ANNALS OF PHARMACY AND PHARMACEUTICAL SCIENCES
Volume 7 | Issue 1 | April, 2016 | 20-24
■ e ISSN-2230-9438
☞DOI:10.15740/HAS/APPS/7.1/20-24
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RESEARCH ARTICLE

Pharmacognostical screening of antibacterial compounds from leaves of *Alstonia scholaris*

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ABSTRACT

Today, when every day a new pathogenic strain of micro-organism is evolving, to combat any and every fatal disease, we need to have a whole new set of drugs, for which that very micro-organism is yet not resistant. India is blessed with numerous medicinal plants, whose secondary metabolites are truly effective in many diseases. *Alstonia scholaris* has long been used in treatment of various disorders in Ayurvedic system of medicine. Standard phytochemical assay on *Alstonia scholaris* leaves extracts have showed that alkaloids, saponins, phenolics were present more in the middle of solvent extracts series. Further we have worked on gram positive and gram negative bacteria both to see our extract's biological activity. There methanolic extract of leaves showed broad spectrum antibacterial activity against tested organisms. Maximum activity was exhibited against *Klebsiella pneumoniae*, *Escherichia coli*, followed by *Staphylococcus aureus*. These results show the possible way out to fight many deadly diseases.

Key words : Medicinal plant, Alstonia, Phytochemistry, Antimicrobial, Zone of Inhibition

How to cite this paper : Biswas, Anindita (2016). Pharmacognostical screening of antibacterial compounds from leaves of *Alstonia scholaris*. *Ann. Pharm. & Pharm. Sci.,* **7** (1) : 20-24.

Article chronicle : Received : *21.01.2016;* **Revised :** *10.03.2016;* **Accepted :** *22.03.2016*

INTRODUCTION

According to WHO, till 1940 mortality rate of many bacteria, such as *Staphylococcus aureus* were really high (Frank *et al.*, 1999). Since new antibacterials have been arising, subsequently drug resistant strains also have been evolving (Bandawane *et al.*, 2010). For an example, initial success of antibiotherapy against *Staphylococcus aureus* was primarily halted due to emergence of penicillin resistant *Staphylococcus aureus*, followed by methicillin resistant *Staphylococcus aureus* (MRSA) and finally on 2002 vancomycin resistant stains have been emerged (Gorak *et al.*, 1999). Therefore, we have two solutions to brawl against these resistant bacteria. First and foremost would be vaccination (Fattom *et al.*, 1996 and Lee *et al.*, 1997). Unfortunately till now we do not have a set of vaccines which could prevent us from all these ermergences. The second option would be new pharmaceuticals and plant could be the best source (Rýos and Recio, 2005 and Olalde, 2005). Secondary metabolites of plants-alkaloids, flavonoids, phenolics are long been used in Indain folklore to treat various diseases (Patrick *et al.*, 2005; Chopra *et al.*, 2009 and Xu *et al.*, 2011). Here, we would like to combine our long practiced traditional way of treatments Ayurveda, Siddha with latest quarter of science. We have references of Saptaparna

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(*Alstonia scholaris*, family-Apocynaceae) in as a wonder drug in traditional Siddha and Ayurvedic systems of medicine (Harbone, 1984). Family Apocynaceae consists of genera with extraordinarily useful medicinal plants: Vinca- produce anti cancer drug-vinblastin, vincristene (Li *et al.*, 1995), *Rauwolfia serpentina*-produce antipsychotic, antihypertensive indole alkaloid-recerpin (Udupa *et al.*, 1994) and Alstonia. The Alstonia species is rich in alkaloids, flavonoids and phenolics (Gawade and Fegade, 2012), which are used as a tonic, anthelminthic, stimulant, carminative (Jagetia and Baliga, 2005; Arulmozhi *et al.*, 2007 and Gupta *et al.*, 2002) and expectorant (Saxena,1997). The bark decoction of *Alstonia scholaris* is used to treat asthma, hypertension and pneumonia, chronic diarrhyoea, cardiac ailments (Kamt *et al.*, 1997; Baliga *et al.*, 2004 and Kaushik *et al.*, 2011). The latex has activity to cure sores, ulcers and leaves to treat fever, beriberi and dropsy (Channa *et al.*, 2005). In this study we include some excellent results of leaves extract of *Alstonia scholaris* against gram positive and gram negative bacteria like *Klebsiella pneumoniae ATCC 25926*, *Escherichia coli ATCC 25922*, *Staphylococcus aureus ATCC 25923*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis.*

MATERIAL AND METHODS

Materials :

Petroleum ether (Pet E), benzene, n-butanol, methanol, chloroform, acetic acid, acetic anhydrate, ethyl acetate, sulphuric acid, hydrochloric acid, tin and thionine chloride and other crude chemicals are purchased from MERCK, India. Ferric chloride, lead acetate, copper sulphate, potassium sodium tartrate, sodium hydroxide, potassium hydroxide and phenolphthalein and magnesium ribbon were purchased from NICE chemicals.

