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Phytochemical and Pharmacognostic Analysis of *Alstonia Scholaris* (l) R. BR., A commonly available Medicinal Plant in Assam, India

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Abstract

Phytochemical analysis of methanolic extracts of leaf, bark and latex of Alstonia scholaris (L) R.Br. revealed marked variation in overall content of phenolics in leaf, bark and latex extracts. The leaf extract had highest content of overall phenolics followed by bark and latex extracts. In the leaf extract, flavonoids and proanthocyanidins were present in abundance with values observed 89 mgQE/g DW and 92 mgCE/g DW, respectively, whereas the phenolics were only 49 mgGAE/gDW. In the bark extract, level of flavonoids and phenolics were comparatively lower than leaf extract, however proanthocynidins (66 mgCE/g DW) was found significantly higher. Latext extract had lowest content of phenolics (26 mgGAE/g DW), flavonoids (16 mgQE/g DW) and proanthocynidins (21mgCE/g DW). Methanol extracts of A. scholaris leaves, bark and latex extracts exhibited strong antioxidant activities in terms of scavenging DPPH free radicals. Antimicrobial microbial rtesponse have been observed.

Keywords: *Alstonia scholaris*, phytochemicals, antioxidant.

Introduction

Complementary therapies based on herbal medicines are the world's oldest form of medicine and recent reports suggest that such therapies still enjoy vast popularity, especially in developing countries where most of the population does not have easy access to modern medicine[1]. The traditional Indian system of medicine, Ayurveda, which means the science of life, is one of the world's oldest systems of medicines. Ayurveda mainly uses plant-based formulas developed through the experimentation and experiences of doctors for centuries[2]. In current world order, an unexplored reservoir of phytochemical information hidden in nature is rapidly destroyed by deforestation and habitat loses. Traditional herbal medicine is an important component of primary health care system in developing countries like India. They are considered to be safe, effective and inexpensive, for which there is a global trend for the revival of traditional herbal medicine. Screening of medicinal herbs used by different ethnic groups or communities has now become a potential source for isolation of bioactive compound.

Alstonia scholaris (L.) R.Br (Apocynaceae) is an evergreen tropical tree native to Indian sub-continent and South East Asia, having graeyish rough bark and milky sap rich in poisonous alkaloid. This plant is a native of India, Sri Lanka, Pakistan, Nepal, Thailand, Burma, Malaysia, South East Asia, Africa, Northern Australia, Solomon Islands, and Southern China. The plant is a large evergreen tree, growing up to 17–20 m in height, with a straight often fluted and buttressed bole, about 110 cm in diameter. Bark is grayish brown, rough, lenticellate abounding, bitter in taste secreting white milky latex. Leaves are 4–7 in a whorl, coriaceous, elliptic-oblong. Flowers are small, greenish white, many in umbellate panicles, corolla tube is short, very strongly scented. Fruits have follicles, 30–60 cm long. Seeds are papillose with brownish hair at each end. The bark, also called dita bark, is traditionally used by many ethnic groups of North East India and other parts of the world as a source cure against bacterial infection, malarial fever, toothache, rheumatism, snakebite, dysentery, bowl disorder, etc.[3]. The presentwork aimed at analysing the phytochemical content and antioxidant properties of the leaf, bark and latex of the mostly used plant as phyto remedial measure.

Material and Methods

Plant material and extraction: Fresh leaves were collected from *A.scholaris* trees (8-12 feet) grown wild in various places in the locality of Lakhimpur District, Assam. Follicles were collected during the month February to March when the tree is laden of follicles. Follicles were cut with sharp blade and compressed to collect milky white latex in a beaker. Latex was stored at 0-4ºC in refrigerator until used. Bark region were collected from a well grown tree and processed for obtaining powdered bark[4].

A known amount of bark, leaves and follicles (100 g each) were kept in an oven at 40°C for drying. These were powdered by using mortar and pestle. The powder (10 g) of leaves, barks and follicles were separately extracted with methanol (25-50 ml) for 24 h in separate Erlenmeyer flasks. Extraction process was repeated three times and each time the extract obtained were

filtered through 0.45-µm filter paper and collected in a beaker. The extract thus obtained was dried over reflection water bath. Dried extracts were stored at 4 °C.

