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CORRELATION BETWEEN PHYTOCHEMICAL SCREENING AND SOME BIOLOGICAL ACTIVITIES USING PLANT EXTRACTS

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ABSTRACT
In order to correlate phytochemical screening with *in vitro* antibacterial and anthelmintic activities of the ethanolic and methanolic extracts of *Saraca indica* leaves, this study was undertaken. Standard methods were followed for the investigation. Tests for alkaloids, amino acids, carbohydrates, flavonoids, glycosides, saponins, proteins, steroids, tannins and triterpenoides were performed for the phytochemical evaluation of the extracts. While alkaloids, amino acids, flavonoids, glycosides, saponins, tannins and triterpenoids were detected from the ethanolic extract, amino acids, carbohydrates, flavonoids, glycosides, saponins and steroids were found in the methanolic extract. The zone of inhibition was largest when ethanolic extract (100 µg/ml and 200 µg/ml) was used against *Escherichia coli*, whereas it was least in case of *Staphylococcus aureus*. The methanolic extract (100 µg/ml and 200 µg/ml) produced maximum zone of inhibition against *Staphylococcus aureus* and the minimum zone was found in case of *Bacillus subtilis*. Chloramphenicol (positive control) produced maximum and minimum zones of inhibition against *Staphylococcus aureus* and *Bacillus subtilis,* respectively. The methanolic and ethanolic extracts of *Saraca indica* displayed anthelmintic property in a dose-dependant manner. We found that the methanolic as well as the ethanolic extracts had more potent anthelmintic property than the positive control (Piperazine citrate) when the extracts were obtained from both the methods of extraction.

KEYWORDS: *Saraca indica*, ethanolic extract, methanolic extract, phytochemical screening, antibacterial activity, anthelmintic activity,

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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, etc. are obtained from plants[1]. Most of the phytochemicals, secondary metabolites of plants, are physiologically active[2]. Majority of phytochemicals are known to produce therapeutic activities like antibacterial, antifungal, antioxidant, etc[1]. Moreover, the plants are also known to provide a rich source of botanical, anthelmintic and insecticides[3]. Alkaloids, tannins, flavonoids and phenolic compounds are the most important of bioactive constituents of plants. In addition to their use for therapeutic purposes, natural phytochemicals are effective as precursors for the synthesis of novel useful drugs. About 50% of modern drugs are natural products, which play an important role in drug development in Pharmaceutical Industry[1].

It is already known that the beneficial effects of medicinal plant materials typically result from the combinations of secondary products present in the plant. The antimicrobial activities of different plant extracts may reside in a variety of different phytochemicals, such as alkaloids, steroids, tannins, phenols, flavonoids, steroids, resins, fatty acids and gums[4,5]. Systematic screening of them may lead to the discovery of novel active compounds[2]. The selection of crude plant extract for the determination of the antibacterial activity has the potential of being more successful in the initial steps than screening of pure compounds[1]. Moreover, there is enormous increase in several infections in recent times and antibiotic resistance has become an ever-increasing therapeutic problem. This problem and many side effects of synthetic antimicrobials may be overcome by using phytochemicals having antimicrobial activity.

Helminths are recognized as a major problem to livestock production throughout tropics. Most 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. 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 also 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[6]. Some investigators have mentioned the importance of some phytochemicals like alkaloids, glycosides, terpenoids, tannins and flavonoids for showing anthelmintic activity of plants[3,7,8].

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[9].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, sweet, 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[10]. The Asoka tree has many folklorical, religious and literary associations in the religions[11].

Some workers have mentioned that both ethanolic and water extracts of bark of *Saraca indica* are effective *in vitro* against *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris* and *Klebsiella pneumoniae*[12]. In addition, some other researchers have also indicated that those extracts of the leaves of the plant show antibacterial activity only against *Escherichia coli*[13]. Both the methanolic and water extracts of its leaves have been mentioned as effective against *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhimurium*[14]. Considering it as a potential antibacterial agent, we undertook antibacterial activity study of the ethanolic and methanolic extracts of leaves of *Saraca indica*.

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.

On the whole, we decided to correlate phytochemical screening with antibacterial and anthelmintic activities of both ethanolic and methanolic extracts of *Saraca indica* leaves, as several phytochemicals possess antibacterial and/or anthelmintic activities. In order to validate the use of plant products in folk medicine, it is very important to perform such a screening of different plants.

MATERIAL 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[15]. Subsequently, both the extracts were tested for their anthelmintic activity.

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[16]. The yield of ethanolic extract was 4.6gm and that of methanolic extract was 3gm. Both the extracts were dissolved in dimethylsulfoxide (DMSO)[17]. After that, at first, phytochemical screening was performed. Then both the extracts were tested for their antibacterial and anthelmintic activities.

a) Phytochemical screening - 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 5min. While hot, it was filtered and 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[18].

