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Research Article

PHYTOCHEMICAL SCREENING AND ANTHELMINTIC ACTIVITY STUDY OF *SARACA INDICA* LEAVES EXTRACTS

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ABSTRACT
To know the anthelmintic property of leaves of *Saraca indica*, we used both maceration and soxhelet methods of extraction using solvents like ethanol and methanol. Each extract was tested for its anthelmintic activity by following standard method. The ethanolic and methanolic extracts (obtained from both the methods of extractions) of *Saraca indica* displayed anthelmintic property in a dose-dependant manner. In both the methods of extraction, we found that the ethanolic as well as the methanolic extracts were more potent than the positive control as far as anthelminthic property was concerned. To correlate phytochemical screening with anthelmintic activity, phytochemical evaluation of the extracts was also performed. From our result, it may be mentioned that the ethanolic extract was relatively more potent as an anthelmintic agent due to the presence of alkaloids, glycosides, tannins and flavonoids. On the other hand, the methanolic extract was effective as an anthelmintic agent probably due to the involvement of glycosides and flavonoids. The presence of alkaloids, glycosides, terpenoids, tannins and flavonoids seems to be the responsible phytochemical constituents for demonstrating anthelmintic activities of our extracts.
KEYWORDS- *Saraca indica*, ethanolic extract, methanolic extract, phytochemical screening, anthelmintic activity.

INTRODUCTION

Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs[1]. The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries.[2] Several top selling drugs of modern times such as Quinine, Artemisinin, Shikonin, etc. are obtained from plants[1]. Most of the phytochemicals, secondary metabolites of plants, are physiologically active[2]. The plants are known to provide a rich source of botanical, anthelmintic, antibacterials, and insecticides.[3] Helminths are recognized as a major problem to livestock production throughout tropics. Most of the diseases caused by helminths are of a chronic and debilitating in nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms, which results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity. Chemotherapy is the only treatment and effective tool to cure and control helminth infection, as effective vaccines against them have not been developed so far. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites. Herbal drugs have been in use since ancient times for the treatment of parasitic disease in human and could be of value in preventing the development of resistance.[4] Some investigators have mentioned the importance of some phytochemicals like alkaloids, glycosides, terpenoids, tannins and flavonoids for showing anthelmintic activity of plants.[3,5,6]

Saraca indica (Roxb) de wild (Family-Caesalpinaceae) is commonly known as Asoka, Sita Asoka and Haempushpam. It is an evergreen tree which is 9m in height. The flowers are orange yellow in colour and arranged in dense corymbs. It occurs throughout India up to an altitude of 750m in central and eastern Himalayas [7]. Useful parts of the plant are barks, leaves, flowers and seeds. The plant is useful in dyspepsia, fever, burning sensation, colic, ulcer, menorrhagia, leucorrhoea, pimples, etc. The bark, used for the pharmaceutical preparations, is bitter, astringent, refrigerant, anthelmintic, styptic, stomachic, constipating, febrifuge and demulcent. Even the juice of the leaves, mixed with cumin seeds, is used for the treatment of stomachalgia [8]. The Asoka tree is considered sacred throughout India. This tree has many folklorical, religious and literary associations in the religions. Due to its high value and handsome appearance, this tree is found close to the temples throughout India[9].

Although an extensive literature survey does not reveal anthelmintic activity of leaves of *Saraca indica*, the present study was undertaken to investigate the preliminary phytochemical screening and anthelmintic activity of leaves of *Saraca indica* [3]. This was done because it is known that several plants possess phytoconstituents which are responsible for their anthelmintic activity.

MATERIALS AND METHODS

Plant material

The leaves of the plant *Saraca indica* were collected from Chhend, Rourkela, during November 2010. The sample was authenticated by Dr.S. K. Padhi, Botanist, Rourkela Autonomous College, Rourkela. The shade dried leaves were powdered and stored in a desiccator until evaporation.

Preparation of extract

The powdered leaves were passed through a sieve (No.40) and stored in a desiccator. The powdered leaves were extracted by both Maceration and Soxhlet methods.

1) Maceration method**:** The powdered leaves (10gm) of *Saraca indica* were extracted using the maceration method. The powdered leaves were macerated in 60ml of 95% ethanol for 3days at room temperature. The resulting extract was filtered through filter paper (Whatman No.1). The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness. We also followed the same method of extraction using methanol instead of ethanol[10].

2) Soxhlet method**:** The powdered leaves (51gm) of *Saraca indica* were successively extracted using solvents in order of increasing polarity, viz. ethanol and methanol. After extraction, each time the marc was dried and later extracted with the next solvent. Both the extracts were dried by distilling the solvents in a rotary vacuum evaporator[11]. The yield of ethanolic extract was 4.6gm and that of methanolic extract was 3gm. Both the extracts were dissolved in dimethylsulfoxide (DMSO)[12]. After that, at first, phytochemical screening was performed. Then both the extracts were tested for their anthelmintic activity.

a) Phytochemical screening- [13]

Following chemical tests were performed for testing different chemical groups present in both the extracts:

Alkaloids

Mayer's test:-To 2-3ml of the extract, few drops of the Mayer's reagent

(1.36gm of Mercuric chloride and 5gm of Potassium iodide in 100ml distilled water) were added. Formation of a cream colour precipitate indicated the presence of alkaloids.

Amino acids

Millon's test:-To 2ml of the test extract about 2ml of Millon's reagent (Mercury nitrate) were added. White precipitate indicated the presence of amino acids.

Carbohydrates

Molish test:-To 2ml of the test extract, at first, few drops of alcoholic α-napthol were added. Then through sides of test tube, few drops of concentrated sulphuric acid were mixed with it. Purple to violet colour ring appeared at the junction indicated the presence of carbohydrates.

