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Research Article

Phytochemical Screening and Antibacterial Activity Studies on the Crude Leaf Extract of *Solanum sisymbriifolium*: Traditional Ethiopian Medicinal Plant

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Ethiopians have used medicinal plants for centuries. In some cases, it is important and the only treatment source. However, these plant species have not been fully studied. In addition, society is inevitably losing knowledge of traditional medicinal plants as society adopts new lifestyles. Consequently, the objective of this research was to determine the phytochemical components and antibacterial activity of *Solanum sisymbriifolium*, a traditional medicinal plant used in Ethiopia to treat arthritis. Phytochemical analyses were performed on the leaf extracts to identify the bioactive constituents. The results of this study indicated that the plant contains carbohydrates, phenols, flavonoids, alkaloids, proteins, steroids, saponins, and terpenoids. Tannins and anthraquinones were absent. Alkaloids and terpenoids' presence in the leaves of these plants is a potential bioactive for bacterial inhibitors. At optimal conditions (62°C, 72 hr, and 1 mm particle size), the maximum extraction yield is 38.538.5 ± 1.15%. Crystals obtained from aqueous methanol extracts were subjected to FT-IR, and the compound spectrum showed a characteristic absorption band for the N-H group at 3500 cm^{-1} and 1700 cm^{-1} for the C = O group, and the medium intensity at 1236 cm^{-1} indicates a C-O stretching. Sharp absorption at 707 cm^{-1} is inductive for = C-H bending. According to agar disc diffusion tests, plant extracts of 50 mg/mL produced 14.04 mm growth inhibition zones of *Bacillus subtilis*. Phytochemical and antibacterial studies of *Solanum sisymbriifolium* indicated that the plant is a source of highly valued compounds for the preparation of medications.

1. Introduction

As reported by the WHO, a medicinal plant is a bioactive plant that can be used for remedial purposes or that is a precursor to the production of chemical and pharmaceutical products [1]. Since ancient times, plants have long been the main source of medicine for about 80% of the population [2, 3]. In literature such as Vedas and the Bible, the wide spread use of herbal medicines and health care products obtained from commonly used traditional herbs and plants is linked to the popularity of herbal medicine and its medical properties [4]. From earliest times itself, plants were used for treatment of disease without knowledge about the compounds present and their mode of action. Over the centuries, societies around the world have developed their own tradition to make sense of medicinal plants and their uses. For example, more than 70% of Ethiopia population uses traditional medicine, and more than 95% of medicines are made of plants [5, 6].

The wide spread use of herbal medicines and health care preparations is obtained from commonly used traditional plants. It has been raised due to the occurrence of natural products with medicinal properties. Not only this, but also, (i) they have practical experience and positive beliefs about traditional medicine. (ii) They have a limited ability to acquire and afford current healthcare services [6, 7].

However, traditional healers and the indigenous community believe that medicinal plants must be kept secret if

they are to be effective. If healers wish to share their wisdom, they usually choose one curious and wiser family member to whom they impart it verbally. These beliefs and practises have been the reason for the fast disappearance of medicinal plants before the scientific community reached. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments [8]. Numerous researches have been carried out recently in various nations to demonstrate its effectiveness. A lot of plants have been employed for their antibacterial properties, which are the result of compounds produced during the plant's secondary metabolism. These products are known by their active substances, for example, the alkaloids, tannin, saponin, and anthraquinone compounds are a significant increase in the study of medicinal plants as a remedy for various forms of diseases and disorders [9].

In this sense, medicinal plants with historically recognised bioactive ingredients offer promising prospects for future research and drug development [10].

Solanum sisymbriifolium is an important flowering plant species with multiple uses. In many Ethiopian communities, it is one of the most widely used species for traditional medicinal treatment. It has scarlet fruits and yellow flowers. It can be distributed in wild near homes, overgrazed areas, wastelands, and roadside areas. In the present study, phytochemical screening and antibacterial activities studies are presented on the crude leaf extract of *Solanum sisymbriifolium*.

