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Research Article

Chemical Profiles of Herbal Compress and Their Persistence Affected by Steaming and Storage Time

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The steamed herbal compress is one of the well-known traditional medicines used to relieve pain and stress and to promote emotional and physical well-being. Although it has several therapeutic benefits due to its chemical compounds, there are insufficient reports on the effect of steaming of the herbal compress to promote the release of active compounds and its shelf life. Hence, this study aims at analysing the effect of various steaming processes and storage conditions on the chemical composition of most commonly used herbs such as kaffir lime, lemongrass, plai, soap pod, tamarind, and turmeric. The herbs were extracted with solid-liquid and Soxhlet-mediated extraction using 95% ethanol, hexane, and water as solvents. The analysis of chemical profiles of herbs indicated that Soxhlet extraction is the best extraction procedure based on the extraction yield and abundance of eluted compounds. The herbs were then steam-processed according to the traditional practice of using herbal compress with water alone, and water in combination with 5% ethanol or 5% coconut oil to analyse the effect of steaming. The results indicated that a steaming process can promote the release of bioactive compounds from herbs. The effect of storage was also investigated by storing herbs for 1 day and 7 days at 4°C. The finding suggested that storage has resulted in changes in the bioactive composition of herbs pointing to the necessity of modifying storage conditions to eliminate the loss of beneficial compounds. Thus, this study can be helpful for product development to improve the quality of products by modifying their steaming or storage conditions.

1. Introduction

Years before modern medical facilities have come into existence, humans were dependent on herbs and herbal products for treatments and curing diseases. These practices later merged with their beliefs and cultures and are now termed traditional medicines. According to WHO, traditional medicine is a compilation of knowledge, skills, and practices developed from indigenous theories, beliefs, and experiences of different cultures, which they have used for diagnosis, treatment, and prevention of many diseases or to improve their health [1]. WHO estimates that about 70–80% of people in developing countries follow traditional medicine for their healthcare needs [2]. Traditional medicine uses herbal or animal parts and minerals in its treatment procedures. The most commonly used are herbal medicines, which are produced from a wide variety of herbs, herbal parts, or products derived from them as the active ingredient in their medications [3]. Herbal medicines are nowadays used among populations of Western countries as well due to their beneficial effects. However, many countries do not have governmental policies to promote and approve traditional medicine as a part of their healthcare system, although their citizens are dependent on it. Southeast Asian countries, especially Thailand, have national policies that are strongly supported by government and private sectors to promote the use of traditional medicine in the official social security system. For example, the Thai government recommends the usage of crude or purified extract of *Andrographis paniculata* (Burm.f.) Wall. ex Nees for the treatment of the early stage infection of COVID-19 for the purpose of reduction in viral propagation [4, 5].

Among various Southeast Asia's traditional medicines, herbal compress has attained worldwide attention for muscle pain relief treatments and recreation purposes. In Thailand, the herbal compress is also listed among the National List of Essential Medicines and Champion products for export goods. An herbal compress consists of herb mixtures, wrapped in a cheesecloth to form a round-shaped ball. In general, this compress is steamed with water before pressing on user's skin. Steaming can lead to the release of active compounds and essential oils from herbal materials. This hot herbal compress when applied to the affected areas of the body can relieve joint pains, muscular sprains, inflammations, etc. [6, 7]. The health benefits of using hot herbal compress could be due to the combined effects of mild-heat pressing on the targeted areas (to increase blood flow), therapeutic effects of the herbal ingredients, and the relaxation effect provided by the essential oils and alcohols [6]. The types of herbs used in each compress depend on the treatment, availability of the herbs, and composition of each recipe [6]. However, most of the compress formulations contain plai, turmeric, and camphor as an ingredient in it [6]. These herbs are reported to have several health benefits. For instance, plai has been reported to use in treating sprains, musculoskeletal pain, and bruises. It is also reported to have anti-inflammatory [8] and antioxidant effects [9]. Similarly, curcumin is also known for its anti-inflammatory and antioxidant activity [10]. Camphor has been traditionally used to treat many diseases and to relieve muscle pains, because it has antimicrobial, antiviral, anticancer, and several other health benefits [11].

There have been several studies comparing the efficacy of hot herbal compress and modern medicinal treatments. A study on the comparison of the effectiveness of hot herbal compress and treatment with typical diclofenac for patients with myofascial pain syndrome proved that hot herbal compress is an effective alternative treatment [12]. Another study was carried out on osteoarthritis patients for 3 weeks to compare the efficacy of Thai massage with herbal compress with oral treatment of ibuprofen, and it showed that the clinical efficacy of herbal compress was similar to oral ibuprofen [13]. Several research studies have identified bioactive chemical compounds in herbal compositions of herbal compress. For example, plai contains compounds such as terpinen-4-ol and sabinene, which has antimicrobial activity along with sesquiterpene compounds such as zerumbone, which gives it antifungal activity [14]. Likewise, turmeric contains curcumin, which is responsible for its anticancer, anti- inflammatory, and antioxidant activities. In spite of this, there are other compounds such as α-turmerone and β-turmerone, which adds to the antimicrobial, anti-inflammatory, and antioxidant activity [15]. Similarly, kaffir lime is also reported to have antibacterial, antioxidant, and antimicrobial activities due to the presence of compounds such as sabinene, linalool, and γ-terpinene [7]. However, to our knowledge, there is no scientific evidence and report for chemical compounds existing in herbal compress before and after steaming. Additionally, as a product in the global market, the herbal compress is exported to the global market in the dried form of ready-to-use product, for example, in a retort sealed bag or in a tin can, but no study has been performed to analyse the remaining active compounds in it. Therefore, this study focussed on the analysis of chemical profiles of 6 types of herbs that are common candidates in herbal compress formulations by using different types of solvents. In addition to this, the effect of steaming herbs with water or in combination with ethanol and coconut oil was also investigated. Moreover, the effect of storage time on herbs used in herbal compresses was also studied. This study will provide a guideline to the best practices for the application of herbal compress product by retaining its maximum active chemical compounds for therapeutic treatment.

