IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2020 Vol.:19, Issue:4

Evaluation of *In-Vitro* Anti-Lice and Anthelmintic Study of Methanolic Barks Extracts of *Alstonia scholaris L.*

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Muhammad Kamal Hossain*[1], Md. Sohanur Rahman[1], Amzad Hossain Siddique[1], Md. Rashadul Alam, Tanwy Chowdhury, Md Ziaur Rahman

Assistant Professor
Department of Pharmacy
Faculty of Basic Medical and Pharmaceutical Sciences
University of Science and Technology Chittagong.
Bangladesh

Submission: 23 October 2020
Accepted: 29 October 2020
Published: 30 November 2020

Keywords: *Alstonia scholaris*; anthelmintic; anti-lice

ABSTRACT

Alstonia scholaris (L.) R. Br. is an ethnomedicinal plant used traditionally in the treatment of various illnesses. The present investigation aimed to elucidate the *in-vitro* evaluation of the anthelmintic and anti-lice activity of methanolic extract of *Alstonia scholaris* (MEAS). Average mortality method and death & paralysis count method were followed for anthelmintic and anti-lice activity, respectively. Treatment with MEAS showed dose-dependent mortality on anti-lice activity when compared to standard agent benzoate 20%. Besides, the oral administration of MEAS revealed promising anthelmintic activity on higher doses as compared to standard drug. Taken together, it can be concluded that MEAS can be a source for the treatment of both anti-lice and anthelmintic activity.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

Plants produce various bioactive phytochemicals which can be grouped under two categories; primary and secondary metabolites. Primary metabolites include proteins, carbohydrates, amino acids and chlorophyll while polyphenols, alkaloids, terpenoids are some examples of secondary metabolites (A Ghani *et al*, 2003).Secondary metabolites are the chemicals that are not required for the immediate survival of the plant but synthesized to increase the survival of the plant by allowing it to interact with pathogens, herbivores insects and environment. Secondary metabolites such as alkaloids, glycosides, flavonoids ,steroids, saponins and terpenoids play an important role in the protection of the plant from environmental stress, attacks of pathogens and insect pests. Due to presence of these bioactive phytochemicals, plants provide a source of medicine since historic times and now these are an important part of all the world's pharmaceuticals and serve as starting material for drug development. Phytochemicals are reported to have various health promoting effects. Studies also suggest that these natural phytochemicals modulate various molecular signal transduction pathways, involved in the phenomenon of inflammation, thereby preventing the onset of various chronic diseases like cancer, atherosclerosis, neuro degradation, obesity, articular rheumatism, skin aging and diabetes. Most phytochemical function as antioxidants in vitro and they can reduce oxidative stress and inflammation which are involved in the progression of type II diabetes mellitus.(AL Ramma *et al,* 2003). Plants are used for variety of purposes. The history of natural product is relatively old and dates back to the time when the early man became conscious of his environment. Cultured and civilized man is said to have been on earth for some two or three million years and he has struggled for his life during the greater portion of the era.(JH Maiden *et al*,,1889) Thousands of years' effort, by examination much has thought him to differentiate between useful and harmful plants. Since then herbs have been used in all cultures as an important source of medicine. A new report by UK-based Botanic Gardens Conservation International warns that many plants and organisms are being lost through the destruction of rainforests, coral reefs and other natural habitats. This trend will affect the discovery of cancer drugs, according to the report (AL Ramma *et al*,,2003).

"Many medicinal plants are being destroyed at an unprecedented rate and are threatened with extinction," said Belinda Hawkins, author of the study, "Plants for Life: Medicinal Plant Conservation and Botanic Gardens," which was published in January. "The destruction of

plant species is occurring at a rate unmatched in geological history. Current extinction rates are at least 100 to 1,000 times higher than natural background rates, with a quarter of the world's coniferous trees in jeopardy, and as many as 15,000 medicinal plants threatened," the author writes.