2. Materials and Methods

2.1. Collection and Preparation of Plant Materials. The leaves of *Solanum sisymbriifolium* were collected from the Endabagerima area, at points of latitude 14°09′49.2″N and longitude 38°56′19.3″E Adwa, Tigray, Ethiopia. Botanical identification of the plant was done by Mr. Abadi Gebreyesus Hndya at Mekelle University-Botany Laboratory. The experiment was conducted in the Department of Health Sciences Laboratory of Mekelle University and the Adigirat Drug Factory. The leaves were collected from the *Solanum sisymbriifolium* plant from the herbal garden. The leaves were thoroughly rinsed with tap water and then with deionised water. The water-rinsed dried leaves were air dried for 3 days in stainless-steel sieve and finally put in to oven at 37°C for 2 days. The moisture content of sample was analysis using moisture analyser with 5 hr intervals. The dried sample (5% moisture content) was pulverised into powder with a vibrating mill and passed through 1, 1.5, and 2 mm sieve sizes to remove fabric particles and stored in refrigerator.

2.2. Extraction of Plant Materials. The different particle sizes (1, 1.5, and 2 mm) with a total of 25 g of *Solanum sisymbriifolium* powder were weighted using an expert pro electrical balance and inserted into the Soxhlet extractor. 80% v/v of methanol was used as extraction solvent. The experimental design was setup with Design-Expert version 11.1.0 software. Response surface methodology (RMS)–Box-Behnken (BB) (Table 1) and crude extracts were filtered using Whatman No. 1 filter paper. Upon observing the homogeneity of the data, contents, the methanol-based Soxhlet extraction, and filtration procedures were repeated three times to increase the extraction and filtration efficiency. Using the developed regression model, the process factors were optimised to produce the best percentage of extracted yield. The % yield is calculated according to Abdisa and Kenea [11]. Based on the above analysis, the extraction temperature (56-68°C), extraction time (48-72 h), and particle size (1–2 mm) were fed to the software, and the experimental design layouts are given in Table 1.

Table 1: Experimental results (mean ± SD, $n = 3$) of *Solanum sisymbriifolium* leaf extract in % yield.

Std	Run	Factor 1 A: temperature °C	Factor 2 B: time Hr	Factor 3 C: particle size mm	Response 1 Yield %
6	1	68	60	1	37.75 ± 0.58
16	2	62	60	1.5	35 ± 2.31
10	3	62	72	1	38.5 ± 1.15
15	4	62	60	1.5	35 ± 0.58
11	5	62	48	2	35 ± 0.58
2	6	68	48	1.5	35.5 ± 1.15
3	7	56	72	1.5	37 ± 0.58
7	8	56	60	2	35.25 ± 0
14	9	62	60	1.5	35 ± 1.15
4	10	68	72	1.5	38.25 ± 0.12
12	11	62	72	2	35.25 ± 0
13	12	62	60	1.5	35 ± 2.31
9	13	62	48	1	34.5 ± 1.15
5	14	56	60	1	36 ± 2.31
8	15	68	60	2	35.25 ± 0
1	16	56	48	1.5	35.75 ± 0.58
17	17	62	60	1.5	35 ± 0

2.3. Box-Behnken Design for Optimisation. The response surface methodology– (RMS–) Box-Behnken (BB) design has been applied to optimise extraction parameters with respect to high yield. This study focuses the effects of three variables such as temperature (56–68°C), particle size (1–2 mm), and time (48–72 hr) on the crude yield. Each variable was considered at three levels. Table 1 shows the ranges and levels of each factor. The response surface methodology– (RMS–) Box-Behnken (BB) design matrix consisting of 17 run trials involving three variables, each variable at three levels, was obtained using Design-Expert software. Therefore, 17 experiments with different combinations of temperature, particle size, and time were conducted according to the BB, and the response was recorded [12].

