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Preliminary Phytochemical Screening and *In Vitro* Evaluation of Anthelmintic Activity on *Alstonia scholaris* R. Br. (Apocynaceae) Leaves Extract

Dr. Nilip Kanti Deb¹, Dr. Jhuma Deb², Soumya Saha^{*3}, Doyel Mukherjee⁴, Saheli Chakraborty⁵, Sourav Chakraborty⁶

¹Professor, Department of Pharmacognosy, Vidyasagara Pharmaceutical College of Education, Shimurali, West Bengal, India. Pin. – 741248. E-mail: nilipkdeb@gmail.com

²Professor, Department of Pharmacognosy, Netaji Subhas Chandra Bose Institute of Pharmacy, Tatla, Chakdaha, Nadia, West Bengal, India. Pin. – 741222. E-mail: jhumaniladi@gmail.com

³Assistant Professor, Department of Pharmaceutical Chemistry, Netaji Subhas Chandra Bose Institute of Pharmacy, Tatla, Chakdaha, Nadia, West Bengal, India. Pin. – 741222. E-mail: soumya_saha@yahoo.com

⁴Assistant Professor, Department of Pharmacognosy, Netaji Subhas Chandra Bose Institute of Pharmacy, Tatla, Chakdaha, Nadia, West Bengal, India. Pin. – 741222. E-mail: doyelmukherjee98@gmail.com

⁵Assistant Professor, Department of Pharmacognosy, Netaji Subhas Chandra Bose Institute of Pharmacy, Tatla, Chakdaha, Nadia, West Bengal, India. Pin. – 741222. E-mail: sahelichakraborty112@gmail.com

⁶Assistant Professor, Department of Pharmacology, Global college of pharmaceutical technology, Krishnagar, Nadia, West Bengal, India. Pin. – 701101. E-mail: souravchakraborty2606@gmail.com

Correspondence Author

Soumya Saha^{*}

Assistant Professor, Dept. of Pharmaceutical Chemistry

Netaji Subhas Chandra Bose Institute of Pharmacy, Tatla, Chakdaha, Nadia, West Bengal, India. Pin. – 741222.
Mail Id. – soumya_saha@yahoo.com Ph. No. – 7501995012

Abstract

In recent times, there has been an increase in the validation of traditional medicinal practices involving medicinal plants, evident from numerous ethnomedicinal papers. *Alstonia scholaris* R. Br. (Apocynaceae), stands out among the various plants with promising ethnomedicinal properties, particularly in claims against Helminthes. Helminthes are primarily parasitic worms that typically infect animals including humans. The present study therefore aims to validate the ethnomedicinal claims of this plant against Helminthes. Moisture content, Ash & Extractive value, preliminary phytochemical screening and Anthelmintic activity of chloroform, ethanolic and aqueous extract was carried out on *Pheretima posthuma* against the reference drug Albendazole.

The presence of carbohydrates, alkaloids, amino acids, flavonoids, tannin and protein was observed. Significant Anthelmintic properties were observed at higher concentration. Different extracts caused paralysis in 34.32 min, 17.29 min and 24.28 min whereas death time observed was 85.35 min, 33.32 min. and 42.31 min in 80 mg/ml concentration respectively against the reference drug at 40 mg/ml concentration has shown paralysis at 10.07 min and occurrence of death at 21.34 min. The results suggest that further studies may be carried out to isolate and evaluate the active constituents responsible for the activity.

Keywords: *Alstonia scholaris* R. Br., *Pheretima posthuma*, Preliminary phytochemical studies, Flavonoids, Anthelmintic.

Introduction

Helminthic infections are among the most common infections in human beings, affecting a large proportion of the world's population. In developing countries, they pose a large threat to public health and contribute to the prevalence of anaemia, malnutrition, eosinophilia and pneumonia. Although the majority of infections due to worms are generally limited to tropical countries, they can occur to travellers, who have visited those areas and some of them can be developed in temperate climates too [1]. The Helminthes which infect the intestine are cestodes e.g. Tapeworms (*Taenia solium*), Nematodes e.g. hookworm (*Ancylostoma duodenale*), Roundworms (*Ascaris lumbricoids*) and Trematodes or Flukes (*Schistosoma mansoni* and *Schistosoma hematobolium*) and Earth worms (*Pheretima posthuma*). The diseases originated from parasitic infections causing severe morbidity include lymphatic filariasis, onchocerciasis and schistosomiasis which can affect most populations in endemic areas with major economic and social consequences. Helminthes also affect millions of livestock resulting in considerable economic losses in domestic and farm yard animals because of limited availability and affordability of modern medicines. Most of the world's population depends to a greater extent on traditional medical remedies. The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in tropical developing countries, including India. It is in this context that the people consume several plants or plant-derived preparations to cure helminthic infections [2].

