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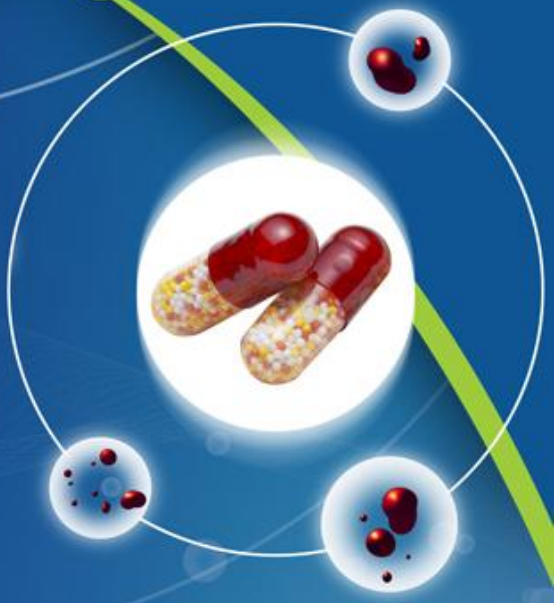

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Research Article

PHARMACOLOGICAL SCREENING OF *ALSOTONIA SCHOLARIS*

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ABSTRACT

A large number of medicinal plants are claimed to possess anthelmintic property in traditional systems of medicine and are also utilize by ethnic groups worldwide. The development of anthelmintic resistance and the high cost of conventional anthelmintic drugs led to the evaluation of medicinal plant as an alternative source of anthelmintice. In the current study were conducted to determine the possible anthelmintic and antibacterial effects of leaves of Alstonia scholaris.

Key words: Alstonia scholaris, saptparna, anthelmintic activity, antibacterial activity.

INTRODUCTION

Saptaparni is a medium-to-large evergreen tree with a dense crown and a straight cylindrical bole. In Sanskrit Saptaparna means "seven leaves", and is based on the fact that four-to-eight simple leaves; more often seven, occur in a circle attaching node around the stem. The species is native to India. The genus *Alstonia* was named by Robert Brown (1773-1858), the famous Scottish botanist. The genus comprises about 45 species inhabiting tropical and subtropical Africa, Central America, Southeast Asia, and Australia. In Ayurveda It is used as a bitter and as an astringent herb for treating skin disorders, malarial fever, urticaria, chronic dysentery, diarrhea, in snake bite and for upper purification process of Panchakarma. The Milky juice of the tree is applied to ulcers.[1]

Biological sources:- *Alstonia scholaris* is popularly known as Saptaparni or Devils tree.

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Family:- Apocynaceae R.Br.

Synonyms:- Chatian (Hindi), Chhatim (Bengali), Maddale (Kannada), Saptaparna, Saptaparni (Sanskrit, 'seven-leaved'), Devil tree, Blackboard Tree, Milkwood Pine, White Cheesewood (English).[2]

BOTANIC DESCRIPTION

Leaves

Leaves are 7 in a whorl, coriaceous, bluntly acuminate, dark green above and pale beneath. Leaf stalk is 11.5 cm long, the lamina is elliptical or elliptical lanceolate, glabrous or sparsely hairy, tapering towards the base and 11.5-23 x 4.7-5 cm is the size. Upper surface is dark green; the lower surface is green white. The tip of the leaf is rounded or shortly pointed, tapering towards the base.[3]

Bark

Bark is rough, tessellated corky grey to grey white and contains whorled branches. The outer blaze is cream to yellowish in color with abundant, milky latex that flows rapidly when cut.[5]

Flowers

Greenish white flowers in umbrellately branched manner. They are 710 mm long, white, cream or green. The tube is hairy lobes sparsely or densely pubescent; 1.54 mm long, the left margins overlapping, strongly perfumed.[4]

Fruits

Fruit a pendulous, two lobed, dehiscent follicles, brown or green, dry or wood, spindle shaped, 1532 cm long, 46 mm in diameter. The trees are often deciduous at irregular intervals. They do not flower at every leaf change, but only after marked periods of dry weather. The large branches provide favorable nesting sites for wild bees. Pollination is by insects; when flowering, butterflies and bees often surround trees. The fruits open on the tree and the seeds, which have a tuft of silky hairs at each end, are dispersed by wind.[3]