2.4. Phytochemical and FTIR Analysis. Standard methods described by Harborne [13] were used to perform the qualitative phytochemical screening of plant extracts such as

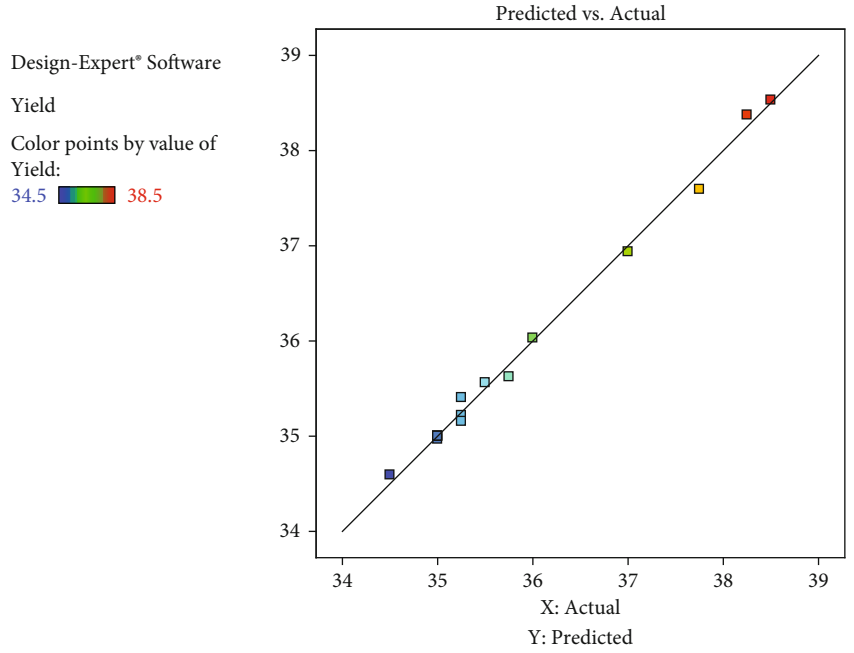

Figure 1: Graph of measured vs. predicted responses.

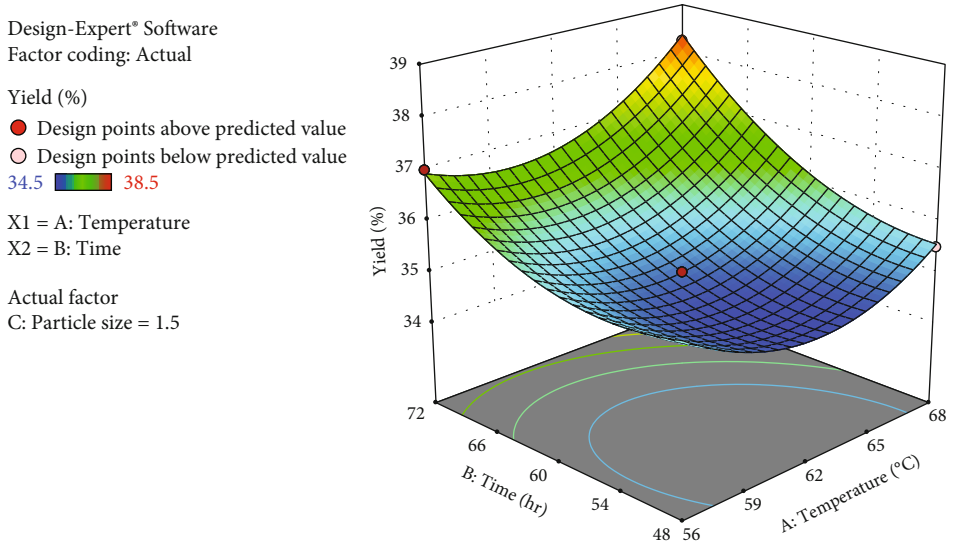

Figure 2: Three-dimensional response surface plots for extract yield as a function of temperature and extraction time.

tannins, terpenoids, anthraquinones, proteins, alkaloids, phenols, carbohydrates, phlobatannin, saponins, flavonoids, and steroids [14–22]. The crude extract was accurately weighed and consistently mixed with KBr salt using a mortar and compressed into a thin pellet. The pellet was analysed using a Shimadzu FTIR spectroscope from 4500 to 400 cm^{-1} with a resolution of 4 cm^{-1} to detect the characteristic peaks.