Alstonia scholaris R. Br., (Apocynaceae), commonly known as "Saptaparni" or "devil tree" is a perennial, evergreen, herbaceous medicinal plant well known for its traditional uses. This plant is a local of India along with South East Asian palm-ruled woodlands and in Notophyll forests. The species can be developed in an assortment of climatic conditions going from dry tropical to sub-temperate in the red alluvial soil having optimum air circulation [3]. The active constituents reported in this plant include alkaloids, flavonoids, phenols, glycosides, tannins, saponins, lignin and carbohydrates [4]. A number of pharmacological activities are reported in this plant like anti-diabetic, anti-inflammatory, anti-oxidative, anti-bacterial, anti-microbial, anti-fungal, anti-spasmodic, ant nociceptive, anti-viral, diuretic, and anthelmintic and urinary tract disorder [5]. Traditionally the powdered bark is used for curing malaria, diarrhea, and dysentery [6]. The leaves are used for curing cough, chronic bronchitis, asthma, fever and other respiratory infections [7]. From Literature review it is evident that different parts of *Alstonia scholaris* like leaves, ripe fruits, milky juice, latex, bark, flower, roots as well as the decoction of the whole plant is traditionally used as an aphrodisiac, stomachic,

dyspepsia and in Helminthiasis [6, 8, 9]. Because of its remarkable traditional uses, the present study is aimed at providing scientific evidence to the ethnomedicinal claim for its uses as an anthelmintic.

Materials and Methods

Plant Materials:

Fresh leaves of the plant *Alstonia scholaris* R. Br. was collected from the Medicinal Garden of Netaji Subhas Chandra Bose Institute of Pharmacy, Tatla, Chakdaha, Nadia, West Bengal, India, during the month of January 2024 and authenticated by Dr. Suchandra Samanta Mandal, M.Sc. (Botany), KU, M.Phil. (Education), KU, Assistant Professor, K. Bed College, Krishnanagar, West Bengal, India.

Chemicals and reagents:

The chemicals, solvents and reagents used in the study were of standard analytical grade obtained from S.D Fine Chem Ltd., Mumbai and Loba Cheme, Mumbai.

Preparation of extract:

The plant material was dried thoroughly in shade condition at room temperature. It was then subjected to size reduction. 250 gm of the plant material was defatted with petroleum ether. The marc was thoroughly dried and subjected to cold maceration process with different solvent (12 hrs. each) like chloroform, ethanol and water. The extracts obtained were dried at temperature below 40° C to obtain concentrate of the crude extract [10, 11]. The extract was kept in a suitable container with proper labelling inside a desiccator for further use.

Phytochemical Screening:

Different qualitative chemical tests were performed on the crude extracts to identify the various active constituents that are present in different extracts of *Alstonia scholaris* R. Br. Preliminary Phytochemical screening was carried out using standard procedures [12].

Physicochemical Parameters:

Physicochemical constants such as the moisture content, percentage of total ash, acid insoluble ash and acid soluble ash; extractive values like alcohol and water-soluble extractive values were determined according to the methods described in Indian pharmacopoeia [13, 14].

Pharmacological evaluation

Animals:

Pheretima posthuma (Adult Indian earth worms) of about 5-7 cm long and 0.3-0.4 cm in width were used for the present study.

Standard drug:

To compare the test results, Albendazole 400 mg Tablet (Glaxo Smith Kline) was used as standard.

Determination of Anthelmintic Activity:

The anthelmintic activity was investigated on mature Indian earthworm *Pheretima posthuma*, which shares morphological and physiological similarities with human intestinal roundworm parasites [15].