Figure – 1: Saptaparnai Tree

MORPHOLOGY OF LEAVES

Leaves are 4 -7 in a whorl, coriaceous, bluntly acuminate, dark green above and pale beneath. Leaf stalk is 1 -1.5 cm long, the lamina is elliptical or elliptical -lanceolate, glabrous or sparsely hairy, tapering towards the base, 11.5 - 23 x 4 - 7.5cm is the size. Upper surface is dark green; the lower surface is green -white. The tip of the leaf is rounded or shortly pointed, tapering towards the base.[4,5]

HELMINTHIASIS AND ANTHELMINTICS

Anthelmintics or antihelminthics are drugs that expel parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. They may also be called vermifuges (those that stun) or vermicides (those that kill). They are used to treat people or animals that are infected by helminths, a condition called helminthiasis.[6]

Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes.

Anthelmintics are medications used to eradicate parasitic worms (helminthes) from the human body. Helminth infections are one of the most common infections, affecting a large proportion of the world mainly in tropical regions. In developing countries they pose a large threat to public health, and leading to malnutrition, anemia, eosinophilia (a higher than normal level of the white blood cell), and pneumonia. The worms that cause infection in man generally include the roundworms, the tapeworms and the flukes.[7]

The roundworms

Roundworms are worms that can infest the human digestive tract, specifically the small intestine. They are parasites and use the human body to stay alive, feed and reproduce. They live in the human small intestine and their eggs are passed out with stools. Infections are transmitted through ingestion of food or water contaminated with their eggs. Contaminated sources such as dirty hands, flies and other insects can also be the transmission media. Symptoms commonly seen with roundworm infections are nausea, abdominal pain, intermittent diarrhea and perianal itching. In most people, roundworm infections do not cause any noticeable symptoms. People most commonly see their doctor until they seen a worm in their stools.

The tapeworms

Tapeworm infection is caused by ingesting food or water contaminated with their eggs or larvae (tiny young worms). If you ingest tapeworm larvae, they develop into adult tapeworms in your intestines (intestinal infection). Intestinal tapeworm infections are usually mild; the usual symptoms are nausea and weakness. Some people with tapeworm infections do not need treatment, for the tapeworm exit the body on its own. People most commonly go for treatment until they seen a worm in their stools.

The flukes

There are 4 categories of fluke infections which are pathogenic in man: the infections of the blood, the intestines, the liver and the lung. People usually become infected with fluke worms by swimming or washing in fresh water that contains fluke worms. Symptoms are usually only seen in heavy infections and commonly include fever, pain, and eosinophilia.[6,7]

In the current study, in-vitro experiments were conducted to determine the possible anthelmintic and antibacterial effects of leaves of *Alstonia scholaris.*

MATERIALS AND METHOD

Plant material

The plant materials (seeds) of Alsotonia *Scholaris* were collected Mandsaur, Madhya Pradesh and positively identified and confirmed by the botanist in Dr. S. N. Mishra, HOD, KNK Horticulture College, Mandsaur, Madhya Pradesh.

Herbarium was submitted in Department of Pharmacognosy at Mandsaur Institute of Pharmacy, Mandsaur, India. [Identification No.MIP/ P, cology/2015/507].

Extraction method

The dried powder material (leaves) (100g). The powder was defatted with petroleum ether and extracted with hydroalcoholic solvent (50% alcohol& 50% water) through soxhlet extraction techniques at 35°C for about 24 hours. The extract was dried at water bath and the percentage yields of the extracts were found to be 14%. The concentrated hydroalcollic extracts then tested for the identification of various active constituents.[8-11]

ANTHELMINTIC ACTIVITY

Experimental worms

The earthworm was used for evaluating the anthelmintic activity the earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. The earthworm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings; used for anthelmintic activity.

