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Phytochemical screening and evaluation of antimicrobial activity of *Pterolobium stellatum* root extract

Abstract

Background: Medicinal plants have been used in the treatment of numerous diseases as they possess potential biological activities. *Pterolobium stellatum* is a native medicinal plant whose leaves and root parts are used in different African countries for the treatment of diseases such as tuberculosis, diarrhea, pneumonia, goiter, epilepsy, tumor, snake bites, stomachache, headache, and rheumatic pain. Even though it is used traditionally for the management of several diseases, the antimicrobial activity of the root part has not been exhaustively studied. Thus, the overall objective of the study was to carry out a phytochemical screening test and evaluate the antimicrobial activity of the root extract of the plant against some pathogenic microorganisms.

Methods: The shade-dried root part of the plant was extracted by 70% ethanol and the extract was partitioned into three fractions using three solvents (hexane, chloroform, and ethyl acetate) by liquid-liquid extraction technique. The extract was subjected to phytochemical screening tests. The antibacterial and antifungal activities of ethanolic crude extract and fractions were assessed using agar well diffusion and disc diffusion methods, respectively, against four bacterial strains (*Staphylococcus aureus, Bacillus cereus*, *Escherichia coli* and, *Salmonella typhi*) and one fungal strain (*Candida albicans*).

Results: Phytochemical screening of the extract revealed the presence of phenols, flavonoids, glycosides, tannins, saponins, and terpenoids. The extract and fractions displayed promising antibacterial and antifungal activities against tested microorganisms. The ethyl acetate fraction exhibited the highest antibacterial and antifungal activities against *Bacillus cereus* (20 mm) and *Candida albicans* (11 mm) followed by the chloroform fraction which showed better antibacterial activity against *Staphylococcus aureus* (12 mm) and *Bacillus cereus* (17 mm).

Conclusion: Overall, the root of *Pterolobium stellatum* possesses some secondary metabolites. The root extract and fractions of the plant exhibited remarkable antibacterial and antifungal activities against tested strains. The study corroborates the ethno-medicinal use of the plant and acclaims its consideration as a possible source of antimicrobial agents for the treatment of bacterial infections. Bioactivity-guided isolation and characterization of the active principles will be recommended.

Keywords: Phytochemical screening, Antimicrobial activity, *Pterolobium stellatum,* liquid-liquid extraction

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Introduction

Traditional medicine is the total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, that are used to maintain health, as well as to prevent, diagnose, and treat various illnesses.[1] Medicinal plants are claimed to possess antibiotic properties and are also used comprehensively by tribal people worldwide. They have been known to alleviate various diseases in traditional medicine.[2] According to World Health Organization (WHO), 60% of the world's population relies on herbal medicine and about 80% of the population in developing countries depends almost totally on it for their primary healthcare needs.[3] Socioeconomic conditions and the unavailability of modern medicine are the main reasons for people to rely on traditional medicine.[4] Ethiopians have used traditional medicines for many centuries, the use of which has become an integral part of the different cultures in Ethiopia, due to cultural acceptability, efficacy against certain diseases, and economic affordability.[5]

WHO reports that infectious diseases are responsible for over 50% of deaths worldwide, occurring mainly in tropical and developing countries.[6] To date even though a wide range of synthetic and semi-synthetic antibacterial agents are available for the control of infectious diseases, infectious disease complications remain an important cause of mortality and morbidity among hospitalized patients.[7] The emergence of multidrug-resistant strains is a serious threat and makes chemotherapy more difficult. Moreover, the current cost of most of the chemotherapeutic agents is unaffordable to the public especially in developing countries.[8] Thus, there is an urgent and constant need for the exploration of cheaper, effective, new plant-based drugs with better bioactive potential.[9]

Pterolobium stellatum is a native species distributed throughout tropical and subtropical regions of Africa, particularly in countries like Sudan, Ethiopia, Kenya, Eritrea, Zambia, Tanzania, and Yemen.[10] Ethnobotanical surveys have revealed that the roots, leaves, and flowers of *Pterolobium stellatum* were used for the treatment of various illnesses including tuberculosis, diarrhea, pneumonia, peptic ulcer, goiter, and epilepsy in African traditional medicine.[11-14] In Kenya, its root decoction is used to treat stomachache and pneumonia.[12]

