induction [11] and the inhibition of Akt induces memory impairments in passive avoidance task [12] and radial arm maze task [13]. Accumulating researches have shown that scopolamine decreases the phosphorylation of both Akt and ERK in the brain of scopolamine-treated mice [14, 15].

Myrrh, *Commiphora myrrha* or *Commiphora molmol* Engler, belongs to the family Burseraceae. It is found in abundance in the dry and arid regions of Ethiopia, Somalia, and Northern Kenya [16] and also habitat in some Asian countries [17]. It exists as a large shrub or a small tree which yields a yellow nonvolatile gum resin. It has been used since ancient times for the treatment of inflammatory diseases, gynecological diseases, wounds, pain, obesity, and hemiplegia [18]. Myrrh is heavily composed of water-soluble gum (30–60%), alcohol-soluble resins (25–40%), and small proportion of essential oil (3–8%) [19]. The characteristic constituents of myrrh oil include furanosesquiterpenes such as furanoelemanes, furanoeudesmanes, and furanogermacrenes. Previous studies demonstrated that the extracts of Myrrh had anti-inflammatory and analgesic effects [20]. The ethanol, petroleum ether, or water extracts of myrrh reduced acetic acid-induced writhing response and formalin-induced paw swelling along with the decreased levels of inflammatory factor prostaglandin E_2 (PGE_2) in mice. It has also been reported that sesquiterpenes isolated from the resins of myrrh showed neuroprotective effects against MPP^+ induced neuronal cell death in SH-SY5Y cells [21]. However, the molecular mechanism of its neuroprotective effects entirely remains to be elucidated. In addition, the effect of myrrh on memory has not been reported yet. Therefore, we tested whether the aqueous extracts of myrrh resin (AEM) ameliorated memory impairments and found that oral administration of AEM improved scopolamine-induced memory impairments using passive avoidance task and Y-maze test. Furthermore, AEM reversed scopolamine-decreased phosphorylation of Akt and ERK in mice hippocampus, suggesting the potential role of Akt and ERK in AEM-improved memory impairments.

2. Materials and Methods

2.1. Chemical Material. (−)-Scopolamine hydrobromide (scopolamine) and 9-Amino-1,2,3,4-tetrahydroacridine hydrochloride hydrate (tacrine) were purchased from Sigma. Scopolamine and tacrine were dissolved in 0.9% saline solution for animal administration.

2.2. Extract Preparation. The resin of myrrh was purchased from Dong Kyung Pharm. Co. located at 128, Yangnyeong-dong-gil, Dongdaemun-gu, Seoul, Republic of Korea. This resin was authenticated by Professor Ju at the School of Korean Medicine, Woosuk University, Samrye, Jeonbuk, Republic of Korea. A voucher specimen (HP-2014-12) of this material was deposited in the herbarium of Hanpoong Pharm & Foods Co. Ltd., Jeonju, Republic of Korea. The resin of myrrh (500 g) was extracted with hot water (10 L) for 3 h and filtrate was evaporated under reduced pressure to give residues (AEM; 188 g; 37.6 w/w%). AEM was prepared in 0.9% saline solution for animal administration.

2.3. Extraction and Isolation of (2E,5E)-6-Hydroxy-2,6-dimethylhepta-2,4-dienal. AEM (50 g) was suspended in H_2O (1 L) and partitioned with EtOAc (1.5 L) to give EtOAc (MYE) and aqueous fraction (MYW). The MYE fraction was fractionated using a silica gel column chromatography, eluted with hexane in EtOAc (3 : 1–1 : 1, stepwise), and 50% chloroform in methanol to provide five subfractions (MYE1–5). The MYE2 subfraction was subjected to a reversed phase (RP) C_{18} column chromatography, eluted with methanol (40%–70%, stepwise) in water to provide (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal. The structure of this compound was identified from the analysis of NMR data with a comparison of its spectral data to those reported in the literature [22]. NMR spectra were recorded in CD_3OD with a JEOL JNM ECP-400 spectrometer, and the chemical shifts were referenced relative to the residual solvent peaks (δ_H/δ_C = 3.30/49.0).

(2*E*,5*E*)-6-Hydroxy-2,6-dimethylhepta-2,4-dienal: ^{1}H NMR data (400 MHz, CD_3OD) δ: 1.34 (6H, s, H-7, H-9), 1.82 (3H, d, J = 1.2 Hz, H-8), 6.42 (1H, d, J = 15.6 Hz, H-5), 6.78 (1H, dd, J = 15.2, 11.2 Hz, H-4), 7.01 (1H, d, J = 11.2 Hz, H-3), 9.40 (1H, s, H-1). ^{13}C NMR data (100 MHz, CD_3OD) δ: 8.8 (C-8), 28.3 (C-7, C-9), 70.3 (C-6), 121.4 (C-4), 137.2 (C-2), 149.3 (C-5), 151.5 (C-3), 195.8 (C-1).

2.4. High-Performance Liquid Chromatography (HPLC). HPLC analysis was performed on a Waters 2695 series HPLC instrument equipped with a sample injector and a photodiode array UV/Vis detector (PDA) (Waters, United States). For all HPLC analysis, a CAPCELL PAK C18 UG120 (4.6 mm × 250 mm; 5 μm, SHISEIDO Co., Japan) column was used as the stationary phase. Samples were prepared that contain 5 mg/mL concentration of AEM and 0.1 mg/mL concentration of (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal. Injection volumes for AEM and (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal were 50 uL and 10 uL, respectively. The mobile phase was composed of water (containing 0.1% formic acid) (A) and acetonitrile (B), with a gradient elution method: 0–5 min, 10% B; 5–15 min, a linear gradient from 10% B to 20% B; 15–60 min, a linear gradient from 20% B to 30% B; 60–80 min, a linear gradient from 30% B to 100% B; 80–90 min held at 100% B. Flow rate was 0.7 mL/min, and the peaks were detected at 270 nm. For coinjection analysis, 5 uL of (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal (0.1 mg/mL) was coinjected with 50 uL of AEM (5 mg/mL).

2.5. Animals. Six-week-old male ICR mice weighing 25 to 30 g were purchased from Orient Co. Ltd., Republic of Korea. Mice were housed six per cage and were maintained in

temperature 20 ± 3°C under a 12/12 hr. light/dark cycle and adapted for 1 week before proceeding with treatment. Commercial pellet feed and water were allowed *ad libitum*. Animal handling and all the animal experiments were performed strictly adhering to the ethical guidelines of Institutional Animal Care and Use Committee at the Wonkwang University, Republic of Korea.

2.6. Y-Maze Test. The Y-maze is black, polyvinyl plastic maze consisting of three identical arms (40 cm × 3 cm × 12 cm). Spontaneous alternation [23] was tested as described previously [24]. In brief, mice were orally administered with AEM (62.5, 125, and 250 mg/kg) or tacrine (10 mg/kg) and injected with scopolamine (1 mg/kg, i.p.) or vehicle after 30 min. Each mouse was placed in the center of the Y-maze 30 min later and was allowed to explore freely through the maze during an 8 min session. The sequence and total number of arms entered were recorded as described before [25]. An entry is counted to have occurred when all four limbs are within the arm. The spontaneous alternation score (%) for each mouse is defined as the ratio of actual number of alternations to the possible alternation number (total number of entries – 2) multiplied by 100. The total number of entries into arms was assessed as a parameter representing locomotor activity [26, 27].

2.7. Passive Avoidance Task. The test was performed according to the method previously described [28]. In brief, assessment of acquisition and retention of the passive avoidance task were carried out using identical light and dark compartment (20 cm × 20 cm × 20 cm) (Jeungdo Bio and Plant Co. Ltd.) with an electrifiable grid floor separated by an entrance (5 cm × 5 cm) shutter. Mice underwent an acquisition trial and a retention trial 24 hours afterward. For the acquisition trial, the mouse was initially placed in the light compartment. After an acclimatization period of 10 s, the shutter was opened and it was closed after complete entry of the mouse into the dark compartment and an electrical foot shock (0.5 mA, 3 s) was delivered through the grid floor. Then mice were returned to their home cage. One hour before the acquisition trial, mice were administered with myrrh extracts (62.5, 125, and 250 mg/kg, p.o.), tacrine (10 mg/kg), or saline. After 30 min, scopolamine (1 mg/kg, i.p.) or vehicle was injected to induce memory impairment. After 24 h of acquisition trial, the mice were again placed in the light compartment for the retention trial and the latency time to enter the dark compartment was recorded and described as step through latency. The retention trial was set a limit of 300 s as cut-off time.