2. Materials and Methods

2.1. Collection and Processing of Herbs. The study has used 6 different types of herbs for extraction as summarized in Table 1. The herbs were purchased from the local market in Bangkok, Thailand, in the year 2017, and the experiments were conducted within one week of purchase. These herbs were washed and chopped into small pieces and sieved to approximately 2 mm size. The herbs were then dried in a hot air oven at 60°C for 24 h.

2.2. Extraction Procedure. Extraction of chemical compounds from the herb was carried out using three types of solvents: 95% ethanol, hexane, and water. Herbal samples were subjected to (1) different steaming processes and (2) different storage times before extraction (Figure 1). To select the selection method for the analysis of chemical profiles of herbal samples, two types of extraction methods were conducted in this work: solid-liquid extraction and Soxhlet extraction.

The solid-liquid extraction of the chemical compounds from different herb samples was using different solvents (water, hexane, and 95% ethanol). The mixture of dried herbal sample and solvent with a ratio of 1 : 8 (w/v) with different solvents was added to a screw-capped Erlenmeyer flask. The flask was placed upon a magnetic stirrer with a rotational speed of 200 rpm. The extraction process was performed at 30°C for 24 h. After the extraction process, the extracted samples were filtered using Whatman No. 1 filter paper. The solvents were evaporated using an rotary evaporator with the temperatures lesser than their normal boiling point. Under the condition of reduced pressure in the rotary evaporator combined with the rotation of the flask to increase the surface area of solvent, the boiling point of solvent will be reduced than that of its normal. Hence, the solvents water, hexane, and ethanol were evaporated at 80°C, 40°C, and 50°C, respectively.

Table 1: Herbs used in experiments for the analysis of the bioactive component and its source.

Herbs	Source part of plant
Plai (*Zingiber montanum*)	Rhizomes
Turmeric (*Curcuma Longa* Linn.)	Rhizomes
Lemongrass (*Cymbopogon citrates*)	Trunk
Kaffir lime (*Citrus hystrix*)	Shell
Tamarind leaves (*Tamarindus indica* Linn.)	Leaf