Procedure

The animals were divided in to seven groups containing three earthworms in each group. Hydroalcoholic extracts of *Alstonia scholaris* were dissolved in normal saline to get 10, 20,30,40,50 mg/ml concentration. The reference standards and extract solution were prepared freshly before starting the experiment. Albendazole was used as standard, where normal saline solution used as control. All the earthworm were released into 10 ml of respective formulation as follows: vehicle control (normal saline), albendazole (10ug/ml), and hydro alcoholic extract (10, 20,30,40,50 mg/ml).[11-14]

Observation was made for the time taken to paralyze or death of individual worms. Paralysis was said to occur when the worms do not receive any sense even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color; when dipped in warm water. [15-18]

Antibacterial activity

Antibacterial activities of hydroalcohilc extracts of leaves were assessed against Gram+ve (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-ve (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria by agar well diffusion method.[6]

The culture plates were prepared by first sterilizing the nutrient agar (36 gm in 1000 ml) in an autoclave at 121 °C at 15 lb for 15 minutes and then by pouring 20 ml of media into sterilized Petri dishes. 1 ml inoculums suspension was spread uniformly over the agar in Petri dishes. Wells were made by sterile cork borer (6 mm) in each plate. Extracts was added aseptically into the well. Plates were incubated at 37 °C for 24 hrs.

After incubation, microbial growth was observed in the Petri dishes. The antibacterial activity was expressed as the mean of diameter of the inhibition.[11,14,16]

RESULT

Phytochemical screening

The extract was screened for the presence of various phytochemical constituents:-

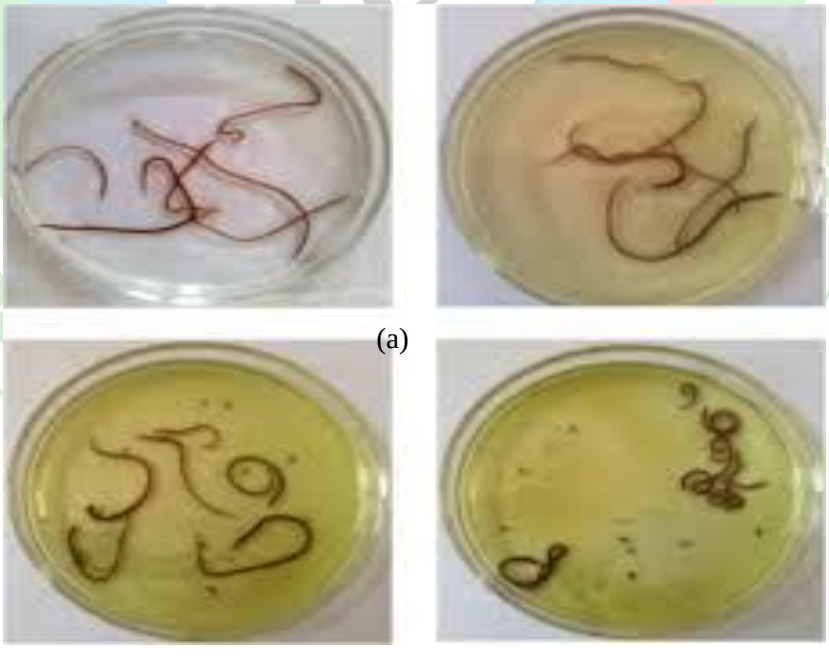
- Test for carbohydrates: Molish test-To 2-3ml. of extract added few drops of alpha-naphtol solution in alcohol, shaken and added conc. H_2SO_4 from side to it. Violet ring at the junction indicates the presence of carbohydrates.
- Test for starch: Iodine test-Mixed 3 ml. test solution & few drops of dilute Iodine solution. Blue colour indicates presence of starch.
- Test for reducing sugars: Fehling test-Mixed 1ml. Fehling A & 1ml. Fehling B, boiled for 1 min. Added equal volume of test solution. Heated in boiling water bath for 5-10 min. Yellow or brick red precipitation indicates the presence of reducing sugars.
- Test for alkaloids: Dragendorff's test-To 2-3 ml. extract added few drops of dragendorff's reagent. Orange brown ppt. indicates presence of alkaloid.
- Test for flavonoids: To small quantity of residue added lead acetate solution. Yellow ppt. indicates flavonoids.
- Test for saponins: Foam test-Shaken dry extract with water. Persistent foam indicates saponins.
- Test for steroids: Salkowski test-To 2 ml. of extract added 2 ml. chloroform & 2 ml. conc. H_2SO_4 then shaken well. Chloroform layer appearing red & acid layer showing greenish yellow fluoresence indicates steroids.
- Test for phenolic compounds: Added lead acetate solution to 2-3 ml. of extract, white ppt. indicates phenolic compounds.
- Test for proteins: Xanthoprotein test-Mixed 3 ml. test solution with 1 ml. conc. H_2SO_4.White ppt. indicates presence of proteins. Adding NH_4OH turns ppt. orange.[3,5,12]

