Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com

Research Article

PHARMACOLOGICAL INVESTIGATION OF THE CHLOROFORM EXTRACTS OF *ALSTONIA SCHOLARIS* (L.) R.BR

Sayema Khanum*

Lecturer, Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh

*Corresponding Author Email: tamanna061@hotmail.com

DOI: 10.7897/2277-4572.03198

Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 28/12/13 Revised on: 03/01/14 Accepted on: 09/02/14

ABSTRACT

Chloroform extracts of *Alstonia scholaris* leaves (CLAS) and stem barks (CSAS) have been evaluated for their analgesic, antimicrobial, antioxidant and cytotoxic activity. Acetic acid induced writhing method was used for evaluating analgesic activity. Both the extracts had reduced pain in dose dependent manner, at all the tested doses (200 and 400 mg/kg body weight). Maximum writhing inhibition (75.33 %) was observed at 400 mg/kg dose of CSAS while for CLAS, 400 mg/kg dose exhibited 70.13 % inhibition. The inhibitory effect of indomethacin (45.92 %) was lower than that of the highest dose of CLAS and CSAS. Antimicrobial activity of the extracts was evaluated against various Gram-positive, Gram-negative bacteria and fungi using disk diffusion technique. The average zone of inhibition exhibited by extract was found 10-14 mm and Kanamycin (30 µg/disc) was used as standard. Antioxidant potentiality of the extracts was investigated on DPPH scavenging activity and the IC_{50} value was found 47.72 µg/ml, 62.03 µg/ml and 45.77 µg/ml for CSAS, CLAS and standard ascorbic acid, respectively. Cytotoxic study was done by brine shrimp lethality bioassay and compared with LC_{50} (8.90 µg/ml.) values of standard vincristin sulphate as a positive control. The cytotoxicity exhibited CLAS and CSAS were promising with LC_{50} value of 10.21 µg/ml and 9.12 µg/ml, respectively. These results suggest into the plant extracts could be used as a potential therapeutics in many pathological conditions.

Keywords: Acetic acid induced writhing, antimicrobial activity, antioxidant, free radical, brine shrimp lethality bioassay.

INTRODUCTION

Phytochemical aspects of most medicinal plants have been known and used since time memorial[1,2]. Ethanobotanical advantages conferred by these plant based products have surpassed the chemical counter parts owing to their lesser side effects and more potent therapeutic effect. Natural products continue to play the most significant role in the drug discovery and development process[3]. Hence it is a demanding need of the hour to study the various pharmacologically valuable aspects of these medicinal plants. *Alstonia scholaris* (Apocynaceae) also called 'Shaptaparna' or 'Devil's' tree is a large evergreen tree. It is commonly found in the subtropical regions of South Asia and Africa and is native to Bangladesh[4-9]. Almost all parts of the plants are used in medicine. Bark of *A. scholaris* possess spectrum of pharmacological activity ranging from bitter, astringent, thermogenic, febrifugic, digestive, laxative, antipyretic, anthelmintic to galactogoguic and cardiotonic properties, therefore used in fevers, malarial fevers, abdominal disorders, dyspepsia, leprosy, skin diseases, asthma, bronchitis, cardiopathy, helminthiasis etc.[10-13]. In folklore medicine, Latex obtained from the exudates of the tree has been in application for ulcers, sores, tumors and in rheumatoid pain; as well as mixed with oil and dropped into ear for earache[4,14,10]. Juices of the leaves and tincture of the bark act in certain cases as a powerful galactogogue. The drug is also used in cases of snake-bite[9,15,10]. Fruits are useful in syphilis and epilepsy and also used a tonic, anti periodic and anthelmintic[16]. Methanolic extracts of roots and flower have exhibited potent antimicrobial activity[5,6]. Leaves are used in the treatment of beri-beri, congestion of liver, dropsy and ulcers. In the China, the leaves have been historically used in 'dai' enthopharmacy to treat chronic respiratory diseases[17]. The leaf extract developed as a commercially available traditional Chinese medicine, used to release tracheitis and cold symptom[18]. In the quest of searching plants having significant pharmacological and biological activities in Bangladesh, therefore the present study is carried out to investigate the crude chloroform extracts of leaves and stem bark of *Alstonia scholaris* for its analgesic, antimicrobial, antioxidant and cytotoxic activities.