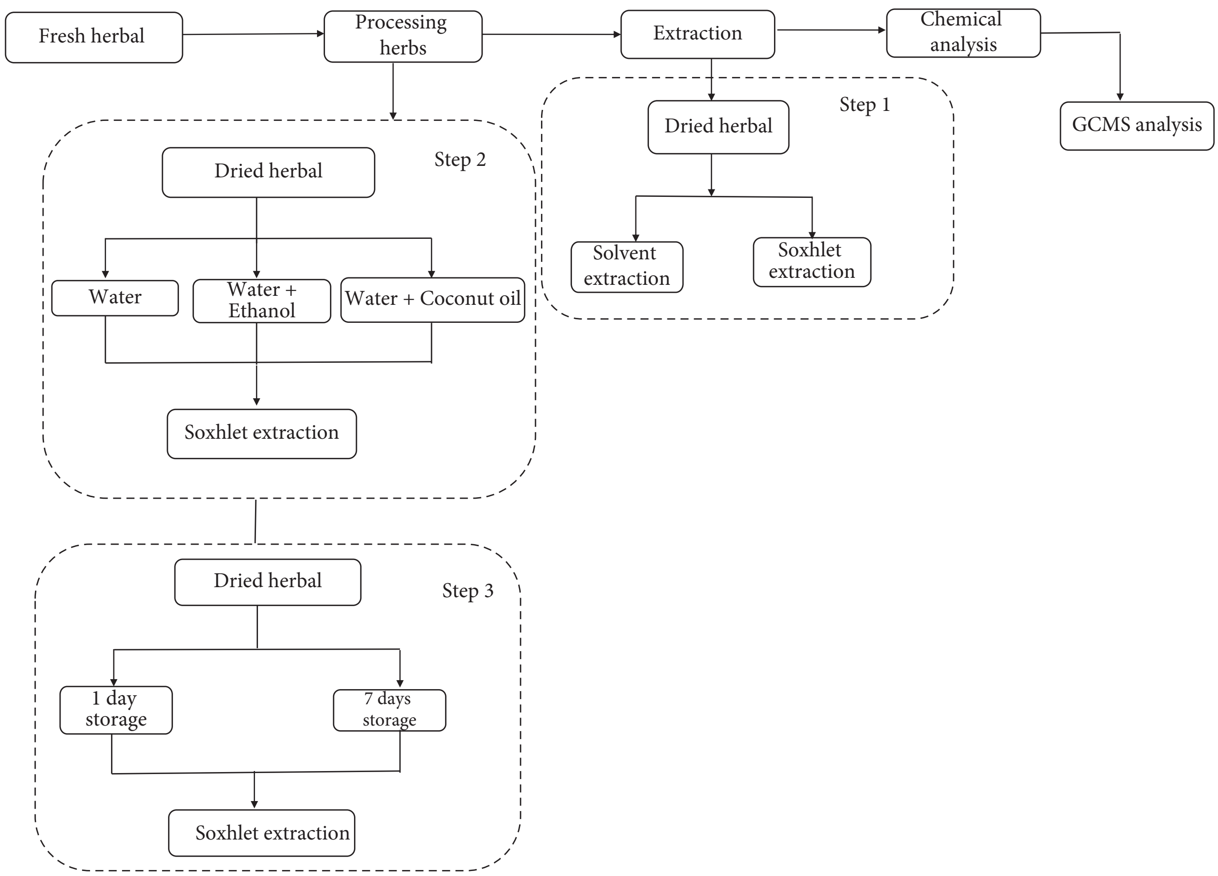

Figure 1: Flowchart of the procedure.

The extract was collected and weighed to determine the yield for different sets of treatments.

For Soxhlet extraction, 5% w/v of the dried herbal sample was added in a cellulose thimble and then placed in the Soxhlet apparatus. The solvents were filled in the round-bottom flask, and the sample container connected to a condenser was placed on top of the round-bottom flask. The Soxhlet apparatus was heated within water bath at controlled temperatures of 100°C, 70°C, and 80°C for water, hexane, and ethanol, respectively, to convert solvents to vapor for extraction. The extraction process with reflux was performed for 4 h. The solvent was recovered under vacuum conditions after the extraction process by subjecting the solvent in the round-bottom flask to a rotary evaporator. The temperature of the bath for water, hexane, and ethanol was maintained at 80°C, 40°C, and 50°C, respectively. The extract was collected and weighed to determine the yield for different sets of studies using different solvents.

Due to conventional uses of Thai's herbal compress, the product is heated before application to users by steaming with water, ethanol, or coconut oil. In this study, the dried herbal samples were separately steamed in water, 5% v/v ethanol in water, and 5% v/v coconut oil in water by placing them in an aluminium steamer tray containing water. The steamer was heated in cooking oven by using water steam as a medium for heat transfer to the sample placed in the steamer for 5 minutes, and the effects of steaming on the chemical profiles were observed. After steaming, the herbal sample was subjected to the Soxhlet extraction method to recover chemical compounds for further analysis. To study the effect of the storage time of herbal compress on the chemical profiles, the dried herbal sample was stored in a controlled-temperature fridge at 4°C for 7 days based on the general user's manual of the herbal compress product. After testing on storage time, all herbal samples were extracted by using the Soxhlet extraction method. All chemical compounds were recovered by the rotary evaporator and further analysed by GC-MS analysis.

2.3. Gas Chromatography-Mass Spectroscopy Analysis (GC-MS Analysis). The chemical profiles of herbal extracts obtained from solvent extraction were analysed for bioactive compounds using GC-MS (Model: GCMS-QP2020 Gas Chromatograph Mass Spectrometer, Make: Shimadzu) equipped with a DB-Wax fused capillary column ($30\,m \times 0.25\,mm$ and $0.25\,\mu m$ film thickness). For each sample analysis, $1\,\mu L$ (5 mg/mL) of crude extracts was injected into GC-MS using helium as carrier gas at a flow rate of 0.69 ml/min with a split ratio of 1 : 20. The oven ramping temperature program was set at 50°C to 250°C. Initial temperature was set at 50°C with the ramping of temperature to 100°C at a rate of 3°C/min. Furthermore, the temperature was increased to 150°C at a rate of 15°C/min and 250°C at a rate of 5°C/min. The total ion chromatograms were used to calculate the relative percentage of the separated compounds by a computerized integrator [16].

3. Results and Discussion

The chemical compositions of six different types of herbs (kaffir lime, lemongrass, plai, soap pod, tamarind, and turmeric) were studied in this work. These 6 types of herbs are common ingredients in different herbal compress products in Thailand as they are local-grown herbs with suggested ayurvedic and traditional therapeutic benefits. Before focussing on the changes in chemical profiles of herbs due to steaming and storage time, a selection of the extraction method is necessary to achieve unbiased comparison on the effect of each treatment on chemical profile. In this work, two extraction methods, solvent extraction and Soxhlet extraction, were selected because these two methods were dependent on different mechanisms. Solvent extraction in this work was conducted at room temperature (30°C) with long extraction time (24 h) based on assumption to reduce the loss of chemicals with a low boiling point, whereas Soxhlet extraction has higher extraction efficiency due to higher temperature and reflux effect.

3.1. Comparison of Chemical Profiles Obtained from Solvent Extraction and Soxhlet Extraction. To achieve the best coverage of chemical profiles of herbal extracts, 3 different solvents, namely, 95% ethanol, hexane, and water, were selected in this work. The extraction was carried out by solvent extraction and Soxhlet extraction methods, and chemical profiles from GC-MS analysis were compared to understand the best extraction method. Among the different solvents used for extraction, 95% ethanol has resulted in maximum extraction yield from all herbs in both extraction methods, followed by hexane and water. The extraction yield from 6 types of herbs in solvent extraction using 95% from 1.263% to 1.632%, whereas the yield varied from 14.46% to 38% in Soxhlet extraction. Using hexane as a solvent, the yield was within the range of 0.55% to 0.73% and 1.6% to 6.3% in the solvent extraction and Soxhlet extraction, respectively. Extraction with water had an extraction yield ranging from 1.287% to 1.532% and 11.2% to 29% using solvent-assisted and Soxhlet-assisted extraction methods, respectively. This result confirms the point that the Soxhlet extraction can provide more extraction efficiency in less time due to the heat-assisted phenomenon [17, 18]. Figure 2 depicts the extraction yield obtained from each herb using two different extraction methods.