2.8. Western Blot Analysis. At the end of Y-maze tests, the mice were sacrificed by cervical dislocation and hippocampus was isolated, dissected, and homogenized in RIPA buffer (150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCL, and 2 mM EDTA) supplemented with a protease and phosphatase inhibitor cocktail (Roche). Protein concentrations were determined using BCA assay kit (Thermo Scientific). Equal quantities of the protein (15–30 μg) were subjected to SDS-PAGE in 10% gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). The blots were then incubated with antibodies specific for Akt, phospho-Akt (S473), ERK1/2, and phospho-ERK1/2 (T202/Y204) (Cell Signaling) (dilution 1 : 1000), followed by the corresponding secondary antibodies and finally developed using chemiluminescent reagents (Thermo Scientific). The relative intensities of specific protein bands were determined by densitometric scanning of images using ImageJ computer-assisted image analysis system.

2.9. Immunohistochemistry. The mice were anesthetized using 20% urethane (1 g/kg, i.p.) and perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed, postfixed in 4% paraformaldehyde, and dehydrated with 30% sucrose solution. Coronal sections (30 μm) of mice brain were made through the hippocampus with a cryostat microtome and then immunostained using a R.T.U. Elite ABC kit (Vector laboratories). In brief, the sections were incubated in 0.3% hydrogen peroxide (H_2O_2) and washed in PBS. After 1 h incubation with blocking serum (2.5% horse serum), the sections were incubated with antibody directed against phosphorylated ERK, followed by biotinylated secondary antibody and then avidin-biotin-peroxidase complex mixture. The antigen-antibody complex was visualized by DAB chromogen (ImmPACT DAB Peroxidase Substrate Kit, Vector Laboratories). The brain sections were mounted, air-dried, dehydrated, cover slipped, and observed under a light microscope (Nikon, TS100). Photomicrographs were taken from hippocampus CA3 regions at ×20 magnification.

2.10. Statistics. All data are expressed as the mean ± SEM and the presented figures are representative of the series of experiments. Statistical significance of differences between test conditions were determined using one-way analysis of variance (one-way ANOVA) with Tukey's post hoc test for comparing multiple sets of data. A value of $p < 0.05$ was considered as significant.

3. Results

3.1. HPLC Analysis of AEM and Identification of a Major Component. HPLC analysis of AEM obtained from the above elution method provided a major peak at 24.97 min (Figure 1(a)), which matched the retention time of (2*E*, 5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal (**1**). To further confirm the presence of **1** in AEM, the marker compound **1** was coinjected with AEM, and the resulting HPLC chromatogram (Figure 1(c)) showed that the area of the major peak has been increased without changes in retention time, peak width, and peak shape compared to the HPLC chromatogram of AEM. Therefore, the major component in AEM was assigned as (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal based on retention time matching and coinjection analysis.

3.2. Effect of AEM on Scopolamine-Induced Memory Impairment in the Y-Maze Test. We first evaluated the effects of AEM on short-term memory function using Y-maze task.

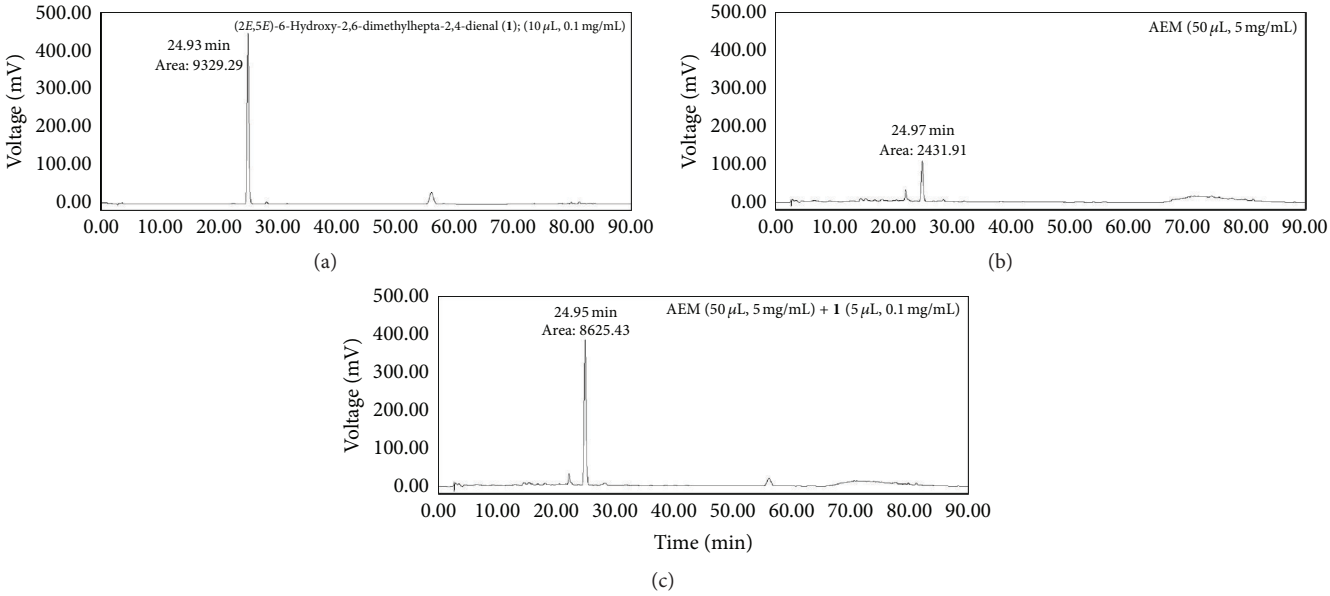

Figure 1: HPLC chromatograms of (a) (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal, (b) AEM, and (c) AEM coinjected with (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal. HPLC analysis was performed as described in Materials and Methods.

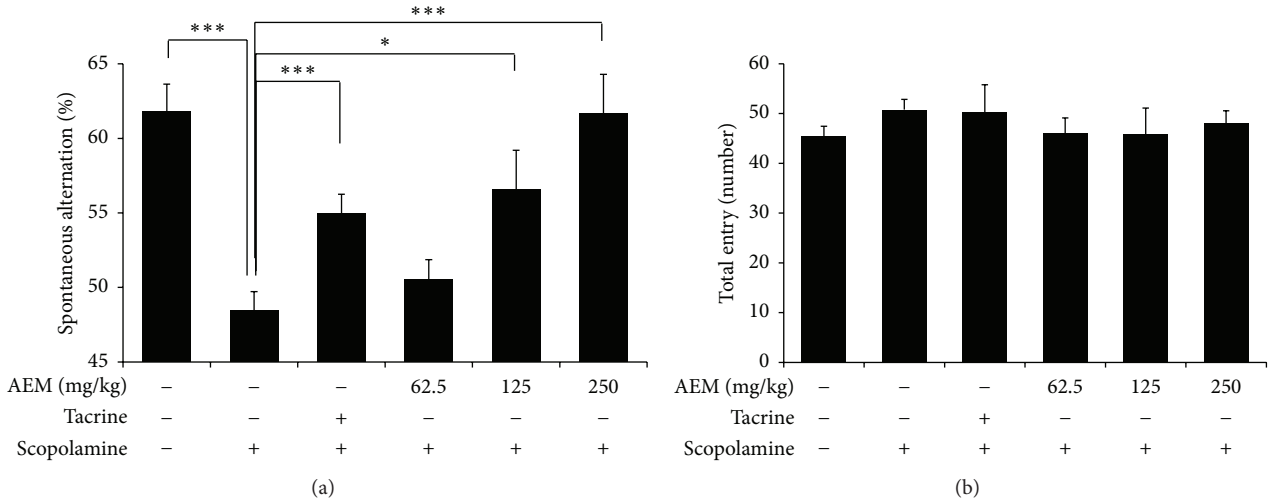

Figure 2: Effect of AEM on scopolamine-induced memory impairments in the Y-maze test. The mice under different groups were administered with equivalent volume of saline, tacrine (10 mg/kg, p.o.), or AEM (62.5, 125, and 250 mg/kg, p.o.) for seven days. Scopolamine (1 mg/kg, i.p.) was given to all the groups except control group 30 min before trial. The spontaneous alternation score (a) and numbers of arm entries (b) were recorded. Data are represented as mean ± SEM ($n = 6 \sim 9$) and the results are considered to be statistically significant at $^{*}P < 0.05$ and $^{***}P < 0.001$.