2.5. Antibacterial Activities of the Extract. The antimicrobial activity of the crude extract under optimal conditions was tested against *Bacillus subtilis*. The strain was obtained from Mekelle University's College of Veterinary Medicine's Veterinary Microbiology Laboratory. The bacteria colonies were inoculated in liquid nutrient broth and cultured at 37°C in the evening and agitated at 200 rpm. Then, each broth culture was adjusted to fit the McFarland half-turbidity standard to obtain around 1×10^8 CFU/mL [23]. Likewise, Mueller-Hinton media were prepared according to the procedures given by its manufacturer as growth media for the agar disc diffusion assay [24–26]. To ensure that the Mueller-Hinton agar medium is not polluted, plates with the medium were developed and maintained in evening. Similarly, 6 mm filter paper discs were prepared from sterile Whatman No. 1 filter paper using a paper punch. M-H media plates were distributed using plate spread technology using 100 microlitres of 12 hr bacteria culture and dried for a

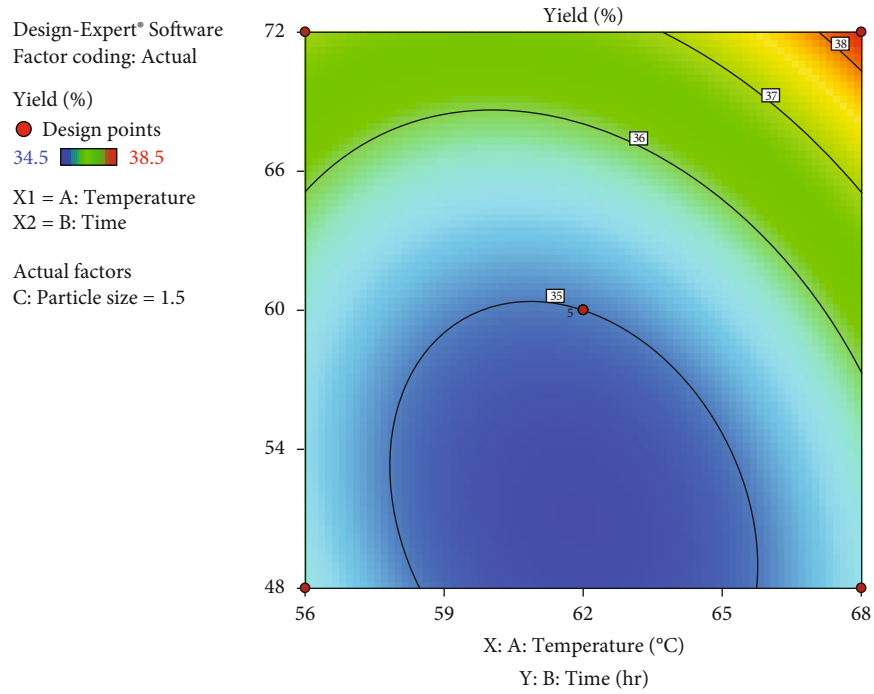

Figure 3: Two-dimensional response surface contour plots for extract yield as a function of temperature and extraction time.

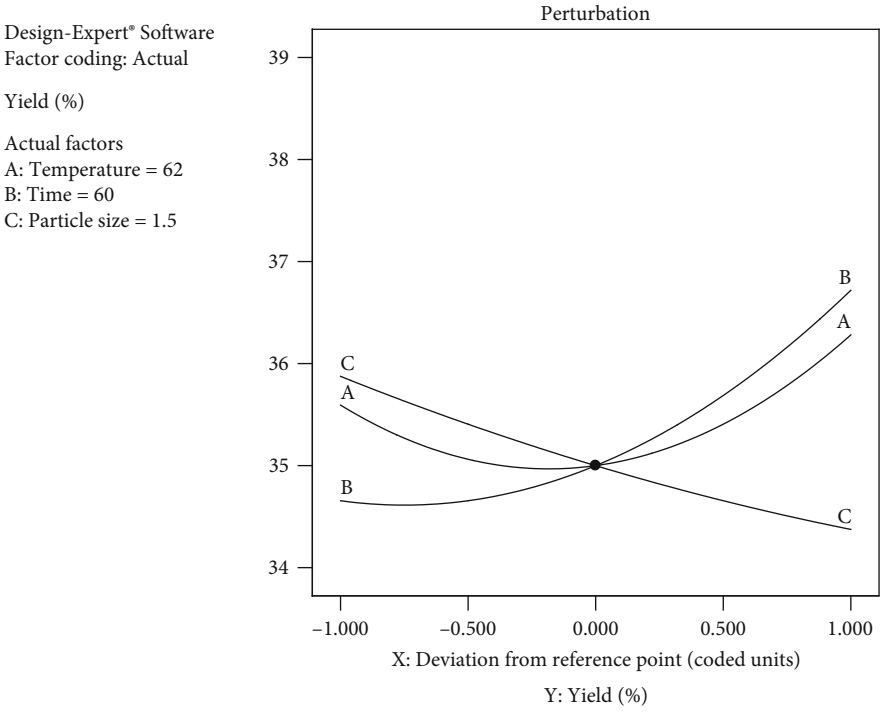

Figure 4: Perturbation vs. extract yield as a function of temperature and extraction time.

