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PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITY OF THE FRUITS OF *ALSTONIA SCHOLARIS*

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ABSTRACT

The present paper is aimed at the phytochemical analysis and antibacterial activity of the fruits of *Alstonia scholaris*. The findings of the work revealed that the solvent extract of plant showed the presence of phytochemical constituents like alkaloids, carbohydrates, phenolic compounds, terpenoids, cardiac glycosides and flavanoids in major amounts while fixed oils and fats, saponins and steroids in lesser amounts. These phytochemicals have reported to have conferred the necessary bioactivity to the plant like the antibacterial activity. However the test of antibacterial activity showed dose dependent inhibition of bacteria with maximum antibacterial activity in case of benzene and butanol solvent inhibiting *Lactobacillus lactis* and *Staphylococcus aureus* respectively. However least antibacterial activity was seen in case of hexane extract and aqueous and methanolic extract showed overall higher activity compared to other extract. The results of antibacterial activity are quite similar to standard antibiotic chloramphenicol thus indicating the possible cure of bacterial pathogenic diseases using these plant extracts in future.

Keywords: *Alstonia scholaris*, antibacterial activity, pathogenic, phytochemical analysis.

INTRODUCTION

The use of traditional plant based medicine for curing diseases has been known since ages. The whole plant or their parts have been put to various ethno botanical advantages treating diseases ranging from diabetes to cancer (Misra *et al*., 2011). Bearing fewer side effects, these medicines have an edge over the conventional chemical based drugs. These phytomedicines, also known as traditional medicines or herbal medicines are used commonly in developing countries like India and China and account for 30-40% of the total medicinal consumption (Thankamani *et al*., 2011a). Owing to the potency of plant based herbal drugs in the treatment of diseases, it is necessary to look for new avenues where potential of these therapeutic interventions can be harvested.

Alstonia scholaris(Apocynaceae) also known as Saptaparna is an evergreen tree found in south Asia and is used in treatment of various diseases(Satyavati *et al*., 1987). It is one of the few well studied plants in the family. Almost all the plant parts have been put under therapeutic investigation to find out the pharmacological properties it holds. The leaves, bark and root have been reported to have anti-microbial properties while flowers and fruits are still under investigation. The bark is used against chronic diarrhoea, dysentery, and bowel movements (Nadkarni, 1976). Leaves are used against beri-beri, congestion of liver, Dropsy and ulcers. The latex is useful as an application for ulcers, sores, tumours, and in rheumatoid pain (Daniel, 2006). Methanolic extracts of the leaf, stem, flowers and root bark extracts

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have been reported as potent antimicrobial agents (Misra *et al*., 2011; Khan *et al*., 2003; Thankamani *et al*., 2011b). Therefore present study was carried out for the preliminary investigation of the phytochemicals present in the fruits and also to evaluate its anti-microbial properties.

MATERIALS AND METHODS

Collection of Plant Samples

The fresh plant of *Alstonia scholaris* was collected from VIT University campus, Vellore and was authenticated by Plant Biotechnology Department. The wet weight of the plants was taken and was then separated into different parts viz. Leaves, Stem Bark, Roots, Fruits and Flowers. These plant parts were washed under running water to remove dirt which might interfere during the further analysis and were then subjected to shade drying for about 10 days.

Processing of Plant Materials

The dried fruit was taken separately and pulverized using an electric blender to obtain a fine powder.

Solvent Extraction

Pulverized fruit parts were subjected to successive solvent extraction in Soxhlet apparatus using solvents in increasing order of polarity like hexane, benzene, butanol, methanol and water respectively. 50g of the powdered fruit was taken and subjected to extraction with 175ml of suitable solvent for 12hrs. The extracts were concentrated using vacuum distillation to obtain semi-solid or solid extracts and weight of each extract was obtained. The fruit weighed 280 gm and it was reduced to 70 gm on drying. This yielded a total of 36.4 gm of solids hexane (14.2g%), benzene (5.7g%), butanol (7.7g), methanol(8.5g%)and water(15.7g%). The concentrated extracts were stored at 20^0 C for further investigation.

Phytochemical Analysis of Plant Extracts

The dried and concentrated extracts were dissolved in Di methyl sulphoxide (DMSO) except water extract which was dissolved in distilled water (Beyer and Walter, 1997).

The plant extracts of all solvents were used for the phytochemical analysis(Khyade and Vaikos, 2008) for the identification of various classes of chemical compounds using standard protocols(Harbone,1973; Kumar *et al.*,2009)- alkaloids(Wagner's test),Proteins and Amino acid (Ninhydrin test), Carbohydrates(Fehling's test), Phenolic compounds(Ferric Chloride test), Terpenoids (Salkowski's test), Flavanoids(an intense yellow colour produced by NaOH turns colourless by an acid), Cardiac Glycosides (Kellar Kiliani test), Fixed oils and Fats (Spot test), Steroids(red colour in the upper layer and yellow with green fluorescence in the sulphuric acid layer with the addition of chloroform followed by conc. $H_2SO_{4)}$, Saponins (appearance of foam upon shaking with water).

Tested Microorganisms

Pathogenic bacteria used for the antimicrobial activity were maintained at the VIT Collection Centre. These included *Staphylococcus aureus, Bacillus cereus, Lactococcus lactis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.* Sub-culturing of these bacterial cultures was done in order to maintain pure isolates (Khyade and Vaikos, 2008). A sterile loop full of tested organism was inoculated in peptone water (HIMEDIA) and incubated for 1hour before being tested for antibacterial property.