MATERIALS AND METHODS

Drugs and Chemicals

Acetic acid (Merck, Germany), Indomethacin (Square Pharmaceuticals Ltd.), Tween-80 (BDH Chemicals Ltd), Normal saline solution (0.9 % NaC1) (Beximeo Infusion Ltd.), DPPH (1, 1-diphenyl, 2-picryl hydrazyl) (Sigma Chemical Co., USA), Dimethyl sulfoxide (DMSO) (Merck, Germany) etc. were used for conducting the tests.

Test animals

Healthy Wister rats of either sex weighing about 135-150 g were used for the experiment. They were collected from the Animal Resource Branch of the "International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B)". They were kept in standard environmental condition (at 24.0 ± 0°C temperature and 55-65 % relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDRB formulated rodent food and water. The set of rules followed for animal experiment were approved by the institutional animal ethical committee[19].

Instruments

The molecular absorption spectra and absorbance at specific wavelengths were recorded with a HACH DR 4000U UV-visible spectrophotometer equipped with quartz cells of 1-cm light path.

Collection and preparation of plant material

The fresh plant *Alstonia scholaris* (leaves and stem) were collected from Chauddagram, Comilla, Bangladesh on October, 2011. The plant was identified by the taxonomist of

Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and a voucher specimen was deposited in the herbarium unit (accession number DACB: 37498). The sun dried powdered leaves and stems (250 mg) of *A. scholaris* were macerated in 500 ml of 99.8 % chloroform (Merck, Germany) separately. After 3 days of occasional shaking and stirring the solutions were filtered using filter cloth and Whatman® filter paper No. 1. The resulting filtrates were then evaporated in water bath maintained at 45°c to dryness and thus a gummy concentrate of greenish colored extract. Finally, about 45.23 g Chloroform extract of leaves of *Alstonia scholaris* (CLAS) and about 30.22 g Chloroform extract of stem bark of *Alstonia scholaris* (CSAS) was found.

***In vivo* analgesic activity test**

Acetic acid induced writhing method

The analgesic activity of the crude Chloroform extracts of CLAS and CSAS were studied using acetic acid induced writhing model in rat[20]. At first, thirty six animals were divided into six groups with six mice in each.

Group I: Treated with vehicle (1 % Tween 80 in water, 10 ml kg^{-1} (p.o.)
Group II: Received Indomethacin (10 mg/kg) body weight (p.o.)
Group III and Group IV: Treated with 200 and 400 mg kg^{-1} body weight (p.o.) of CLAS, respectively.
Group V and Group VI: Treated with 200 and 400 mg kg^{-1} body weight (p.o.) of CSAS, respectively.

The test samples and vehicle were administered orally 30 minutes before intraperitoneal administration of 0.7 % v/v acetic acid but Indomethacin (reference drug) was administered orally 15 minutes before injection of acetic acid. After an interval of 5 minutes, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 minutes. Full writhing was not always accomplished by the animal; this incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated groups was compared to that of a control group. Samples having analgesic activity will reduce number of writhes of treated mice. The percent inhibition (% analgesic activity) was calculated by

$$\% \text{ inhibition} = \{(A-B)/A\} \times 100$$

Where, A = Average number of writhing of control per group;
B = Average number of writhing of test per group

Antimicrobial assay

Microorganisms

Antimicrobial activity was tested against *Bacillus megaterium*, *B. subtilis, B. cereus, Staphylococcus aureus, Sarcina lutea, Vibrio mimicus*, *V. parahemolyticus, Pseudomonas aeruginosa, Escherichia coli, Shigella boydii, S. dysenteriae, Salmonella paratyphi, Saccharromyces cerevaceae*, *Candida albicans* and *Aspergillus niger*. These microbial strains were isolated from clinical samples and obtained as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Determination of the diameters of inhibition zone

The crude chloroform extracts of leaves and stem bark of *Alstonia scholaris* were tested *in vitro* for antimicrobial activity by the standard disc diffusion method[21,22] against the bacteria. Solutions of known concentration (500 µg/10 µl) of the test samples were made by dissolving measured amount of the samples (50 mg) in 1 ml of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances (500 µg/disc) using micropipette and the residual solvents was completely evaporated. Discs containing the test materials were placed on to nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4°C) for 16 hours to allow maximum diffusion of the test materials and kanamycin. The plates were then incubated at 37°C for 24 hours to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the mean value was taken.

