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Research Article

Physico-Phyto and Chromato Graphic Analysis of *Aviccinia alba* and *Laguncularia racemosa* Leaves

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ABSTRACT

To investigate the physicochemical analysis, preliminary phytochemicals screening, along with TLC analysis and anti-bacterial activities of mangrove plants leaves of *Aviccinia alba* and *Laguncularia racemosa*. *A. alba* crude drug were showed the Physicochemical characteristic such as; extractive value (5.00%), water soluble extractive value (7.20%), loss on drying weight (12.43%), total ash value (14.00%), acid insoluble ash value (3.10%) and water soluble extractive value (3.90%). Similarly, physicochemical values in *L. racemosa* 8.50% extractive value, 6.45 % water soluble extractive value, 8.45 % of loss on drying weight, 16.22% total ash value, 3.40 % acid insoluble ash value and 3.40 % water soluble extractive values. Phytochemical analysis of methanol extracts of *A. alba* (AME) and *L. racemosa* (LME) revealed the presence high amount of tannins and phenolic compounds. TLC analysis revealed five clear distinct bands or spots by AME showed with their Rf values range from 0.2 to 0.75. Whereas LME showed seven unclear compound spots with varying Rf values range from 0.2 to 0.80. Methanol extracts of both plants were strongly inhibited against with *Staphylococcus aureus, Streptococcus pyogenes* as gram negative bacteria and a much broader spectrum of action than *Pseudomonas aeruginosa* and *E. coli* as gram positive bacteria. These studies will help in setting down Pharmacopoeia standardisation and determining the quality and purity of bioactive compounds. Further investigation is being advocated for the identification of lead molecule with pharmacological significance.

Keywords: *Aviccinia alba, Laguncularia racemosa*, Physicochemical parameters, Extractive value, Ash value, TLC

INTRODUCTION

Plant based metabolites are widely used in traditional practices around the world. Plants are rich source of secondary metabolites which has been used for the treatment of various diseases for ages[1]. Like any other plant communities, the mangrove plants have also been reported for their traditional uses[2]. Mangrove plants are the unique plant communities inhabiting the estuarine and intertidal regions of both tropical and subtropical coasts regions. These are salt tolerant plant communities comprising of trees, herbs, shrubs and grass[3]. Mangrove plant inhabit an extremely challenging environmental abiotic stress condition enumerated by high salinity, water logging condition, high and low tides of water, high temperature, low oxygen, low nutrition, muddy anaerobic soil and strong wind conditions where other plants cannot grow. Along with these biotic stress factor the insects, microorganisms and other anthropological condition also contribute a large in developing the biotic stress to these unique plant community[4]. However, these mangrove plants adapted well to these ecological undesired condition by the synthesis of novel chemical compounds that offer protection to these plants against various biotic and abiotic stresses mentioned above. A number of these phytocompounds have significant pharmacological properties and being used traditionally for treatment of number of ailments[5]. For centuries mangroves have been used for food, feed and medicinal purpose in different parts of the world. They are well known to produce natural metabolites with diverse biological activities[6]. The present investigation was focused on *Aviccinia alba* and *Laguncularia racemosa* for their physicochemical analysis, phytochemicals constituents, antimicrobial activities and TLC analysis in order to search for possible identification of bioactive compounds.

MATERIALS AND METHODS

Plants collection and identification

Fresh leaves of *Aviccinia alba* and *Laguncularia racemosa* were collected separately in the month of September, 2014 from Nizampatnam, Guntur district, A.P, India. The taxonomic identification of the plants was confirmed by the Department of Botany, Acharya Nagarjuna University, Guntur.

Preparation and extraction of the plants material

Leaves of *A. alba* and *L. racemosa* were dried at room temperature and crushed to fine powder in a mechanical grinder, weighed and stored in air tight container for further analysis. The crude drug material was used for the analysis of physicochemical properties and extraction. 15 grams of powdered material was macerated separately with 150 ml of methanol (polar solvents) and stored in airtight conical flask for 48 hours. Plant extract was filtered through double layered muslin cloth and sterile Whatman

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No1filter paper. Extraction was carried at 40-60ºC in soxhlet apparatus and then concentrated under reduced pressure by using rotary evaporator. Finally, all the extracts were stored at 4 ºC used for further study of physico-phyto-chemical analysis.