Ethiopia is one of the richest African countries in traditional medicines and is associated with indigenous knowledge which is mainly related to the presence of many ethnic groups and unique cultures.[15] In this country, the root of *P. stellatum* is used to remove retained placenta during delivery, to avoid tumors, treat snake bites and to treat stomachache, headache, and rheumatic pain. According to some ethnobotanical studies, the crushed leaves of the plant are mixed with butter and applied as a paste around the neck to treat goiter and powdered root cream was used to treat external hemorrhoids. The whole plant juice is given orally for one month to treat epilepsy and neuralgia.[16]

Despite the wide use of medicinal plants in traditional health care, the work that has been done to evaluate the efficacy of Ethiopian traditional medicinal plants is not extensive, so studies done on such areas might help address relevant information about traditional herbal preparations. The antimicrobial activity of root extracts of *Pterolobium stellatum* has not been exhaustively studied although it is used traditionally for the treatment of different infectious diseases. Therefore, the present study was focused on phytochemical screening of the root extract of the *Pterolobium stellatum* and evaluation of its antimicrobial activities.

Materials and methods

Chemicals

The main chemicals and reagents that were used include 70% ethanol, ethyl acetate, n-hexane, chloroform, gentamicin, DMSO, distilled water, hydrochloric acid, sodium hydroxide, Wagner Reagent, Mayer reagent and benzene, glacial acetic acid, iodine solution, sulfuric acid, ferric chloride, ammonium, and acetic anhydride. All chemicals used in this experiment were of analytical-grade reagents.

Equipment

The following equipment and materials were used: separatory funnel, metal stand, Whatman filter paper, electrical balance, desiccators, oven, Erlenmeyer flask, measuring cylinder, rotary evaporator, round bottom flask, conical flask, beaker, grinding machine, and test tube.

Plant materials

The root of *Pterolobium stellatum* (Figure 1) was collected on April 9 and April 26, 2022, in two rounds from Seto kebele, Jimma town which was located in the Jimma zone, Oromia region at a distance of 346 km from Addis Ababa. It was identified and authenticated by a botanist at Jimma University.

Figure 1 Picture of the root part of *Pterolobium stellatum* (by Samuel Teshome, April 2022).

Preparation of plant extract

The collected root was washed or cleaned from dirt and soil with water and dried at room temperature in the open air under shade for about 3 weeks at the pharmaceutical analysis laboratory (School of Pharmacy). The plant roots were spread out and regularly turned over to avoid rotting. Thereafter, 530g dried roots were weighed using an electrical balance and were ground into a course by using a grinding machine and stored at room temperature. Then 500g of course powder was macerated in 70% ethanol for a period of 72 hours with occasional shaking. This was repeated two times and the extracts obtained were filtered through the Whatman filter paper (No 1). Then after the filtrate was concentrated and organic solvent was removed by evaporation by using a rotary evaporator machine at a temperature not exceeding 40 OC. Finally, the extract was stored in airtight glass containers for further analysis.

Liquid-liquid extraction of crude extract

To further concentrate the active metabolites and improve the antimicrobial activity, the 70% ethanolic root extract of the plant was further fractionated by using a solvent partition in different polarities of solvents. Three types of organic solvents, namely hexane, chloroform, and ethyl acetate were selected to partition crude extract into individual fractions using the technique of liquid–liquid extraction.[17] The ethanolic crude extract for fractionation was suspended in 250 ml of ethanol and distilled water (ethanol: water at the ratio of 7:3) and then extracted with 100 ml n-hexane. The separatory funnel was shaken vigorously and the solution was left for phase separation after extraction. The organic phase (hexane soluble portion) was withdrawn and the remaining aqueous phase was extracted thrice with n-hexane (Figure 2). Similar fractionation process was also carried out to prepare chloroform and ethyl acetate fractions. The organic fraction of n-hexane, chloroform, and ethyl acetate were combined individually and air dried at room temperature and stored in a beaker closed with aluminium foil until used in bioactivity assay.

Figure 2 Liquid–liquid extraction of extract.

Phytochemical screening test

The 70% ethanolic root extract of *P.stellatum* was subjected to qualitative chemical screening tests for identification of various classes of secondary metabolites such as alkaloids, steroidal compounds, terpenoids, phenolic compounds, flavonoids, glycosides, quinines, cardiac glycosides, tannins and saponins employing standard procedures.