As shown in Figure 2(a), scopolamine (1 mg/kg, i.p.) significantly decreased the percentage of spontaneous alternation. The scopolamine-induced reduction of spontaneous alternation was significantly restored by the treatment with AEM (125 and 250 mg/kg) in a dose-dependent manner, suggesting the improved memory. The average spontaneous alternation of 125 and 250 mg/kg AEM was higher than that of 10 mg/kg tacrine. The total number of arm entries between the groups was not different suggesting that locomotion activity was not affected by scopolamine, tacrine, or AEM treatment (Figure 2(b)).

3.3. Effect of AEM on Scopolamine-Induced Memory Impairment in the Passive Avoidance Task. Passive avoidance task was performed for testing the effect of AEM on scopolamine-induced memory impairment. As shown in Figure 3(a), the latency was not different between any of the groups during the acquisition trial. In the retention trial, the latency time of the scopolamine-treated group for entering the dark compartment was significantly shorter than the control group, indicating memory impairment. The latency of retention trial reduced by scopolamine treatment was ameliorated with treatment of AEM (62.5, 125, and 250 mg/kg) in

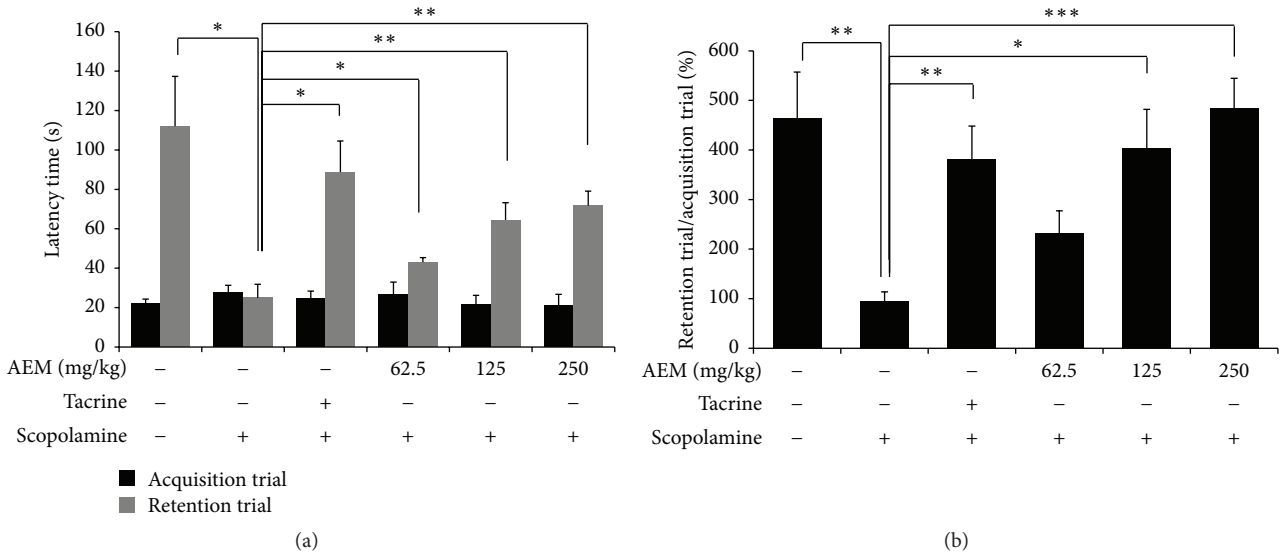

Figure 3: Effect of AEM on scopolamine-induced memory impairments in the passive avoidance task. The mice under different groups were administered with equivalent volume of saline, tacrine (10 mg/kg, p.o.), or AEM (62.5, 125, and 250 mg/kg, p.o.) for six days. Scopolamine (1 mg/kg i.p.) was given to all the groups except control group 30 min before acquisition trial. At 24 h after acquisition trial, a retention trial was performed 1 h after oral administration of saline, tacrine, or AEM. Latency time in the acquisition trial and retention trial (a) was recorded and the percentage ratio of retention trial to acquisition trial in each mouse (b) was calculated. Data are represented as mean ± SEM ($n = 7 \sim 9$) and the results are considered to be statistically significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

a dose-dependent manner. In the percentage ratio of retention trial to acquisition trial, AEM treatment significantly reversed the scopolamine-induced reduction of latency time (Figure 3(b)).

3.4. Effect of AEM on the Phosphorylation of Akt and ERK1/2 in the Hippocampus. To elucidate the molecular mechanisms underlying the memory enhancing effect of AEM, we have examined the phosphorylation of Akt and ERK1/2 in the lysates of mice hippocampus. Western blot analysis clearly showed that scopolamine decreased Akt phosphorylation at serine 473 site and ERK1/2 phosphorylation at threonine 202 and tyrosine 204 sites. AEM (125 and 250 mg/kg) significantly reversed the scopolamine-suppressed phosphorylation of Akt and AEM (250 mg/kg) recovered the phosphorylation of ERK1/2 (Figure 4). In the Immunohistochemistry, scopolamine treatment decreased the immunoreactivity for the phosphorylated ERK in the hippocampal CA3 region (Figure 5). This reduction was recovered by AEM administration.

4. Discussion

In the present study, we demonstrate for the first time that the treatment of AEM ameliorates scopolamine-induced memory impairments. This effect was observed in the passive avoidance task and Y-maze test in mice. Furthermore, we found that AEM reversed the scopolamine-decreased phosphorylation of both Akt and ERK in the hippocampus of mice brains.

First, we estimated AEM with (2*E*,5*E*)-6-hydroxy-2,6-dimethylhepta-2,4-dienal as a representative constituent by HPLC. (2*E*,5*E*)-6-Hydroxy-2,6-dimethylhepta-2,4-dienal has been reported to be isolated from *Erechtites hieracifolia* [22], *Alpinia oxyphylla* [29], and Labdanum oil [30], but this compound was revealed as a component of myrrh for the first time in this paper.

The reduction of spontaneous alternation in the Y-maze test is known to represent short-term memory impairment in rodents [31]. In addition, retention latency in the passive avoidance task is considered to show the formation of long-term memory [32]. In the retention trial the latency time taken for mice to move into the dark compartment is increased owing to the knowledge of electric foot shock a day beforehand. In this study, the oral administration of AEM (125 and 250 mg/kg) increased spontaneous alternation more than that of 10 mg/kg of tacrine in Y-maze test (Figure 2(a)), suggesting its great efficacy in short-term memory deficit. In passive avoidance task, AEM treatment significantly increased the latency time in the retention trial in a dose-dependent manner, although the latency time in AEM groups was less restored than that in tacrine group (Figure 3). These results implicated the potential of AEM as a palliative in AD.