The anthelmintic method was carried as per the method of Pal et al., with minor modifications. Thirteen groups each containing six earthworms of approximately equal size were released into 10 ml of desired formulation. Each group was treated with chloroform, ethanol, aqueous extract and Albendazole (40, 60 and 80 mg/ml) in normal saline with 5% DMF and in vehicle alone acting as control. Time for paralysis was noted when no movement could be observed with a slight pin prick method. Time for death of individual earth worms was recorded when the worms showed no movement either by vigorous shaking or by dipping in warm water [15-17].

Result and Discussion

Qualitative Chemical Tests of the Extracts:

Preliminary phytochemical analysis of different extracts indicated the presence of alkaloids, Glycosides, flavonoids, carbohydrates, tannins, proteins and steroids (**Table 1**).

Table 1: Qualitative Chemical Tests of the Extracts

Test		Chloroform Extract	Ethanolic Extract	Aqueous Extract
Alkaloids		+	+	+
Amino Acids	Millon's Test	+	+	+
Carbohydrates	Molisch's Test	+	+	+
	Barfoed's Test	+	+	+
	Test for Pentoses	+	+	+
Flavonoids	Alkaline reagent Test	+	+	+
	Zinc hydrochloride Test	+	+	+
Glycosides	General Tests	-	+	-
	Boritrager's Test	-	-	-
	Modified anthraquinones Test	-	+	-
	Froth formation Test	-	+	+
Tannins	Chlorogenic acid Test	+	+	+
	Ferric Chloride Test	+	+	+
	Gelatin Test	+	+	+
Proteins	Xanthoproteic Test	+	+	+
Steroids	Libermann-Burchard Test	+	+	+
	Salkowski Test	+	+	+

[“+” = Present and “-” = Absent]

Physicochemical characters:

The Moisture content in the leaves was found to be 05.35 % w/w. The total ash calculated is 15.85 % w/w, acid insoluble ash is 4.35 % w/w and Water soluble Ash is 11.50 % w/w. The leaf shows higher extractive values in alcohol (8.22 %) w/w than that of water (5.35 %) w/w. The moisture content of the drug is not high, thus it discourages bacteria, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash and water soluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence of or absence of foreign inorganic matter such as metallic salts or silica. The results are depicted in **Table 2**.

Table 2: Physicochemical characters

Serial No:	Parameters	Result (in w/w %)
1	Moisture content	5.35 % \pm 0.031
2	a. Total Ash value	15.85 % \pm 0.031
	b. Acid insoluble Ash	4.35 % \pm 0.011
	c. Water soluble Ash	11.50 % \pm 0.001
3	90% Alcohol soluble extractive	8.22 % \pm 0.071
4	Water soluble extractive	4.20 % \pm 0.045

Anthelmintic Activity:

The result of Anthelmintic Activity is given in **Table 3**. and the results are plotted in **Figure 1**. The result of Anthelmintic activity on Indian earthworm *Pheretima posthuma* revealed that both the ethanolic and aqueous extract at different concentration has shown paralysis effect and has caused death of earthworms. Significant Anthelmintic properties were observed in a dose dependent manner. The mean \pm SEM values [statistical analysis] were calculated for the extract.

The ethanolic extract of *Alstonia scholaris* R. caused paralysis in 17.29 min and death in 33.32 min. in 80 mg/ml concentration whereas aqueous extract of the plant also exhibited moderate effect in case of paralysis and death against standard. The aqueous extract caused paralysis in 24.28 min and death in 42.31 min. in 80 mg/ml concentration. The reference drug Albendazole at 40 mg/ml concentration has shown paralysis at 10.07 min and occurrence of death at 21.34 min.

Table 3: Anthelmintic Activity of Different Extract of the Leaves of the Plant *Alstonia scholaris* R. Br.

Group Treatment Dose			Reaction time in (minutes)	
			Time taken for paralysis (P)	Time taken for death (D)
Control	Normal Saline	-	0.00	0.00
Standard	Albendazole	40 mg/ml	10.07±0.05	21.34±0.03
		60 mg/ml	7.38±0.21	15.56±0.02
		80 mg/ml	5.54±0.03	11.67±0.04
Chloroform extract		40 mg/ml	70.35±0.03	135.41±0.22
		60 mg/ml	50.23±0.27	105.94±0.06
		80 mg/ml	34.32±0.14	85.35±0.05
Ethanol extract		40 mg/ml	30.58±0.06	50.64±0.04
		60 mg/ml	21.38±0.03	43.09±0.01
		80 mg/ml	17.29±0.05	33.32±0.16
Aqueous extract		40 mg/ml	45.32±0.04	78.38±1.22
		60 mg/ml	30.45±1.15	61.16±0.21
		80 mg/ml	24.28±0.09	42.31±0.06

Results are expressed as mean ±SEM of three observations. P- Paralysis; D- Death

mg: milligram, ml: millilitre.