Screening of Antibacterial Properties

Plant extracts were tested for their antibacterial activity against Gram positive and Gram negative strains by well diffusion method (Khyade and Vaikos, 2008; Kavanagh, 1972). Freshly prepared culture plates were used which were prepared by pouring 20ml of nutrient agar (HIMEDIA) in Petri plates. A sterile well borer (8 mm) was used to make wells in each plate for the extracts. Using a sterile swab, inoculum was spread on agar plates to get a uniform distribution of bacteria.100µl of each extract was added into the wells (Ates and Erdoúrul, 2003; Ramesh *et al*., 2010) and incubated over night at 37^0 C. The concentrations of plant extract are presented (Table 1). The antibacterial activity was observed next day as evidenced by the zone of inhibition surrounding the well.

RESULT AND DISCUSSION

The results of phytochemical analysis of different solvent extracts of fruits of *Alstonia scholaris* are presented in Table 2. The fruits demonstrated the presence of alkaloids in major amounts in the methanol extract while them being absent in hexane, benzene and butanol. Carbohydrates are present more in the benzene extract as compared to methanol and water whereas the presence of amino acids is more prominent in butanol extracts as compared to other solvents. Phenolics and tannins are absent in all the extracts with an exception of being present in traces in the butanol extract. Terpenoids are present in almost all the extracts except the benzene extract. Cardiac glycosides are also a major constituent in all extracts of the fruit unlike that of other parts in the plant where it is almost absent (Misra *et al*., 2011). Saponins are present in traces in all solvent extracts and absent in the hexane extract. Except in water extract, flavanoids are present in all fruit solvent extracts. Steroids are only present in the benzene fraction followed by small amounts in the butanol fraction. Fixed oils and

fats are present more in hexane than in polar solvents like methanol and water and absent in benzene and butanol. The presence of these phytochemical constituents are found to be comparable to the work done by us on other parts of *Alstonia scholaris* (Misra *et al*., 2011; Thankamani *et al*., 2011b).

Results of anti-microbial activity with respect to different fruit extracts of *Alstonia scholaris* are presented in Table 3. All the extracts except hexane of the fruit showed high anti microbial activity. The benzene and the butanol extracts produced the highest activity with a zone of 25mm against *Lactobacillus lactis* and *Staphylococcus aureus*. The significant activity of the butanol extract against *Salmonella typhi* can be noted here (23mm). The methanol fraction was effective against *Staphylococcus aureus* more than the other bacteria. The water fraction on the other hand showed high activity against *E.coli*. The anti microbial assay of the fruit suggests that the fruit can possibly be used in the treatment of malaria. Similar results were obtained in our work done on different parts of *Alstonia scholaris* in which polar solvents, methanol and water was found to inhibit a wide range of pathogenic bacteria. Other researches have also shown similar anti microbial effect of different solvent extracts of the plant (Khan *et al*., 2003). The data presented can be inferred to have comparable antimicrobial activity of plant extracts to the standard antibiotic chloramphenicol with DMSO as control (Misra *et al*., 2011; Khyade and Vaikos, 2008).

Table 1. Concentration of plant extract of *Alstonia scholaris* in different solvent

Solvents	Concentration of Extract (mg/100 µl)
Hexane	100
Benzene	40
Butanol	54
Methanol	60
Water	110

Table 2. Phytochemical screening of extracts of fruits of *Alstonia scholaris**

S.No.	Plant constituents	Hexane	Benzene	Butanol	Methanol	Water
1.	Alkaloids	--	--	--	++++	+++
2.	Carbohydrate	--	++++	+ +	+++	+++
3.	Proteins and amino acids	++	+++	++++	+++	++
4.	Fixed oils and fats	++++	--	--	+++	++
5.	Phenolic compounds	--	--	+++	--	--
6.	Terpenoids	++	--	+++	++++	+++
7.	Cardiac Glycosides	+++	++++	++++	++++	++
8.	Steroids	--	+++	++	--	--
9.	Saponins	--	++	++	++	++
10	Flavanoids	+++	+++	+++	+++	--

*Weak (+), moderate (++) strong (+++) very strong (++++), absent (--)

Table 3. Determination of Antibacterial activity of fruits of *Alstonia scholaris*

S.No.	*Test Organisms*	Diameter of Zone of inhibition(mm)#					
		H	B	Bu	M	W	Chl
1	*Staphyloccocus aureus*	10	23	25	24	20	16
2	*Bacillus cereus*	15	18	20	22	20	23
3	*Lactococcus lactis*	13	25	19	18	15	21
4	*Pseudomonas aeruginosa*	0	16	15	17	18	20
5	*Escherichia coli*	14	18	20	19	24	21
6	*Salmonella typhi*	0	15	23	17	20	22

Values are average of triplicate

H,Hexane extract ;B, Benzene extract; Bu, Butanol extract ; M, Methanol extract; W, Water extract; Chl, Chloramphenicol.

CONCLUSION

The phytochemical analysis of fruit extracts showed the presence of carbohydrates, amino acids, cardiac glycosides and flavanoids as major phytochemical constituents followed by alkaloids and terpenoids. Solvent extracts used against gram positive and gram negative bacteria revealed that the methanol and water fractions of fruit showed significant activity against all the organisms tested. The present work and similar studies on *Alstonia scholaris* revealed that gram positive bacteria are more susceptible to the extracts as compared to gram negative bacteria. Thus the medicinal properties of the plant can be attributed to the presence of these phytochemicals but detailed research with purified fractions needs to be carried out to reach a firm conclusion about their therapeutic significance.

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