Several medications for AD treatment such as donepezil and rivastigmine caused some side effects such as hepatotoxicity, nausea, and diarrhea. Therefore, it is important to find relatively safe agents with few or no side effects. For the safety evaluation, AEM was administered at dose level of 200 mg/kg daily for 7 days in SD rats. Mortality, clinical signs, changes in body weight, hematology (WBC, RBC, Hgb, Hct, and PLT), serum chemistry (ALT, AST, PT, and APTT), gross observation, and organ weights were monitored in accordance with OECD guidelines. AEM did not produce treatment related signs of toxicity or mortality in any of

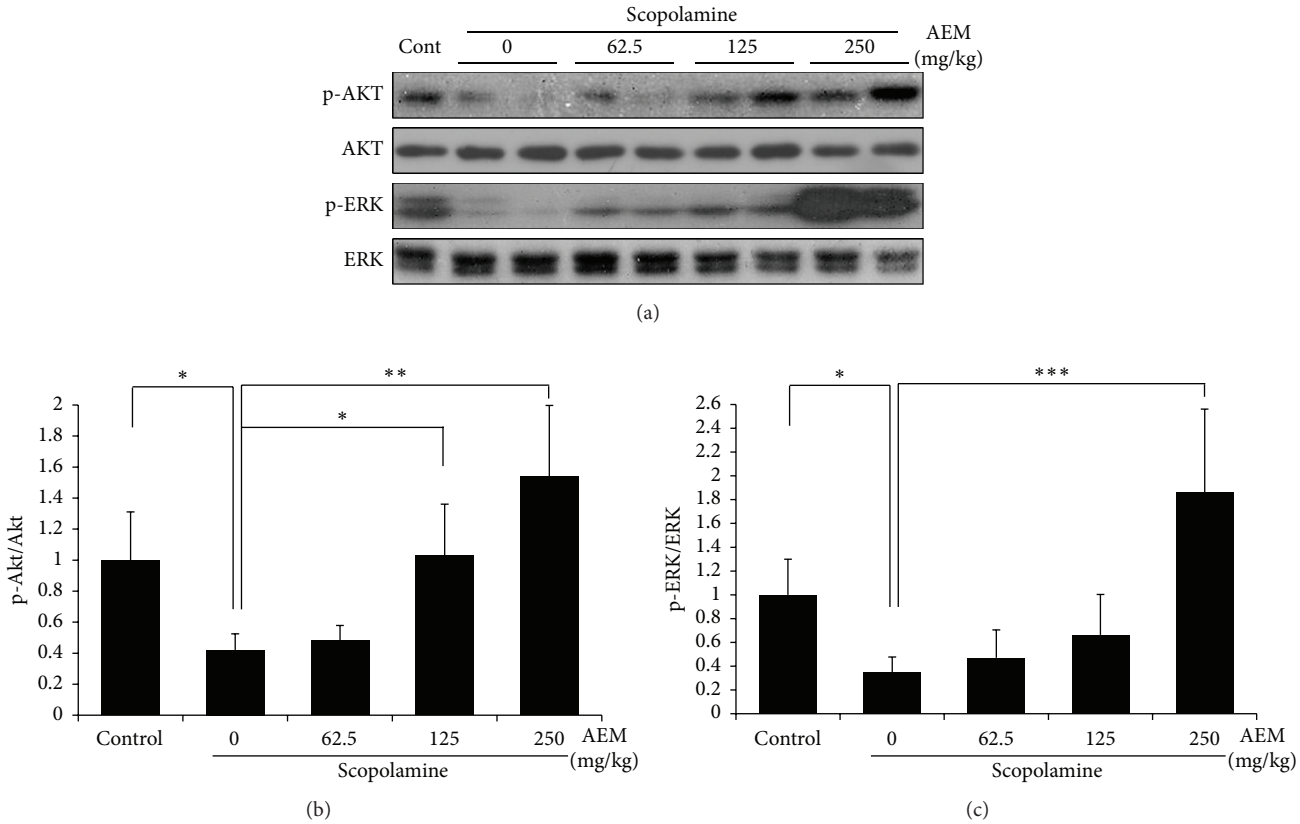

Figure 4: Effect of AEM on Akt and ERK phosphorylation in brain hippocampus. The hippocampus dissected from the randomly selected mice under different groups was used for Western blot analysis. The protein levels of phosphorylated Akt, total Akt, phosphorylated ERK, and total ERK were detected using specific antibodies. The representative blot (a) and the graph of quantification (b and c) were shown ($n = 3$). Quantifications were performed using densitometry. The results were normalized to total Akt or total ERK and expressed relative to the phospho-Akt or phosphor-ERK level of scopolamine-treated group. Each bar shown is the mean fold increase above control ± SEM and the results are considered to be statistically significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

the animals tested during the observation period (see Supplemental information in Supplementary Material available online at http://dx.doi.org/10.1155/2015/925432). Therefore, no observed adverse effect levels (NOAEL) were established for 200 mg/kg AEM in rats under the conditions of this study.

One of the important findings in this study is to show that AEM increased ERK and Akt activation in brain hippocampus. Western blot analysis (Figure 4) showed that AEM increased Akt and ERK phosphorylation in the hippocampus, compared to the scopolamine group. Furthermore, Immunohistochemistry (Figure 5) clearly showed that scopolamine treatment decreased ERK phosphorylation and AEM restored the reduction of ERK phosphorylation in mice hippocampus. Scopolamine inhibits the cholinergic neurotransmission through blocking mAChR. The activation of mAChR in the neurons leads to the elevation of intracellular calcium level, phosphoinositol turnover that activates ERK [9]. The activation of ERK1/2 is necessary for the establishment of long-term potentiation (LTP), which is associated with neuronal plasticity and development of memory [33]. It was reported that NMDA treatment or HFS-induced LTP increased ERK phosphorylation and PD098059, a MEK inhibitor, attenuated the induction of LTP in hippocampal CA1 area [34]. ERK is involved in the LTP-dependent transcriptional regulation through activation of transcriptional factor CREB [10, 35] in hippocampal dentate gyrus. Furthermore, ERK plays a crucial role for the induction of translation via phosphorylation of translation factors eIF4E, 4EBP1, and ribosomal protein S6 in the late LTP phase [36]. In addition, the activation of mAChR has been shown to induce Akt phosphorylation and thereby the inhibition of apoptosis in diverse cell types including neurons [37]. The potentiated action of acetylcholine through the injection of AChE inhibitors was reported to increase acute Akt phosphorylation in mice hippocampus [38]. Accordingly, AEM-induced activation of Akt and ERK in the hippocampus could be one of the molecular mechanisms underlying the memory enhancing effect of AEM.

In accordance with our findings, a wide range of natural product extracts, phytochemicals, and synthetic compounds have been reported to enhance memory in scopolamine-induced impairments through ERK and/or Akt activation. Stigmasterol, phytosterol present in foods, or α-amyrin and β-amyrin isolated from *Angelica keiskei* have been found

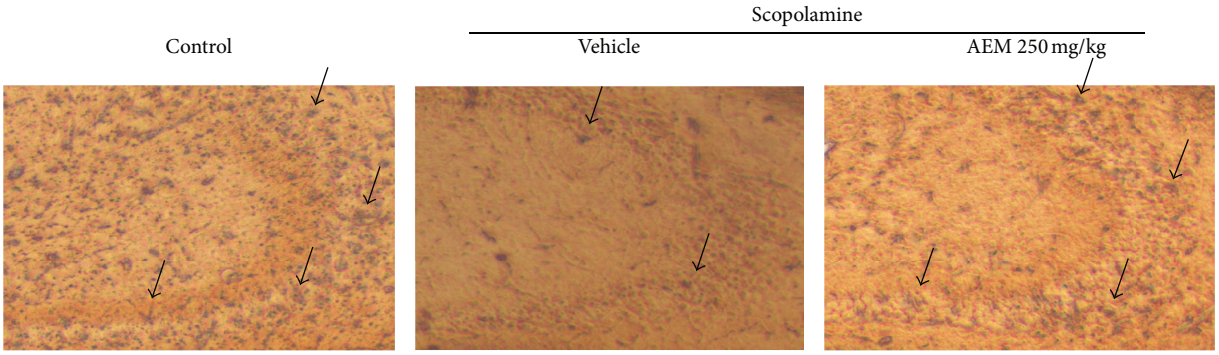

Figure 5: Effect of AEM on the phosphorylation of ERK in the brain CA3 region. The mice from each group were perfused 30 min after scopolamine injection. The brain sections were immunostained with antibody specific for p-ERK using the Elite ABC kit and then visualized with DAB chromogen. The black arrows indicate p-ERK antigen-antibody complexes. The representative images are shown ($n = 4$).

to recover scopolamine-induced memory impairments in mice through enhanced ERK signaling in the hippocampus [28, 39]. Similarly, it has been reported that the scopolamine treatment decreased the phosphorylation of Akt and ERK and agmatine or honokiol reversed scopolamine-induced reduction of phosphorylated Akt and ERK in the brain and ameliorated memory impairments [14, 15, 40].

