INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 3, p. 260-265, 2022

RESEARCH PAPER OPEN ACCESS

Preliminary screening of phytochemical contents, antioxidant and antimicrobial activity of *Alstonia scholaris* (L.) R. Br collected in the Darrang area of Assam, India

Lakhya Jyoti Gogoi[1], Sristisri Upadhyaya[2], Ranjan Sarma[3], Sahabuddin Ahmed[4*]

[1]*Department of Medical Lab & Molecular Diagnostic Technology, Mangaldai College, Darrang 784125, Assam, India*

[2]*Department of Botany, Dergaon Kamal Dowerah College, Golaghat, 785614, Assam, India*

[3]*Department of Physics, Mangaldai College, Darrang, 784125, Assam, India*

[4]*Department of Botany, Mangaldai College, Darrang, 784125, Assam, India*

Key words: *Alstonia scholaris* (L.) R. Br., phytochemical, antioxidant, antimicrobial.

http://dx.doi.org/10.12692/ijb/20.3.260-265 Article published on March 30, 2022

Abstract

The current study was carried out to investigate the phytochemical contents, antioxidant activity, total phenolic content, total flavonoid content, and antimicrobial activity of *Alstonia scholaris* (L.) R. Br. A preliminary phytochemical study was performed using conventional procedures. In crude aqueous, methanol, chloroform, and hexane extracts, total phenolic content, total flavonoid content, and antioxidant activity were measured spectrophotometrically. A well diffusion approach was used to assess in vitro antimicrobial activity against seven Microbial Type Culture Collection (MTCC) bacterial species and two MTCC fungus species. Saponin, tannin, flavonoid, phenol, terpenoids, cardiac glycoside, steroid, and alkaloid were found in the preliminary phytochemical study, but not anthraquinone and reducing sugar. The methanol extract fared better than the other extracts. The methanolic extract exhibited clear antimicrobial action against *Staphylococcus epidermidis*, *Proteus vulgaris* and *Penicillium chrysogenum*, whereas hexane extract showed modest activity against *Penicillium chrysogenum*. Using petroleum ether hot extract, petroleum ether showed better antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Penicillium chrysogenum*, respectively. The greatest percentages of inhibition for DPPH and ABTS were 82.00±0.000 and 80.50±0.000 percent for methanol extracts, respectively. According to the current study, *Alstonia scholaris* (L.) R. Br includes both primary and secondary metabolites, which contribute to its traditional usage as a food and medicinal.

* **Corresponding Author:** Sahabuddin Ahmed ✉ dr.sahab1970@gmail.com

Introduction

Medicinal plants are a significant natural alternative to synthetic drugs, and their use in both traditional and modern medicine is gaining popularity. (Karthishwaran and Mirunalini, 2012).Traditional medicine has been proven to be beneficial, and approximately 60% of rural residents rely on it for their main healthcare. (WHO, 1978 and Akinyami *et al.*, 2000). The medicinal properties of plant bioactive constituents such as alkaloids, tannins, flavonoids, and phenolic compounds have a distinct physiological effect on the human body. (Tariq *et al.*, 2013 and Sangeetha *et al.*, 2014). The presence of phytochemical elements in medicinal plants makes them effective for both healing and curing human ailments (Nostro *et al.*, 2000). Herbalists, healers, spiritualists, hunters, and farmers have employed traditional medicine as a primary health care option at the community level for generations. Alkaloids, which are present in medicinal plants, are utilized as anesthetics (Herouart *et al.*, 1998). Terpenoids have a number of key pharmacological properties, including anti-inflammatory, anticancer, antiviral, anti-malarial, cholesterol synthesis inhibition, and antimicrobial properties (Mahato and Sen, 1997).

Alstonia scholaris (L.) R. Br (Apocynaceae) is an evergreen tropical tree endemic to India and Southeast Asia with grey rough bark and milky sap high in toxic alkaloid. This plant is indigenous to India, Sri Lanka, Pakistan, Nepal, Thailand, Burma, Malaysia, South East Asia, Africa, Northern Australia, the Solomon Islands, and southern China. (Nadkarni, 1976 and Sathyavathi, 1987) Many ethnic groups in North East India and other areas of the world have traditionally utilized the bark, also known as dita bark, as a source remedy for bacterial infection, malarial fever, toothache, rheumatism, snakebite, diarrhea, bowl problem, and other ailments. Latex is also used to treat coughs, sores, and fevers. The purpose of this study was to evaluate the phytochemical composition, total phenol content, total flavonoid content, antioxidant and antimicrobial potential of *Alstonia scholaris* (L.) R. Br. collected from Darrang district, Assam, India.