Collection of plant materials and preparation of extracts :

The fresh leaves of *A. scholaris* were collected on from lower part of 20 feet heighted plant. Plant samples were washed with tap water and shade dried for twelve days. The dried plant material was grounded into fine powder using a grinder. 120 g of powdered material was extracted in soxhlet extraction apparatus with 1 lit of Pet E on the same day and continued for 10 hour. After

Table 1 : Solvent extraction

Solvent used	Weight of the leaves before extraction	Weight of the leaves after extraction	Extracts	Weight of the extracts obtained
Petroleum ether	80g	79.27g		4.208 g
Benzene	79.87g	79.31g		3.864 g
n-Butanol	79:31g	78:62g		3.982 g
Methanol	78:62g	77:49g		2.48g
	79.19g	78.34g		6.57g
Water	76:98g	76.08g		3.71g
	77.76g	76.48g		4.56g

extraction, the solvent with extract was allowed for distillation for recovery of solvent. The same method was followed for the other solvents – 'benzene, n-butanol, methanol, water' extraction as well.

Phytochemical assay :

We had followed standard methods to detect qualitative presence of various components like phenolics, tannins, saponins, phytosterol, alkaloids, flavonoids in our five extracts (Harbone, 1984).

Antibacterial assay :

All the bacterial strains were grown upto log phase and plated to determine antibacterial activity by pour plate method. The air dried extracts was weighed and dissolved into sterile water to make different concentration and zone of inhibition were measured after 14hr. incubation at 37ºC. Each experiment was repeated at least 3 times and the mean diameter of zone of inhibition for each dose was calculated.

RESULTS AND DISCUSSION

During solvent extraction maximum extract was obtained from methanol (polarity 5.1), followed by water (polarity 9). Fair amount of extract was obtained in case

Table 2 : Phytochemical assay

Tests	Solvent used							
	Petroleum ether	Xylene	Benzene	n-Butanol	Methanol	Acetonytryl	Acetic acid	Water
Phenolics	+	-	++	++	+++	++	-	-
Tannins	-	-	-	-	+	+	++	+++
Saponins	-	-	-	-	+	++	+	+++
Carbohydrates	-	-	-	-	-	-	+	+
Phytosterols	+++	++	+++	+	++	-	-	-
Alkaloids	-	+	+	++	+++	++	++	-
Oils	+++	+	++	+	+	-	-	-
Flavonoids	-	-	-	+	++	-	-	+
Tarpenoids	-	+	-	-	+	+	-	-

Table 3 : Antibacterial activity

Micro-organisms	Doses	Zone of inhibitions (mm)							
		Petroleum ether	Xylene	Benzene	n-Butanol	Methanol	Acetonitryl	Acetic acid	Water
Proteus mirabilis	A	10	15	12	21	25	23	22	-
	B	-	12	11	20	24	18	20	-
	C	-	10	-	19	22	16	19	-
Enterococcus	A	13	13	15	18	23	20	17	15
faecalis	B	11	11	13	17	20	16	13	14
	C	10	-	11	15	18	15	12	12
Bacillus cereus	A	15	11	17	20	21	19	21	17
	B	12	-	14	16	19	14	20	-
	C	10	-	13	15	18	10	17	-
Staphylococcus	A	14	18	16	15	24	18	13	14
aureus	B	12	14	15	14	17	15	11	12
	C	11	11	14	13	16	14	10	11
Klebsiella	A	15	18	28	23	30	22	-	-
pneumoniae	B	13	16	27	19	21	17	-	-
	C	11	15	25	17	20	15	-	-
Escherichia coli	A	13	11	15	19	27	18	-	-
	B	12	-	14	18	24	15	-	-
	C	-	-	13	16	22	11	-	-
Bacillus subtilis	A	11	12	13	15	21	18	18	13
	B	-	-	12	14	18	13	16	12
	C	-	-	10	13	14	11	15	11