b) *In vitro* antibacterial activity study using agar well diffusion method- In order to determine the antibacterial activity of the ethanolic and methanolic extracts of *Saraca indica*, the nutrient agar well diffusion method, as described by Schillenger and Luke (1989), was performed[19]. Sterile nutrient agar medium was inoculated with 0.1ml of fresh overnight nutrient broth culture of *Staphyloccus aureus* (approx.10^7CFU/ml) and poured into sterile Petridishes. In each plate, four wells of 6mm in diameter were punched using a sterile borer and the plates were allowed to dry for 5min. In one well 50µl of ethanolic extract was poured. In other wells, 50µl of methanolic extract, chloramphenicol (positive control) and DMSO (negative control) were delivered separately. The same procedure was followed in cases of other two strains such as *Bacillus subtilis* and *Escherichia coli*. Both the extracts were used at 100 µg/ml and 200 µg/ml concentrations[19]. The concentration of chloramphenicol was 100µg/ml[15]. After holding the plate at room temperature for 2 hours to allow diffusion of the extracts and controls into the nutrient agar medium, the plates were incubated at 37^0C for 24hrs. Then they were examined for inhibition of the bacterial growth around the wells. The diameters of the zones of inhibition in each case were measured[19].

c) Anthelmintic activity- The suspension of both the extracts 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 was 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[20]. 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[21].

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 zone of inhibition was largest when ethanolic extract (100µg/ml and 200µg/ml) was used against *Escherichia coli*, whereas it was least in case of *Staphylococcus*

aureus. The methanolic extract (100µg/ml and 200µg/ml) produced maximum zone of inhibition against *Staphylococcus aureus* and the minimum zone was found in case of *Bacillus subtilis*. Chloramphenicol produced maximum and minimum zones of inhibition against *Staphylococcus aureus* and *Bacillus subtilis,* respectively (Table 2). Against those three strains, no zone of inhibition was observed in case of DMSO.

The methanolic and ethanolic extracts of *Saraca indica* displayed anthelmintic property in a dose-dependant manner. The extracts obtained from both the methods of extraction, we found that the methanolic as well as the ethanolic extracts had more potent anthelmintic property than the positive control (Piperazine citrate) (Tables 3 and 4).

DISCUSSION

Like Setharam et al[13] we found that ethanolic extract of the plant was active against *Escherichia coli.* But unlike Setharam et al[13] we observed that ethanolic extract of the plant was also effective against *Staphylococcus aureus*. Like Pal et al[14] we also saw that the methanolic extract was active against *Bacillus subtilis*. Moreover, we found that this extract was more effective in cases of *Staphylococcus aureus* and *Escherichia coli*. Although our extracts were inferior to the positive control as far as zones of inhibition were concerned, the differences between the zones of inhibition produced by the positive control and the extracts against *Escherichia coli* and *Bacillus subtilis* were not remarkable. Even at low concentration (100µg/ml), both the extracts showed antibacterial activity. Those extracts at 200µg/ml produced good antibacterial activity against *Escherichia coli* and *Staphyloccus aureus*. From the above mentioned results, it may be concluded that both the extracts possess antibacterial activity.

It is known that due to the presence of several phytochemical constituents like alkaloids, flavonoids, glycosides, saponins, tannins, steroids etc., the plant extract generally show antibacterial property[4,5]. From our result, it may be mentioned that methanolic extract was relatively more potent against *Staphylococcus aureus* may be due to the presence of steroids, which was absent in case of the ethanolic extract. On the other hand, the ethanolic extract was more effective against *Escherichia coli* appeared to be due to the involvement of alkaloids and tannins which were present in this extract only. In addition, the methanolic extract was effective against different strains of bacteria, which was expected to be due to the presence of flavonoids, glycosides, saponins and steroids, whereas the antibacterial efficacy of ethanolic extract was probably due to alkaloids, flavonoids, glycosides, saponins and tannins (Tables 1 and 2). Our preliminary phytochemical studies revealed the presence of flavonoids, glycosides and saponins as the chemical classes which were present in both the extracts. So, the leaves of *Saraca indica* are rich in alkaloids, flavonoids, glycosides, saponins, tannins and steroids. These phytochemicals probably confer antimicrobial activity on the leaf extracts.

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 (Tables 1, 3 and 4). The presence of alkaloids, glycosides, tannins and flavonoids seems to be the responsible phytochemical constituents for demonstrating anthelmintic activities of our extracts[3,7]. It is known that 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 tracts of host animal or glycoprotein on the cuticle of the parasite and cause death[6]. Moreover, it is also possible that alkaloids may act on CNS and cause paralysis of *Pheritima posthuma* worms[3].

Like ours, similar studies should be carried out to identify the actual phytochemical constituents that are present in the crude drug extracts of this plant, which are responsible for antibacterial and/or anthelmintic activities. Thorough investigation should be performed for this purpose. Systematic screening of plants (using crude plant extracts instead of taking pure compounds) may lead to the discovery of novel active compounds.

As has been mentioned, it is very important to perform such a screening of different plants in order to validate the use of plant products in folk medicine. 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

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

'+' = Present; '-' = Absent

Table 2: Antibacterial activity pattern of the extracts of *Saraca indica* leaves and controls

Microorganism	Zone of inhibition after 24 hrs(in mm)					
	Ethanolic Extract		Methanolic Extract		Chloramphe-nicol	DMSO
	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml	100 µg/ml	
Staphylococcus aureus	12.6	14.3	19.3	23.3	36	0
Bacillus subtilis	13.2	16.6	12.3	15.3	24	0
Escherichia coli	13.3	18.3	13.5	15.6	24.5	0

Table 3: 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 4: 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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