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Evaluation of Phytochemical and Antimicrobial activity of *Premna integrifolia* leaf extract

[1] Jayashri B Uppin, [*2] Gajanana R Naik
[1] Department of PG Studies and Research in Biotechnology, Gulbarga University, Kalaburgi, Karnataka, India
[2] Professor, Dept. of Biotechnology, Gulbarga University, Kalaburgi, Karnataka, India

Abstract
The present study was carried out to illustrate the relative evaluation of phytochemical constituents and antimicrobial activity of *Premna integrifolia* leaves with ethanol, methanol and chloroform extract. A preliminary study of Phytochemical analysis was carried out. Obtained results reveal that the presence of alkaloids, tannins, flavonoids and glycosides in these leaves extract. The antimicrobial activity was tested using disc diffusion method against Styphylococcus. Aureus, *Escheria.coli*, *Bacillus* subtilis, *Bacillus pumilis, Lactococcus lactis, Klebiella pnemoniae, Proteus mirabilis, Salmonella typhi,* Methicillin resistant *S.aureus, and* fungal cultures like *Candida albicans, Aspegillus niger* using Tetracycline as antibiotic standard. The outcome showed that the antimicrobial activity were effective in plant extract. From the current investigation, it can be concluded that *Premna integrifolia* have significant towards antimicrobial activity and potent Phytochemical constituents.

Keywords: *Premna integrifolia,* phytochemical constituents, disc diffusion, antimicrobial activity

1. Introduction
Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and large number of diverse types of plants grows in different parts of the country. The use of medicinal plants and plant products could be traced as far back as beginning of human civilization. Herbal medicine is a still mainstay of about 75-80% of the whole population and major part of traditional therapy involves the use of plant extract and their active constituents [1] Among medicinal plant of Verbenaceae Family *Premna serratifolia linn Premna integrifolia,* otherwise known as "Agnimanth" in ayurvedic system of medicine, is a small sized tree or large shrub up to 9 meter in height with comparatively short trunk and numerous branches lantoceolate bark, spinous large branches and yellowish brown woody aromatic roots, leaves simple, opposite sometime whorled elliptic-ovate, membranous, primary lateral nerves 4-6 pairs, flowers are small [2].
In order to overcome antimicrobial resistance issue, it is crucial to discover new antimicrobial agents in the pharmaceutical pipeline in order to replace currently available antimicrobials. Now a day's multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This result in the need of higher dose use with increased risk of drug toxicity or consideration to change the regimen. In addition to this problem, antibiotics are sometime associated with adverse effect on the host including hypersensitivity and allergic reactions, because of this reason it is necessary to develop alternative drugs for the treatment of infectious disease [3] Many herbs are used as antimicrobial agents which inhibit the growth of microorganism by unknown mechanism other than that of known antibiotics [4]. The present study includes phytochemical analysis and antimicrobial activity of *Premna integrifolia*. This plant is used as traditional medicinal plant because of their anticancer, antimicrobial, anti-inflammatory and hepatoprotective properties [5-7]. The present study was performed to evaluate Antimicrobial activity and phytochemical analysis of ethanol, methanol and chloroform extract of leaf *of Premna integrifolia* against some pathogenic microorganisms. From the above results it was show that methanol extract exhibit promising activity towards pathogenic organisms.

2. Materials and methods
2.1 Plant collection
The Leaves of *P.integrifolia* were procured from Botanical Garden of Basaveshwar Science College, Bagalkot. Karnatak, India The leaves were identified and authenticated by Dr. S.A. Kappali Associate Professor, Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka-India. The plant material wash under running tap water; air dried and then homogenized to fine powder.The powder was stored in airtight container at -20°C for further studies.

2.2 Preparation of extracts
About 100 g dried leaves were coarsely powdered and subjected to successive extraction by Soxhlet extractor. The extraction was done with 99% Ethanol; methanol and chloroform. Each time the plant material was dried and later extracted with solvents. The extracts were concentrated by rotary vacuum evaporator and evaporated to dryness.

2.3 Phytochemical screening
The extracts were analyzed for the presence of phenolic compounds, flavonoids, terpenoids, saponins, alkaloids, cardiac glycosides and protein [8].

2.3.1 Ferric chloride test
Each extract (50 mg) was dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. Bluish black colour indicated the presence of phenolic compounds.

2.3.2 Alkaline reagent test
Few drops of sodium hydroxide were added into the extracts to give intense yellow colour. The disappearance of colour after addition of dilute hydrochloride acid showed the presence of flavonoid.

2.3.3 Salkowski's test
The extract (0.5 mg) was added with few ml of chloroform followed by concentrated sulphuric acid to form a layer. Reddish brown colour at the interface indicated the presence of Terpenoids.

2.3.4 Froth test
Each extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. The development of two cm layer of foam indicated the presence of Saponins.

2.3.5 Wagner's test
About 50 mg of extracts was stirred with few ml of dilute hydrochloric acid and filtered. Then, few drops of Wagner's reagent were added at the side of the test tube. The formation of reddish-brown precipitate showed the presence of alkaloids.

2.3.6 Keller-Kiliani's test
A small amount of extract (50 mg) was treated with 2 ml of glacial acetic acid containing one drop of 5% anhydrous ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid, a brown ring at interface is characteristic of cardinal dedeoxysugar. The appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides.