Antioxidant study by DPPH free radical scavenging activity

The ability of ethanolic extracts of leaves and stem bark of *A. scholaris* to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described by Hasanuzzaman *et al.,*[23]. The percentage inhibition activity was calculated from the following equation.

$$\text{Percentage of inhibition} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (µg/ml) versus % inhibition.

Cytotoxic activity

The cytotoxicity was conducted using brine shrimp lethality test following the method of Meyer *et al*[24]. The brine shrimp eggs were placed in 1 liter of sea water, aerated and hatched for 48 hours at 37°C to become nauplii. After 48 hours, ten brine shrimp nauplii were placed in a small container filled with seawater. CLAS and CSAS, serially diluted with DMSO (Dimethyl sulfoxide), were then added to the container. The mortality of brine shrimp was observed after 24 hours of treatment was given. Vincristine sulphate was used as positive control. The lethal concentration (LC_{50}) of the test samples after 24 hours was determined by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis.

RESULTS

Analgesic activity

Acetic acid induced writhing method

The results showed that the pain relief was achieved significant in dose dependent manner, at all test doses (200 and 400 mg/kg body weight) as shown in Table 1. Maximum writhing inhibition (75.33 %) was observed at 400 mg/kg dose of CSAS while for CLAS, 400 mg/kg dose exhibited 70.13 % inhibition. The inhibitory effect of indomethacin (45.92 %) was lower than that of the highest dose of CLAS and CSAS.

Antimicrobial screening

The chloroform extracts of *A. scholaris* were screened against twelve human pathogenic bacteria to check antibacterial activities by disc diffusion method. The extracts showed mild to moderate activity against tested pathogenic bacteria which was shown in Table 2. CSAS showed moderate antibacterial activity against tested pathogenic bacteria. CLAS showed good antibacterial activity against Gram (-ve) pathogenic bacteria with an average zone of inhibition of 10-14 mm. This extract was found very active against *Shigella dysenteriae* (14 mm) and *Shigella boydii* (13 mm). No activity was found against *E. coli*, *B. subtilis*, *S. paratyphi* for both the extracts. The antifungal activity of CLAS and CSAS against tested fungus was shown in Table 3. Mild activity was found against *Sacharomyces cerevaceae* and no activity was found against *Aspergillus niger* and *Candida albicans*.

DPPH free radical scavenging activity

DPPH is most common stable radical commonly used in antioxidant assays. The percentage (%) scavenging of DPPH free radical was found to be concentration dependent i.e. concentration of the extract between 5-100 µg/ml greatly increasing the inhibitory activity (Figure 1). The IC_{50} of CSAS and CLAS was found 47.72 µg/ml and 62.03 µg/ml, respectively (Table 4). But both extracts had a lower scavenging activity than the standard ascorbic acid (IC_{50} = 45.77 µg/ml), which was used as standard.

Cytotoxic activity

In cytotoxic test activity, % mortality increased gradually with the increase in concentration of the test samples of both the extracts (Figure 2). Vincristine sulphate (VS) was used as positive control and the LC_{50} value was found 8.90 µg/ml. LC_{50} values obtained from the best-fit line slope were 10.21 µg/ml and 9.12 µg/ml for CLAS and CSAS respectively (Table 5).