minute before surface moisture was extracted. The filter paper disc was infused with a concentration of 100 mg/mL curd extract. The disc was then placed on the agar plate using a stick and distributed for an hour at normal temperature. The plates were then stored in the laboratory for 2 hr at room temperature. Finally, the plates were incubated at 37°C for 24 hr [27]. After incubation, plates are observed, and the diameter of the inhibition area is measured using digital electronic measuring devices. Bacterial cultures with an inhibitory area greater than or equal to 7 mm in diameter were considered resistant to extracts [2].

Table 2: Overall report of actual versus predicted value of extract yield.

Run order	Actual value	Predicted value	Residual value	Leverage	Internally studentized residual	Externally studentized residuals	Cook's distance	Influence on fitted value	Standard order
1	37.75	37.59	0.1562	0.750	2.500	7.071[(1)]	1.875[(2)]	12.247[(2)]	6
2	35.00	35.00	0.0000	0.200	0.000	0.000	0.000	0.000	16
3	38.50	38.53	-0.0313	0.750	-0.500	-0.471	0.075	-0.816	10
4	35.00	35.00	0.0000	0.200	0.000	0.000	0.000	0.000	15
5	35.00	34.97	0.0313	0.750	0.500	0.471	0.075	0.816	11
6	35.50	35.56	-0.0625	0.750	-1.000	-1.000	0.300	-1.732	2
7	37.00	36.94	0.0625	0.750	1.000	1.000	0.300	1.732	3
8	35.25	35.41	-0.1562	0.750	-2.500	-7.071[(1)]	1.875[(2)]	12.247[(2)]	7
9	35.00	35.00	0.0000	0.200	0.000	0.000	0.000	0.000	14
10	38.25	38.37	-0.1250	0.750	-2.000	-2.828	1.200[(2)]	-4.899[(2)]	4
11	35.25	35.16	0.0937	0.750	1.500	1.686	0.675	2.920[(2)]	12
12	35.00	35.00	0.0000	0.200	0.000	0.000	0.000	0.000	13
13	34.50	34.59	-0.0937	0.750	-1.500	-1.686	0.675	-2.920[(2)]	9
14	36.00	36.03	-0.0312	0.750	-0.500	-0.471	0.075	-0.816	5
15	35.25	35.22	0.0312	0.750	0.500	0.471	0.075	0.816	8
16	35.75	35.62	0.1250	0.750	2.000	2.828	1.200[(2)]	4.899[(2)]	1
17	35.00	35.00	0.0000	0.200	0.000	0.000	0.000	0.000	17

3. Results and Discussion

All experiments in this study were performed in triplicate, and the results were mean values. From Table 1, it was observed that the optimum yield is 38.538.5 ± 1.15% that was obtained at run three.

The plot of the actual versus predicted response (Figure 1) were observed to fit in a straight line. It is assumed that the suggested quadratic model is appropriate and effective for the optimisation of process variables. The regression model equation gave precise description of the experimental data, in which all points are very similar to the line of perfect fit. The three-dimensional response surface plots and two-dimensional contour plots are useful tools for anticipating the effects of two factors on the response at the same time and for identifying the optimum values of the independent variables for obtaining the maximum response.

Figures 2 and 3 represent the effects of temperature and extraction time on the extraction yield while the particle size was kept constant. The equation in terms of actual factors (Equation (1)) can be used to create predictions about the response.

Perturbation disturbance graph (Figure 4) indicates the relationship between extraction temperature (A), extraction time (B), and particle size (C) with yields (Y). Both extraction temperature (A) and extraction time (B) increased, particle size could decrease, and yield of the plant extract increased. The extraction yield is highly affected by extraction time (B).

