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PHYTOCHEMICAL PROFILING AND PHARMACOLOGICAL EVALUATION OF A HERBAL EXTRACT WITH ANTIDEPRESSANT POTENTIAL

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ABSTRACT

Depression is a prevalent neuropsychiatric disorder that significantly impacts quality of life and is often associated with high morbidity. Current antidepressant therapies are limited by delayed onset of action, side effects, and incomplete remission, highlighting the need for alternative therapeutic approaches. Herbal medicines, owing to their phytochemical diversity and multi-target actions, are being increasingly investigated for their neuroprotective and antidepressant potential. The present study aimed to evaluate the phytochemical composition, cytotoxicity, and antidepressant activity of a selected herbal extract. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which are known to possess neuroactive and antioxidant properties. Cytotoxicity studies (MTT assay) revealed the extract to be safe at therapeutic concentrations. Pharmacological assessment was conducted using validated animal models of depression, including the forced swim test (FST) and tail suspension test (TST). The extract demonstrated significant antidepressant-like activity, reflected by reduced immobility time compared to control, and its efficacy was comparable to standard antidepressant drugs. The findings suggest that the antidepressant effect may be attributed to the synergistic action of bioactive phytoconstituents, possibly mediated through modulation of monoaminergic pathways and antioxidant defense mechanisms.

1. INTRODUCTION

The emergence and spread of drug-resistant microbes has threatened the activity of available drugs and remains the major cause of treatment failure.1–3 The burden of morbidity and mortality has been inclined towards developing countries due to the increased prevalence of risk factors associated with economic transition.4,5 Antibiotics that were once thought to be miracle cures are now unable to treat resistant bacteria. Numerous multidrug-resistant microbes have been identified as virulently dangerous bacteria.6,7 Over recent years, the number of new approved antimicrobial medicines has dropped greatly, and the supply of effective antimicrobials is anticipated to run out shortly.3,8,9 Traditionally used medicinal plants represent the ancient and remain an indispensable source of novel and effective pharmaceutical products. The capacity to use active substances derived from plants or their synthetic equivalents in medicine has improved with the development of phytochemistry and pharmaceutical chemistry. This is due to the fact that medicinal plants have a greater variety and novelty of chemicals than any other sources.

Africa has an immensely rich biodiversity and knowledge base in the use of plants to treat various ailments, including infectious diseases. In fact, the World Health Organization (WHO) estimates that due to their easy availability, low cost, and socio-cultural background, over 80% of the population in sub-Saharan Africa relies solely on traditional medicine derived from plants for their primary health-care needs.9–12 However, these resources have hardly been investigated scientifically. In Ethiopia, some of the studies presented on medicinal plants were limited to an

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ethnomedical survey, and the results were listed with incomplete descriptions.13–15

In various regions of Ethiopia different plant species are traditionally utilized for the treatment and prevention of both human and animal illnesses. Justicia schimperiana (Hochst. ex Nees), Croton macrostachyus (Hochst. ex Delile), Albizia gumifera (J.F.Gmel.) C. A. Sm, Clematis hirsuta Guill. and Perr, Solanum nigrum L, Dodonaea angustifolia L.f., Crinum abyssinicum Hochst. ex A. Rich, Dracaena steudneri Engl., Pycnostachys abyssinica Fresen, Trichilia dregaeha Sand, are the most commonly used medicinal plants by TMPs.17–21 Therefore, it is of paramount importance to focus on antimicrobial drug discovery from medicinal plants, particularly from those which are widely used by traditional healers for the mitigation of infectious diseases.

2. MATERIALS AND METHODS

Study Design

Qualitative phytochemical screening and in-vitro antimicrobial investigation were conducted from 1st June to 31st September 2021.

Plant Material

A comprehensive ethnomedicinal survey was conducted in southwest Ethiopia. Based on the information from the traditional healers and evidence of traditional use value frequency index, twelve medicinal plants were selected and their plant materials were collected from Ilu Aba Bor Zone forest, Oromia, southwest Ethiopia (34° 52’ 30” E to 36° 5’ 30” E longitudes and 7° 27’ 30” N to 8° 49’ 30” N latitude). The selected plant species include Justicia schimperiana (Hochst. ex Nees) root, Croton macrostachyus (Hochst. ex Delile) stem bark, Albizia gumifera (J.F.Gmel.) C. A. Sm stem bark, Clematis hirsuta Guill. and Perr. whole part, Solanum nigrum L. fruit, Dodonaea angustifolia L.f. leaf, Crinum abyssinicum Hochst. ex A. Rich root bulb, Dracaena steudneri Engl. root, Pycnostachys abyssinica Fresen root, Trichilia dregaeha Sand stem bark, Momordica foetida Schumach. et Thonn leaf, and Thalictrum rhynchocarpum Quart.-Dill. and A.Rich. root. The collected plant samples were allowed to dry at room temperature under the shade; their identification was carried out by a botanist; and the voucher specimens (P1/2021-P12/2021) have been deposited at Mattu University Herbarium.