Material and methods

Plant Material Collection

The entire plants parts *Alstonia scholaris* (L.) R. Br was collected from Darrang district, Assam, India and were authenticated and deposited at the Department of Botany, Mangaldai College, Mangaldai. Fresh plants were picked, cleaned in tap water, and then dried in the shade at room temperature before being homogenized into a fine powder. Before being filtered using Whatman No 1 filter paper, the powder was macerated for 48 hours in a range of solvents, including ethyl acetate, methanol, chloroform, and hexane. The filtrate was evaporated in a water bath at a constant temperature of 72°C, yielding a highly concentrated extract. The crude extract was dissolved in DMSO and kept in the refrigerator until needed to make a final concentration.

Phytochemical Analysis

The tannin, saponin, flavonoid, phenol, cardiac glycoside, alkaloid, anthraquinone, trapezoid, steroid, and reducing sugar of *Alstonia scholaris* (L.) R. Br was determined using the colorimetric procedures described in Jovale Vincent V. *et al.*, 2014.

Antioxidant Activity

The scavenging activity of the sample for DPPH and ABTS free radicals was determined using the technique described by Stanojevice *et al.*, 2009.

DPPH radical scavenging activity

0.5 ml of extract solutions (1mg/ml) were taken and made up the volume to 3 ml with methanol. 0.15 ml of freshly prepared DPPH solution was added, stirred and left to stand at room temperature for 30 minutes in dark. The control contains only DPPH solution in methanol instead of sample while methanol served as the blank (negative control). Absorbance was noted at 517 nm using UV-Vis spectrophotometer (Systronics, Model 2202). The percentage inhibition calculated as DPPH radical scavenging activity (%) = [(Abs control - Abs sample /Abs control.] x 100 where, Abs control is the absorbance of DPPH radical + methanol, Abs sample is the absorbance of DPPH radical + sample extract/standard.

ABTS radical scavenging activity

Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using a spectrophotometer. The percentage inhibition calculated as ABTS radical scavenging activity (%) = [(Abs $_{control}$ - Abs $_{sample}$ /Abs $_{control}$.] x 100 where Abs $_{control}$ is the absorbance of ABTS radical in methanol; Abs $_{sample}$ is the absorbance of ABTS radical solution mixed with sample extract/standard.

Antimicrobial activity study

The antimicrobial test was performed using a 6mm borer and the agar well diffusion technique reported by Nair *et al.* 2008. The extract's activity was assessed by measuring the width of its Zone of Inhibition (ZOI).

Determination of antimicrobial activity

The antimicrobial activity was determined by the agar well-diffusion method. One litre of nutrient broth was prepared by dissolving 13g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the media completely.

The medium was dispensed as desired and sterilized by autoclaving at 15lbs pressure (121°c) for 15 minutes. Petriplates containing 20ml nutrient medium were seeded with 24hr culture of bacterial strains. 20µl bacterial culture was transferred to nutrient agar plates and spread. Wells were punched by using 5mm borer and approximately 60µl of the different solvent extract was placed in the wells. Incubated at 4°c for 2 hours and then 37°c for 24 hours. The diameter of the Inhibition Zones was measured in mm.

Selected strains for antimicrobial study

Strains were collected from the Institute of Microbial Technology's (IMTECH) Microbial Type Culture Collection (MTCC) in Chandigarh, India. The study used five Gram-positive bacterial strains: *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615), and *Proteus vulgaris* (MTCC744); two Gram-negative strains: *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439) and two fungal strains: *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947).

Standard antibiotics

For comparison of ZOI with the sample, standard antibiotics such as Chloramphenicol (C) 30mcg and Clotrimazole (CC) 10mcg were used for bacterial and fungal strains.

