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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING, QUANTITATIVE ESTIMATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF ALSTONIAMACROPHYLLA STEM BARK

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ABSTRACT

The phytochemical analysis for the bark of the *Alstoniamacrophylla*has been studied in this work.*Alstoniamacrophylla* is an Indian plant which is used in Ayurveda for treatment of many diseases. Especially the bark of the plant used for treating skin disorders, malarial fever, urticaria, chronic dysentery, diarrhoea, in snake bite and for upper purification process of Panchakarma. Antimicrobial property of the different extracts (Acetone, Methanol and water) has been studied for various gram positive and gram negative bacteria (*Staphylococcus aureus, Pseudomonas aurisinosa, Clostridium vivax, Micrococcus varians, Streoptococci, Bacillus subtilis*). All bark extracts has shown the antimicrobial property against all tested bacterial strains and it reveals that the bark of the plant has rich with the antioxidants. The presence of alkaloids, saponins, steroids, flavonoids and phenolic compounds has been identified and quantitatively these compounds are estimated by spectrophotometry. This study supports the Ayurvedic use of bark of the *Alstoniamacrophylla*for treatment of various diseases caused by bacteria.

Keywords: Alstoniamacrophylla, Spectrophotometer, anti-microbial activity, anti-oxidants.

INTRODUCTION

Plants and herbal extracts have formed important position in modern medicine, due to their chemical and medicinal contents found in natural from. Their secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities. Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global health problem. This compelled the scientists to search out new drugs from plant origin (Khoobchandani et al.,2010). Plant derived antimicrobial compounds might inhibit bacteria through different mechanisms and provide clinical values for the treatment of infections caused by resistant microbes (Stein et al., 2005). Neman et al., (2003) reported that majority of antibacterial drugs in clinical uses are from natural origin. There is a need to evaluate the herbs (1352, Muhammad gulfraz et al) scientifically for their antimicrobial activity against the antibiotic-resistant microorganism in order to develop new drug from plant origin (Simoes et al., 2009). The vast majorities of antibiotics used today are produced by microorganisms, yeasts or fungi, which belong to the vegetable kingdom. Higher plants mainly produce antimicrobial compounds for their defense mechanism against infections constituting cellular metabolism.

Alstonia macrophylla is a member of Apocyanaceae family. It occurs in a variety of habitats but is most often found in India, Afghanisthan .The common name of this tree is devil tree,Batino.

Classification:

Kingdom: Plantae

Subkingdom: Tracheobianta

Super Division: Spermatophyte

Division: Magnoliophyta

Class: Magnoliopsida

Sub Class: Astride

Order: Gentian ales

Family: Apocyanaceae.

Common name: Devil Tree, Batino

Figure 1: Plant and bark of Alstonia macrophylla

Alstonia macrophylla is a medium-sized tree, growing up to 20 meters high. Bark is smooth. Branches are 4-angled. Leaves are in whorls of three, oblong-obviate, 10 to 30 centimeters long, 5 to 7 centimeters wide, and short-stalked. Flowers are small, yellowish-white, borne on short, terminal cymes. Calyx is small. Corolla is tubular, 1 to 1.5 centimeters long, lobed towards the top. Fruit is a double follicle, pendant, long and slender, 20 to 40 centimeters long. Seeds are small and very flat, with deep-brown hairs, especially along the edges.

Due to its medicinal value different parts of the Alstonia macrophylla has been studied and they exhibit different activities like Antimicrobial activity, CNS Depressant Activity, Cytotoxic activity, Antipyretic Activity, Anti-inflammatory Activity, Sperm-motility Inhibition Activity, Antiplasmodial / Vasorelaxant Activity, Antibacterial, Chemomodulatory Activity and Antiprotozoal Activity.

MATERIALS AND METHODS

Instrumentation:

soxhlet apparatus is used for the extraction of phytoconstituents from the plant powder. TECHCOMP Double beam UV-Visible Spectrophotometer with Hitachi software, standard Quartz cuvetts with lid is used for measuring the absorbance. All the chemicals and reagents used were LR grade and were purchased from Merk chemicals PVT LTD, Mumbai.

Tested bacterial strains:

Staphylococcus aureus, Pseudomonas aurisinosa, Clostridium vivax, Micrococcus varians, Streoptococci, Bacillus subtilis.

Collection of plant material:

The plant Alstoniamacrophylla was collected from forest nearer to Maredumilli, East Godavari, AP, India. The bark is peeled out from the tree by making incisions, the collected bark is dried in shady conditions, the dried bark is taken and powered, the powdered bark is then stored in the suitable conditions (air tight, light resistant containers).

Extraction procedure:

The powdered material is weighed in a selected quantity and is subjected to soxhelt extraction using, Acetone, Methanol and Water in successive mode respectively for 48 hrs. The solvent was recovered using Rotary Vacuum Evaporator and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of Phytochemical screening, and Anti-microbial Evaluation.