Test for terpenoids: 5 ml of extract was mixed with chloroform (2 ml) and 3 ml of sulfuric acid was added to form a layer. A reddish-brown coloration of the interface was an indication of terpenoids.[18,19]

Test for tannins: About 2 ml of the extract was stirred with 2 ml of distilled water and a few drops of 10 % $FeCl_3$ solution were added. The formation of a green precipitate was an indication of the presence of tannins.[19,20]

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Test for alkaloids: To a few milliliters of filtrate, 2-3 drops of Mayer's reagent (Potassium mercuric iodide solution) were added along the sides of the test tube. The formation of a white or creamy precipitate indicates the presence of alkaloids.[19,21,22]

Test for flavonoids: To 2 ml of extract, a few drops of sodium hydroxide solution were added to a test tube. The formation of an intense yellow color that became colorless with the addition of a few drops of dilute HCl indicated the presence of flavonoids.[19,22,23]

Test for saponins: About 0.5 g of extract was dissolved and 2 mL was taken to the test tube which was diluted with 2 ml of distilled water. Then this was vigorously shaken for about 3-4 minutes. A 1 cm persistent foam for 10 min was indicative of the presence of saponins.[19,24]

Test for phenols: About 2 ml of extract was taken, add few drops of 5% $FeCl_3$ solution, and observed for coloration. The formation of a bluish-black or dark green color indicates the presence of a phenol compound.[19,20]

Test for steroids: a red color was produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added to it, indicating the presence of steroids.[19,20,25]

Test for cardiac glycosides: To 2 ml of extract, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlaid with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.[19,26]

Test for glycosides: To 2 ml amount of extract was mixed with 1 ml water and was shaken well. Then aqueous solution of NaOH was added. Yellow color appeared which indicates the presence of glycosides.[19,23]

Test for Quinone: 2ml extracts were treated with concentrated HCl appearance of green coloration indicates the presence of Quinone.[19]

Standard antibiotics

Gentamicin was used as positive control for the antibacterial susceptibility test and clotrimazole was used as positive control for the antifungal activity test; whereas DMSO serves as a negative control in both tests.

Bacterial and fungal strains

The tests were carried out against two Gram-positive bacterial strains (*Staphylococcus aureus, Bacillus cereus*), two Gram-negative bacterial strains (*Escherichia coli, Salmonella typhi*) and one fungal strain (*Candida albicans*). All the standard bacterial and fungal strains were obtained from Microbiology laboratory, biology department, at Jimma University (JU).

Antimicrobial activity tests

Antibacterial activity test

Well agar diffusion method was used to evaluate the antibacterial activity of the *P.stellatum* root extracts. Muller Hinton Agar (MHA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. Wells (6mm diameter and about 2 cm apart with 4mm depth) were made in each of the plates using a sterile cork borer. Stock solution of crude plant extract and fractionated extracts were prepared at a concentration of 500mg/ml in different beakers by DMSO. About 100 µl of different concentrations of plant solvent extracts were added by micropipette into the wells and allowed to diffuse at room temperature for 2 hours. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zone (ZI) was measured using a transparent ruler and results were reported in millimeters (mm).

Antifungal activity test

Antifungal activity test was assayed against *Candida albicans* strain using disc diffusion method. Potato dextrose agar (PDA) medium was filled in petri dishes (diameter 90 mm) and activated for 24 hours. After that, the fungal strain was seeded on a plate by using a sterile cotton swab. The surface of the medium was allowed to dry for about 3 min. Whatman No.1 filter paper discs (6 mm in diameter) were impregnated with 100 µl of different test extracts (500mg/ml) and then placed with the help of sterile forceps on the media on the surface of these agar plates. Clotrimazole (positive control) and DMSO (negative control) were also applied to the disc simultaneously. The plates were then incubated at 28ºC for 48 hours. After the incubation period, the antifungal activity was evaluated by measuring the inhibition zones and data were recorded.

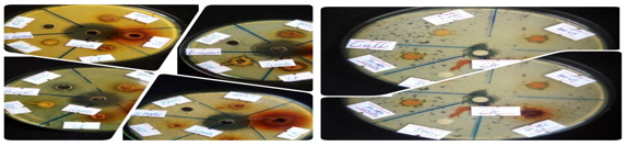

Figure 3 Antibacterial and antifungal activity tests.