From Table 2, we observed that the differences in actual value and predicted value in each run are small means that the model is significant [28]. The differences between the actual values and predicted values are indicators for models that are acceptable or not. If the differences are more, the model is invalid, whereas if the differences are very small, the models are significant. Finally, after omitting the AB and AC terms, the simpler quadratic regression model was proposed. The linear effects of temperature and time are significant and positive, which means that by increasing them, it is possible to increase the extraction yield.

$$Y = 35 + 0.34A + 1.03B - 0.75C + 0.38AB + 0.94A^2 + 0.69B^2 + 0.13C^2. \quad (1)$$

According to ANOVA, extraction was significantly influenced by extraction temperature, particle size, and extraction time variables.

The Shimadzu FTIR spectrum analysis confirmed the presence of 3500 cm^{-1} N-H starching functional groups. 2980.75 cm^{-1} indicated C-H stretching with the medium appearance of alkane compound. Symmetric extension of HC (CH_2) was found at absorption of 2860 cm^{-1}, the H-C aldehyde absorption band 2750 cm^{-1}. 3500 cm^{-1} indicates primary stretching of the amine medium of H-C with single-bond region. 2250 cm^{-1} is C-C medial alkyne acetylenic with triple bond region [28]. 1750 cm^{-1} is C = O starch ester with strong double bond region. 700 cm^{-1} is cis-C-H out-of-plane bend alkene with strong fingerprint's region. 986.11 cm^{-1} is for C=C twisting [29]. The phytochemical analysis of the crude extract (Table 3) confirmed the presence of proteins, phenols, saponins, flavonoids, alkaloids, steroids, phlobatannins, carbohydrates, and terpenoids. Tannins and anthraquinone were absent. The presence of phenolics in plants indicates that plants are sources of antimicrobial agents [22]. Flavonoids and phenolics showed that

Table 3: Qualitative phytochemical screening results.

No.	Test	Result
1	Carbohydrates	+
2	Alkaloids (Mayer test)	+
3	Terpenoids	+
4	Steroids	+
5	Flavonoids	+
6	Tannins	-
7	Saponin	+
8	Phenol	+
9	Anthraquinone	-
10	Phlobatannins	+
11	Protein	+

+ = present; - = absent.

the plant has potent antioxidant activity or free radical scavengers [30]. Alkaloids in medicinal plants have been reported as an important antimicrobial and analgesic drug alkaloids and terpenoids which have a potential antibacterial activity in medicine [31]. Saponins are known to produce an inhibitory effect on inflammation [32]. Saponins are essential in the treatment of cough and in the controlling of soreness of the upper respirational region. Furthermore, plant-based saponins serve primarily as tonics for the heart and have been documented to prevent diabetics and inhibit fungi growth [33]. Terpenoids have been found to be useful in the prevention of antimicrobial, antifungal, antiparasitic, antiallergenic, and anti-inflammatory properties. Phlobatannins have been reported to possess astringent properties [18].

The antimicrobial activity of the crude extract under optimal conditions was tested against standard strains of *B. subtilis* bacteria. According to the diffusion tests of the agar disc, 50 mg/ml of crude extract under optimal plant conditions produced bacterial growth inhibition zones of 14.04 mm in diameter, which were found to be more effective. Bacterial cultures with an inhibition zone of ≥7 mm in diameter were remarkable antimicrobial activities [2].

4. Conclusions

The optimal extraction yield achieved under optimal conditions of 62°C, 72 h, and 1 mm particle size is 38.538.5 ± 1.15 %. The findings of this study demonstrate that plants contain significant bioactive substances, such as terpenoids and alkaloids, which prevent the growth of bacterial strains [31]. The results suggest that the leaf of *Solanum sisymbriifolium* contains secondary metabolites, indicating that the plant is the source of highly valued compounds for drug preparation.

Data Availability

The data that was used to support the study are available in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest that could have appeared to influence the work reported in this paper.

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References

[1] K. Karunamoorthi, M. Phil, K. Jegajeevanram, J. Vijayalakshmi, and E. Mengistie, "Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings," *Journal of Evidence-Based Complementary & Alternative Medicine*, vol. 18, no. 1, pp. 67–74, 2013.