Antimicrobial assay: Antimicrobial activities of methanolic extracts of leaves, follicles and latex were assessed against few fungal infectants such as *Aspergillus niger, Erisiphe gramineae, Alternaria solani* and *Fusarium oxysporum.* The culture plates were prepared by first sterilizing the nutrient agar (36 gm in 1000 ml) in an autoclave at 121°C at 15 lb for 15 minutes and then by pouring 20 ml of media into sterilized Petri dishes. 1 ml inoculums suspension was spread uniformly over the agar in Petri dishes using sterile glass rod. Wells were made by sterile cork borer (6 mm) in each plate. Extracts 100 µl (at concentration of 50, 100 mg/ml) was added aseptically into the well. Simultaneously, a control with Ampicillin was also run. Plates were incubated at 37°C for 24 hrs. After incubation, microbial growth was observed in the Petri dishes. The antimicrobial activity was expressed as the mean of diameter of the inhibition.

Phytochemical analysis: Total content of phenolics, flavonoids and proanthocynidins were determined as per procedure of Liu *et al.*[5]. Total phenolic contents were expressed as mg gallic acid equivalent (GAE)/g dry weight of the sample. Flavonoids content was measured by the method of Jia *et al*[6]. Total flavonoids content was expressed as milligrams of quercetin equivalent (QE)/g dry weight of sample. Proanthocyanidin content was measured according to the method of Manikandan[7]. Total proanthocyanidins content was expressed as milligrams of catechin equivalent (QE)/g dry weight of sample.

DPPH assay: The antioxidant activity of the each sample extract was assessed by the ability of the extract to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Liu et.al,2008). DPPH free radical scavenge activity was monitored by measurement of decline in absorbance at 517 nm. Butylated hydroxyanisole (BHA) was used as the standard compound.

Results and Discussion

Phytochemical constituents: Total phenolics content including flavonoids and proanthocyanidins of barks, leaves and latex extracts are presented in table-1. The results revealed marked variation in overall content of phenolics in leaf, bark and latex extracts. The leaf extract had highest content of overall phenolics followed by bark and latex extracts. In the leaf extract, flavonoids and proanthocyanidins were present in abundance with values observed 89 mgQE/g DW and 92 mgCE/g DW, respectively, whereas the phenolics were only 49 mgGAE/gDW. In the bark extract, level of flavonoids and phenolics were comparatively lower than leaf extract, however proanthocynidins (66 mgCE/g DW) was found significant. Latext extract had lowest content of phenolics (26 mgGAE/g DW), flavonoids (16 mgQE/g DW) and proanthocynidins (21mgCE/g DW).

Anti microbial properties: Results of the present study reveals varied level of antimicrobial qualities of the different plant parts of *A. scholaris*. Concentration at the rate of 100mg/ml showed greater inhibition for all extracts. However the latex extract showed highest inhibition percentage in all fungal organisms. Growth of *Aspergillus niger* was significantly inhibited by extracts of all the three parts.

Table-1
Phenolics content of methanolic extracts of leaves, bark and latex of A.scholaris

Sl. No	Phyto-constituents	Plant parts		
		Leaves	Bark	Latex
1	Phenolics (mg GAE/g)	49.66±1.52	38.63±1.51	26.0±2.0
2	Flavonoids (g QE/g)	89.33±1.35	35.86±01.64	16±1.0
3	Proanthocyanidins (mg CE/g)	92.33±1.65	66.81±1.75	21.33 ±1.35

Table-2
Antimicrobial properties of methanolic extracts of leaves, bark and latex extract of Alstonia scholaris

Extract/ standard	Concentration	Zone of inhibition (mm)			
		Aspergillus niger	Alternaria solami	Erisiphe graminis	Fusarium oxysporeum
Leaves	50	12	06	06	08
	100	16	10	10	12
bark	50	11	15	09	10
	100	15	17	11	17
Latex	50	10	17	10	12
	100	21	18	14	16
Ampicillin	10	23	16	18	23

S.E.M = 0.687

DPPH free radical scavenging activities: Antioxidant profiles of methanol extracts of leaves, follicles and latex in terms of their ability to scavenge DPPH free radicals are depicted in figure-1. Among the extracts tested, leaf extract displayed most potent antioxidant activity (60-80%) followed by bark (40-70%) and latex (30-46%). However, leaf and bark extracts exhibited similar antioxidant (60-75%) activities at concentrations, 20 and 50 mg/ml.