Results

Phytochemical screening

As shown in Table 1, the phytochemical screening of the 70% ethanolic root extract of *P.stellatum* revealed the presence of secondary metabolites such as tannins, saponins, flavonoids, phenols, terpenoids, and glycosides while alkaloid, quinine, and cardiac glycosides were absent (Table 1). The pharmacological activity of a plant can be predicted by the identification of the phytochemicals.

Table 1 Result of phytochemical screening test

Phytoconstituents	Results
Terpenoids	+
Tannins	+
Alkaloids	–
Flavonoids	+
Saponins	+
Phenols	+
Steroids	–
Cardiac glycosides	–
Glycosides	+
Quinine	–

Key: (+), positive; (–), negative

Antimicrobial activity Test

Anti-bacterial activity test

The antibacterial activity test of *P.stellatum* root extract was done for four bacterial strains which were evaluated based on the diameter of the clear inhibition zone in millimeters by well agar diffusion method (Figure 3). The below table shows the antibacterial and antifungal activities of the 70 % ethanolic crude extract, hexane fraction, chloroform fraction, and ethyl acetate fraction (Table 2).

Table 2 Antimicrobial activity test of crude extract and fractionated solvent extracts

Test samples and standards	Zone of inhibition (mm)				
	Bacterial strains			Fungal Strain	
	S. aureus (+)	*B. cereus* (+)	*E. coli* (-)	*S. typhi* (-)	*C. albicans*
Ethanol extract	7	14	8	NI	10
Hexane fraction	11	11	NI	NI	7
Chloroform fraction	12	17	7	NI	10
Ethyl acetate fraction	10	20	10	NI	11
Gentamicin	28	28	25	27	NT
Clotrimazole	NT	NT	NT	NT	13
DMSO	NI	NI	NI	NI	NI

Key: NI, no inhibition; NT, not tested; (+), gram-positive; (-), gram-negative bacteria

Antifungal activity test

The antifungal activity test of *P. stellatum* root extract was done for one fungal strain which was evaluated based on the diameter of the inhibition zone by disc diffusion method. The above table shows the antifungal activity test result of the 70 % ethanolic crude extract and the three fractions (hexane, chloroform, and ethyl acetate) (Table 2 and Figure 3). Except hexane fraction, the other test samples displayed promising antifungal activities against *Candida albicans* with inhibition zones ranging between 7mm and 11mm while the standard drug revealed an inhibition zone of 13 mm.

Discussion

The phytochemical analysis is very important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants. Further, it provides the base for targeted isolation of compounds and to perform more precise investigations.[19] The phytochemical screening test of the ethanolic root extract of *Pterolobium stellatum* revealed the presence of tannins, phenols, flavonoids, saponins, glycosides, and terpenoids. The results are in line with previous studies.[11] These secondary plant metabolites have versatile medicinal properties, which may be a reason for the use of the plant material for different health problems traditionally. They are known to be biologically active and play significant roles in the bioactivity of medicinal plants because the medicinal values lie in these phytochemical compounds which produce a definite and specific action on the human body.[27] The results of the phytochemical test on this plant are in good agreement with other studies conducted on the leaf part of the plant.[28] Slight variation in phytoconstituents could be due to environmental factors, area of sample collection, solvent used, and different methods. Also, the research done on the root of *P.stellatum* revealed the presence of terpenoids, saponins, and tannins in 80% methanol extract[11] which was the same as with that of the present study except for the absence of flavonoids.

Phytochemicals exert antimicrobial activity through different mechanisms. For instance, flavonoids exhibit a wide range of biological activities which include antimicrobial, anti-inflammatory, anti-angiogenic, analgesic, anti-allergic, cytostatic, and antioxidant properties. Flavonoids in the human diet may reduce the risk of various cancers. The antibacterial activity of flavonoids has been reported to be a result of their ability to form complexes with bacterial cell walls, extracellular and soluble proteins.[29] Flavonoids and tannins are phenolic compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, there might be a great potential for potent antioxidant capacity.[30] Similarly, phenolic compounds have been accompanied by antimicrobials.[31]