After the analysis of yields of chemical compounds, the profiles of chemical compositions obtained from the solvent extraction and Soxhlet extraction methods were analysed qualitatively by GC-MS. Several compounds were eluted by both extraction methods from each of the herbs. Among the eluted compounds, few compounds with bioactivity were selected and are listed in (Tables S1 and S2). Most of the eluted compounds belong to terpenes, which have been previously reported with various activities such as antioxidant, anti-inflammatory, and antimicrobial activities [19]. Among the various extracted terpene derivatives, D-limonene is a compound that is extracted from 5 out of 6 herbs studied, except for soap pod. Its presence is detected either in ethanolic or hexane extracts of herbs from both extraction methods (Tables S1 and S2). D-Limonene is a monoterpene with a characteristic odour of lemon, and it is used in the food industry as a flavouring agent. In addition to this, this compound is reported to have antioxidant, anti-inflammatory, and antidiabetic effects, and several other health benefits for humans [20]. The anticarcinogenic effects of D-limonene are also studied against lung cancer [21] and gastric cancer [22]. Along with D-limonene, there are few other compounds found common in solvent extraction and Soxhlet extraction of herbs. These include terpinene-4-ol and terpineol in kaffir lime; geraniol, junenol, and globulol in lemongrass; α-terpinyl acetate and tetradecane in plai; and caryophyllene and β-bisabolene in turmeric. All these compounds exhibit bioactivity, which is demonstrated in published research.

Terpinen-4-ol and α-terpineol, terpenoid derivatives identified in kaffir lime and turmeric extracts, are isomers of terpineols. Terpinen-4-ol is reported to have anti-inflammatory, antimicrobial, antiviral, and anticancer activities [23, 24]. α-Terpineol is previously studied and proved to have antioxidant, anticancer, anti-ulcer, and anti-hypertensive effects [25]. In addition to this, α-terpineol is also reported to have antimicrobial activity against oral microbes [26]. Globulol has been identified in lemongrass extract in this work, and it showed an antimicrobial compound from the ethanolic extract of *Eucalyptus globulus* fruit [27]. α-Terpinyl acetate in plai has exhibited anticholinesterase activity, and it has proved to be a neuroprotective agent in in vitro studies [28]. Tetradecane is identified in extracts of plai, lemongrass, and soap pod, and it is reported to have antifungal and antibacterial activities [29, 30]. Caryophyllene and β-bisabolene are sesquiterpenes that are identified in essential oils extracted from kaffir lime, turmeric, lemongrass, and plai. These compounds are reported to have an anticancer effect. Caryophyllene exhibited antiproliferative effects against colorectal cancer along with its antioxidant and antimicrobial activities [31]. β-Bisabolene has been