There have been few studies showing the effect of myrrh in the neuronal cells and the brain. The sesquiterpenes isolated from the resins of *C. myrrha* were reported to show neuroprotective effects against MPP^+ induced neuronal cell death in SH-SY5Y cells [21]. In addition, we previously reported that 1β, 6α-dihydroxyeudesm-4(15)-ene, a sesquiterpene isolated from AEM, blocked lipopolysaccharide-induced inflammation by inhibiting the production of nitric oxide and PGE2 and by suppressing the protein expression of inducible nitric oxide synthase and cyclooxygenase-2 in BV2 microglial cell [41]. Although it has not been reported yet that myrrh has memory enhancing effects, *C. wightii,* another plant of the genus *Commiphora,* improved scopolamine- and streptozotocin-induced memory deficits [42]. *C. wightii* treatment also caused the reduction of AChE activity and increment of GSH levels in the mice brains. Taken together, myrrh might have the potential benefits to alleviate various neurodegenerative diseases. To clarify this issue, further *in vivo* and *in vitro* studies will be needed.

5. Conclusion

Our study showed for the first time that AEM reverses the scopolamine-induced memory impairments in mice using the passive avoidance task and Y-maze test. Furthermore, AEM treatment upregulated Akt and ERK phosphorylation in the hippocampus of mice brain, suggesting that the memory improving effects of AEM treatment might be mediated at least partially through Akt and ERK activation. On the basis of our results, AEM is likely to be registered as a new promising candidate for the treatment of memory impairments.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Samrat Baral and Du-Hyong Cho contributed equally to this study.

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Research Article

Ethnomedicinal Plants Used by Traditional Healers to Treat Oral Health Problems in Cameroon

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Objectives. The objective of the study was to determine the therapeutic methods used by traditional healers to treat oral diseases in Cameroon. *Methods.* A total of 200 traditional healers with a mean age of 50.4 ± 14.2 years from all the provinces of Cameroon were studied using questionnaires. Information elicited was the local names of the medicinal plants used for the management of oral problems, their routes of administration, and methods of usage. Identification of live or dried plants or photographs of sample of the plants was done by a taxonomist. *Results.* The majority of the participants were males urban dwellers aged 41–50 years, 112 (56.0%) practice as herbalists and 56 (28.0%) were trained on medications preservation, 77(56.6%) treat diseases inside or outside the mouth, and 9.0% reported being specialist in oral diseases treatment. Of the 52 plants identified, 48 are used in the management of toothache, sore throat, mouth sores, abscess, broken tooth and jaw, tooth sensitivity, mouth thrush, dental caries, gingivitis, sinusitis, tonsillitis, xerostomia, oral syphilis, oral cancer, TMJ pain, halitosis, and tooth bleaching and 4 plants are used for dental extraction. Roots, leaves, and bark were the parts of plants used and some minerals as adjuncts. *Conclusion.* The study provides comprehensive information on therapeutic methods employed by traditional healers for the treatment of oral diseases.

1. Background

Plants produce chemicals as primary and secondary metabolites which have beneficial long-term health effect and also are used effectively to treat diseases. Specifically, it is the secondary metabolites that exert therapeutic actions in humans. It has been stated that more than 30% of the entire plant species, at one time or another, are used for medicinal purposes necessarily due to the amount and type of secondary metabolite they contain [1]. These drugs of plant origin have saved lives of many residents of developing countries because of their good values in treating many infectious and noninfectious chronic diseases.

In spite of the overwhelming influences and the dependence on modern medicine and tremendous advances in synthetic drugs, large segments of the world population depend on drugs from plants [1]. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs, and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plants as sources of medicines for a wide variety of human ailments [2–5]. According to World Health Organization (WHO), over three-quarters of the world population rely on plants and their extracts for healthcare needs [6].

Poverty, inadequacy of health services, shortage of health workers, and rampant shortage of drugs and equipment in existing health facilities make traditional medicine an important component of healthcare in Africa. Traditional healers were reported as first choice healthcare providers when they faced health problems in Ethiopia due to their efficacy and dissatisfaction with modern medicine [7]. In many of the developing countries, the use of plant drugs is increasing because modern life saving drugs are beyond

the reach of their countries although they spend 40–50% of their total wealth on drugs and healthcare. As a part of the strategy to reduce the financial burden on the developing countries, it is obvious that an increased use of plant drugs will be followed in the future [1, 2].

The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective, and economical comes from the rise in disease incidence particularly in developing countries, increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics, opportunistic infections in immune-compromised individuals, and financial considerations in developing countries [4, 5]. For example, bacterial resistance to most (if not all) of the antibiotics commonly used to treat oral infections (penicillin and cephalosporins, erythromycin, tetracycline and derivatives, and metronidazole) has been documented [8]. These drugs also alter oral microbiota and have undesirable side effects such as vomiting, diarrhea, and tooth staining [3]. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment.

Traditional Chinese medicines have been used to treat some of these orofacial problems for more than 2000 years [9]. Zheng and colleagues reported that Chinese traditional medicines are effective in treating oral diseases including recurrent aphthous stomatitis, oral lichen planus, leukoplakia, and Sjögren's syndrome but remarked that most of them lacked standard criteria of posttreatment assessment and laboratory evidence [9]. It has also been reported that traditional Chinese medicine and naturopathic medicine resulted in significantly greater reduction of pain and psychosocial interference in temporomandibular disorders than the state-of-the-art specialty care [10].

Hollist (2004) [11] reported that about 10 different oral/dental conditions are treatable with plants in traditional health practice, namely, toothache/decay, gingivitis, ulcerative gingivitis, angular stomatitis, mouth ulcers, swollen tonsil, oral thrush, tonsillitis, and black tongue. The result of a study in the Tanga Region of Tanzania showed that dental patients are commonly treated by traditional healers and more than half of inhabitants with toothache sought treatment from traditional healers, where they had all been treated with local herbs. This health seeking behavioral pattern was not altered by the establishment of modern emergency oral care in rural health centers and dispensaries did not influence the villagers' use of the traditional healers [12]. More than half of the Malaysian aborigines (56.8%) used traditional medicine for relief of orofacial pain [13].

There is paucity of literature in traditional therapeutics in the treatment of orofacial diseases in Cameroon. Few studies in Cameroon showed that native herbs are common self-medicament for oral diseases and that traditional healers are involved in tooth extractions [14] and the treatment of other oral diseases like the oral manifestations of HIV/AIDS [15]. It was also documented that Cameroonians use herbs for self-medication for oral health problems [16]. Hence, the objective of this study was to determine the therapeutic methods used by traditional healers to treat oral diseases.

2. Methods

2.1. Study Design. This study set to determine the therapeutic methods used by traditional healers to treat oral diseases was conducted as a cross-sectional study.

2.2. Study Setting. This study was conducted in all the 10 regions of Cameroon. This is a central African country with a population of 21.7 million, without dental school, and has about 220 practicing dentists revealing a low dentist population ratio of about 1 : 10,000.

2.3. Participants. This study included all forms of traditional healers that belong to and participate in the regional activities of their association.

2.4. Instrument. A self-administered questionnaire was used to elicit information from traditional healers. Those traditional healers who were unable to read or write were interviewed and their responses captured. Information elicited was demography of the traditional healers, the local names of the medicinal plants/products used for the management of orofacial problems, and their routes of administration and methods of usage.

2.5. Procedure. Traditional healers assembled by their regional head in the monthly meetings were contacted and the purpose of the research was explained to them. Those that consented were recruited and studied. They were administered the questionnaire. Plants samples were requested from traditional healers who could present life plants. The plant samples and products were collected from the traditional healers either fresh or in dried forms and photographs depicting the habit of plants that could not be harvested were taken. These plants samples were subsequently taken to the Department of Botany at the University of Dschang, Cameroon, for identification by a taxonomist.

2.6. Ethical Concerns. Authorization to conduct this research on traditional healers in all the regions of Cameroon was obtained from the Ministry of Higher Education and Scientific Research of Cameroon and the Senate Research Ethics Committee of the University of the Western Cape (UWC), South Africa. Written informed consent was obtained from traditional healers who agreed to participate in the study.

2.7. Data Analysis. Data obtained were analyzed using the Statistical Package for Social Sciences (SPSS version 17.0, SPSS Inc., Chicago, IL, USA) and summarized using descriptive statistics and presented as frequencies and percentages.