Preliminary phytochemical screening:

The extracts of the dry powdered leaves of Alstoniamacrophyllawere analyzed for the presence of various phytoconstituents like carbohydrates, reducing sugars, monosaccharide (Evans, 1996), Tannins

(Evans, 1996), Saponnins (Evans, 1996) Flavonoids, Terpenes /steroids , Alkaloids(Evans, 1996), Anthraquinones, cardiac glucosides and amino acids were identified using standard phytochemical procedures.

Quantitative Estimation of Alkaloids:

To 1ml of Methanolic extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

Quantitative Estimation of flavanoids:

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Quantitative Estimation of Saponins:

Methanolic and water extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60^0c for 10min, absorbance was measured at 544nm against reagent blank. Diosgeninis used as a standard material and compared the assay withDiosgeninequivalents.

Quantitative Estimation of Steroids:

1ml of Methanolic extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Quantitative Estimation of Phenoilc Compounds:

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR)**.** In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of

milligrams of catechol per gram of dry weight and the standard graph was shown in figure 1 and results are shown in table B.

Antimicrobial activity by disc plate method:

Authentic pure cultures of bacteria namely Bacillus subtilis, Bacillus(+ve), bacillus(_ve), streptococci (+ve), mono cocci (+ve), staphylococcus auriusare obtained from department of Microbiology, Lydia college of Pharmacy.

Antimicrobial activity of different solvent extracts was determined by disc diffusion method on nutrient agar medium. Seeded broth containing test organism was inoculated on plates of solidified nutrient agar and spread uniformly. Filter paper discs were prepared with a diameter of 3mm. In every plate Acetone, methanolic and water extracts containing discs are placed. The treatments also included filter paper discs immersed in sterilized distilled water, Acetone and Methanol separately which served as control. oneanti bacterial tablet were purchased from local market and concentrations were prepared same as extract at their respective recommended dosage were tested for comparative efficacy. The plates were incubated for 24 h. at 37°C and zone of inhibition around the wells were measured in mm (millimeter). For each treatment six replicates were maintained. The data was subjected to statistical analysis; results can be shown in table C.

RESULTS AND DISCUSSION

Preliminary phytochemical screening results reveals that the bark extract of Alstoniamacrophyllashow many types of chemical constituents. Among different solvents used for extraction in a series, methanolic extract showed +ve results for many numbers of chemical compounds. It contains Steroids, Saponins, Alkaloids, Carbohydrates, flavonoids and phenolic compounds. The summery results for the preliminary phytochemical screening of different solvent extracts of Alstoniamacrophylla are shown in the table 1.

S.No	Sec. Metabolites	Test names	Acetone	Methanol	Water
1.	STEROIDS:	A)Salkowski test B)Lieberman-Buchard's Test:	-ve	+v e	-ve
2.	Triterpenes:	A)Salkowski Test: B)Lieberman-Buchard's Test: C)Ischugajiu Test: D)Brickorn and Brinar Test	+v e	-ve	+v e
3.	Saponins	A)Foam test: B)Haemolysis test	-ve	+v e	+v e
4.	Alkaloids:	A)Mayer's test: B)Dragendroff's Test: C)Wanger'sTes: D) Hager's Test	-ve	+v e	-ve
5.	Carbohydrates	A)Fehling's Test: B)Molisch's Test: C)Barfoed's Test: D)Benedict's Test	+v e	+v e	+v e
6.	Flavonoids:	A) Ferric chloride test B) Lead acetate test	-ve	+ve	+ve
7	Phenolic compounds	A) Ferric cyanide test B) Gelatin test	-ve	+v e	-ve

Table 1: Phytochemical screening for extracts of bark

S.NO	Chemical constituent	Standard	Methanolic extract	Water extract
1	Alkaloids	Atropine	42.69µg	--------
2	Saponin	Diosgenin	114.05µg	30.03 µg/
3	phenolic compound	Catechol	68.42µg	71.52µg
4	Steroids	Cycloartenol	111.51µg	------
5	Flavanoids	Catechin	126.05µg	95.63µg

Table 2: Quantitative estimation of phytoconstituents

Antimicrobial activity studies confirm that different solvent extract of Alstoniamacrophylla show high inhibitory effect on the growth of different pathogenic microorganisms under the study. Results were shown in table 2. All the plant extracts show inhibition activity for the growth of microorganisms under study. Among the extracts methanolic extract show more effect on the growth of the microorganisms then compared to other extracts. Water extract show comparatively least effect on the growth of the microorganisms. The presence of large amount of phytochemical in different solvent extracts of Alstoniamacrophylla are may be reason for antimicrobial activity.