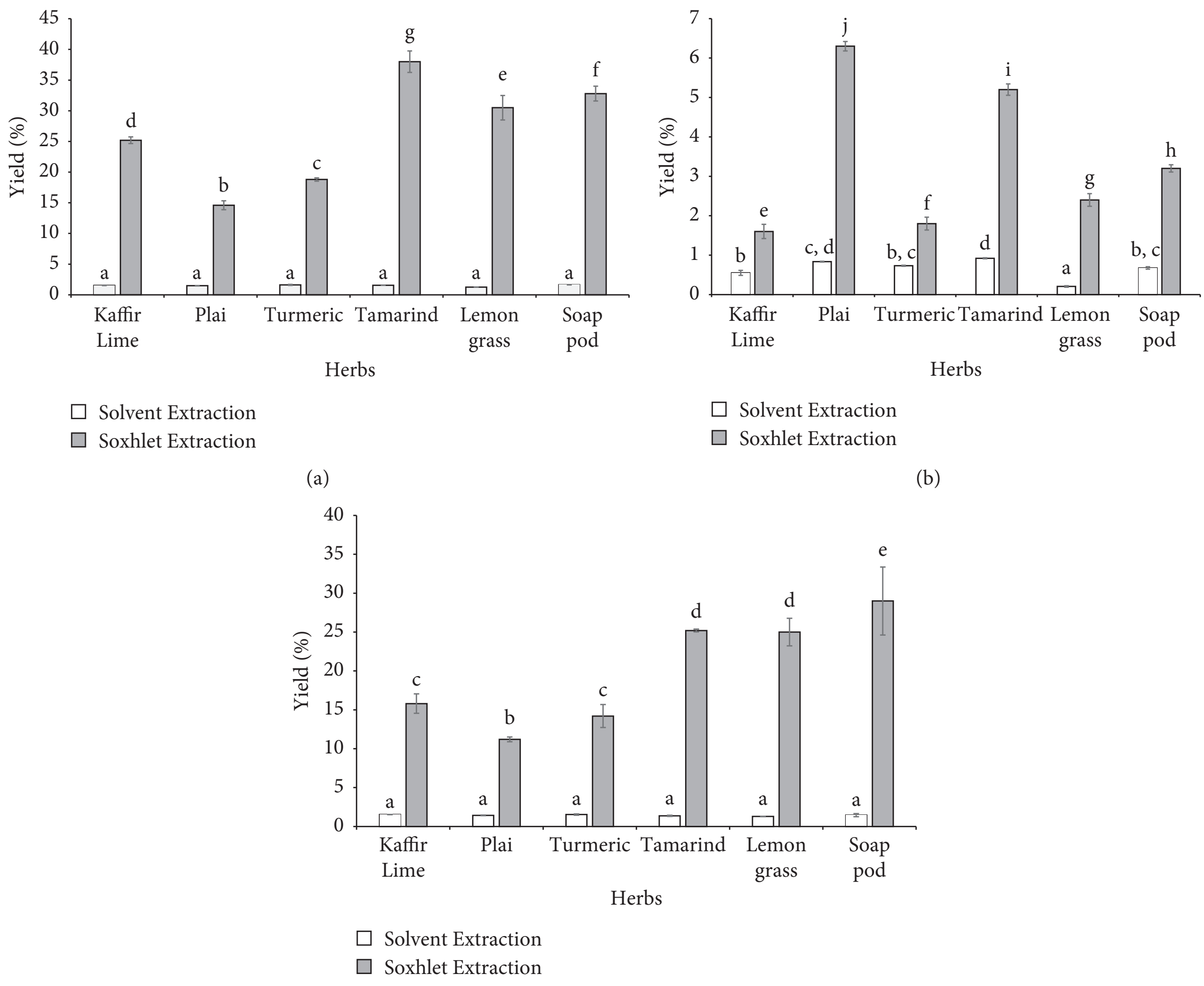

Figure 2: Extraction yield from different herbs using (a) 95% ethanol, (b) hexane, and (c) water. *Different letters above each bar represent different subsets of alpha. All values are average of 3 values obtained after the extraction of sample in triplicate.

studied as an anticancer agent for breast cancer in in vitro and in vivo studies conducted on human and murine mammary tumour cells [32].

Among the other sesquiterpenoid alcohol compounds extracted from herbs, α-cadinol is the most abundant compound in the ethanolic extract of the lemongrass extracted by solvent extraction. α-Cadinol is the active compound found in twig oil of *Litsea acutivena*, with antimicrobial activity [33]. Pentadecane and heptadecane are the other hydrocarbons extracted from herbs along with tetradecane. This pentadecane is the major hydrocarbon that is reported to have antimicrobial activity [30]. 5-Hydroxymethylfurfural was present in both ethanolic and water extracts of soap pod. It is identified to have antioxidant activity by scavenging free radicals and antiproliferative activity against human melanoma cells [34]. Few other compounds such as α- and β-pinene, sabinene, and β-myrcene were also eluted by solvents in soap pod, tamarind, and turmeric. Pinenes are generally known for their antimicrobial activity and are used to treat respiratory disease [19]. β-Myrcene has anti-inflammatory activity along with analgesic and sedative effects [19]. Based on the listed bioactive compounds extracted by using solvent extraction and Soxhlet extraction, it is observed that these chemical profiles of 6 types of herbs were different in terms of qualitative and quantitative. For example, most of terpene derivatives of kaffir lime and lemongrass were obtained from ethanolic extracts of solvent extraction, but these compounds were identified in hexane extracts of Soxhlet extraction. However, when compared with their extraction yield and abundance of eluted compounds, Soxhlet extraction shows to be more efficient method in extracting compounds than solvent-mediated extraction with the shorter extraction time. The maximum extraction yield was obtained from tamarind, plai, and soap pod when extracted with 95% ethanol, hexane, and water, respectively in Soxhlet-mediated extraction. This was almost 24-fold, 7.5-fold, and 20-fold more extraction than solvent-mediated extraction from tamarind, plai, and soap pod, respectively. Hence, in the following studies to analyse the effect of different treatment and storage processes, extraction was carried out using Soxhlet extraction only.

3.2. Comparison between Different Steam Processing of Herbs. Generally, in hot herbal compress treatment, the herbs are steamed with water before their application on user's skin. To understand the effect of steaming on herbs on the availability of active compounds, all the herbs were subjected to steaming with different solvents, including water, 5% ethanol, and 5% coconut oil in water for 5 minutes. The steamed herbs were then extracted using 95% ethanol, hexane, and water in the Soxhlet apparatus, and the eluted compounds were analysed by GC-MS. The profiles of eluted compounds were similar to the compounds eluted from solvent and Soxhlet extraction procedures. However, the different steaming processes resulted in variations in the abundance of the compounds in the herbs.

The extraction yield of herbs after the steaming process has also changed when compared with the extraction yield after Soxhlet extraction (Figure 3). The herbs when steamed with water alone had an extraction yield in the ranges of 9% to 33.8%, 1.8% to 4%, and 6% to 24.4% when extracted with 95% ethanol, hexane, and water, respectively. Steaming by using ethanol, hexane, and water as media for heat transfer resulted in an extraction yield of 7.2% to 31.6%, 2% to 6.2%, and 8.8% to 38.8% respectively. The extraction yield varied from ranges of 11% to 27.4%, 1.8% to 11%, and 7.8 to 32% after steaming with water and oil during extraction with 95% ethanol, hexane, and water, respectively. These extraction yields obtained after steam processing were different from the extraction yield of Soxhlet-mediated extraction. Steaming with water in combination with ethanol and oil has shown an increase in extraction yield when extracted using hexane and water as solvent. The Soxhlet extraction yield using hexane was 1.6% to 6.3%, whereas after steaming with water and coconut oil, the extraction yield increased to 1.8% to 11%. Similarly, the extraction yield was 11.2% to 25.2% when extracted with the Soxhlet apparatus using water as solvent, whereas the extraction yield showed an increase of 8.8% to 38.8% when steamed with water and ethanol. Even though the extraction yield was maximum using water as solvent after the steaming process, a greater number of compounds were eluted using hexane as solvent. To our knowledge, there is no report previously that steaming of herbal compress promotes the release of bioactive compounds. The steaming process can cause the starch molecules in the herbs to dissolve and form gel-like substances after undergoing gelatinization and retrogradation. This gel-like structure can protect the molecules present inside the herb [35]. This could be one possible reason for increasing extraction yield and the number of eluted compounds after steaming.