Determination of extractive value

An accurately weiged 5gm of the air-dried coarse leaves powder of *A. alba* and *L. racemosa* were macerated with 100 ml of methanol in a closed flask for 24 hours with shaking frequently during the first 6 hours and allowed to stand for 18 hours. There after it was filtered rapidly. 25ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, dried at 105˚C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the shade-dried pants powder. The percentage of methanol soluble extractive value was calculated with reference to the shade-dried pants powder[7].

Determination of water soluble extractive value

An accurately weight 5gm of the air-dried coarse leaves powder of *A. alba* and *L. racemosa* were macerated with 100 ml of water in a closed flask for 24 hours with shaking frequently during the first 6 hours and allowed to stand for 18 hours. There after it was filtered rapidly. 25ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, dried at 105˚C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the shade-dried pants powder[8].

Loss on drying weight

An accurately weighed quantity of the shade-dried coarsely powder drug of *A. alba* and *L. racemosa* leaves powders were taken in a tarred glass bottle. The crude drugs were heat at 105˚c in oven and weighed. The procedure was repeated till a constant weight obtained. The moisture content of the sample was calculated as percentage with reference to the shade-dried material[9].

Determination of total ash value

About 3 grams of air dried *A. alba* and *L. racemosa* leaf powder were taken separately and scatter on the bottom of the tarred platinum silica crucible dish. Incinerate by gradually increasing the temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed and repeated for constant value. The percentage of total ash was calculated with reference to air dried drug[10].

Determination of acid insoluble ash value

The acid insoluble ash value was obtained by *A. alba* and *L. racemosa* total ash. The total ash was boiled for 10 minutes with 25 ml of dilute HCl. The insoluble matters were collected an ashless filter paper than washed with hot water after ignited and weighed. The percentage of acid insoluble ash was collected with reference to air dried drug[11].

Determination of water soluble ash value

The water insoluble ash value was obtained by *A. alba* and *L. racemosa* total ash. The total ash was boiled for 10 minutes with 25ml of water. The insoluble matter was collected on ashless filter paper then washed with hot water after ignited and weighed. The percentage of acid insoluble ash was collected with reference to air dried drug[12].

Preliminary phytochemical screening

A portion of crude extracts of methanol of *A. alba* and *L. racemosa* were subjected to preliminary qualitative phytochemical screening for the presence of alkaloids, saponins, phytosterols, phenols, tannins, flavonoids, steroids, terpinoids and cardiac glycoside[13].

Thin layer chromatography analysis (TLC)

The TLC plates were trimmed to strips and the position of the origin marked by a straight line. The methanol extract was spotted on the origin and put in a lidded tank containing a solvent system. The procedure was repeated with in various solvent systems, hexane: methanol ratios 9:1, 9:0.5 and 9.8:0.2 was used for *A. alba* methanol extract and hexane: ethyl acetate, solvent systems ratios of 8:2, 9:0.5 and 9.5:0.5 for *L. racemosa* methanol extract, until good resolution was noticed. The resolution viewed under UV light[14].

Anti-bacterial activity

Antibacterial activities of both plants leaves methanol extracts were being tested separately against four human pathogens, *Streptococcus progenies, Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa* (Gram-positive and Gram-negative) by using agar well diffusion method. A stock solution of the extract was prepared by dissolving 100mg of each extract in 100ml of 1% (v/v) DMSO. 24 hours a broth bacterial culture was used for assay. Autoclaved Muller-Hinton Agar medium was cold then 1ml of bacterial suspension (10^6CFU/ML) was mixed within 5ml of medium. Wells were punched on the seeded plates using sterile borer (8 mm). The extract was dispensed into each well using sterile micropipette. Antibacterial activity was compared with standard streptomycin. The activities are determined by measuring the diameter of zone of inhibition[15].