Materials, Chemicals and Reagents

Materials

Beakers, conical flask, measuring cylinders (different size), glass funnels, glass stirrer, cotton wool, spatula, bunsen burner, top mettler weighing balance, test tubes, stainless steel tray, thermostat water bath, oven, aluminum foil paper, hand gloves, mortar and pestle, analytical weighing balance, test-tube holder, refrigerator, meter rule, bottles, cabinet tripod stand, wire gauze, capillary tubes, filter paper, autoclave, UV box with UV lamp, and TLC paper.

Chemicals and Reagents

Analytical standards of chloroform, methanol, n-hexane ethyl acetate (Lneos Solvents Belgium), ferric chloride, HCl, Mayer-Wagner reagent (2.5 gm of iodine is dissolved in 12.5 gm of KI2 with 250 mL of distilled water), magnesium ribbon, NaOH, sulfuric acid, potassium ferricyanide (K2Fe (CN6)), dimethyl sulfoxide (DMSO) (Mettler-Toledo India Pvt. Ltd), Mueller Hinton Broth (Thermo ScientificTM), gentamycin (Bactigen FDC Limited) and clotrimazole (Glenmark Pharmaceuticals Ltd). Jimma University Laboratory of Drug Quality (JuLaDQ), organic chemistry, and microbiology labs of Jimma University provided all the chemicals and reagents.

Test Organism

Four bacterial strains; Klebsiella pneumoniae (ATCC 700603), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC43300), Escherichia coli (ATCC 25922), and one fungal strain, Candida albicans (ATCC 90028) were obtained from Ethiopian Public Health Institute (EPHI) and used to examine the antimicrobial activity of the plant extracts.

Extraction and Fractionation

The air-dried and pulverized plant materials were extracted with chloroform/methanol 1:1 (v/v) three times for 24 hours each. The extracts were concentrated using a rotary evaporator at a temperature of 40°C to obtain crude extracts, which were subjected to phytochemical screening and antimicrobial evaluation. The crude extracts were suspended in water and further partitioned successively with n-hexane, chloroform, and methanol. Each fraction of the plant extracts were then concentrated using rotary evaporator; scanted and dried by putting it in warm mental mantle using desiccator to remove the solvent residue based on previous studies.

Phytochemical Screening

The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) present in the extracts following standard protocols.

Test for Tannins

About 200 mg of the plant extract was boiled with 10 mL of distilled water; and 0.1% Ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

Test for Alkaloids

The plant extract was dissolved in 100 mL of water, filtered, and cooked in steam with 2 mL of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids.

Test for Saponins

About 0.5 milliliters of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of foam confirmed the presence of saponins.

Test for Flavonoids and Glycosides

200 mg of the plant extract was mixed with 10 mL of ethanol and filtrated. Two mL of the filtrate, concentrated HCl, and magnesium ribbon were mixed. The formation of a pink or red color indicates the presence of flavonoids. Adding 1 mL of distilled water and NaOH to 0.5 mL of crude extract, the formation of a yellowish color indicated the presence of glycosides.

Test for Steroids

About 1 mL of the crude extract was combined with 10 mL of chloroform and 10 mL of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

3. RESULT

The Ethnomedicinal Information of Selected Plants

Among the twelve plant species selected, five (42%) were trees and three (25%) were herbs. The roots of the plants were the most commonly used, followed by stem bark. The selected plant species were usually utilized to treat different perceived infections. Justicia schimperiana, A. gumifera, S. nigrum, C. abyssinicum traditionally used for the treatment of neglected tropical diseases such as leishmaniasis, trypanosomiasis, onchocerciasis and scabies. C. hirsuta, C. macrostachyus, and T. rhynchocarpum were claimed to be used for the treatment of gastrointestinal infections. And C. abyssinicum, D. steudneri and M. foetida were used the treatments of wound infections. The majorities of the TMPs provide the plant materials as fresh, crushed or powdered and applied on the affected part or administered orally by mixing them with milk, honey, butter, coffee, or water (Table 1)