Table S3 shows the bioactive compounds eluted after steaming and extraction using hexane. Even though there were several compounds eluted after the steaming process, few compounds with bioactivity were selected from them and listed in Table S3. Most of the eluted compounds belong to terpenes. Among the listed bioactive compounds, β-sesquiphellandrene and terpinen-4-ol are present with maximum abundance in plai. Terpinen-4-ol is reported to have antimicrobial and anti-inflammatory activities. In addition to this, it is identified as an important component in tea tree oil responsible for its fungicidal activity in treating candidiasis [36]. β-Sesquiphellandrene, β-, and β-curcumene along with other phytocompounds present in turmeric are previously reported to show antioxidant and anti-inflammatory activities [37]. β-Sesquiphellandrene is also identified to have anticancer potential after isolating from curcumin [38]. Linalool is another bioactive compound isolated after steaming from the herb. Linalool is a monoterpene with analgesic, anti-inflammatory, and antioxidant properties [19]. It is also studied to have antimicrobial action against oral bacteria [26]. Caryophyllene, humulene, and germacrene are sesquiterpenes [39]. Caryophyllene and germacrene D exhibits antimicrobial activity [40]. In addition to this, caryophyllene and humulene are also reported to have an anticancer effect [31] and anti-inflammatory activity [19]. Heneicosane is a compound that has the potential to be an antimicrobial agent [30]. Citronellal was identified as a main component in the essential oil from leaves of *Citrus grandis* and was reported to have antibacterial activity against *S. aureus* [41]. Among the three different ways of steaming, steaming with water alone has eluted 27 bioactive compounds from herbs, when extracted with hexane, whereas steaming with 5% ethanol or 5% coconut oil eluted 20 bioactive compounds each from herbs. This implies that herbs when steamed with water can elute more types of compounds and would be beneficial in hot herbal compress treatment. However, steaming with 5% ethanol or 5% coconut oil promotes the elution of some important compounds with bioactivities, such as β-myrcene and 4-carene (kaffir lime), terpinen-4-ol and α-terpineol (lemongrass), β-pinene and γ-terpinene (plai), and humulene, β-curcumene, and β-sesquiphellandrene (turmeric). Therefore, it is the first scientific proof that uses steaming process on herbal compress to promote the release of bioactive chemicals from herbs to user. Also, combinations of 5% ethanol or 5% coconut oil in steaming with water could also promote the elution of beneficial chemicals for herbal compress treatment.

3.3. Comparison between Different Storage Conditions of Herbs. Traditionally, herbal compress is prepared by mixing of dried herbs in cheesecloth and the product is suggested to keep in room temperature, with no limit of time suggested. Currently, for export purpose, dried herbal compress is stored in a tin can, retort bag, or simple sealed plastic bag. Previously long storage of herbs is previously reported to affect the microbial growth in the products and bioactivity of herbs for treatment on users [42–44]. However, the analysis of chemical compositions of herbal compress during storage is not yet published. In this study, the herbs were evaluated for the effect of storage on herbs after storing the herb for 1 day and 7 days at 4°C. The stored herbs were extracted with 95% ethanol, hexane, and water using the Soxhlet apparatus. Figure 4 displays the extraction yield of herbs after storing at different periods of time.

The herbs when extracted using 95% ethanol after 1-day storage have an extraction yield of 8.2% to 35.4%. The extraction yield varied from 8% to 28.4% when the herbs are