RESULTS AND DISCUSSION

Physicochemical analysis

Physicochemicaly Standardisation of crude drugs plays a very important role in identifying the purity and quality of drugs. Crude drug contains therapeutically desired portion and complex mixture of many active secondary metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and saponnins, phenolics and phytosterols. Unwanted material present in the drug substance can be eliminated by using of selective solvent. Physicochemical Standardisation of crude drugs of *A. alba* and *L.racemosa* were reveals which includes methanol soluble extractive value, water soluble extractive value, loss on drying, total ash, acid insoluble ash, and water soluble ash were investigated and the results were presented (Tables 1, Figure 1).

Methanol extractive value

Alcohol soluble extracts are one of the tools for standardization of crude drugs. Here we used methanol for determination of alcohol soluble extracts values. Methanol extractive values of *A. alba* showed 5.00% and whereas *L. racemosa* showed 8.50 % respectively results showed in table (Tab 1). Alcohols are ideal solvents for extraction of various chemicals like tannins, resins. Therefore, extraction methods are frequently employed to determine

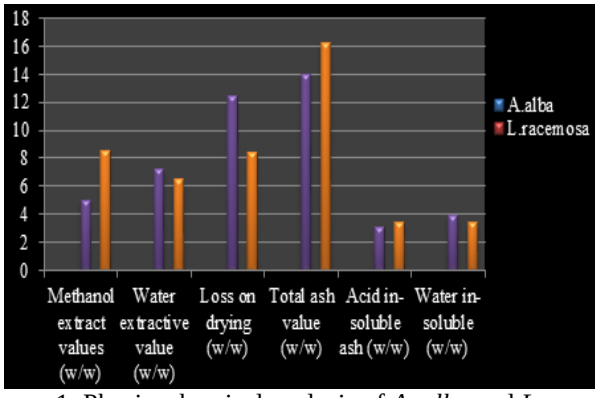

Figure 1: Physicochemical analysis of *A. alba* and *L. racemosa*

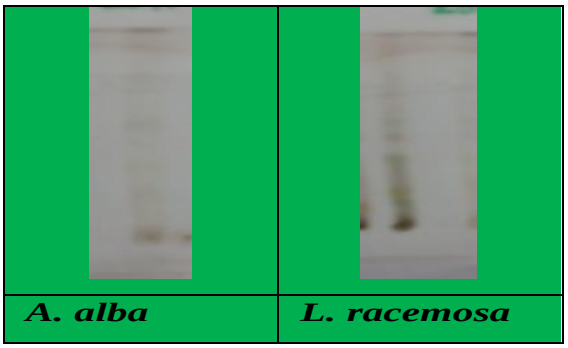

Figure 2: TLC analysis of methanol extracts of *A. alba* and *L. racemose.*

the approximate resin content of drug and useful further evaluation of a crude drug gives an idea about the nature of chemical constituents present in it and further useful for estimation of chemical constituents, soluble in that specific solvent used for extraction[16,17].

Water soluble extractive value

Determinations of water soluble extractive value are used for evaluating crude drugs which are not readily estimated by other means. *A. alba* and *L. racemosa* showed water extractive values 7.20% and 6.45% respectively. These extract values obtained by exhausting crude drugs are indicative of approximate measure of their chemical constituents. Extractive values applied to the drugs which contain water soluble active constituents such as tannins, sugars, plant acids, mucilage and glycosides. The water soluble extractive value can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the process of drying and storage[18-20]. The water soluble extractive value was indicating the presence of sugar, acids and inorganic compounds. Alcohol soluble extractive values indicated the presence of polar chemical constituents like phenols, alkaloids, steroids, glycosides and flavonoids. *A. alba* water extractive value of 7.20% showed that water permeates the cells of the aerial parts and thus, a better extractive compared to alcohol with extractive value of 5.0%. Whereas in *L. racemosa* water soluble extractive value is less 6.45% compared to alcohol soluble extractive value of 8.50% which shows that much water not permeates the cells of the aerial parts of *L. racemosa* compared to A. alba. These results clearly indicated that in *L. racemosa* percentage of extractive yield was high and highly soluble in alcohol than water.