3. Results

A total of 200 traditional healers with a mean age of 50.4 ± 14.2 years from all the provinces (regions) of Cameroon participated in this study. The majority 48 (24.0%) were aged 41–50 years. Males 138 (69.0%) were more than females 62

(31.0%). More than a third 64 (32.0%) had a primary school education, 57 (28.5%) secondary education, 47 (23.5%) a tertiary education, and 32 (16.0%) only informal education. A total of 110 (55.0%) of the participants resided in urban areas. More than half 112 (56.0%) of the traditional healers practice as herbalist, as diviners 28 (14.0%) and both herbalism and divination 60 (30.0%). Fifty-six (28%) of the traditional healers were trained on how to preserve their medications. Seventy-seven (56.6%) participants indicated treating disease inside or outside the mouth but only 9.0% reported being specialist in treating oral diseases only (Table 1). The mean number of patients seen in a week was 13 ± 8 with majority of them 49 (25.0%) treating 13–16 patients weekly (Table 2).

A total of 52 plants were identified, 32 of which are used in the management of oral problems in form of toothache, sore throat, mouth sores, mouth ulcers, bullous lesion, abscess, broken tooth, dentine sensitivity, mouth thrush, dental caries, gingivitis, sinusitis, tonsillitis, dry mouth, oral syphilis, and oral cancer (Table 3), 16 used for specific oral diseases (Table 4), and the remaining four used for dental extraction (Table 5). Roots, leaves, and bark were most common parts of identified plants used for treatment of oral problems (Table 6). The minerals used as adjunct were calcium carbonate, alum, bicarbonate solution, and white and yellow sulphur (Table 7).

4. Discussion

Medicinal plants play an essential role in primary healthcare as they are used to treat wide varieties of oral diseases because they possess antibiotic and anti-inflammatory properties. However, the main drawbacks of tradition health practices endogenous uses include incorrect diagnosis, imprecise dosage, low hygiene standards, the secrecy of some healing methods, and the absence of written records about the patients [17]. There is a common perception that traditional healers do not have any equipment for the diagnoses of oral pain or evaluate posttreatment pain assessment thereby depending on the signs and symptoms. However, Ndenecho had observed that the diagnosis of disease by traditional healers is not limited to direct observation and tests. Divination was also used as 44.0% of the participants practiced it. Preservation and storage of medicinal plants products is a major problem facing traditional medicine in many developing countries like Cameroon. In this study, only 56 (28.0%) of the traditional healers were trained on medication preservation. This invariable means that the postharvest storage and processing technologies are poor and need to be developed to produce the value added to finished products that may be directly utilized by the industry. The establishment of training to facilitate the production of good quality, safe, and efficacious therapeutic is therefore necessary.

The secondary metabolites of plants possess medicinal properties and these medicinal qualities of plants have influenced their price for centuries [1]. This study documented 52 plants used in the management of various forms of oral problems and dental extractions. The majority of the identified plants were used in the management of oral pain mainly toothache. This may not be unconnected with the fact that attention seeking for toothache is very high; thereby every care provider equips their arsenal with best and effective treatment option. Laboratory analyses of several medicinal plants have been showing that they have antimicrobial and anti-inflammatory components as primary or secondary metabolites. This study confirms why most of the oral diseases treated were related to pain and infections.

The resolution of toothache from pulpitis occurs because many medicinal plants cause pulp necrosis and mummification of the pulp tissue. However, this resolution is temporary because further dental caries leads to reinfection of dental pulp tissues and consequent reoccurrence of pain. The 10 different oral conditions (toothache/decay, gingivitis, ulcerative gingivitis, angular stomatitis, mouth ulcers, swollen tonsil, oral thrush, tonsillitis, and black tongue) treatable with plants in traditional health practice [11] documented in the literature were also noted in this study except for black tongue. However, other additional uses like oral syphilis, oral cancer, tooth bleaching, halitosis, and dental extraction reported in this study reflect the comprehensive and nationwide spread of the study.

Other studies in Cameroon have indicated that, apart from their specific uses for dental treatments, some of these plants are used for other medical treatments and as food as well [18]. Most of the plants in this study are used as food *or* as food additives and they include *Canarium schweinfurthii, Vernonia amygdalina, Anacardium occidentale, Cocos nucifera, Allium sativum, Citrus sinensis, Carica papaya, Allium cepa, Ricinodendron heudelotii, Mangifera indica, Zea maize,* and *Syzygium aromaticum*. This is in line with other studies carried out by Dibong et al. [19] and Din et al. [20] who found out that some of these plants and their parts such as roots, rhizomes, tubers, leaves, stem, wood, bark, flowers, seeds, and fruits are used in various purposes in their daily life. Cloves (*Syzygium aromaticum*), known locally in Cameroon as "clue de geof" because of their nail-like presentation, are a common food spice that has natural antiviral, antimicrobial, antiseptic, and antifungal agent with aphrodisiac and circulation-stimulating capacities [21, 22]. Approximately, 72–90% of the essential oil extracted from cloves has eugenol which has a variety of uses in dentistry (pulp dressings, cavity liners, and dry socket dressing) [21]. The clove has also been used in India and China, for over 2,000 years to control both tooth decay and counter bad breath [21]. It has been used extensively in dental care for toothache, sore gums, and oral ulcers relief. Gargling with clove oil can also aid in sore throat conditions and bad breath [22]. In this study, it is used locally to treat toothache by direct application of moist paste to a painful tooth or mouth sore. *Allium sativum* also called garlic is a common food spice and medicinal plant. The paste made from the bulb is used by direct application to a painful tooth to relieve toothache and treat gingivitis. It has broad spectrum antibacterial, antiviral, and fungal activity [23]. Although less effective and cytotoxic to periodontal tissues compared to chlorhexidine, garlic has a high antibacterial effect against human dental plaque microbiota (*Streptococcus mutans, S. sanguis, and S. salivarius; Pseudomembranous aeruginosa and lactobacillus* spp.) even at very low concentrations [24]. The side effect of

Table 1: Sociodemographic characteristics of the participants.

Characteristics	Male *n* (%)	Female *n* (%)	Total *n* (%)
Age (years)			
≤20	1 (0.7)	0 (0.0)	1 (0.5)
21–30	8 (5.8)	7 (11.3)	15 (7.5)
31–40	23 (16.7)	15 (24.2)	38 (19.0)
41–50	35 (25.4)	13 (21.0)	48 (24.0)
51–60	30 (21.7)	9 (14.5)	39 (19.5)
61–70	29 (21.0)	16 (25.8)	45 (22.5)
>70	12 (8.7)	2 (3.2)	14 (7.0)
Educational attainment			
Informal	23 (16.7)	9 (14.5)	32 (16.0)
Primary	51 (37.0)	13 (21.0)	64 (32.0)
Secondary	33 (23.9)	24 (38.7)	57 (28.5)
Tertiary	31 (22.5)	16 (25.8)	47 (23.5)
Residence			
Urban	82 (59.4)	28 (45.2)	110 (55.0)
Rural	56 (40.6)	34 (54.8)	90 (45.0)
Type of practices			
Herbalist	74 (53.6)	38 (61.3)	112 (56.0)
Diviners	18 (13.0)	10 (16.1)	28 (14.0)
Herbalist & diviners	46 (33.3)	14 (22.6)	60 (30.0)
Apart from your training, were you taught how to measure your medicine and how to preserve them?			
Yes	37 (26.8%)	19 (30.6%)	56 (28.0%)
No	101 (73.2%)	43 (69.4%)	144 (72.0%)
Treat disease inside or outside the mouth			
Yes	77 (56.6%)	32 (53.3%)	109 (55.6%)
No	59 (43.4%)	28 (46.7%)	87 (44.4%)
Specialist in treating mouth disease			
Yes	12 (8.7%)	6 (9.8%)	18 (9.0%)
No	126 (91.3%)	55 (90.2%)	181 (91.0)
Total	138 (69.0)	62 (31.0)	200 (100.0)

Table 2: Mean number of patients seen weekly by the participants.