Table – 1: Phytochemical screening

Phytochemical constituents	Test	Result
Carbohydrates	Molish test	(+)
Starch	Iodine test	(-)
Reducing sugars	Fehling test	(-)
Alkaloids	Dragendorff test	(+)
Flavonoids	Lead acetate test	(+)
Saponins	Foam test	(+)
Steroids	Salkowski test	(-)
Phenolic compounds	Lead acetate test	(+)
Proteins	Xanthoprotein test	(-)

Anthelmintic activity

In vitro anthelmentic activity result in the predominant effect albendazole on the worm is to cause flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperploarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. Observation was made for the time taken to paralyze or death of individual worms. Paralysis was said to occur when the worms do not receive any sense even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color shown in.



(a)

(b)

Figure – 2: Showing time of paralysis (a) showing time of death (b)

Table – 2: Anthelmintic activity of hydroalcohollic extract of leaves of *Alstonia scholars*

S. No	Groups	Concentration	Time taken for paralysis in sec.	Time taken for death in sec.
1	Control (normal saline)	(0.9%Nacl)	289.64±15.8	338.33±11.99
2	Standard (Albendazole)	85 mg/ml	4.33 ±33	6.6 ± 33
3	Hydroalcohollic Extract	10 mg/ml	71.66 ± 0.7	87.66 ±0.7
4		20 mg/ml	67.66 ±0.7	75.66 ±0.7
5		30 mg/ml	57.33 ±0.3	76.33 ±0.3
6		40 mg/ml	47.33 ±0.3	67.33 ±0.3
7		50 mg/ml	38.33 ±0.3	57.33 ±0.3

ANTIBACTERIAL ACTIVITY

Plates were examined after 24 hrs for clear zone of inhibition. All measurements were taken in mm. The disc diffusion method was used to determine the growth inhibition of bacteria by the plant extracts. Discs containing different concentration of dissolved plant extract and prepared in sterile condition. Nutrient agar medium was prepared, sterilized, cooled and poured in to sterile petri dishes to a depth of 4 mm about 25 ml/plate to solidify. Pure cultures of the test organism were used to inoculate the petri dishes. This was done by spreading the inoculums on the surface of the prepared nutrient agar plate using sterile cotton swabs which have been dipped in the diluted suspension of the organism. The discs were then aseptically placed evenly on the surface of the inoculation and gently pressed down to ensure contact using a pair of forceps. The plates were finally incubated at 37ºC for 18-24hrs. The plates were examined after 24 hrs for clear zone of inhibition. All measurements were taken in mm.

Table -3: Antibacterial activity of *Alstonia scholaris* in hydroalcohllic extract

S. No	Extracts /Standard	Concentration (mg/ml)	Zone of inhibition (mm)			
			S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
1	leaves		-	-	-	-
2		50	13	12	9	11
3		100	18	15	17	13

CONCLUSION

The pharmacological screening of extract shows the presence of carbohydrates, alkaloids, saponnins, flavonoids. In this investigation the hydroalcohollic extract of leaves *Alstonia scholaris* linn. were used to evaluate anthelmintic activity by using the

above model. The present study of hydroalcohollic proves its anthelmintic property.

In the current investigation the hydroalcohlic extract of the *A. scholaris* leves was found to be active on test bacteria. Demonstration of antibacterial activity of *A. scholaris* against the test bacteria is a possible indication of newer antibacterial agents.

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