Flavonoids

Alkaline reagent test:-To 2ml of the test extract, few drops of sodium hydroxide solution were added. At first, intense yellow colour was formed, which was subsequently turned to colourless, on addition of few drops of dilute acid indicated the presence of flavonoids.

Glycosides

Borntrager's test :- The test extract was boiled with 1ml of sulphuric acid in a test tube for 5minutes. While hot it was filtered, then it was cooled. Shaking of the mixture was done with equal volume of chloroform. Two layers of solution were formed. The lower layer of chloroform was separated. Then that layer was shaken with half of its volume of dilute ammonia. Production of a rose pink to red colour suggested the presence of glycosides.

Saponins

Froth formation test :-Two millilitre of the extract was shaken vigorously with water in a test tube.

Formation of persistent foam indicated the presence of saponins.

Tannins

Gelatin test :- To 2ml of the extract, 1% gelatin solution containing 10% sodium chloride was added. Formation of a precipitate suggested the presence of tannins.

Proteins

Warming test :- Two millilitre of the extract was heated in a boiling water bath. Proteins get coagulated due to heating.

Steroids and Triterpenoids

Salkowski test :- The test extract was treated with few drops of concentrated sulphuric acid. Red colour at lower layer indicated the presence of steroids, whereas formation of yellow colour at the lower layer suggested the presence of triterpenoids.

b) Anthelmintic activity- The suspension of both the extracts, obtained from the maceration and the soxhlet methods, was prepared in DMSO to obtain 1, 2.5 and 5% concentrations. Solutions of similar concentrations of the standard anthelmintic drug like Piperazine citrate (as positive control) were also prepared in distilled water.

For our study DMSO and distilled water were used as negative controls.

Two millilitre of each concentration of both methanolic and ethanolic fractions and Piperazine citrate were diluted to 10ml separately with normal saline and poured into Petridishes. Nine groups of approximately equal size of earthworms, consisting of six in number in each group, were released into each Petridish. The anthelmintic activity was evaluated by adopting the standard method [14]. Adult Indian earthworms *Pheritima posthuma* were selected for the study because of their anatomical and physiological resemblance with the intestinal round worm parasite of human being [15].

RESULTS

For preliminary phytochemical screening of the extracts, we performed tests for alkaloids, amino acids, carbohydrates, flavonoids, glycosides, saponins, proteins, steroids, tannins and triterpenoides. While in case of ethanolic extract, alkaloids, amino acids, flavonoids, glycosides, saponins, tannins and triterpenoids were detected, amino acids, carbohydrates, flavonoids, glycosides, saponins and steroids were found in the methanolic extract (Table 1).The methanolic and ethanolic extracts of *Saraca indica* displayed anthelmintic property in a dose-dependant manner. In both the methods of extraction, we found that the methanolic as well as the ethanolic extract were more potent than the positive control as far as anthelmintic property was concerned (Table 2, 3).

DISCUSSION

From our result it may be mentioned that ethanolic extracts (obtained from both the methods of extractions) were relatively more potent as an anthelmintic agent due to the presence of alkaloids, glycosides, tannins and flavonoids. On the other hand, the methanolic extracts (obtained from both the methods of extractions) were effective as an anthelmintic agent probably due to the involvement of glycosides and flavonoids (Table 1). The presence of alkaloids, glycosides, tannins and flavonoids seems to be the responsible phytochemical constituents for demonstrating anthelmintic activities of our extracts[3,5]. Chemically tannins are polyphenolic compounds. Some synthetic phenolic anthelmintics e.g., niclosamide, oxyclosamide and bithinol are shown to interfere with the energy generation in helminths by uncoupling oxidative phosphorylation. It is possible that tannins contained in the extracts produced similar results. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death[4]. It is also possible that alkaloids may act on CNS and cause paralysis of *Pheritima posthuma* worms[3].

To identify the actual phytochemical constituents that are present in the crude drug extracts of this plant which are responsible for anthelmintic activity, should be studied thoroughly. It would be even better to conduct further research on pure chemical constituents of the plant to critically evaluate their activity on many animals[3].

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Table 1: Qualitative analysis of various extracts of *Saraca indica* leaves

Phytoconstituents	Ethanolic extract	Methanolic extract
Alkaloids	-	+
Amino acids	+	+
Carbohydrates	+	-
Flavonoids	+	+
Glycosides	+	+
Saponins	+	+
Protiens	-	-
Steroids	+	-
Tannins	-	+
Triterpenoids	-	+

'+' = Present; '-' = Absent

Table 2: Anthelmintic activity of methanolic and ethanolic extracts (by Maceration method) of *Saraca indica* leaves.

Treatment group	**Concentrations (%)**	**Time taken (seconds)**	
		Paralysis	**Death**
Methanolic extract	1.0	105	180
	2.5	100	155
	5.0	80	150
Ethanolic extract	1.0	130	170
	2.5	75	135
	5.0	60	95
Piperazine citrate	1.0	1920	3000
	2.5	1680	2450
	5.0	780	2220

Table 3: Anthelmintic activity of methanolic and ethanolic extracts (by Soxhlet method) of *Saraca indica* leaves

Treatment group	**Concentrations (%)**	**Time taken (seconds)**	
		Paralysis	**Death**
Methanolic extract	1.0	370	690
	2.5	310	620
	5.0	190	435
Ethanolic extract	1.0	630	945
	2.5	510	770
	5.0	480	650
Piperazine citrate	1.0	1920	3000
	2.5	1680	2450
	5.0	780	2220

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