Phytochemical Screening

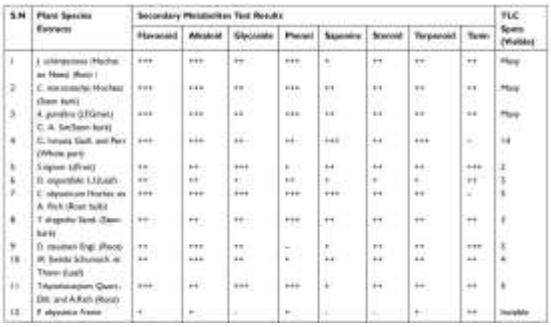
All selected plant extracts were presented with notable positive phytochemical results (Table 2), which were evidenced with remarkable color changes. Flavonoids, alkaloids and phenols were the most abundant classes of compounds in majorities of the screened plants. Flavonoids were exhibited highly positive with significantly visible color change in J. schimperiana root, C. macrostachyus stem bark, A. gumifera stem bark, C. hirsuta whole part, T. dregaeha stem bark, C. abyssinicum

root bulb and T. rhynchocarpum root. Alkaloid was the next most class of compound which presented in C. macrostachyus, C. hirsuta C. abyssinicum and T. rhynchocarpum root. Phenols were the third phytochemicals presented in J. schimperiana, C. macrostachyus, A. gumifera, C. hirsute, C. abyssinicum and T. rhynchocarpum root. Thin layer chromatography also confirmed the presence of different phytochemical components.

Table 1 Ethnomedicinal Information of the Selected Medicinal Plants



Table 2 Phytochemical Screening Results of Crude Extract of Selected Plant Species



Antimicrobial Activities

Among the selected plant species, all fractions of the extracts from T. rhynchocarpum root presented with the greatest efficacy against all tested strains. Particularly, the crude extract of T. rhynchocarpum exhibited with MIC 0.98 µg/mL against K. pneumoniae and P. aeruginosa and 0.48 µg/mL against S. aureus and E. coli which is even greater than that of the control drugs, gentamicin and clotrimazole (Table 3). Extracts from J. schimperiana and C. macrostachyus also demonstrated remarkable activity against tested microbial strains. The chloroform fraction of J. schimperiana root presented the highest activity with MIC of 3.8 µg/mL against S. aureus and E. coli. The crude extract of C. macrostachys exhibited 3.9 µg/mL against K. pneumoniae, 7.8 µg/mL against S. aureus, and E. coli. The chloroform fraction of C. macrostachys also demonstrated the lowest MIC with 7.8 µg/mL against K. pneumoniae, P. aeruginosa and S.aureus. A Kruskal–Wallis/Ddunn's statistical test showed a significant difference between the tested samples and fractions of the plant extracts on MIC with tested plant species (H(df:11) = X2:180.45, p = 0.000) (Table 4).

Table 3 Percentage Yields and Antimicrobial Activities Test of Selected Plant Crude Extract and Different Solvent Fraction

Plant Species Extract	Fraction of the Plant Extract	Antimicrobial Activities (MIC in µg/mL)				
		KP	PA	SA	EC	CA
J. schimperiana (Hochst. ex Hiern)	Crude extract	15.6	7.8	7.8	7.8	15.6
	n-Hexane fraction	31.25	15.6	7.8	7.8	31.25
	Chloroform fraction	15.6	7.8	3.8	3.8	15.6
	Methanol fraction	15.6	7.8	15.6	15.6	31.25

Plant Species Extract	Fraction of the Plant Extract	Antimicrobial Activities (MIC in µg/mL)				
		KP	PA	SA	EC	CA
C. macrostachys Hochst	Crude extract	31.25	31.25	7.8	7.8	31.25
	n-Hexane fraction	62.5	62.5	15.6	15.6	31.25
	Chloroform fraction	31.25	31.25	15.6	15.6	62.5
	Methanol fraction	15.6	15.6	7.8	7.8	31.25
A. gumifera (Forssk.) C. A. Sm	Crude extract	3.9	3.9	7.8	7.8	7.8
	n-Hexane fraction	31.25	125	31.25	31.25	31.25
	Chloroform fraction	15.60	15.6	15.6	15.6	31.25
	Methanol fraction	31.25	31.25	62.50	62.5	62.5
C. hirsuta Gutl. and Perr	Crude extract	7.8	7.8	15.6	15.6	15.6
	n-Hexane fraction	7.8	7.8	15.6	15.6	31.25
	Chloroform fraction	7.8	7.8	7.8	7.8	15.6
	Methanol fraction	31.25	31.25	15.6	31.25	62.5
S.nigrum L.	Crude extract	15.6	31.25	250	250	62.5
	n-Hexane fraction	62.5	250	250	250	125
	Chloroform fraction	62.5	62.5	62.5	125	125
	Methanol fraction	125	125	125	250	62.5
O. engaddita L.f.	Crude extract	15.6	15.6	31.25	31.25	31.25
	n-Hexane fraction	62.5	62.5	62.5	31.25	31.25
	Chloroform fraction	15.6	15.6	62.5	31.25	31.25
	Methanol fraction	15.6	7.8	62.5	62.5	62.5
C. abyssinicum Hochst. ex A. Rich	Crude extract	15.6	15.6	7.8	7.8	15.6
	n-Hexane fraction	31.25	31.25	7.8	7.8	15.6
	Chloroform fraction	7.8	7.8	7.8	15.6	15.6
	Methanol fraction	31.25	31.25	31.25	31.25	31.25
O. schultzii Engl.	Crude extract	250	125	125	125	125
	n-Hexane fraction	500	>500	>500	>500	>500
	Chloroform fraction	250	250	125	125	62.5
	Methanol fraction	250	250	250	250	250
P. abyssinica Fresen	Crude extract	62.5	62.5	31.25	31.25	31.25
	n-Hexane fraction	125	125	125.00	125.00	125.00
	Chloroform fraction	62.5	62.5	31.25	31.25	62.5
	Methanol fraction	31.25	31.25	15.6	15.6	62.5

