

Evaluation of Anti Microbial Antihelminthic Properties and Phytochemical Analysis of Medicinally Important Plant *Saraca indica* Linn

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ABSTRACT

The present study focus on the preliminary phytochemical analysis, antimicrobial and antihelminthic activity of different solvent extracts of leaf and bark of *Saraca indica*. Various phytochemicals present in bark and leaves are extracted using soxhelt apparatus with different solvents. The antimicrobial activity was checked against different human pathogens (MTCC). Antihelminthic activity was checked against *Phertima posthuma*. The phytochemical analysis revealed the presence of various secondary metabolites. Methanol extract of leaf sample showed the highest zone of inhibition against *Bacillus megaterium* (18mm) and *Enterobacter faecalis*(18mm). Ethyl acetate extract of leaf and bark sample showed highest antifungal activity against *Rhizomucor* species(20mm and 19mm respectively).Butanol extract of leaf extract showed potent antihelminthic property(20 min death) The study shown it has promising antimicrobial and antihelminthic property.This study showed that *Saraca indica* , a versatile plant, have many active compounds present which can be successfully used for the development of various potent drugs.

Keywords: *Saraca indica*, anti microbial, MTCC, Anti helminthic, *Phertima posthuma*

INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs¹. Antibacterial extracts from tested plant can be useful in warding off infectious diseases and therefore a compelling reason to suppose that, as anti-infective agents from tested plants are active against human pathogens, it can be assumed that these plants could be useful in warding infectious diseases^{2,3}. Now a day a renewed interest in traditional medicine is observed and there has been an increasing demand for more and more drugs from plant sources. The wide spread use of herbal remedies and health care preparations gained from ordinarily used traditional herbs and medicinal plants have been elevated due to the occurrence of natural products with medicinal properties⁴. There are many approaches to the search for new biologically active principles in higher plants⁵. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics⁶. Hence, plant derived antimicrobial agents have received considerable attention in recent years.

Saraca indica or *Saraca asoka* is a small evergreen tree belonging to the family Caesalpinaceae.It is popularly called Asoka or Ashoka, Ashok, or Sita Ashok. Ashoka is considered sacred tree in India. It occurs throughout India up to an altitude of 750m in central and eastern Himalayas⁷. In traditional medicine, Ashoka is

recommended for treating dysmenorrhea and pregnant women prone to miscarriages⁸. It exhibit various activities such as antitumour⁹, analgesic, antipyretic, fungitoxic, antihelminthic, antidiabetic, larvicidal activity¹⁰, antimicrobial activity, CNS depressant activity, antiulcer activity and anti-inflammatory activity¹¹.

Based on the previous studies, here we aim to evaluate the phytochemical constituents, antimicrobial and antihelminthic activities of the leaf and bark extract of *Saraca indica*.

MATERIALS AND METHODS

Collection extraction and sample preparation: The plant *Saraca indica* was identified and collected from university campus. The collected plant sample (Bark and Leaf) was washed thoroughly under running tap water to remove dust and sand particles. Then it was shade dried for 2-3 weeks, powdered and stored for further use.

In order to extract the active compounds from plant parts such as leaves and bark, 25 g of each dried sample was soaked in 100 ml of solvents such as Benzene, Methanol, ethyl acetate and water. This was kept for 48 hours incubation and filtered using Whatmans No. 1 filter paper. The extract obtained was evaporated completely for dryness. The 2 g of dried sample obtained was dissolved in 10 ml of DMSO and stored for further use.

Phytochemical analysis: Chemical tests were carried out using the extracts obtained (methanol, butanol,ethyl acetate, aqueous) using standard procedures to identify

Table 1: Phytochemical analysis of *Saraca indica* leaf and bark extracts

Sl NO	Compound	Methanol		Butanol		Ethyl acetate		Aqueous	
		leaf	Bark	Leaf	bark	Leaf	Bark	Leaf	bark
1	Alkaloid	+	+	+	+	+	+	-	+
2	Carbohydrate	+	+	+	+	+	+	+	+
3	Cardiac glycoside	+	+	+	+	+	+	+	-
4	Flavanoids	+	-	-	-	+	+	-	+
5	Saponin	+	+	-	+	+	+	+	+
6	Tannins	+	+	-	+	+	+	+	+
7	Terpenoids	-	+	+	+	+	+	+	+

(+ - present, - absent)