[2] D. B. Sbhatu and H. B. Abraha, "Preliminary antimicrobial profile of *solanum incanum* L.: a common medicinal plant," *Evidence-Based Complementary and Alternative Medicine*, vol. 2020, Article ID 3647065, 6 pages, 2020.

[3] G. Gebreyohannes, A. Nyerere, C. Bii, and D. B. Sbhatu, "Investigation of antioxidant and antimicrobial activities of different extracts of auricularia and termitomyces species of mushrooms," *The Scientific World Journal*, vol. 2019, Article ID 7357048, 10 pages, 2019.

[4] L. Hoareau and E. J. DaSilva, "Medicinal plants: a re-emerging health aid," *Electronic Journal of Biotechnology*, vol. 2, no. 2, 1999.

[5] N. Tuasha, B. Petros, and Z. Asfaw, "Medicinal plants used by traditional healers to treat malignancies and other human ailments in Dalle District, Sidama Zone, Ethiopia," *Journal of Ethnobiology and Ethnomedicine*, vol. 14, no. 1, pp. 1–21, 2018.

[6] B. N. Tefera and Y. Kim, "Ethnobotanical study of medicinal plants in the Hawassa Zuria District, Sidama Zone, Southern Ethiopia," *Journal of Ethnobiology and Ethnomedicine*, vol. 15, no. 1, pp. 1–21, 2019.

[7] B. Abera, "Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia," *Journal of Ethnobiology and Ethnomedicine*, vol. 10, no. 1, pp. 1–15, 2014.

[8] M. Thenmozhi and R. Sivaraj, "International journal of pharma and bio sciences phytochemical analysis and antimicrobial activity of Polyalthia longifolia materials and methods," *International Journal of Pharma and Bio Sciences*, vol. 1, no. 3, pp. 1–7, 2010.

[9] F. Othman, M. S. Sadeghian, F. Ebrahimi, and M. Heydari, "A study on sedimentation in Sefidroud Dam by using depth evaluation and comparing the results with USBR and FAO methods," *International Proceedings of Chemical, Biological and Environmental Engineering*, vol. 51, no. 9, p. 6, 2013.

[10] R. Article, "Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes," *Journal of Pharmacy & Bioallied Sciences*, vol. 12, no. 1, pp. 1–10, 2020.

[11] Z. Abdisa and F. Kenea, "Phytochemical screening, antibacterial and antioxidant activity studies on the crude root extract of *Clematis hirsuta*," *Cogent Chemistry*, vol. 6, no. 1, 2020.

[12] S. N. H. Azmi, B. M. H. Al-Jassasi, H. M. S. Al-Sawafi, S. H. G. Al-Shukaili, N. Rahman, and M. Nasir, "Optimization for synthesis of silver nanoparticles through response surface methodology using leaf extract of Boswellia sacra and its application in antimicrobial activity," *Environmental Monitoring and Assessment*, vol. 193, no. 8, p. 497, 2021.

[13] J. B. Harborne, "Metode Fitokimia : Penuntun Cara Modern Menganalisis Tumbuhan," 1987, http://perpustakaan.stifar.ac.id/index.php?p=show_detail&id=448&keywords=harborne.

[14] K. S. Banu and L. Cathrine, "General Techniques Involved in Phytochemical Analysis," *International Journal of Advanced research in Chemical Science*, vol. 2, no. 4, pp. 25–32, 2015.

[15] S. Paul, K. Mukherjee, A. De, A. Bhattacharyya, and S. Basu, "Process optimization for preparation of an antibacterial agent using a combined extract of tulsi and marigold leaves," *IOSR Journal of Environmental Science, Toxicology and Food Technology*, vol. 11, no. 5, pp. 31–35, 2017.

[16] N. Jaradat, F. Hussen, and A. Al Ali, "Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata," *Journal of Materials and Environmental Science*, vol. 6, no. 6, pp. 1771–1778, 2015.

[17] S. Bhaisare Manmohan and S. G. Kunjalwar, "Phytochemical screening of E. *chaetaria*, (Roem. & Schult), Cyperaceae Bhandara District of Maharashtra," *International Journal Life Sciences*, vol. 3, no. 1, pp. 91–95, 2015.