Loss on drying weight

The loss on drying is the loss of weight in percentage from water and volatile matter of any kind that can be driven of under specified conditions. Percentage of weight loss on drying or moisture content was found to be 12.43% in *A. alba* and 8.45% in *L. racemosa*. The results indicate highest loss of drying weight identified in *A. alba* than *L. racemosa* (Figure 1).

Total ash value

The total ash method is designed to measure the total amount of material remaining after ignition, including physiological and non-physiological ash. Ash values are important quantitative standards and criteria to analyse the identity and purity of crude drugs especially in the powder form. Moreover, the total ash of a crude drug also reflects the intensity of care taken in drug preservation, and purity of crude drug. The total ash value content of *A. alba* and *L. racemosa* were 14.0% and 16.22% respectively.

Acid insoluble ash value

Acid insoluble ashes are a part of total ash and measure of the amount of silica present, especially as sand and siliceous earth. The percentage of acid insoluble ash values were 3.10% in *A. alba* and 3.40% in *L. racemosa* were showed. Acid insoluble ash value is frequently necessary to evaluate the purities of crude drug. This ash value indicates contamination with siliceous material. The comparison of this with the total ash value of the sample will differentiate between contaminating minerals and variations of the natural ash of the drug.

Water insoluble ash value

Water insoluble ash values are also a part of total ash. Water soluble ash value of A. alba is 3.90 % and 3.4 0 % in *L. racemosa* were showed respectively. More water in soluble ash value appears in A. alba, whereas L. racemosa less insoluble ash and high in acid insoluble ash.

Preliminary Phytochemical screening

Preliminary phytochemical analysis of methanol extract of *A. alba* (AME) and *L. racemosa* (LME) were revealed the presence important secondary metabolites. Viz. tannins, phenols, glycosides, flavonoids, and phytosterols. Both plant showed highest amount of tannins and phenolic compounds. Whereas alkaloids, saponins were absent, flavonoid and glycosides were trace amount in qualitative analysis. Results were showed in table 1.

Thin layer chromatography

TLC is one of the important tools detecting the adulteration for judging the quality of drugs. If the drug is adulterated there might be appearance of the other compounds present in adulterant, in turn may increase the number of spots. On the other hand, the exhausted or deteriorated drugs may lose the component and the number of spots appeared might be less. Five clear and distinct spots/ bands were visualized in hexane: methanol solvent system at ratio 9.8:0.2, in methanol extracts of *A. alba*. Seven bands were identified in *L. racemosa* is solvent system of hexane: ethyl acetate at ratio of 9.5:0.5. Whereas other two ratios of the

Table 1: Physicochemical properties of methanol extract of *A.alba* and *L. racemosa*

Name of the plant	Methanol soluble Extract values(w/w)	Water Soluble Extractive Value(w/w)	Loss on drying (w/w)	Total Ash value (w/w)	Acid In-soluble ash (w/w)	Water In-soluble ash (w/w)
Aviccinia alba	5.00 %	7.20%	12.43%	14.00%	3.10%	3.90%
Laguncularia racemosa	8.5%	6.45%	8.45%	16.22%	3.40%	3.40%