DISCUSSION

In our present study the analgesic activity of *A. scholaris* extract was assessed by acetic acid induced writhing model. The acetic acid-induced writhing model is extensively used for the determination of analgesic activity because of its sensitivity and response to the molecules at a dose that is not effective in other models[25]. Acetic acid causes algesia by liberating endogenous substances that excite the pain nerve ending and also because of some other pain mediators like as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis[26]. It is also seen that the level of lipoxygenas enzyme in peritoneal fluids is also increased by acetic acid[27]. Substances that prohibit writhings must have significant analgesic activity which may be attributed by the inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition[25]. Several phytochemicals such as flavonoids, tannins, alkaloids etc. have been reported to possess analgesic activity[28]. The preliminary study on *A. scholaris* revealed that the plant is abundant of a wide spectrum of phytochemicals like tannin, carotene, phytosterol, resin, gum, isoflavonoids, alkaloids and saponins[8,15]. These compounds may attribute to the potent analgesic activity of the chloroform extracts of the plant. Bacteria and fungi are responsible for many infectious diseases. But bacteria are becoming resistant to conventional antibiotics[29] at an alarming rate. Antibacterial resistance has created desperate need for the search of new antibacterial[30,31]. The disc diffusion method had shown that the tested chloroform extracts of the steam barks and leaves of *A. scholaris* have moderate antimicrobial activity against the tested organisms. These extracts showed weak potentiality against tested fungus. The DPPH assay is one of the most common and relatively quick methods used for testing radical scavenging activity of various plant extracts[32]. In our present study, the antioxidant activity was determined using this method. Percentage inhibition of DPPH and IC_{50} are parameters widely used to measure antioxidant/free radical scavenging power[33,34]. The results of this study indicated that, the IC_{50} in chloroform extract of stem barks of *A. scholaris* was significantly lower than that of the leaf extract suggesting that the chloroform extract of leaves had better scavenging activity than the steam bark extract. It was reported that several active compounds such as anthrocyanins, saponins, tannins, flavones, and polyphenols etc. are responsible for demonstrating antioxidant activity of plant extracts[35]. *A. scholaris* is proved to be a potent source of flavonoid, saponins and tannins[8,15]. Therefore, it may be said that these compounds may play the significant role in revealing the antioxidant property of the plant extracts. The findings of the brine shrimp lethality bioassay method showed that CSAS possesses better cytotoxic activity in comparison to CLAS. Previous studies have proved that several bioactive compounds like glycosides, alkaloids, flavonoids and saponins show cytotoxic activities due to their diverse chemical compounds[36]. Some of these are present in the plant extract which may be accountable for the cytotoxicity of the plant extracts. However this type of cytotoxicity is non-specific. Thus further studies including animal model should be conducted to test the possible antitumor or anti-carcinogenic activity of the plant extracts.

Table 1: Effect of chloroform extracts of leaves and stem barks of *A. scholaris* on acetic acid induced writhing in rats

Groups	Treatment	Dose	No. of writhing	% Writhing inhibition
Group-I (Control)	1 % Tween 80 in water	10 ml/kg body weight	22.5	
Group-II (Standard)	Indomethacin	10 mg/kg body weight	12.167	45.92
Group-III	CLAS	200 mg/kg body weight	8.92	60.35
Group-IV	CLAS	400 mg/kg body weight	6.72	70.13
Group-V	CSAS	200 mg/kg body weight	8.45	62.44
Group-VI	CSAS	400 mg/kg body weight	5.55	75.33

Here, n = 6, CLAS = Chloroform Leaves extract of *A. scholaris*, CSAS = Chloroform Stem bark extract of *A. scholaris*

Table 2: *In vitro* antibacterial activity of chloroform extracts of *A. scholaris* leaves and steam barks

Test organism	Diameter of zone of inhibition (mm)		
	Chloroform extract of *A. scholaris* (CSAS)	Chloroform extract of *A. scholaris* (CLAS)	Kanamycin (30 µ/disc)
Gram positive bacteria			
Bacillus subtilis	-	-	30
Bacillus megaterium	10	7	32
Bacillus cereus	10	8	33
Staphylococcus aureus	10	8	29
Sereina lutea	7	8	31
Gram negative bacteria			
Vibrio mimicus	12	10	30
V. parahemolyticus	10	11	32
Pseudomonas aeruginosa	7	9	31
Escherichia coli	-	-	22
Shigella dysenteriae	8	14	25
Shigella boydii	10	13	30
Salmonella paratyphi	-	-	31

(-) = No activity

Table 3: *In vitro* antifungal activity of chloroform extracts *of A. scholaris* leaves and steam barks

Test organisms	Diameter of zone of inhibition	
	Chloroform extract of *A. scholaris* (bark)	Chloroform extract of *A. scholaris* (leaf)
Aspergillus niger	-	-
Sacharomyces cerevaceae	8	10
Candida albicans	-	-

(-) = No activity

Table 4: IC_{50} of *A. scholaris* extracts and Ascorbic acid

Sample	IC_{50} (µg/ml)
Ascorbic acid (Standard)	45.77
CLAS	62.0
CSAS	47.7

Table 5: Cytotoxic potential of chloroform extracts of leaves and stem barks of *A. scholaris* along with Vincristine sulphate

Sample	LC_{50} (µg/ml)	Regression Equation	R^2
Vincristine Sulphate	8.90	y = 36.594x + 22.387	0.993
CLAS	10.21	y =39.003x + 14.976	0.971
CSAS	9.12	y =37.524x + 23.635	0.973