2.3.7 Biuret test
Each extract (50 mg) was diluted with distilled water and treated with Biuret reagent. The appearance of pink colour indicated the presence of protein.

2.4 Assay for antimicrobial activity
2.4.1 Disc Diffusion Method
The antimicrobial assay was performed by disc diffusion technique (9-10). Disc diffusion technique is highly effective for rapidly growing microorganisms. The sample solution of the material to be tested was prepared by dissolving a definite amount of material in appropriate solvent to attain a concentration of 50mg/ml. 10 µl of such solution was applied on sterile disc (5mm diameter, filter paper) and allowed to dry off the solvent in an aseptic hood. Thus, such discs contain 500µg of crude extracts Standard antibiotic discs (Tetracycline 30µg $disc^{-1}$) and blank discs (impregnated with solvents) were used as a positive and negative control.). In this study the test discs and standard disc were placed in a Petri dish seeded with particular bacteria then left in a refrigerator at 4°C for 12-18 hrs in order to diffuse the material from the discs to the surrounds media in the Petri dishes. The Petri dishes were then incubated at 37°C for overnight to allow them for bacterial growth. The antibacterial activity of ethanol, methanol and chloroform extracts of leaves of *Premna integrifolia* were then determined by measuring the respective zone of inhibition in mm.

3. Result and Discussion
In the present study, *Premna.integrifolia* leaves with methanol, ethanol and chloroform extract were examined for their phytochemical screening, antimicrobial activity by disc diffusion method against some pathogenic microorganisms.

3.1 Phytochemical analysis
The phytochemical screening of ethanol,methanol and chloroform extract of *Premna.integrifolia* revealed the presence of flavanoids, alkaloids, saponins, glycosoids, phenol and tannins.which was shown in Table 1.

3.2 Assay for antimicrobial activity
The important task of this study is to screen medicinal plants with recognized antibiotic properties. The plants were initially screened for their antimicrobial activity against selected pathogenic microorganisms. The results of zone of inhibitions are presented in Table 2. Crude extracts the Leaves of *Premna integrifolia* showed moderate to good antimicrobial activity against all the tested pathogenic microorganisms.The variations in the sensitivity could also be attributed to the differences in growth rate of the tested organisms, nutritional requirements, temperature and inoculum's size
Based on the present study, we can consider the leaves of *Premna integrifolia* understudied plants to be good sources of antimicrobial activity. The bioactive compounds on the medicinal plants employed contain various secondary metabolites such as phenols, tannins, alkaloids, flavonoids, steroids and glycosides in appreciable quantities. The important task of this study is to screen medicinal plants with recognized antibiotic properties. The plants were initially screened for their antibacterial activity against selected pathogenic bacteria. The results of zone of inhibitions are presented in Table 2. Crude extracts the Leaves of *Premna integrifolia* showed moderate to good antibacterial activity against all the tested pathogenic microorganisms. The highest zone of inhibition was observed for methanol extract of *Premna.integrifolia* show significant activity against Bacillus subtilis and *Klebiella pnemoniae* (19mm) followed by ethanol extract shows good result against salmonella typhi (18mm), chloroform extract shows moderate activity against candida albicans and aspergillus niger(16mm)
The presence and the phytochemical components of the studied plants, the inhibitory zones at which values were effective on the tested organisms, highlights that there were variations in the antimicrobial potency of the plants extracts. The variations in the sensitivity could also be attributed to the differences in growth rate of the tested organisms, nutritional requirements, temperature and inoculum's size [11].

Table 1: Phytochemicals constituents of *P.integrifolia* leaf extract

phytochemicals	Test	Ethanol extract	Methanol extract	Chloroform extract
Alkaloid	Iodine,Wagnore's,Dragandroff's	+, +, -	+, +, -	+, +, -
Flavonoids	Shinoda Test, NaOH Test	+, ++	++, ++	+, ++
Glycosides	K,K test, Conc. H2SO4 Test, Molisch Test	++, ++, ++	++, ++, ++	++, ++, ++
Phenols	Phenol Test	++	++	++
Lignins	Lignin Test	-	-	-
Saponins	Foam Test, Heamolysis Test	+, +	+, +	+, +
Sterols	Salkowaski Test	+	+	+
Tannins	Gelatin Test, Lead acetate Test	++, ++	++, ++	+, +
Anthraquinone	Bomtrager's test	-	-	-
Reducing sugar	Reducing Sugar test	-	-	-

Table 2: antimicrobial activity of *P.integrifolia* leaf extract

Name of the microorganisms	Zone of inhibition in mm			
	PIE	PIM	PIC	STD
Styphylococcus.aureus	17	17	14	19
Escheria.coli	16	18	18	16
Bacillus subtilis	14	19	15	19
Bacillus pumilis	16	18	17	22
Lactococcus lactis	15	18	16	21
Klebsiella pnemoniae	17	19	15	22
Proteus mirabilis	14	16	16	21
Salmonella typhi	18	18	14	20
Methicillin resistant S.aureus,	16	17	14	19

4. Conclusion

We demonstrated that, in the present studies, the investigation of above results reveals the leaves of *Premna integrifolia* are good sources of antibacterial property

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7. Conflict of Interest

We declare that no conflict of interest

8. References

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