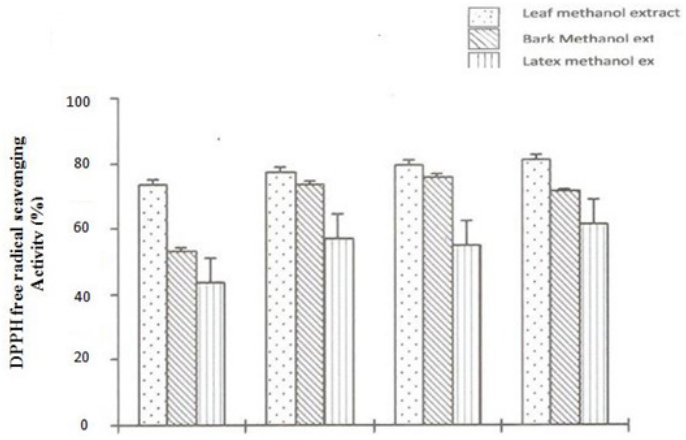

Figure- 1
DDPH free radical scavenging activity of methanolic extracts of leaf, bark and latex of *A.scholaris*

Phytochemical composition and antimicrobial properties have been reported for different plants of medicinal importance. Since awareness towards natural products in healthcare is rapidly increasing, interest in medicinal plants has earned remarkable importance. Plants produce many important compounds such as phenolics and flavonoids which possesses antioxidant and antimicrobial properties[8,9]. Phenolics and flavonoids provide protection against free radicals and regulate various oxidative reactions occurring naturally. Also, they are used to protect food quality mainly by the prevention of oxidative deterioration of constituents of lipids

The present study reports phytochemical composition of leaves, barks and latex of *A. scholaris* which play a major role in their antibacterial and antioxidant properties. Study revealed that *A. scholaris* leaves accumulated high content of phenolics including flavonoids and proanthocynidins whereas bark and latex had comparatively lesser phenolics content. Higher levels of phenolics accumulated in the green tissues (leaves) may be due to higher rates at which photosynthesis proceeds in these parts. Kumar *et. al.*[10] have also been reported significantly higher (80mg/ml) phenolics content in leaf methanolic extract of some medicinal plants. A. scholaris bark methanolic extract also contained significantly higher (46 mg/ml) phenolics content. Variations in the phytochemical compositions of the different plant parts of *A. scholaris* are almost identical with many other plant species[11-13]. Phytochemical contents are reported to be influenced by several other factors such as geographical, genetic, environmental, degree of maturity at the time of harvest.

Phenolics content of the plants/parts are often correlated with their strong antioxidant activities[14]. In the present study, methanol extracts of *A. scholaris* leaves, bark and latex extracts exhibited strong antioxidant activities in terms of scavenging DPPH free radicals and superoxide anions. The hydrogen donating potential is known to be one of the various mechanisms for measuring antioxidant activity. In DPPH assay, the radical scavenging ability of the extract was determined by the DPPH which itself is a stable nitrogen-centered free radical. Here, the leaf extract showed 78 % DPPH free radical scavenging activity, much higher than that reported previously[15]. Higher antioxidant activities of *A. scholaris* plant parts reported here also coincide with the previous study reported significant amount of phyto constituents as well as antioxidant activities of A. scholaris[16] However, very little report is available on antioxidant activity of *A. scholaris* bark and latex methanolic extracts except that by James *et al.*[17], who studied it in flowers and fruits.

Conclusion

Alstonia scholaris is a commonly available plant in Assam and various plant parts are is traditionally used by the local tribes as remedy for a numberof ailments. Phytochemical analysis of methanolic extract of various parts of the plant such as leaves, bark and latex, revealed antimicrobial and antioxidant property of the plant. Further chemical analysis of various parts f the plant is necessary contributing to ethnomedicinal quality of the plant.

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