S.NO	Name of the Organism	Inhibition zone in mm			
		Acetone	Methanol	Water	Standard
1	Staphylococcus aureus	10.32	14.29	10.68	12.96
2	Pseudomonas aurisnosa	11.96	15.24	12.92	15.63
3	Clostridium vivax	10.98	13.79	9.69	12.97
4	Micrococcus varians	11.85	14.57	9.86	15.35
5	Streptococci	10.87	12.39	10.14	11.15
6	Bacillus subtilis	8.96	15.38	11.92	12.34

Table 3: Inhibition zone on the microorganisms of different solvent extracts

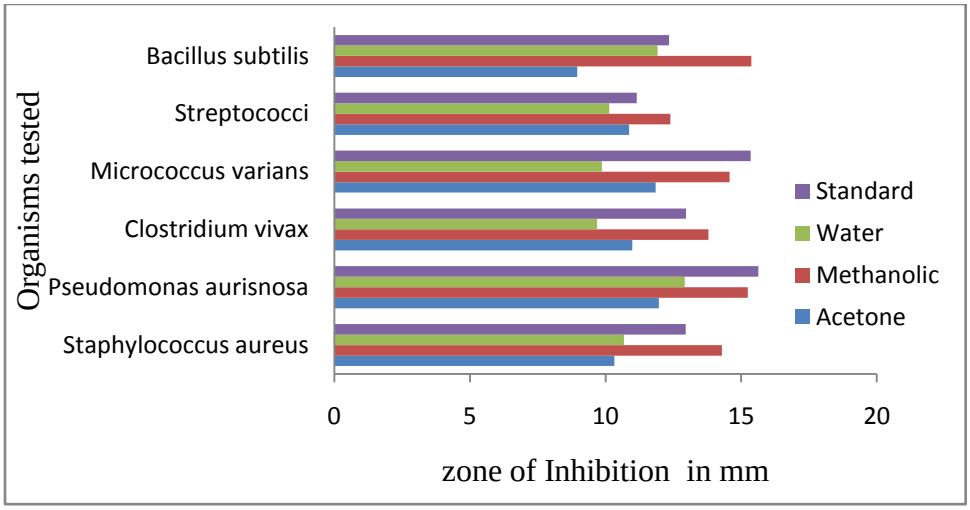
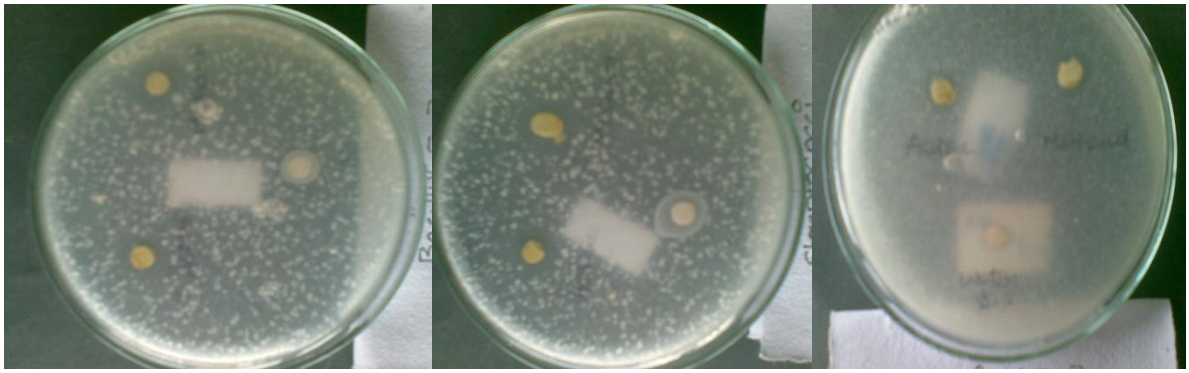
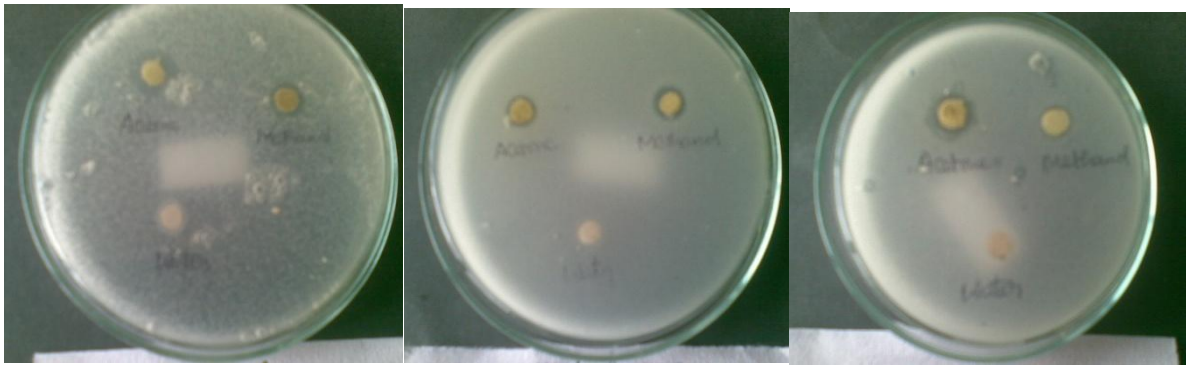


Figure 2: Comparative antimicrobial activities



Pseudomonas aurisinosaClostridium vivaxStreoptococci



Staphylococcus aureus Bacillus subtilisMicrococcus varians

CONCLUSION

Results of antimicrobial tests against different bacteria, determination and estimation of antioxidents studies has reveals that bark of Alstoniamacrophylla has possess different antioxidents like alkaloids, saponins, steroids, phenolic compounds and they have activity against different bacterial strains. This study supports the Ayurvedic use of bark of the *Alstoniamacrophylla* for treatment of various diseases caused by bacteria.

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