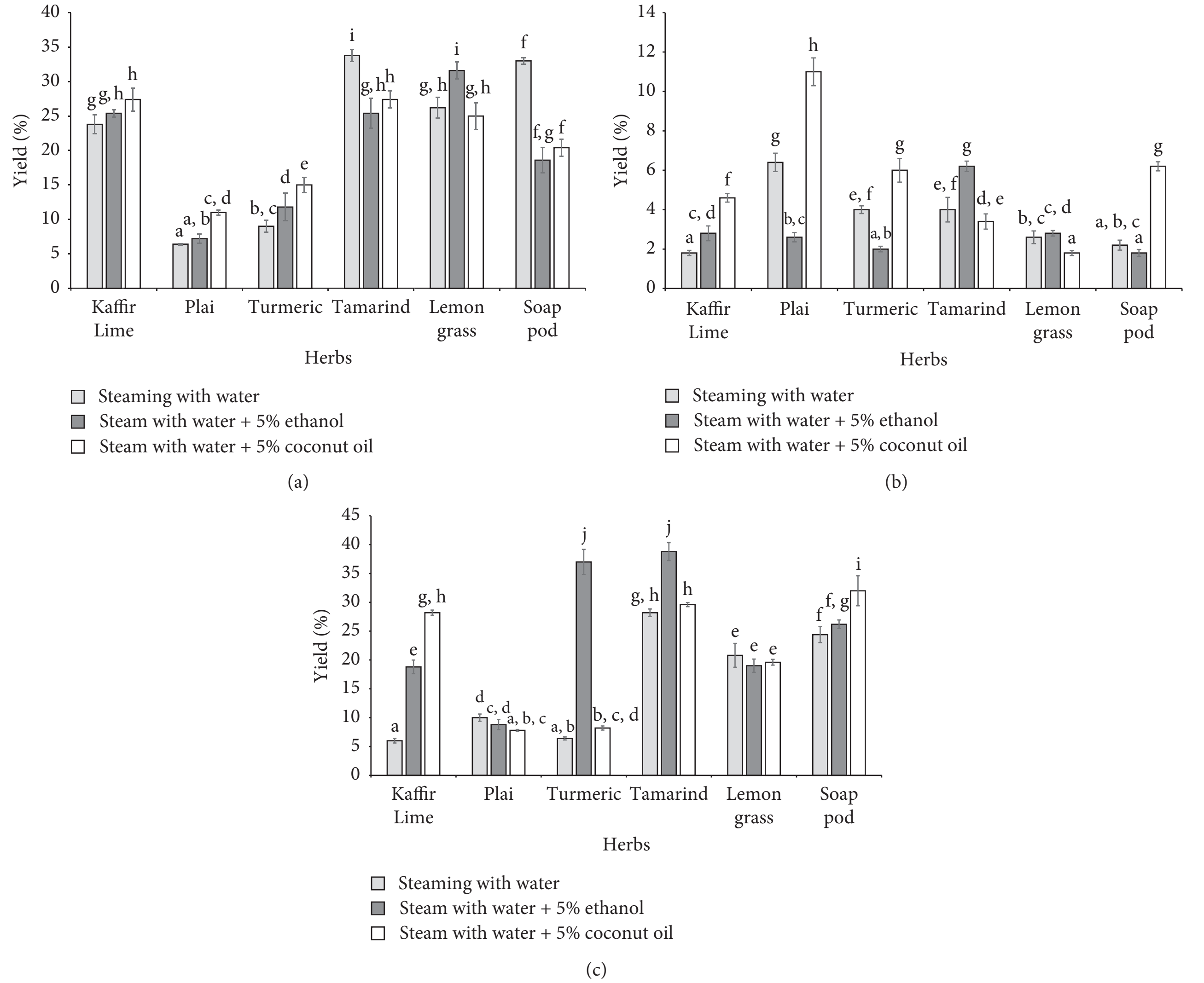

Figure 3: Extraction yield after different steaming processes and extraction with (a) 95% ethanol, (b) hexane, and (c) water. *Different letters above each bar represent different subsets of alpha. All values are average of 3 values obtained after the extraction of sample in triplicate.

stored for 7 days at 4°C. Similarly, the extraction yield was 1% to 6% and 0.6% to 5.2%, when extracted using hexane after 1-day and 7-day storage, respectively. Extraction using water yields 8.2%–28.8% after 1-day storage, whereas the yield decreased to 3% to 16.8% after 7-day storage. This shows that storage has caused a decrease in the extraction yield of herbs.

Among the several compounds eluted after storage for 1 day or 7 days, few bioactive compounds were selected and are represented in Table S4. All these compounds are previously reported to have bioactivities such as antioxidant, anti-inflammatory, or antimicrobial activity. Most of the compounds eluted are similar to the compounds eluted after the steaming process and extraction methods. Similar to the previous results, terpenoids were the main class of eluted compounds. Due to this reason, extraction with water after storage could not elute much bioactive compounds. However, more compounds were eluted when extraction was carried out with 95% ethanol and hexane (Table S4). Moreover, a comparison between eluted compounds displays variation in their content after storage. Some compounds were able to resist the storage condition at 4°C, whereas some compounds were affected due to storage conditions. Few bioactive compounds such as D-limonene and 3-carene from kaffir lime, which were present after 1-day storage, are absent when stored for 7 days. Likewise, some compounds such as trans-α-bergamotene from lemongrass, (+)-4-carene, and γ-terpinene from plai, and β-bisabolene from turmeric show a decrease in their content after storage. Also, few compounds such as terpinene-4-ol in soap pod, D-limonene, and (+)-4-carene in tamarind and humulene in turmeric are eluted only after 7-day storage. In addition to this, some compounds such as γ-terpinene, (+)-4-carene, and cis-sabinene, which was absent in kaffir lime after 7-day storage, were still present in plai after 7-day storage, however, with decrease in its content. Despite this analysis, other compounds such as caryophyllene in lemongrass and turmeric, β-sesquiphellandrene, heneicosane, and guaiacol in turmeric have increased their contents