Plant Species Extract	Fraction of the Plant Extract	Antimicrobial Activities (MIC in µg/mL)				
		KP	PA	SA	EC	CA
T. angustifolia Sond.	Crude extract	250	250	125	125	62.5
	n-hexane fraction	125	125	62.5	62.5	62.5
	Chloroform fraction	125	125	250	250	250
	Methanol fraction	125	125	250	250	250
M. ferrea Schumach. et Thonn.	Crude extract	250	250	125	250	500
	n-hexane fraction	500	500	500	125	500
	Chloroform fraction	15.6	15.6	62.5	125	125
	Methanol fraction	125.00	125	125	125	125
T. rhynchocarpum Quart.-Dill. & A.Rich	Crude extract	0.98	0.98	0.48	0.48	3.9
	n-hexane fraction	7.8	7.8	3.9	3.9	7.8
	Chloroform fraction	1.95	1.95	0.98	0.98	3.9
	Methanol fraction	3.9	3.9	1.95	1.95	3.9
Gentamycin		6.25	12.5	1.5	3.13	
Chloramphenicole						10

Table 4 SPSS Output of Kruskal–Wallis/Ddunn's Report of MIC of Each Extract Against Selected Strain Among Grouping Variable Test Statistics

Grouping Variables	Chi-Square	df	Asymp. Sig.
Plant species	180.45	11	0.000[a]
Fractions of the plant extracts	7.44	3	0.059[a]
Tested Microbial strains	0.123	3	0.989

4. DISCUSSION

Plant extracts have demonstrated high-level activity against pathogens due to the enormous variety of phytochemicals. There are limited detailed examinations of these plants for their potential role as phytochemical entities and antimicrobial therapy.16,20,21 Antibiotic resistance, harmful side effects, and the high costs of synthetic drug development are shifting the focus to plant-derived medicines.4,7,30 This study identified potential plant species traditionally utilized to treat a variety of infections, including tropical infectious diseases, gastrointestinal, skin, and wound infections. The majority of the investigated plants were found to contain different phytochemical classes of compounds including flavonoids, alkaloids, phenols, glycosides, and steroids; which was confirmed by TLC results presented with multiple spots at different RF values. Among screened classes of compounds flavonoids, alkaloid and phenols were the phytochemicals with significant visible color changes. Justicia schimperiana, C. macrostachyus, A. gumifera, C. hirsuta, T. dregaeha, C. abyssinicum and T. rhynchocarpum were the plant species containing flavonoids, alkaloids and phenols. This finding is similar to the findings of other studies elsewhere.

As illustrated in Table 3, most of the evaluated plant extracts demonstrated remarkable activity against selected microbial strains, with the lowest in-vitro inhibitory concentration (<10 µg/mL). The crude extracts of T. rhynchocarpum root demonstrated the greatest activity, with the lowest MIC of 0.48 µg/mL against S. aureus and E. coli and 0.98 µg/Ml K. pneumonia and P. aeruginosa. The finding is consistent with reports indicating the antimicrobial efficacy of this medicinal plant for treatment of microbial infections.30,37 The chloroform fraction of J. schimperiana also demonstrated antibacterial activity with the lowest MIC value of 3.8 g/mL against S. aureus and E. coli. Except pneumoniae and C.albicans its MIC is less than 10 µg/mL, which in line with other similar studies.30,38–40 Extracts from C. acrostachyus also exhibited activity against S. aureus and E. coli. These findings are onsistent with previous report that the plants have antimicrobial activity .

5. CONCLUSION

The study successfully established the phytochemical profile, safety, and antidepressant potential of the investigated herbal extract. The presence of alkaloids, flavonoids, and phenolics correlated with its observed pharmacological activity, while cytotoxicity assays confirmed its safety at effective doses. The extract significantly reduced immobility time in animal models, indicating strong antidepressant-like activity comparable to standard drugs. These findings support the therapeutic potential of herbal formulations as safe and effective alternatives for managing depression. However, further detailed studies including isolation of active constituents, mechanistic exploration, and clinical trials are warranted to validate its efficacy and translate the findings into therapeutic application.

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