[18] M. Yadav, S. Chatterji, S. K. Gupta, and G. Watal, "Preliminary phytochemical screening of six medicinal plants used in traditional medicine," *International Journal of Pharmacy and Pharmaceutical Science*, vol. 6, no. 5, 2014.

[19] A. A. Abdalla, M. I. Mustafa, and A. M. Makhawi, "Phytochemical screening and antimicrobial activities studies of *Acacia nilotica* fruit cover," *bioRxiv*, 2020.

[20] M. Merghem, S. Dahamna, and S. Khennouf, "Polyphenols contents and antioxidant activity of extracts from leaves and flowers of Thymelaea hirsuta," *Journal of Drug Delivery and Therapeutics*, vol. 10, no. 4, pp. 108–111, 2020.

[21] T. Thilagavathi, R. Arvindganth, D. Vidhya, and R. Dhivya, "Preliminary phytochemical screening of different solvent mediated medicinal plant extracts evaluated research," *International Research Journal of Pharmacy*, vol. 10, no. 4, pp. 246–248, 2015.

[22] A. Doss, M. Sc, and M. Phil, "Preliminary phytochemical screening of some Indian medicinal plants," *Ancient Science of Life*, vol. 29, no. 2, pp. 12–16, 2009.

[23] S. Kothari, V. Mishra, S. Bharat, and S. D. Tonpay, "Antimicrobial activity and phytochemical screening of serial extracts from leaves of Aegle marmelos (Linn.)," *Acta Poloniae Pharmaceutica - Drug Research*, vol. 68, no. 5, pp. 687–692, 2011.

[24] E. Karimi, H. Z. E. Jaafar, and S. Ahmad, "Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of labisa pumila benth," *Molecules*, vol. 16, no. 6, pp. 4438–4450, 2011.

[25] M. D. Makut, S. D. Gyar, G. R. I. Pennap, and P. Anthony, "Phytochemical screening and antimicrobial activity of the ethanolic and methanolic extracts of the leaf and bark of Khaya senegalensis," *African Journal of Biotechnology*, vol. 7, no. 9, pp. 1216–1219, 2008.

[26] C. N. Durga, M. Lakshmi, B. Mahitha, T. Madhavi, and N. J. Sushma, "Phytochemical screening and FTIR analysis of Clitoria Ternatea leaves," *International Journal of Scientific and Engineering Research*, vol. 6, no. 2, pp. 287–290, 2015.

[27] A. P. S. A. Da Silva, L. C. Nascimento da Silva, C. S. Martins da Fonseca et al., "Antimicrobial activity and phytochemical analysis of organic extracts from Cleome spinosa Jaqc," *Frontiers in Microbiology*, vol. 7, pp. 1–10, 2016.

[28] I. T. Karabegović, S. S. Stojičević, D. T. Veličković, N. Č. Nikolić, and M. L. Lazić, "Optimization of microwave-assisted extraction and characterization of phenolic compounds in cherry laurel (*Prunus laurocerasus*) leaves," *Separation and Purification Technology*, vol. 120, pp. 429–436, 2013.

[29] A. Bayu, D. Nandiyanto, R. Oktiani, and R. Ragadhita, "How to read and interpret ftir spectroscope of organic material," *Indonesian Journal of Science & Technology*, vol. 4, no. 1, pp. 97–118, 2019.

[30] G. A. Ayoola, H. A. Coker, S. A. Adesegun et al., "Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria," *Tropical Journal of Pharmaceutical Research*, vol. 7, no. 3, pp. 1019–1024, 2008.

[31] A. M. Karande, H. V. Kamble, V. H. Kumbhar, S. R. Kane, C. S. Magdum, and S. R. Kane, "Preliminary phytochemical screening of Glochidion ellipticum," *European Journal of Experimental Biology*, vol. 6, no. 4, pp. 41–45, 2016.

[32] O. A. Aiyegoro and A. I. Okoh, "Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC," *BMC Complementary and Alternative Medicine*, vol. 10, no. 1, 2010.

[33] A. C. Marcus, O. S. Edori, and M. C. Maduagu, "Phytochemical and anti-microbial screening of Phyllantus fratenus and Taraxacuim officinale leaves," *Biochemistry & Analytical Biochemistry*, vol. 8, pp. 1009–2161, 2019.