Mean patients seen in a week			
0–4	14 (10.3)	3 (5.0)	17 (8.7)
5–8	21 (15.4)	12 (20.0)	33 (16.8)
9–12	34 (25.0)	11 (18.3)	45 (23.0)
13–16	32 (23.5)	17 (28.3)	49 (25.0)
17–20	11 (8.1)	5 (8.3)	16 (8.2)
>20	24 (17.6)	12 (20.0)	36 (18.4)

this plant is chemical burns that are usually associated with mouth ulcers and persistent halitosis from its strong aromatic smell. Deodorization of garlic extract resolves much of this drawback of halitosis. Drinking milk and use of mushroom extract, tea catechins, plant extracts containing polyphenol and phenolic derivatives, and honey have been reported to be effective in suppressing the malodour of garlic [25–29]. *Ricinodendron heudelotii* is a plant that produces some oily seeds (Njasang) used for spicing soup. It has been found to have antioxidant and antibacterial properties stronger than most antibiotics [30, 31]. The leaves of *Vernonia amygdalina* (bitter leaf) are used in Cameroon as a green vegetable or as a spice in soup, especially in the popular bitter-leaf soup (ndolé). It is used for calming down toothache by direct application on a cavity. Extracts of the plant have been used in various folk medicines as remedies against helminthic, protozoal, and bacterial infections with scientific support for these claims [32], cancer chemoprevention, and the treatment of diabetes [32]. *Ricinodendron heudelotii* is boiled to make

Table 3: Medicinal plants used for the management of oral problems.

Common names	Scientific names	Parts used	Forms of preparation	Diseases treated	Method of administration
(1)	*Acalypha* sp.	Leaves	Boil	Toothache	Gargling
(2)	*Coleus blumei*	Leaves	Paste	Sore mouth and toothache	Brushing
(3)	*Sida rhombofrica*	Whole	Chewing stick and mouth rinse	Toothache	Gargling/brushing
(4)	*Chenopodium ambrosioides*	Whole	Paste	Toothache	Placing on painful tooth
(5) Masung		Seeds	Paste	Toothache	Directing the smoke from melted paste into the mouth
(6) Atui	*Ancistrocladus abbreviatus*	Bark	Boiled bark	Toothache	Calm down pain after mouth rinse
(7) Bird eye view	*Aspilia africana*	Whole	Paste	Dry mouth and toothache	Brushing
(8) Bitter leaves	*Vernonia amygdalina*	Leaves	Solution	Toothache	Gargling
(9) Blue verbena	*Stachytarpheta angustifolia*	Leaves	Deconction	Toothache	Mouth rinse
(10) Castor bean	*Ricinus communis*	Leaves	Solution	Toothache	Gargling
(11) Clue de geof (clove)	*Syzygium aromaticum*	Dried fruits	Wet paste	Toothache	Pain reliever
(12) Coconut	*Cocos nucifera*	Shell and roots	Powder and whole roots	Toothache and tooth sensitivity	Direct application and boil and gaggle
(13) Cola nut tree	*Cola nitida*	Bark and fruit	Solution and paste	Sore mouth and toothache	Direct application and gargling
(14) Bitter kola	*Garcinia kola*	Bark and seeds	Mouth rinse and grinded paste	Toothache and mouth thrush	Gargling paste applied by rubbing on sore areas
(15) Dibobonji	*Alchornea cordifolia*	Stem/bark	Boil	Toothache	Mouthwash
(16) Esamba	*Pycnanthus angolensis*	Stem, bark, and leaves	Deconction	Toothache	Mouth rinse
(17) Eucalyptus tree	*Eucalyptus saligna*	Leaves	Solution/paste	Halitosis, toothache, and TMJ joint pain	Gargling
(18) Eye fowl	*Spilanthes africana (Asteraceae)*	Flower buds	Whole parts	Mouth odor, broken teeth or jaws, and toothache	Chew fresh or dry until peppery taste is felt Direct application to the cavity for toothache
(19) Garlic	*Allium sativum*	Root	Paste	Gingivitis/toothache	Direct application
(20) Guava leaves	*Psidium guajava*	Leaves	Hot mouth rinse	Toothache and mouth ulcer	Calm down pain and wound healing
(21) Long pepper	*Capsicum frutescens*	Fruits	Paste	Toothache and oral candida	Mouth rinse/gaggling
(22) Maize	*Zea maize*	Whole	Powder	Mouth lesions and bullous lesion	Brushing
(23) Mango plant	*Mangifera indica*	Bark and root/leaves	Solution	Toothache/soft tissue inflammation	Gargling
(24) Medmekube	*Acmella caurlirhiza*	Fruits	Paste	Toothache and abscess	Maceration
(25) Moringa	*Moringa oleifera*	Root	Decoction	Toothache	Mouth rinse
(26) Njansang	*Ricinodendron heudelotii*	Seeds	Paste	Toothache	Apply directly
(27) Pawpaw	*Carica papaya*	Leaf latex and leaf	Solution	Toothache, mouth sores, sore throat, and thrush	Gargling
(28) Peace plant	*Dracaena diesteliana*	Root	Solution	Toothache	Harvest fresh boil and gargle
(29) Pear tree	*Persea americana*	Seed and bark	Deconcoction	Toothache	Gaggling of hot solution
(30) Sun flower	*Arnica montana*	Leaves	Solution	Toothache	Gargling
(31) Sweet potato	*Ipomoea batatas*	Fresh leaves	Wet paste	Acute toothache/inflammation	Direct application
(32) Tobacco	*Nicotinia tobacum*	Dried or fresh leaves	Powder or wet paste	Powder for toothache and paste for tooth bleaching	Direct application of powder into cavity to arrest caries or mummify pulp tissue Direct application of fresh squeezed paste to bleach teeth

Table 4: Medicinal plants specific for oral diseases.

Common names	Scientific names	Parts used	Forms of preparation	Diseases treated	Method of administration
(1)	*Ocimum basilicum*	Leaves	Deconction	Sinusitis	Sniff hot vapour
(2) Bush pepper	*Piper guineense*	Fruits	Paste in water	Dental caries	Mouth rinse and direct application
(3) Orange fruits	*Citrus sinensis*	Leaves	Decoction	Gingivitis	Gargling
(4) Alakata pepper	*Aframomum danielli*	Seed	Paste	Sore mouth	Rubbing
(5) Aloe vera	*Aloe vera*	Leaves	Gel	Gingivitis	Rubbing
(6) Banana plant	*Musa cavendishii*	Roots	Solution	Sore throat	Drinkable
(7) Onion	*Allium cepa*	Leaves	Paste/deconction	Sore throat, toothache, and dental abscess	Direct application for toothache and gargling of hot fluid for sore throat
(8) Black *(bush plum)*	*Canarium schweinfurthii*	Dried fruits	Baked fruits in water	Tonsillitis	Gaggling
(9) Camelina	*Camelina bengalensis*	Whole	Drinkable solution	Dry mouth and thrush	Drinking
(10) Cashew	*Anacardium occidentale*	Unripe fruits	Deconction	Oral syphilis	Mouth rinse
(11) Cypress	*Cupressus bethanis*	Seed/bark	Mouth rinse solution	Bolous lesion, toothache	Gargling/paste for brushing
(12) Echinacea	*Echinacea purpurea*	Leaves/stem	Paste	Toothache, sinusitis, and oral cancer	Calm down pain on direct application
(13) Garden eggplant	*Solanum torvum*	Leaves/roots	Mouth rinse solution	Dry mouth/inflammation	Gargling/drinking
(14) Ginseng	*Lepidium meyenii*	Roots	Mouth rinse	Inflammation	Gaggling
(15) Hibiscus	*Hibiscus esculentus*	Leaves	Deconction	Sore throat	Gaggle hot liquid
(16) Chewing stick	*Garcinia mannii*	Stem	Direct chewing	Arrest caries	Chew stem

Table 5: Medicinal plants for dental extractions.

Common names	Scientific names	Parts used	Forms of preparation	Diseases treated	Method of administration
(1) "Native iodine"	*Arnica montana*	Leaves	Solution	Toothache/fresh wound from extraction	Rubbing (maceration)
(2) Ageratum (king plant)	*Ageratum conyzoides*	Whole	Powder	Toothache/extraction	Brushing/direct application
(3) Cotton tree	*Gossypium arboreum*	Leaves	Hot mouth rinse	Tooth extraction	Gaggling
(4) Bang-api	*Dichrocephala integrifolia*	Whole	Paste	Migraine/toothache	Massaging/tooth extraction

hot mouthwash that is used to treat toothache. There is a traditional belief in Cameroon that whatever can be used as food or whatever animals eat without any harm can safely be used for medicine because of its minimal toxicity. The high usage of herbs among the households is an indication of their abundance [33].