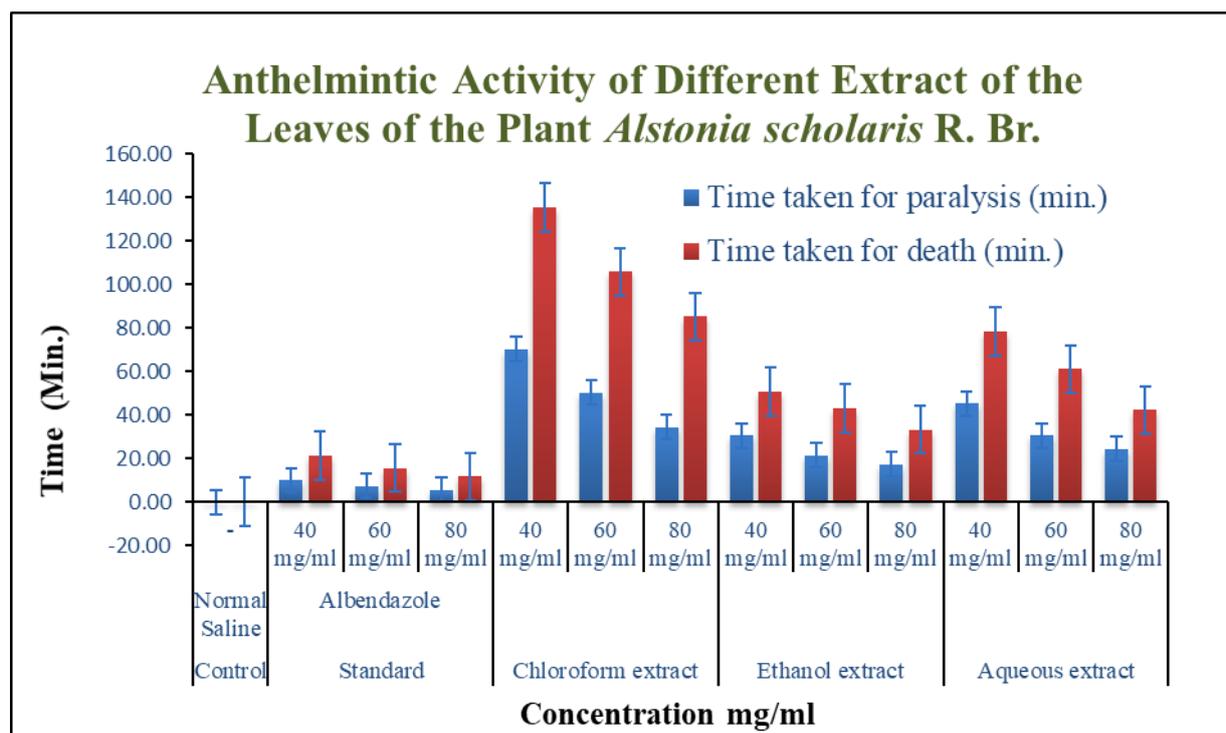


Figure 1: values of paralysis and death time of *Pheretima posthuma* [Indian adult earth worms] plotted against varying concentration of different extract of leaves of *Alstonia scholaris* R. Br. compared with standard (Albendazole).

Conclusion

The present study reveals the presence of various phytochemical constituents like alkaloids, glycosides, flavonoids etc. in the chloroform, ethanolic and aqueous extract of the leaves of *Alstonia Scholaris* R. Br. The study has also confirmed *in vitro* anthelmintic activity against Indian earthworm *Pheretima posthuma* against the standard drug Albendazole suggesting a need to isolate and evaluate the active constituents responsible for the exhibited biological activity. Further studies may also be carried out using *in vivo* model to establish anthelmintic activity. In this context it may be concluded that the present study will initiate further research work on *Alstonia Scholaris* which has many more potential ethnomedicinal claims to be scientifically validated.

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