Tannins have amazing astringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes.[30] They act by iron deprivation, hydrogen bonding, or specific interaction with proteins such as enzymes, cell envelopes, and complex formation with polysaccharides. Herbs that have tannins as their component are astringent and are used for treating intestinal disorders such as diarrhea and dysentery thus exhibiting antimicrobial activity.[32]

Saponins are known to produce inhibitory effects on inflammation. They have also been reported to possess antibacterial properties with their mode of action attributed to their ability to cause leakage of proteins and certain enzymes from bacterial cells.[21]

In this study, the antibacterial activity of root crude extract and fractions of *P. Stellatum* was conducted. The inhibition zone displayed by the crude extract and fractions of the root of the studied plant is depicted in Figure 3 above. The result indicates that all the test samples showed better antimicrobial activity toward the Gram-positive bacteria as compared to Gram-negative bacteria. None of the test samples showed antibacterial activity against the Gram-negative bacterium, *Salmonella typhi*. Hence, *Salmonella typhi* was the most resistant strain whereas *Staphylococcus aureus* and *Bacillus cereus* were the most susceptible bacterial strains. Gram-negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphipathic compounds and multidrug resistance pumps that extrude toxins across this barrier.[33]

The extract and fractions demonstrated varying degrees of antimicrobial effect against the test strains. The difference in antibacterial activity between crude extract and fractions may be due to different compounds from the plant material getting extracted in solvents of different polarities and differences in bacterial strain.[34,35] The potential antimicrobial properties of plants are related to their ability to synthesize several secondary metabolites of relatively complex structures possessing antimicrobial activities.[36] Thus, the antibacterial effect of this plant could be attributed to the presence of secondary metabolites like tannins, phenols, flavonoids saponins, terpenoids, and glycosides that are detected during phytochemical screening tests. These constituents have been reported to be associated with different pharmacological activities.[37]

The ethanolic crude extract showed activity against *S.aureus, B.cereus*, and *E.coli* with inhibition zones of 7 mm, 14 mm, and 8 mm, respectively. The hexane extract exhibited moderate activity against Gram-positive bacteria. The ethyl acetate exhibited relatively better activity against gram-positive bacteria (*Bacillus cereus*) with a maximum inhibition zone of 20 mm. Likewise, the chloroform fraction also revealed promising activity against *S.aureus* (12 mm),

and B.cereus (17 mm). Previous study done on *P. stellatum* revealed that the 80% methanolic root extract was active against different bacterial species.[11] Similarly, the result of ethanolic crude extract was in good agreement with previous reports stated by Tilahun Yet al.,[34] in which the ethanolic leaves extract of the plant displayed the highest antibacterial activity against *Staphylococcus aureus, Escherichia coli, Shigella species, Pseudomonas species, Salmonella species,* and *Streptococcus pyogenes*.

Regarding antifungal activity assessment, three test samples showed better activity towards *Candida albicans* namely; ethanolic crude (10 mm), chloroform fraction (10 mm), and ethyl acetate fraction (11 mm) whilst the standard drug, clotrimazole revealed an inhibition zone of 13 mm. However, the hexane fraction displayed the lowest antifungal activity (7 mm). To the best of our knowledge, there were no studies done on antifungal activity of this plant. In general, the result of the antimicrobial activity of this study was appreciable.

Conclusion

The present study attempted to undertake phytochemical screening and antimicrobial activity of crude extract and fractions. Qualitative phytochemical analysis of ethanolic root extract of *P. stellatum* showed the presence of tannins, phenols, flavonoids, saponins, terpenoids, and glycosides. The ethanolic root extract and fractions have shown significant antibacterial activity against Gram-positive bacteria with ethyl acetate fraction displaying the highest antibacterial and antifungal activities against *Bacillus cereus* (20 mm) and *Candida albicans* (11 mm) while no inhibitory effect on *Salmonella typhi* was observed. It can also be concluded that the partitioning process enhances the antimicrobial efficiency of the crude extract. The results of the study suggest that the root of the plant contains bioactive constituents and affords acceptance to the ethnomedicinal use of the plant. Therefore, the results of this study validate the traditional usage of the studied plant for the treatment of various diseases. Further studies are needed with this plant to isolate and characterize the bioactive compounds responsible for the displayed antimicrobial activity and additional bioassay tests are required by using various types of bacterial and fungal strains.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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