The most commonly used plants in Cameroon for the management of dental infections are *Carica papaya, Psidium guajava*, and *Nicotinia tobacum. Other common plants for treatment of oral problems included Spilanthes africana, Eucalyptus saligna, Moringa oleifera, Capsicum frutescens, Ageratum conyzoides, Dichrocephala integrifolia, Persea americana, Ipomoea batatas, Vernonia amygdalina, Garcinia mannii, Garcinia kola*, and *Arnica montana*.

The seed and the pulp of *Carica papaya* had been shown to treat more than 20 diseases and studies have shown that it is bacteriostatic against common oral microorganisms like *Staphylococcus* spp. [34]. The latex of *Carica papaya* has been shown to reduce the growth of *Candida albicans* by 60%; the fruits when used as topical ulcers dressing had been found to promote desloughing, granulation, and healing. These thereby make it suitable for the treatment of mouth sores such as aphthous ulcers [34]. In this study, whitish latex of *Carica papaya* is applied directly to the affected areas of the tooth to

Table 6: Parts of plant used for treatment.

Part	Frequency (n)	Percent (%)
Leaves	49	48.1
Bark	43	42.1
Whole plant	30	29.4
Fruit	5	4.9
Seed	4	3.92
Mineral	17	16.6
Root	51	59.0
Stem	31	30.2
Total	102	100

Table 7: Minerals used as adjuvants with plants by traditional healers.

Minerals	Form of preparation	Diseases treated	Method of administration
White sulphur	Paste	Toothache	Direct application
Yellow sulphur	Paste	Toothache	Direct application
Calcium carbonate	Paste	Toothache	Direct application
Alum	Grounded powder	Bleeding gums and arrest of postextraction bleeding	Direct application in aqueous solution as mouth rinse
Bicarbonate solution	Aqueous solution	Mouth ulcers	Mouth rinse

cure toothache and the decoction is used for treating mouth sores and oral thrush.

Psidium guajava is a well-known traditional medicinal plant used in various indigenous systems of medicine [35]. The leaves and bark of *P. guajava* tree have long history of medicinal uses, which is still employed today. The root is used in West Africa as a decoction to relieve diarrhea, coughs, stomach ache, dysentery, toothaches, indigestion, and constipation [35]. Stem, bark, and root-bark are astringent. The ethnomedicinal uses include the crushing of the leaves and the application of the extract on wounds, boils, skin, and soft tissue infectious site. *P. guajava* leaf is a phytotherapeutic used to treat gastrointestinal and respiratory disturbances and is used as anti-inflammatory medicine. The leaves are used in USA as an antibiotic in the form of poultice or decoction for wounds, ulcers, and toothache [36, 37]. The leaf extract possesses anticestodal, analgesics, anti-inflammatory, antimicrobial, hepatoprotective, and antioxidant activities [38–41]. The flavonoids content of aqueous extract of *P. guajava* leaves is believed to be responsible for the good antibacterial activity [42]. *Psidium guajava* in this study is used to calm down toothache which may be due to analgesics, anti-inflammatory, and antimicrobial properties.

Nicotinia tobacum locally called tobacco or tabac in Cameroon is used for toothache by direct application of fresh leaves or ground dried leaves mixed with calcium carbonate (a mixture generally called snuff) into an infected tooth to calm down pain. Apart from its use in the management of toothache, it is also used by traditional healers from the northern regions of Cameroon in tooth whitening. Smokeless tobacco is believed to increase the circulation of endorphins which act to alter pain appreciation at different levels within the central nervous system including spinal cord, midbrain, thalamus, and cortex [43].

Spilanthes africana is another plant that is widely used by traditional healers in Cameroon. It is also used as a mouthwash for instant treatment of halitosis due to its peppermint taste and for the treatment of minor fractures of the teeth and alveolar bone; when applied directly to the cavity, it alleviates toothache. The use of *Spilanthes* spp. in the treatment of toothache by direct application by traditional healers had been documented in India [44]. Analgesic and anti-inflammatory activities of different *Spilanthes* spp. have made it useful for the treatment of toothache, mucositis, and sore throat and for relieving pain from boils, cut wounds, and other types of wounds in traditional medicine [44]. *Spilanthes* also possess antipyretic, anticancer, antifungal, and antioxidant activities [44].

Eucalyptus saligna mouthwash gargle is used in Cameroon to treat mainly toothache, sore throat, and halitosis. It has been shown that the essential oil of the leaves of *Eucalyptus globulus* has antimicrobial activity against gram-negative bacteria (*E. coli*) as well as gram-positive bacteria (*S. aureus*) [45, 46] which are found in the oral cavity.

The roots of *Moringa oleifera* were also used to treat toothache in Cameroon by direct application on the tooth cavity. This plant has been found to be specific against *Staph. Aureus.*, Vibrio cholerae, and *Escherichia coli* and have no antifungal activity [47, 48]. Its antibacterial activity is responsible for its ability to calm toothache.

Capsicum frutescens (long pepper) have antibacterial properties against *Staph aureus* [49] and its extracts possess anti-inflammatory and analgesic effects comparable to diclofenac in experimental rat models [50].

Ageratum conyzoides is a herb called African panacea or the king of plants in Cameroon because it treats several diseases. It is used by traditional healers in many countries in the treatment of a wide variety of diseases. The entire parts are used for calming down pain and for tooth extractions by traditional healers. This plant possesses anticancer and antiradical properties which inhibit the growth of many microorganisms and exhibits anti-inflammatory, analgesic, and antidiarrheic properties [51, 52].

Dichrocephala integrifolia is an annual herb that is used by traditional healers in Cameroon for tooth extractions [53]. In in vitro studies, anticancer, antimicrobial, anti-inflammatory, and antioxidant activities of this herb have been noted [54].

Cocos nucifera (coconut) roots are boiled and used as mouth rinse for treating toothache and tooth sensitivity in Cameroon. Decoction obtained from coconut tree is used as mouthwash and gargle [55]. Extracts from this plant have been shown to have antibacterial, antifungal, antiviral, and antioxidant properties [55, 56]. The anticaries effect is attributed to lauric acid obtained from coconut flour, which is sensitive to *Streptococcus mutans* and as a result reduces

plaque bacteria and biofilm and exerts antifungal activities [55, 56].

Persea americana (avocado pear) possess anti-inflammatory and antifungal properties, specific activity against *Mycobacterium tuberculosis, Streptococcus pyogenes, Staphylococcus aureus*, and varieties of fungi [57, 58]. The seeds of this plant are crushed and boiled to constitute a mouth rinse that is used in treating toothache and mouth sores by traditional healers.

Ipomoea batatas (sweet potato) leaves are squeezed and placed into an open cavitation of the tooth to treat toothache. Antimicrobial study has shown that low concentrations of the sweet potato freeze dried extract inhibit the growth of *Streptococcus mutans, S. mitis, Staphylococcus aureus*, and *Candida albicans* in both agar disk and agar well diffusion tests [58]. It has been shown to contain wound healing antiulcers, anti-inflammatory, antimutagenic, and antidiabetic properties [59].

Garcinia mannii locally called chewing stick is a tropical forest tree whose twigs are used as chewing stick or local toothbrush. Locally it has been observed to arrest dental caries or reduce long-term dental pain among people who use them daily. The chemical constituent of this plant reported in the literature is aminoflavone which requires further scientific evaluation [54].

Garcinia kola also known as bitter kola has been referred to as a "wonder plant" because every part of it has been found to be of medicinal importance. In Cameroon, the bark is used as a mouth rinse to stop dental pain. The roots and stems are cut into short chew sticks used for cleaning teeth. *Garcinia kola* seeds are chewed as an aphrodisiac or used to cure cough, dysentery, and chest colds, to prevent and relieve colic, and can as well be used to treat headache in herbal medicine.

Arnica montana also known as sun flower though used for treatment of oral infection has been shown to have slight inhibition of the adherence of the growing cells (19% for *Streptococcus mutans* and 15% for *Streptococcus sobrinus*) and of water-insoluble glucan formation (29%) at these same concentrations [60].

5. Conclusion

The study provides comprehensive information on therapeutic methods employed by traditional healers for the treatment of oral diseases. The identification of the active ingredients of the plants used by these traditional healers and assessment of their efficacy in the treatment may provide some useful leads for the development of new effective drugs in oral disease treatment.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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