MATERIALS AND METHODS

Identifications & Collections

The plant named *Alstonia scholaris* commonly known medicinal plant for its various type of pharmacological effect in human body. This plant was first introduced by Robert Brown of Edinburgh in 1811. The barks of *Alstonia scholaris* were collected for this project work from forest located at Moulovibazar, Sylhet. The barks were then cleaned properly**.**

Extractions

700gm of dried powder of seeds was weighted & taken in two different aspirator bottle. Before placing the powders into the aspirator, the jar was washed properly and then dried.1000ml of solvent i.e. Methanol was added gradually in both of the container. The container with its content was sealed on & kept for 30 days with occasional shaking and stirring. The major portion of the extractable compound of the seeds were dissolved in the solvent. Then the whole mixture was filtered through cotton wool and the filtrate was concentrated by evaporating in dry & clean air. (RA Butt et al,2017).

Collections of head lice

Adults, nymphs, and nits of *P. humanus capitis* were collected from children the age 13 by combing through sections of the scalp using a clean comb. After combing, the lice were carefully removed from the teeth of the comb into plastic boxes. All the subjects had not been treated with any anti-lice products.

Collections of Earthworms

The earthworms Pheretima posthuma were used to study the anthelmintic activity. Earthworms were collected from Nazirhat, Chattogram. The worms were washed with normal saline to remove all the fecal matter. (AL Ramma et al, 2003). The earthworms

resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence where used to study the anthelmintic activity.

Anti-lice Investigations

Average Mortality Method

Methanol extracts of *A.scholaris* were tested for pediculocidal activity by filter paper diffusion method. All the extracts were dissolved in distilled water to obtain 3 different concentrations (5%, 10%, and 20%). After careful selection under a dissecting microscope, the adults and nymphs were identified and separated. All the test organisms in a ratio of 3.6/1.4 (adult/nymph) were divided into 7 groups (5 lice each) and were placed on a filter paper at the bottom of petri dish and kept open. A 0.5 ml of each test samples was poured on the test organisms and allowed to spread as a thin layer of 4 cm2. Group 1 was treated with 0.5 ml distilled water and served as control. Group 2 to group 4 received 0.5 ml of various concentrations of methanol extracts respectively. Group 5 to group 7 were treated with 0.5 ml of 5%, 10%, and 20% of benzyl benzoate 25% (w/v). All the Petri dishes were set aside for 1 hr in a dark chamber at 26 ± 0.5℃ and 70 ± 1% humidity. At the end of 1 hr, the dishes were taken out and applied 0.5 ml of distilled water and further placed in the chamber under the condition mentioned above. After 18 hr, the dishes were observed under a dissecting microscope for any possible movement of lice and absence of any movement were considered dead. All the treatment was triplicate. [(AJS Samuel *et al*,,2009) (C Rossini *et al*,,2008) (S Upadhyay *et al*,,2011)]

Anthelmintic investigations

Death & Paralysis count Method

The anthelmintic activity was carried out on adult earthworm (*Pherithema posthuma*) with minor modifications. The earthworms of equal sizes were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. The worms were divided into two groups of three adult earthworms. Stock solutions (100mg/20ml, 200mg/20ml) of the methanolic extract and albendazole tablets of 200 mg were prepared separately by dissolving each in distilled water as vehicle. Different of concentrations of 5 and 10mg/ml in normal saline were prepared by drawing different volumes from the stock solutions. Groups of earthworms were released into

the petridish containing desired concentration as made above. Group one serve as standard, received standard drug Albendazole tablets and group two for Methanolic extract of Alstonia scholaris barks. (AR Ramma et al, 2003). Observations were made for the time taken to cause paralysis and death of individual worms. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli.

RESULTS

Anti-lice investigations

All the extracts displayed concentration (5%, 10%, and 20%) dependent activity among which methanol extract with 20% concentration showed higher mortality followed by 5% and 10% extracts, respectively and was well comparable with the standard. Water extract in various concentrations showed minimal anti-lice activity (Figure 1).