Table 2: Diameter of Zone of inhibition of *Saraca indica* Leaf and Bark sample

Sl NO	Bacteria	Zone of inhibition (mm)							
		Methanol		Butanol		Ethyl acetate		Drug control	
		Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
1	<i>Pseudomonas aeruginosa</i>	16	11	10	13	9	11	15	11
2	<i>Proteus vulgaris</i>	13	14	15	13	18	10	10	12
3	<i>Klebsiella pneumoniae</i>	11	10	11	13	10	11	11	12
4	<i>Enterobacter aerogens</i>	14	10	16	13	17	10	11	13
5	<i>Bacillus megaterium</i>	18	11	16	17	14	13	13	14
6	<i>Pseudomonas putida</i>	10	11	13	13	11	8	10	13
7	<i>Lactococcus lactis</i>	9	17	14	14	13	13	15	13
8	<i>Bacillus subtilis</i>	15	14	10	17	14	10	12	13
9	<i>Enterobacter faecalis</i>	18	9	15	14	14	10	15	13
10	<i>E.coli</i>	11	10	10	12	18	15	14	13

the constituents as described by Harborne¹². To identify alkaloids wagners and molish's tests were conducted. Keller killani test, sulphuric acid test, forthin test, Ferric chloride test and salkowski tests were conducted to identify cardiac glycosides, flavinoids, saponins, tanins and terpenoids respectively.

Anti microbial screening: The crude extracts of the plant and drug were tested for antimicrobial activity (antibacterial and antifungal) against strains of pathogenic microbes (MTCC). Drug like ciprofloxacin (3µg) and kanamycin (15µg) and DMSO were used as controls.

Antibacterial activity of samples against human pathogens: The crude extracts of the plant and drug were tested for their antibacterial activity by disc diffusion method against pathogenic organism like *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Enterobacter aerogens*, *Bacillus megaterium*, *Pseudomonas putida*, *Lactococcus lactis*, *Bacillus subtilis*, *Enterobacter faecalis*, and *Escherichia coli*. Prepared nutrient agar plates were inoculated with pathogenic organism (0.1ml) by spread plate method. Whatmans no.1 filter paper disc were sterilized and inoculated with the samples and DMSO were kept as negative controls. After incubation at 30°C for 24 hours zone of inhibition was measured.

Antifungal activity of samples against human pathogens: The crude extracts of the plant and drug were tested for their antifungal activity by disc diffusion method against pathogenic organism like *Aspergillus fumigates*, *Aspergillus niger*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Rhizomucor miehei*. Prepared Rose Bengal Agar plates were inoculated with pathogenic organism by spread plate method. Whatmans filter paper were sterilized and inoculated with samples and DMSO

were kept as negative controls. After incubation at 37 °C for two days the zone of inhibition was measured.

Antihelmintic activity: Antihelmintic activity was conducted using *Pheretima posthuma* (Earth worm) of nearly equal size (±8 cm) due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. 2-3 earth worm of nearly equal size were placed in each petridish containing 2 ml (0.4g) sample at room temperature. Observation was made for the time taken for paralysis when there was no movement of any kind except when shaken vigorously and are not revived in normal saline. Time for death were recorded when they lost their mobility even after vigorous movement and also by fading off their body colors.

RESULTS

Phytochemical analysis: Phytochemical analysis showed the presence of alkaloids, carbohydrates and cardiac glycosides in methanol, butanol and ethylacetate extract of both bark and leaf samples as shown in Table 1.

Antibacterial activity: Methanol extract of leaf sample showed the highest zone of inhibition against *Bacillus megaterium* and *Enterobacter faecalis* (18 mm). Ethyl acetate extract of leaf also showed high zone of inhibition against *Proteus vulgaris* and *E.coli*. In bark sample butanol extract showed highest zone of inhibition against *Bacillus megaterium* and *Bacillus subtilis* (17mm). Methanol extract of bark also showed high zone of inhibition against *Lactococcus lactis*. All other samples obtained almost equal range of inhibition zone (13mm-16mm). Results are shown in Table 2.

Anti fungal activity: Ethyl acetate extract of leaf sample showed highest antifungal activity against *Rhizomucor* species with a zone of inhibition 20 mm. Methanol extract of leaf also showed high zone of inhibition against