Result and discussion

Table 1 summarizes the quantitative analysis of phytoconstituents in the plant sample. Saponin, tannin, flavonoid, phenol, trapezoid, cardiac glycoside, steroid, and alkaloid were found in preliminary phytochemical investigation. The lack of anthraquinone, and reducing sugar in the sample was verified in this analysis. Khan *et al.*, 2020 also describe the presence of carbohydrates, alkaloids, steroids, glycosides, tannins, flavonoids, saponins, terpenoids, and phenol.

Table 1. Phytochemical present in extracts of *Alstonia scholaris* (L.) R. Br.

Sl. No.	Parameter	Method	*Alstonia scholaris* (L.) R. Br
1	Tannin A)$FeCl_3$ test B)$PbAc_3$ test	$FeCl_3$ test $PbAc_3$ test	+ +
2	Saponin	Frothing test	+
3	Flavonoid	Alkaline reagent test	+
4	Phenol	$FeCl_3$test	+
5	Alkaloid	Dragendorff's test	+
6	Anthraquinone	Lye test	-
7	Cardiac glycoside	Keller-Kilani test	+
8	Trapenoids	Salkowski test	+
9	Steroid	Salkowski test	+
10	Reducing sugar		-

('+') indicates presence; while ('-') stands for absence

The presence of acubins/iridoids, alkaloids, coumarins, flavonoids, leucoanthocyanins, phlobatannins, reducing sugars, simple phenolics, steroids, saponins, and tannins in the leaves of *Alstonia scholaris* (L.) R. Br was reported by qualitative screening of phytochemical components (Khyade and Vaikos, 2009). The alkaloids fraction of *Alstonia scholaris* (L.) R. Br leaf has anti-tussive, anti-asthmatic, and expectorant actions, and it might be a potential starting material for respiratory illness medication development. Picrinine, the principal anti-tussive and anti-asthmatic ingredient, might be used in quality monitoring of *Alstonia scholaris* (L.) R. Br leaf products. (Shang *et al.*, 2010).

Table 2. TPC, TFC and antioxidant activities of different solvent extract of *Alstonia scholaris* (L.) R. Br.

Sample Extract	TPC (mg catechol equivalent/gm dry material)	TFC (mg quercetin equivalent/gm dry material)	Antioxidant activity (% inhibition in mg/ml)	
			DPPH radical scavenging activity	ABTS radical scavenging activity
Ethyl acetate	05.22 ± 0.002	34.10 ± 0.000	82.00 ± 0.000	77.80 ± 0.000
Methanol	11.22 ± 0.000	54.20 ± 0.000	80.20 ± 0.000	80.50 ± 0.000
Chloroform	06.55 ± 0.000	33.34 ± 0.001	76.34 ± 0.000	69.45 ± 0.000
Hexane	03.50 ± 0.000	31.10 ± 0.000	60.50 ± 0.000	62.30 ± 0.000

Due to the presence of secondary metabolites with promising pharmacological effects, the methanol extract of dita bark may contribute to antidepressant, anti-inflammatory, and thrombolytic actions. (Khan *et al.*, 2020). In terms of antioxidant activity, ethyl acetate and methanolic extract were efficient DPPH and ABTS radical scavengers, respectively (Table 2). The Total Phenol Content (TPC) and Total Flavonoid Content (TFC) were determined to be the highest in the plant's methanolic extract, at 11.22±0.000 and 54.20±0.000, respectively. Against *Staphylococcus epidermidis* and *Proteus vulgaris*, the chloroform and methanolic extracts had a clear antimicrobial effect (Table 3).

Table 3. Antimicrobial activities (ZOI in mm) of different solvent extract of *Alstonia scholaris* (L.) R. Br.