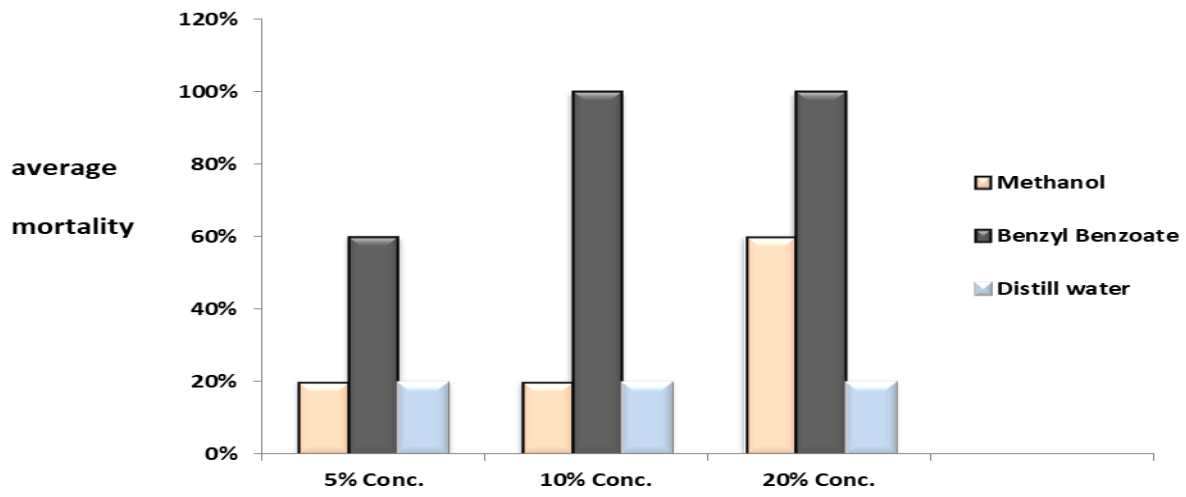

Figure No. 1: Effects of *A. scholaris* barks extracts against *P. humanus capitis*

Table No. 1: Effects of *A.scholaris* barks extracts against *P. humanus capitis*

Test Sample	Average Mortality
Distilled Water (.5 ml)	**20%**
5% Extract	**20%**
10% Extract	**20%**
20% Extract	**60%**
Benzyl Benzoate (5%)	**60%**
B.Benzoate (10%)	**100%**
Benzoate (20%)	**100%**

Anthelmintic Investigations

All the extracts displayed concentration (5 mg/ml, 8 mg/ml, and 10 mg/ml) dependent activity among which methanol extract with 10 mg/ml concentration showed early death & paralysis followed by 5 mg/ml and 8 mg/ml extracts, respectively and was well comparable with the standard. (Figure 2).