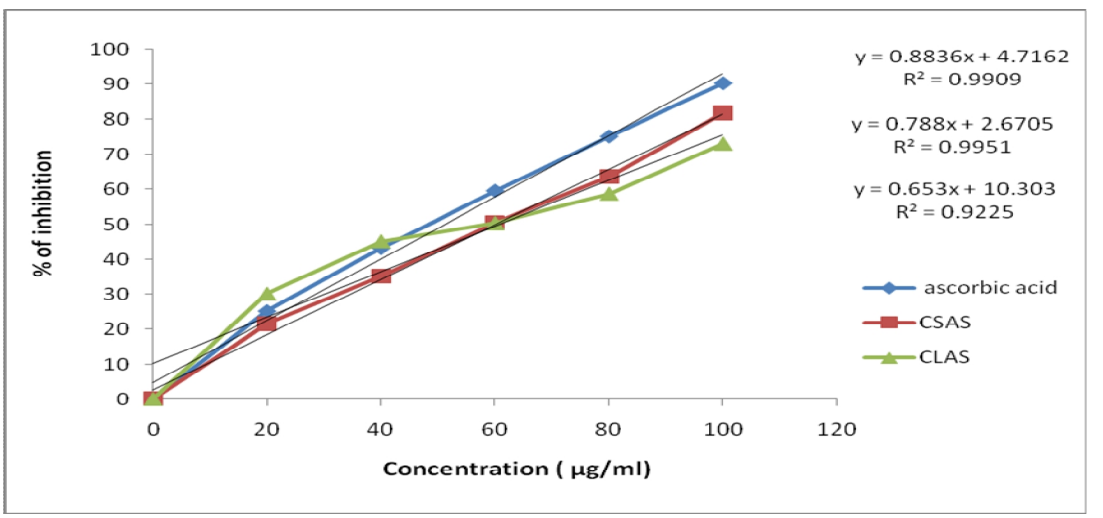

Figure 1: DPPH free radical scavenging activity of CLAS and CSAS extracts of *A. scholaris* along with ascorbic acid

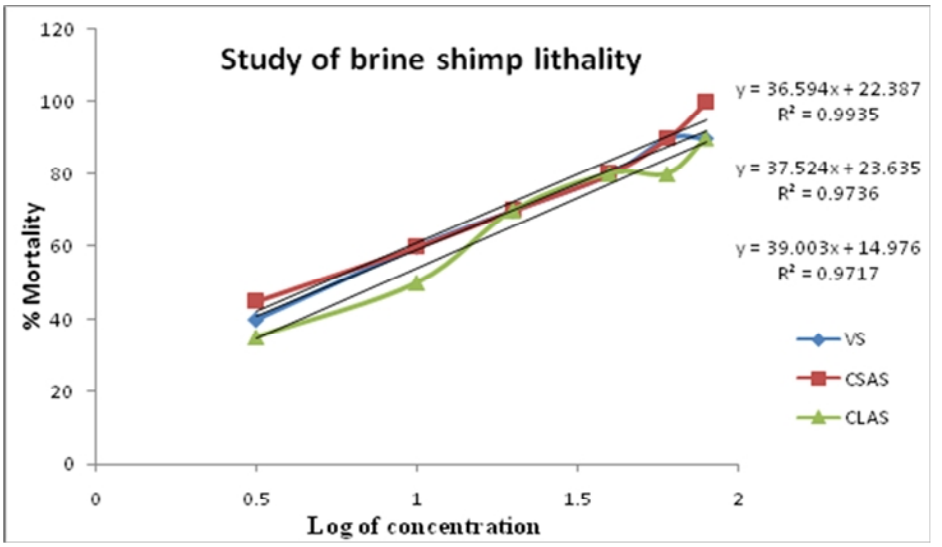

Figure 2: Determination of LC_{50} values for standard and crude chloroform extracts of steam barks and leaves of *A. scholaris* from linear correlation between logarithms of concentration versus percentage of mortality

CONCLUSION

Our preliminary pharmacological studies on the chloroform extract of *A. scholaris* leaves and stem barks provide in part scientific support for the use of this species in traditional medicine, particularly in various ailments related to pain. The non prescription use of medicinal plants is cited today as an important health problem, in particularly their toxicity to the kidney. So, if the plant extract found to show significant antimicrobial activities must take into account acceptable levels of toxicity. Therefore, further pharmacological investigations are required to understand the underlying mechanism of these pharmacological activities.