Sample extract	Bacterial strains							Fungal strains	
	B. subtilis	*B. cereus*	*S. aureus*	*S. epidermidis*	*P. vulgaris*	*E. faecalis*	*E. coli*	*P. chrysogenum*	*C. albicans*
Cold extract									
Ethyl acetate	-	-	-	-	-	-	-	9	-
Methanol	-	-	-	7	8	-	-	10	-
Chloroform	-	-	-	7	9	-	-	-	-
Hexane	-	-	-	-	-	-	-	-	-
Hot extract									
Petroleum ether	10	-	8	-	-	-	-	8	-
Standard									
Chloramphenicol 30mcg	15	-	-	30	-	8	-	-	-
Clotrimazole 10mcg	20	10	14	20	8	-	26	11	32

Hexane and methanolic extract both had antifungal efficacy against *P. chrysogenum*. The hot extract of petroleum ether, on the other hand, has stronger antimicrobial action against *Bacillus subtilis*, *Staphylococcus aureus*, and *Penicillium chrysogenum* than the cold extract. The antimicrobial activities of the extracts were equivalent to those of the conventional antimicrobial agents Chloramphenicol (C) 30mcg and Clotrimazole (CC) 10mcg. (Marjorie, 1999) revealed that methanol extracts of *Alstonia scholaris* (L.) R. Br had a greater range of action, being active to both gram-positive and gram-negative organisms when compared to chloroform and acetone, although petroleum ether had no inhibition. The discrepancies in the reported activity of the various extracts might be attributable to the active components' varying degrees of solubility in the solvents used. It has been shown that different

solvents have differing levels of solubility for certain phytochemical components. The present study shows that both primary and secondary metabolites, antioxidant activities, and antimicrobial activities are present in *Alstonia scholaris* (L.) R. Br. A plant's secondary metabolite synthesis might change qualitatively and quantitatively depending on its growing environment. In fact, this issue must be addressed effectively in the case of medicinal plants, and it is critical in the quality monitoring of crude pharmaceuticals. The study also provides a scientific foundation for the medicinal use of *Alstonia scholaris* (L.) R. Br. collected in the Darrang district of Assam.

Conclusion

The current study discovered that *Alstonia scholaris* (L.) R. Br contains both primary and secondary metabolites, which contribute to its traditional usage as a food and medicinal. The presence of tannins and alkaloids chemicals was considered to cause antimicrobial action. Plant additions containing bioactive chemicals must be able to modulate signaling pathways in cellular and molecular systems (Singh *et al.*, 2016). Many plants include tannins and secondary metabolites of alkaloids, which have antimicrobial and anti-parasitic properties (Islamy, 2019 and Li Q. *et al.*, 2019). Tannins hindered the development of several yeasts, bacteria, fungi, and viruses (Chung *et al.*, 1998). According to Toshiya *et al.*, 2012 naturally produced flavonoids have the ability to serve as radioprotection. Primary research must be improved and expanded. In order to do additional study on this extract, we would employ an animal model.

Acknowledgement

The authors are grateful to the Department of Biotechnology, Government of India, which financed the Institutional Level Biotech Hub at Mangaldai College for providing the essential resources.

Conflict of interest

The authors state that they do not have any conflicts of interest.

References

Akinyemi KO, Coker AO, Bayagbon C, Oyefolu AOB, Akinside KA, Omonigbehin EO. 2000. Antibacterial screening of five Nigerian medicinal plants against *S. typhi* and *S. paratyphi*, Journal of the Nigerian Infection Control Association **3(1),** 1-7.

Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. 1998. Tannins and human health: a review, Critical Review in Food Science and Nutrition **38(6),** 421-464. https://doi.org/10.1080/10408699891274273

Herouart D, Sangwan RS, Fliniaux MA, Sangwan-Norreel BS. 1988. Variations in the leaf alkaloid content of androgenic diploid plants of *Daturainnoxia*, Planta Medica **54,** 14-17. PMID: 17265195. https://doi.org/10.1055/s-2006-962320.

Islamy RA. 2019. Antibacterial activity of Cattle fish sp. (Cephalopoda) ink extract against Aeromonas hydrophila, Majalah Obat Tradisional **24(3),** 184-188. https://doi.org/10.22146/mot.45315

Jovale Vincent Tongco V, Remil Aguda M, Ramon Razal A. 2014. Proximate analysis, phytochemical screening and total phenolic and flavonoid content of Philippine bamboo *Schizostachyum lumampao*, Journal of Chemical and Pharmaceutical Research **6(1),** 709-713.