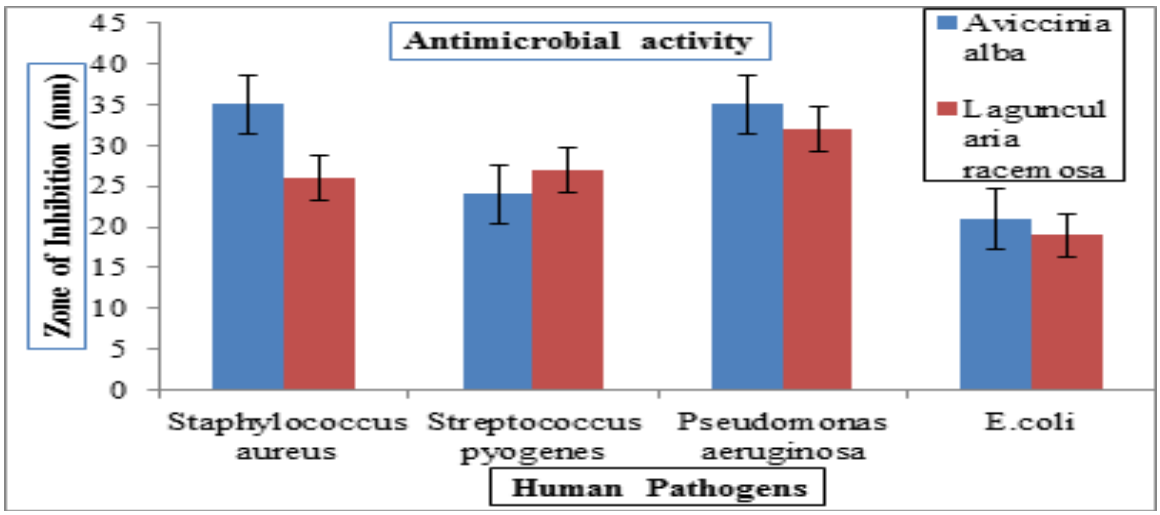

Figure 3: Anti-bacterial activity of methanol extracts of leaves of *A. alba* and *L. racemose.*

Table 2: Presence of different phytochemicals in methanol extracts of leaves of *A. alba* and *L. racemose.*

Name of plant	*Aviccinia alba*	*Laguncularia racemosa*
Plant part	Leaves	Leaves
Solvent	Methanol	Methanol
Colour of extract	Dark green	Dark green
Alkaloids	-	-
Tannins	+++	+++
Phenols	+++	+++
Glycosides	+	+
Flavanoids	+	+
Saponins	-	+
Phytosterols	++	++

+++ = High amount, ++ = Relatively high amount, + = Trace amount, - =Absent

solvent systems unclear spots were observed in both of plants (Fig 2).

Anti-bacterial activity

Antibacterial activity of methanol extract of *A. alba* (AME) and *L. racemosa* (LME) was studied at a concentration of 25µg/ml against 4 human pathogenic bacterial strains of which are two gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two gram-negative (*Pseudomonas aeruginosa* and *E. coli*). Anti-bacterial activity of the both the extracts and their potency were quantitatively evaluated by the appearance of inhibition zone and zone diameter was measured (mm). AME showed maximum inhibition against *Staphylococcus aureus* (35mm), followed by *Pseudomonas aeruginosa*(33mm), *Streptococcus pyogenes*(24mm) and minimum zone of inhibition observed in *E. coli* (21mm). Similarly, LME showed variation antibacterial activity against *P. aeruginosa*(32mm), *S. pyogenes* (27mm), *S. aureus*(19mm) and *E. coli*(18mm) with respectively. Both plant extracts were strongly inhibited with gram negative bacteria and a much broad spectrum of action compared with gram positive bacteria. Results showed in Fig3.

CONCLUSION

Mangrove plants are the unique plant communities inhabiting an extremely challenging environmental condition. These mangrove plants adapted well to undesired hostile condition by the synthesis of novel bioactive chemical compounds against to various stresses factors. In recent years, ethno-botanical and traditional uses of natural compounds, especially of plant origin medicine much attention as they are well tested for their efficacy and generally believed to be safe for human use. The quality, purity of herbal drugs is the sum of factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. Determination of physicochemical parameters of crude drug is essential in misidentification, mishandling, adulteration and to maintain proper standards. Our result demonstrates various physic-phytochemicals characteristics of *A. alba* and *L. racemosa* were reveals such as like water soluble, methanol soluble extractive values and total ash values were checked quantitatively. High extractive values and total ash values were identified in *L. racemosa* than *A.alba*. Phytochemical analysis AME and LME showed relatively high amount of tannins and phenolic compounds. Both AME and LME extracts strongly inhibited gram negative bacteria and a much broader spectrum of action than the gram positive bacteria. These studies will help in setting down Pharmacopoeia

standardisation and determining the quality and purity of bioactive compounds. Further investigation is being advocated for the identification of lead molecule with pharmacological significance.

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