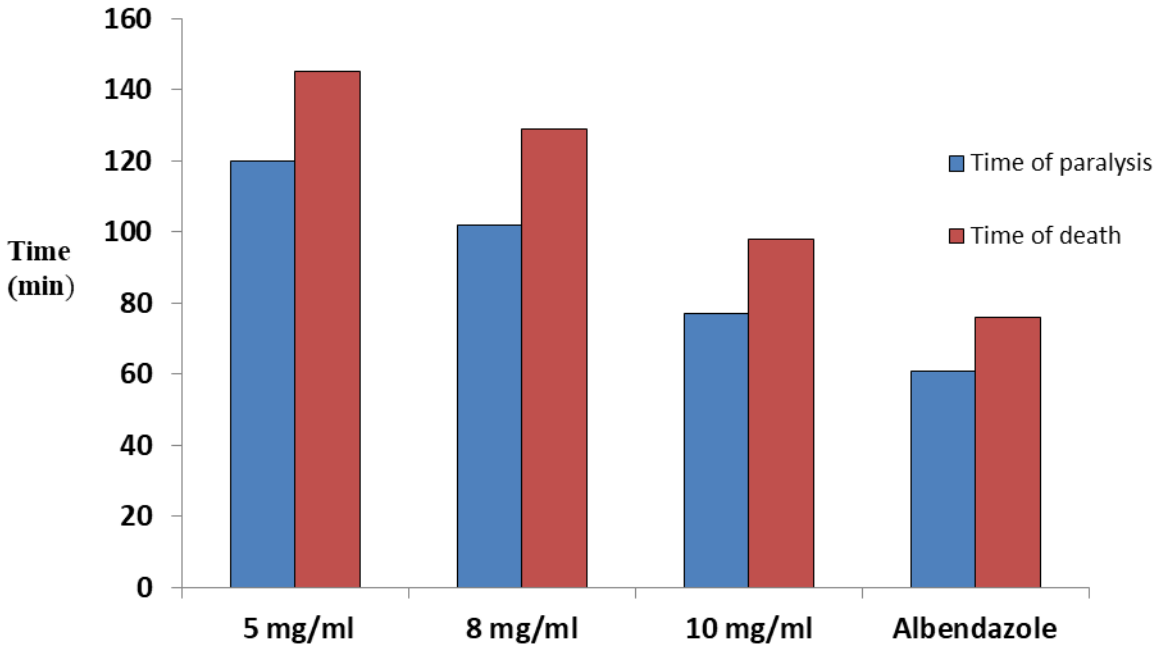

Figure No. 2: Effects of *A. scholaris* barks extracts against *P. Posthuma*

Table No. 2: Effects of A.scholaris barks extracts against *P. Posthuma.*

Treatment	Conc. (mg/ml)	Time of Paralysis (min) (TP)	Time of Death (min) (TD)
Extract (1)	**5**	**120 ± 8**	**145± 4**
Extract (2)	**8**	**102±3**	**129±6**
Extract (3)	**10**	**77±5**	**98±7**
Albendazole	**10**	**61±4**	**76±3**

DISCUSSION

The findings of this study showed moderate anti-lice activities of methanol extract of A. scholaris. Penetration of extracts into the alimentary tract of lice could be ignored since all the extracts was applied on lice placed on the filter paper which also subsequently avoided immense dissemination of active constituents into the cuticle when the compound is directly applied to the insect skin. Additionally, the lice was not exposed in an enclosed environment with the petri dish kept open which limits the possibility of volatile agents getting absorbed through the spiracles. For synthetic pediculocidal agents, the residue which remains in the head even after rinsing with water gives an enhanced control against lice but also noted for the development of resistance for lice. Natural extracts from medicinal plants has been noticed for its safe and effective use, and appearance of resistance patterns were minimal due to its different mode of action. Among all the extracts concentration, 20% conc. exhibited the maximum effects and moderately inhibited nymph emergence. Hence, the results obtained from this research may present a promising scenario for using *A.scholaris* bark extract as an effective alternative for treating human head lice.

Again in case of anthelmintic investigations of methanolic extract of *Alstonia scholaris* barks showed moderate anthelmintic activity at higher concentration than lower concentration of barks extracts. Among all the extracts concentration, 10 mg/ml conc. exhibited the maximum effects and result to early death and paralysis other than 5 mg/ml and 8 mg/ml. Hence, the results obtained from this research may present a promising scenario for using *Alstonia scholaris* bark extract as an effective alternative for anthelmintic purposes only at higher concentration.

CONCLUSIONS

There are presence of moderate anti-lice activity at higher concentration of methanolic extract of *Alstonia scholaris* barks where less effects at lower concentrations. And also there are presence of moderate to less anthelmintic activity at high concentration to low concentration of methanolic extract of *Alstonia scholaris* barks. So, our future motive is to find out a comparative study for all in-vitro & in-vivo approaches of the methanolic extracts of the *Alstonia scholaris* barks**.**

ACKNOWLEDGEMENT

This research is supported by Pharmacy Research Group, Department of Pharmacy, University of Science and Technology Chittagong, Bangladesh.