ACKNOWLEDGEMENTS

The work was financially supported by the department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh.

REFERENCES

1. Misra CS, Pratyush K, Sagadevan LDM, James J, Veettil AKT and Thankamani V. A comparative study on phytochemical screening and antibacterial activity of roots of *Alstonia scholaris* with the roots leaves and stem bark. International Journal of Research in Phytochemistry and Pharmacology 2011; 1(2): 77-82.
2. Misra CS, Pratyush K, Sagadevan LDM, James J, Veettil AKT and Thankamani V. Analgesic Activity Of The Methanolic Extract of *Alstonia scholaris*. Int. Res. J. Pharm 2011; 2(8): 117-118.
3. Newman DJ and Cragg GM. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod 2007; 70: 461-477. http://dx.doi.org/10.1021/np068054v
4. Antony M, James J, Misra CS, Sagadevan LDM, Veettil AKT and Thankamani V. Anti-mycobacterial activity of the plant extracts of *Alstonia scholaris*. International Journal of Current Pharmaceutical Research 2012; 4(1).
5. Thankamani V, James J, Veettil AKT and Sagadevan LDM. Phytochemical screening and anti microbial activity of *Alstonia scholaris* flowers (L) R.BR. Fam: Apocynaceae. International Journal of Pharmaceutical Research And Development 2011; 3(3): 172-178.
6. Thankamani V, Pratyush K, Misra CS, James J, Sagadevan LDM, Veettil AKT. FT-IR Analysis and *in vitro* Cytotoxicity Assay of Methanolic Extract of Roots of *Alstonia scholaris*. International Journal of Institutional Pharmacy and Life Sciences 2011; 1(1): 53-67.
7. Khyade MS and Vaikos NP. Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br. African Journal of Biotechnology 2009; 8(22): 6434-6436.
8. Pratap B, Chakraborthy GS, Mogha N. Complete Aspects of *Alstonia Scholaris*. International Journal of Pharm Tech Research 2013; 5(1): 17-26.
9. Ganjewala D and Gupta AK. Study on Phytochemical Composition, Antibacterial and Antioxidant Properties of Different Parts of *Alstonia scholaris* Linn. Advanced Pharmaceutical Bulletin 2013; 3(2): 379-384.
10. Nadkarni AK. Dr KM Nadkarni's Indian Materia Medica, Vol. 1, Popular Prakashan, Bombay, India; 1976. p. 80-83.
11. Kirtikar KR and Basu BD. Indian Medicinal Plants, Vol. 1, Lalit Mohan Basu, Allahabad, India; 2002. p. 111-4.
12. Arulmozhi S, Mazumder PM, Narayan LS, Thakurdesai PS. Analgesic, anti-inflammatory and anti-ulcerogenic activities of fractions from *Alstonia scholaris*. Pharmacologia 2012; 3(5): 132-137. http://dx.doi.org/10.5567/pharmacologia.2012.132.137
13. Arulmozhi S, Mazumder PM, Narayan LS, Thakurdesai PA. *In vitro* antioxidant and free radical scavenging activity of fraction from *Alstonia scholaris* Linn. R.Br. International Journal of Pharm Tech Research 2010; 2(1).
14. Daniel M. Medicinal plants: Chemistry and Properties, USA: Science Publisher; 2006. p. 24-25. http://dx.doi.org/10.1201/b11003
15. Dey A. *Alstonia scholaris* R.Br. (Apocynaceae): Phytochemistry and pharmacology, a concise review. J Appl Pharm Sci 2011; 1(6): 51-7.
16. Pankti K, Payal G, Manodeep C, Jagadish K. A phytopharmocological review of *Alstonia scholaris*: A panoramic herbal medicine. Int. J. Res. Ayurveda Pharm 2012; 3(3): 367-7.
17. Compiling Group of Yunnan Traditional Chinese Medicine, Yunnan Traditional Chinese Medicinal Plant. Yunnan People's Press, Kunming; 1977.
18. Shangb JH, Caia XH, Fenga T, Zhaob YL, Wangb JK, Zhangc LY, Yanc M, Luo XD. Pharmacological evaluation of *Alstonia scholaris*: Anti-inflammatory and analgesic effects. Journal of Ethno pharmacology 2010; 129: 174–181. http://dx.doi.org/10.1016/j.jep.2010.02.011
19. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16(2): 109-110. http://dx.doi.org/10.1016/0304-3959(83)90201-4
20. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. Bri J Pharmacol Chemother 1964; 22: 246-249. http://dx.doi.org/10.1111/j.1476-5381.1964.tb02030.x
21. Bauer AW, Kirby WMM Sherries and M Tuck. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol 1966; 36: 493-496.
22. Rahman MS and MA Rashid. Antimicrobial activity and cytotoxicity of *Eclipta prostrate*. Oriental Pharm. Exp. Med 2008; 8: 47-52. http://dx.doi.org/10.3742/OPEM.2008.8.1.047
23. Hasanuzzaman M, Ali MR, Hossain M, Kuri S, Islam MS. Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of *Averrhoa bilimbi* (fruits). Int Curr Pharm J 2013; 2: 92-96. http://dx.doi.org/10.3329/icpj.v2i4.14058
24. Meyer BN, NR Ferrigni, JE Putnam, JB Jacobsen, DE Nicholsand and JL Mclaughlin. Brine shrimp; a convenient general bioassay for active