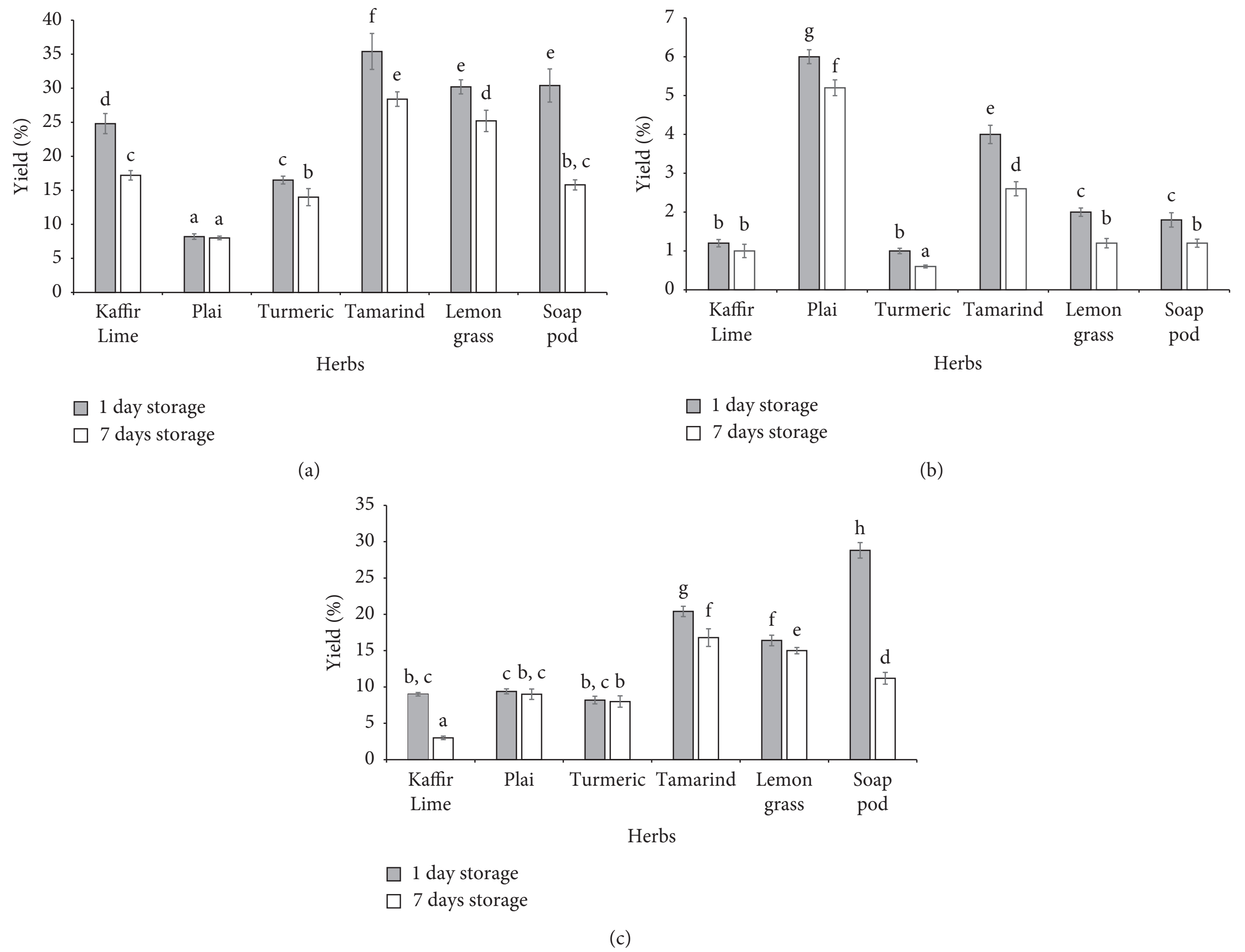

Figure 4: Extraction yield after storage of herbs and extraction using (a) 95% ethanol, (b) hexane, and (c) water. *Different letters above each bar represent different subsets of alpha. All values are average of 3 values obtained after the extraction of sample in triplicate.

after storage. This points that storage time can influence the chemical composition of herbs [42]. Long-time storage can also cause the degradation of bioactive compounds [45]. This can lead to an absence or decrease in the bioactive compound on storage. Thus, this study can confirm that the storage of herbs can influence its contents and may also affect its bioactivity. Therefore, it is necessary to develop the new packaging or new storage method that are different from conventional packages or methods to prolong the storage time of the product to maintain the bioactive compounds.

4. Conclusion

Several studies have been conducted on medicinal herbs to identify the bioactive compounds and their bioactivity to utilize them in various treatments. However, this study has focussed on comparing different extraction procedures, steaming processes, and storage conditions of the herbs used in an herbal compress. The study could reveal that Soxhlet extraction could extract more compounds in less time than solvent extraction. Most of the extracted compounds were bioactive with previously reported antimicrobial, anti-inflammatory, antioxidant, and anticancer activities. Among the steaming processes studied, steaming with water was identified as the best steaming process as it could elute more bioactive compounds from herbs. The study could also prove that the storage of herbs plays an important role in the contents and its abundance in the herbs. Long-time storage can alter their chemical composition and might affect their bioactivity potential also. From this study, it can be concluded that steaming the herbs with water without prior storage can be beneficial in hot herbal compress treatment as it produces more bioactive compounds, which are beneficial for human health. Moreover, this study shows the necessity to develop new packaging or storage methods for herbs to maintain their bioactivity after long-time storage.

Data Availability

The profiles of chemicals from herbal extract data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Supplementary Materials

The results of GC-MS analysis are included as supplementary information. Table S1: bioactive compounds eluted from 6 types of herbs by solvent extraction. Table S2: bioactive compounds eluted from 6 types of herbs by Soxhlet extraction. Table S3: bioactive compounds eluted by different steaming processes. Table S4: bioactive compounds eluted in storage studies. (*Supplementary Materials*)

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