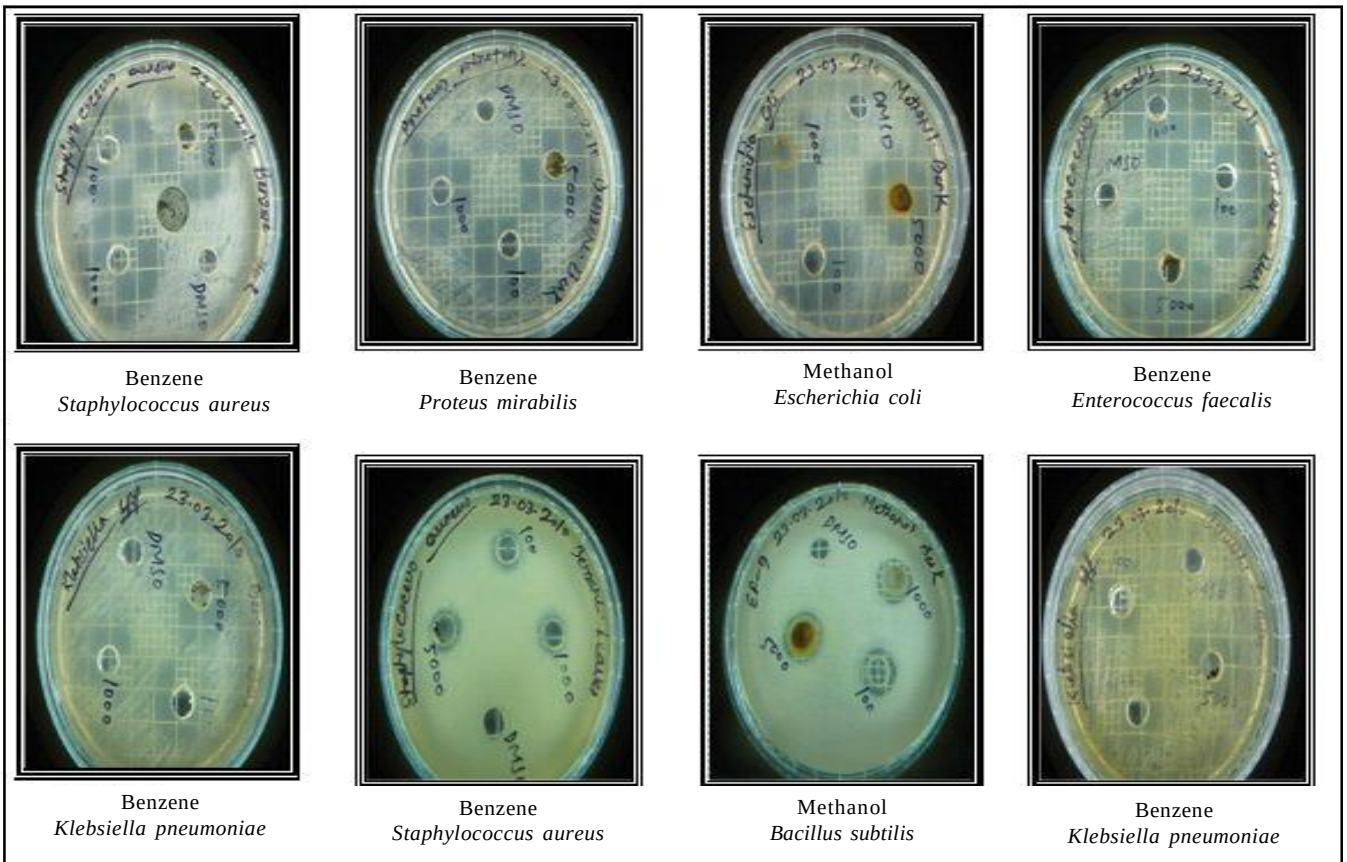

Fig. 1 : Antibacterial activity of leaves extract

of zero polar solvent (petroleum ether).

Phytochemical assay was performed following standard methods and the result shown in Table 2 where '+' and '-' explains the presence and absence of corresponding phytochemical, respectively. It is evident from the Table 1 that methanolic extract contains alkaloids and phenolics maximally, moderate amount of phytosterols, flavonoids and saponins, trace amount tannins.

Leaves extract of Alstonia scholaris showed broad spectrum of antimicrobial activity. Methanol extract of leaves showed activity against all the experimental bacterial strains. n-Butanol extract of leaves also showed broad spectrum of activity. Benzene extract of leaves showed huge zone inhibition against experimental strain of *Klebsiella pneumoniae* (Table 3 and Fig. 1).

(Here A stands for 1000µg, B stands for100µg, C stands for 10µg of doses, respectively).

Conclusion :

In present study, excellent medicinal properties was observed from Indian sub continental plant *Alstonia scolaris*. Phytochemicals like alkaloids, phenolics were observed in the extracts all most all extracts. Whereas among five extracts from leaves, methanol contain maximum number of phytochemicals like phenplics, tannins, alkaloids, flavanoids, saponins etc. The n-butanol, methanol extracts showed extra-ordinary antimicrobial activity against *Klebsiella pneumonia* ATCC 25926, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis.* These results initiate the further study to produce new set of antibacterials to combat many deadly deadly bacterial diseases of todays world.

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