Khyade MS Vaikos NP. 2009. Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br. African Journal of Biotechnology **8(22),** 6434-6436. https://doi.org/10.5897/AJB2009.000-9489

Karthishwaran K, Mirunalini S. 2012. Assessment of the antioxidant potential of *Pergulariadaemia* (Forsk.) Extract *in vitro* and *in vivo* experiments on hamster buccal pouch carcinogenesis, Asian Pacific Journal of Tropical Disease **31(2),** 509-516. https://doi.org/10.1016/S2222-1808(12)60212-6

Li Q, Zhao YL, Long CB, Zhu PF, Liu YP, Luo XD. 2019. Seven new veratramine type alkaloids with

potent analgesic effect from *Veratrumtaliense*, Journal of Ethanopharmacology **244,** 112137. https://doi.org/10.1016/j.jep.2019.112137

Molly Antony, Darsan Menon B, James Joel, Dev Lipin MS, Arun K, Thankamani V. 2011. Phytochemical analysis and antioxidant activity of *Alstoniascholaris*, Pharmacognosy Journal **3(26),** 13-18. https://doi.org/10.5530/pj.2011.26.3

Mohammad Forhad Khan, Faisal Bin Kader, Mohammad Arman, Suhel Ahmed, Chadni Lyzu, Shahenur Alam Sakib, Shaifullah Mansur Tanzil, AFM Irfan Uddin Zim, Md. Abdus Shukur Imran, Tommaso Venneri, Barbara Romano Md. Areeful Haque, Raffaele Capasso. 2020. Pharmacological insights and prediction of lead bioactive isolates of Dita bark through experimental and computer-aided mechanism, Biomedicine & Pharmacotherapy **131,** 110774. https://doi.org/10.1016/j.biopha.2020.110774

Marjorie MC. 1999. Plant products as anti-microbial agents, Clinical Microbiology Reviews **12(4),** 564-582.

Mahato SB, Sen S. 1997. Advances in tri terpenoid research 1990-1994, Phytochemistry **44,** 1185-1236. https://doi.org/10.1016/S0031-9422(96)00639-5

Nair R, Chandra S. 2008. Antimicrobial activity of *Terminalia catappa*, *Manilkara zapota* and *Piper betel* Leaf Extract, Indian Journal Pharmaceutical Sciences **70(3),** 390–393. https://doi.org/10.4103/0250-474X.4301

Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA. 2000**.** Extraction methods and bio autography for evaluation of medicinal plant antimicrobial activity, Lett Appl Microbiol **30,** 379-384. https://doi.org/10.1046/j.1472765x.2000.00731.x

Nadkarni AK. 1976. Indian Materia Medica, 3rd ed. Mumbai, India: Popular Press.

Singh A, Dayal R, Ojha RP, Mishra KP. 2016, Phytocomponents of *Argyreiaspeciosa* (Linn. f) Confer radioprotection, Journal of Radiant and Cancer Research **7,** 99-102. https://doi.org/10.4103/jrcr.jrcr_11_17

Shang JH, Cai XH, Zhao YL, Feng T, Luo XD. 2010. Pharmacological evaluation of *Alstonia scholaris*: anti-tussive, anti-asthmatic and expectorant activities. J Ethnopharmacol **129(3),** 293-8. PMID: 20381600. https://doi.org/10.1016/j.jep.2010.03.029

Stanojevic L, Stanojevic M, Nikolic V, Nikolic L, Ristic J, Canadano Vic-Brunet J, Tumbas V. 2009. Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L extract, Sensors (Basel) **9(7),** 5702-5714. https://doi.org/10.3390/s90705702

Sangeetha M, Preethi S. 2014. Qualitative and quantitative analysis of bioactive compounds in *Morinda citrifolia* fruit, Indian journal of advances in plant research **16,** 5-8.

Sathyavathi GV, Gupta AK, Tandon N. 1987. Medicinal Plants of India 2, New Delhi, India: Indian Council of Medical Research.

Toshiya K, Testuya T, Akira H, Takuji T. 2012. Cancer chemoprevention through the induction of apoptosis by natural compounds", Journal of Biophysical Chemistry **3,** 156-173. https://doi.org/10.4236/jbpc.2012.32018

Tariq AL, Reyaz AL. 2013. Quantitative phytochemical analysis of traditionally used medicinal plant *Terminaliachebula*, International Research Journal of Biotechnology **4(5),** 101-105.

WHO. 1978. The Promotion and development of traditional medicine. World Health Organization. Technical Report Series No. 622.2.