Table 3 Diameter of Zone of inhibition of *Saraca indica* leaf and bark

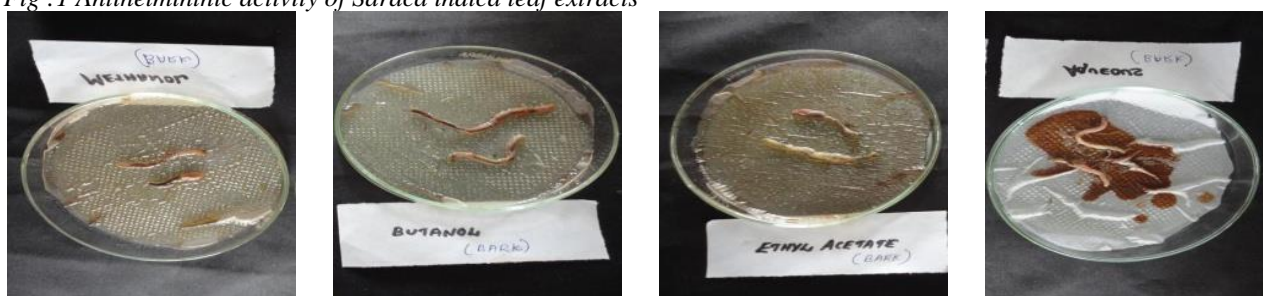
Sl NO	Fungi	Diameter of zone of inhibition(mm)					
		Methanol		Butanol		Ethyl acetate	
		Leaf	Bark	Leaf	Bark	Leaf	Bark
1	<i>Aspergillus fumigatus</i>	12	13	9	14	10	11
2	<i>Aspergillus niger</i>	8	11	10	9	12	9
3	<i>Candida albicans</i>	16	9	15	14	11	11
4	<i>Candida glabrata</i>	13	10	16	16	11	11
5	<i>Candida tropicalis</i>	19	17	10	10	15	13
6	<i>Rhizomucor miehei</i>	16	13	14	11	20	19

Table 4: Antihelminthic activity of *Saraca indica* leaf and bark

Sl NO	Name of earthworm	Extract	Time in minutes			
			For paralysis		For death	
			Leaf	Bark	Leaf	Bark
1	<i>Pheretima posthuma</i>	Methanol	15	17	26	40
2		Butanol	10	17	20	45
3		Ethylacetate	20	26	33	45
4		Aqueous	110	50	180	150



Butanol Methanol Ethyl acetate Aqueous
Fig :1 Antihelminthic activity of *Saraca indica* leaf extracts



Methanol Butanol Ethyl acetate Aqueous
Fig :2 Antihelminthic activity of *Saraca indica* bark extracts

Candida tropicalis. In Bark sample ethyl acetate extract showed highest zone of inhibition against *Rhizomucor* species (19mm). All other samples showed almost equal range of inhibition zone (10mm- 16mm). Results are shown in Table 3.

Anti helminthic activity: Butanol extract of leaf sample exhibited less time for paralysis and death for *Pheretima posthuma*. In bark, methanol extract of leaf sample exhibited less time for paralysis and death for *Pheretima posthuma*. Aqueous extract of leaf show the maximum time for paralysis and subsequent death. Results are shown in Table 4 ,fig 1 and fig 2

DISCUSSION

Plants have a great potential for producing new drugs of great benefit to mankind. The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. It has been reported that leaves of *saraca indica* are known to contain carbohydrates, proteins, tannins and saponins¹³ and barks contains glycosides, steroids, saponins, carbohydrates and tannins¹⁴. In the present study the preliminary phytochemical analysis of *Saraca indica* reveals the presence of many biologically active compounds, such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins and terpenoids.

The stem bark extract of *saraca indica* demonstrated significant antimicrobial activity against various human pathogens¹⁵. The present study exhibited powerful antibacterial activity with a maximum inhibition zone 17mm,18mm,19mm, 20mm against *Bacillus megaterium*, *Enterobacter faecalis*, *Bacillus subtilis*, *Proteus vulgaris*, *Ecoli.*, *Lactococcus lactis*, *Candida tropicalis* and *Rhizomucor* species.

It was reported that ethanolic extract of leaf of *Saraca indica* induced antihelminthic activity¹⁶. From our result it may be mentioned that the butonolic leaf extract (20 minute for death) and methanolic bark extract (40 minute for death) of *Saraca indica* were relatively more potent as an antihelminthic agent due to the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terepenoids. The results showed that the leaf and bark of *Saraca indica* contain a number of chemical ingredients, which may be responsible for the various pharmacological actions although their specific roles remain to be investigated. Proper chemical and biological investigation, understanding of the mechanism of action, development of the structure activity relationship and high yield production by plant tissue culture of these drug promote their use against various infectious diseases as such or there semi synthetic analogues.

CONCLUSION

This work confirms that *Saraca indica* is an medicinally important plant containing many biologically active compounds that can be used effectively against numerous diseases. Further research is needed for the development of a safe, more economic and site specific drug from this plant.

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