REFERENCES

1. A Ghani: Medicinal Plants of Bangladesh: Chemical Constituents & Uses. Asiatic Society of Bangladesh, Dhaka, Second Edition 2003;500-504
2. AJ Paul, MD Adnan, M Mojumder et al. Anthelmintic activity of Piper sylvaticum Roxb. (family: Piperaceae): In vitro and in silico studies. Clinical Phytoscience, 2018, 4(1), 17
3. AJS Samuel, S Radhamoni, R Gopinath et al. In vitro screening of anti-lice activity of Pongamia pinnata leaves. 2009, 47(4), 377
4. AL Ramma, T Bahorun, A Crozier. "Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits". Journal of the Science of Food and Agriculture.2003, 83(5): 496–502. doi:10.1002/jsfa.1365.
5. Arulmozhi, Papiya, Purnima et al; Pharmacological Activities of Alstonia scholaris linn. (Apocynaceae) - A Review, Pharmacognosy Reviews,2007;1,1,163-170
6. Bundy DA. Immuno epidemiology of intestinal helminthic infection,the global burden of intestinal nematode disease. Trans R Soc Trop Med Hyg 1994; 88: 259-261.
7. C Rossini, L Castillo, A Gonzalez. Plant extracts and their components as potential control agents against human head lice. Phytochemistry Reviews 2008, 7 (1), 51
8. Chen, Jie & Craven, Lyn A., "Syzygium", in Wu, Zhengyi; Raven, Peter H. & Hong, Deyuan (eds.), Flora of China (online), eFloras.org, retrieved 2015-08-13
9. GC Jagetia, MS Baliga. Evaluation of anticancer activity of the alkaloid fraction of Alstonia scholaris (Sapthaparna) in vitro and in vivo. Phytother. Res. 2006,20(2),103-109
10. JH Maiden, Turner, Henderson. The useful native plants of Australia: Including Tasmania. Turner and Henderson, Sydney. 1889, Food and Drug Subdivisions.
11. JH Shung, XH Cai, YL Zhao et al, Pharmacological evaluation of Alstonia scholaris: anti-tussive, anti-asthmatic and expectorant activities. J. of Ethno-pharmacology 2010,129(2),174-181
12. Kulkarni, Juvekar, Archana et al. Effect of Alstonia scholaris (Linn.) R. Br. on stress and cognition in mice. Indian j. Exp. Biol. 2009; 47-52
13. MS Baliga. Alstonia scholaris Linn R Br in the treatment and prevention of cancer: past, present, and future. Integr. Cancer Ther. 2010,9 (3),261-269
14. M Saxena, J Saxena, R Nema et al. Phytochemistry of medicinal plants. J. of Pharmacognosy & Phytochemistry, 2013, 1(6)
15. MS Baliga. Review of the phytochemical, pharmacological and toxicological properties of Alstonia scholaris Linn. R. Br (Saptaparna) Chin. J integer. Med. 2012,1-14

16. MS Khyade, DM Kasote, NP Vaikos. Alstonia scholaris (L.) R. Br. and Alstonia macrophylla Wall. ex G. Don: A comparative review on traditional uses, phytochemistry and pharmacology. J. Ethno-pharmacology, 2014,153(1),1-18
17. Pratap, Chakraborthy, Nandini. Complete aspects of Alstonia scholaris. Pharmtech, 2013, Vol-5,17-26, ISSN 0974-4304
18. RA Butt, M Sadiq; IU Haq et al. "Jamun seed and fruit extract attenuate hyperglycemia in diabetic rats".Asian Pacific Journal of Tropical Biomedicine. 2017, 7(8): 750–754. doi:10.1016/j.apjtb.2017.07.006
19. S Jahan, R Chaudhary, PK Goyal. Anticancer activity of an Indian medicinal plant, Alstonia scholaris, on skin carcinogenesis in mice. Integr. Cancer Ther. 2009, 8(3), 273-279
20. SJ Lee, SA Cho, SS An et al. Alstonia scholaris R. Br. significantly inhibits retinoid-induced skin irritation in vitro and in vivo. Hindawi, 2012, 11
21. S Upadhyay, AK Ghosh, VJ Singh. Anti-lice activity of Abrus precatorius Linn (Family- Fabaceae) seed oil. Egyptian Dermatol J 2011,7(2), 4