plant constituents. Planta. Med 1982; 45: 31-34. http://dx.doi.org/10.1055/s-2007-971236
25. Muhammad N, Saeed M, Khan H. Antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. BMC Comple Alter Med 2012; 12: 59-63. http://dx.doi.org/10.1186/1472-6882-12-59
26. Khan H, Saeed M, Gilani AUH, Khan MA, Dar A, Khan I. The antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models. J Ethno pharmacol 2010; 127: 521–527. http://dx.doi.org/10.1016/j.jep.2009.10.003
27. Duarte I, Nakamura M, Ferreira S. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz J Med and Bio Res 1988; 2: 341-345.
28. Zulfiker AHM, Rahman MM, Hossain MK, Hamid K, Mazumder MEH, Rana M. *In vivo* analgesic activity of ethanolic extracts of two medicinal plants - *Scoparia dulcis* L. and *Ficus racemosa* Linn. Biol and Med 2010; 2: 42-48.
29. Kumarasamy KK, Toleman MA, Walsh TR, Bangari J, Butt F, Balkrishnan R *et al*. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. The Lancet Infectious Disease 2010; 10(9): 597. http://dx.doi.org/10.1016/S1473-3099(10)70143-2
30. Ghosh A, Das BK, Roy A, Mandal B and Chandra G. Antibacterial activity of some medicinal plant extracts. Journal of Natural Medicine 2008; 62: 259. http://dx.doi.org/10.1007/s11418-007-0216-x
31. Vento S and Cainelli F. The need for new Antibiotics. The Lancet 2010; 375(9715): 637-638. http://dx.doi.org/10.1016/S0140-6736(10)60265-6
32. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J Food Compos Anal 2007; 20: 337-345. http://dx.doi.org/10.1016/j.jfca.2006.07.003
33. Qian H and Nihorimbere V. Antioxidant power of phytochemical from *Psidium guajava* leaf. Zheijiang Uni Sci 2004; 5(6): 676-683. http://dx.doi.org/10.1631/jzus.2004.0676
34. Olaleye SB, Oke JM, Etu AK, *et al*. Antioxidant and anti-inflammatory properties of a flavonoid fraction from the leaves of *Voacanga africana*. Nigerian J Physiol Sci 2004; 19(1, 2): 69-76.
35. Firdaus M, Prihanto AA, Nurdiani R. Antioxidant and cytotoxic activity of *Acanthus ilicifolius* flower. Asian Pac J Trop Biomed 2013; 3: 17-21. http://dx.doi.org/10.1016/S2221-1691(13)60017-9
36. Vital PG and Rivera WL. Antimicrobial activity, cytotoxicity, and phytochemical screening of *Voacanga globosa* (Blanco) Merr. leaf extract (Apocynaceae). Asian Pac J Trop Med 2011; 4: 824-828. http://dx.doi.org/10.1016/S1995-7645(11)60202-2

Source of support: Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
Sayema Khanum. Pharmacological investigation of the chloroform extracts of Alstonia scholaris (L.) R.BR. *J Pharm Sci Innov*. 2014;3(1):14-19 http://dx.doi.org/10.7897/2277-4572.03198