

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY STUDY OF *SARACA INDICA* LEAVES EXTRACT

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ABSTRACT

To know the role of different phytochemicals in relation to the *in vitro* antibacterial activity of both the ethanolic and methanolic extracts of *Saraca indica* (Family-Caesalpinaceae), present study was conducted. Following standard methods, phytochemical screening of each extract was performed. For antibacterial activity study, both the extracts were used at 100 µg/ml and 200 µg/ml concentrations. The largest zone of inhibition was produced when ethanolic extract (100 µg/ml and 200 µg/ml) was used against *Escherichia coli*, whereas the zone was least in case of *Staphylococcus aureus*. However, methanolic extract (100 µg/ml and 200 µg/ml) gave maximum zone of inhibition against *Staphylococcus aureus* and it was minimum in case of *Bacillus subtilis*. Although our extracts were inferior to the positive control (chloramphenicol) 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. So, it may be noted that both the extracts, even at lower concentration, possess antibacterial activity. From the results of both the studies, it may be concluded that the ethanolic extract showed antibacterial activity was probably due to the presence of alkaloids, tannins, saponins, flavonoids and glycosides. On the other hand, due to different phytochemicals such as steroids, saponins and glycosides, the methanolic extract indicated antibacterial activity. Preliminary phytochemical studies revealed the presence of glycosides and saponins as the phytochemical classes present in both the extracts.

KEY WORDS- *Saraca indica*, methanolic extract, ethanolic extract, phytochemical screening, antibacterial activity.

INTRODUCTION

Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs¹. The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries². Several top selling drugs of modern times such as Quinine, Artemisinin, Shikonin, etc. are obtained from plants¹. Most of the phytochemicals, secondary metabolites of plants, are physiologically active². Majority of phytochemicals are known to produce therapeutic activities like antibacterial, antifungal, antioxidant, etc. 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 Industry¹.

Recently, several infections have increased enormously and antibiotic resistance has become an ever-increasing therapeutic problem. Natural products of some plants may possess a potential source of antimicrobial agents with possibly novel mechanisms of action. They are effective in the treatment of infectious diseases while

decreasing many of the side effects that are often found in synthetic antimicrobials. Therefore, in order to validate their use in folk medicine, it is very important to perform a screening of these plants. This evaluation is also useful to show their active principle by isolation and characterization of their constituents. Systematic screening of them may lead to the discovery of novel active compounds². 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 compounds¹.

Considering the above mentioned importance of phytochemical screening along with the antibacterial activity of its extracts, we used extracts of *Saraca indica* leaves. *Saraca indica* (Roxb) de wild (Family-Caesalpinaceae) is commonly known as Asoka, Sita Asoka and Haempushpam. It is an evergreen tree which is 9 m 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 Himalayas³. Useful parts of the plant are barks, leaves, flowers and seed. 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 stomachalgia⁴. 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 India⁵.

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^{6,7}.

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*⁸. In addition, some other researchers have also indicated that those extracts of the leaves of the plant show antibacterial activity only against *Escherichia coli*⁹. Moreover, both the methanolic and water extracts of its leaves are effective against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*¹⁰. Considering it as a potential antibacterial agent, we undertook antibacterial activity study of the ethanolic and methanolic extracts of leaves of *Saraca indica*.

Since several phytochemicals possess antibacterial activity, we decided to correlate phytochemical screening with antibacterial activity of both ethanolic and methanolic extracts of *Saraca indica* leaves.

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 dessicator 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 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 evaporator¹¹. The yield of ethanolic extract was 4.6 gm 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 antibacterial activity.

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

Alkaloids

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

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

Amino acids

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

Carbohydrates

Molish test-To 2 ml of the test extract, at first, few drops of alcoholic α -naphthol 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 2 ml 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 1 ml of sulphuric acid in a test tube for 5 minutes. 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 the lower 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 2 ml 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, and formation of yellow colour at the lower layer suggested the presence of triterpenoids¹³.

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 performed¹⁴. Sterile nutrient agar medium was inoculated with 0.1 ml of fresh overnight nutrient broth culture of *Staphylococcus aureus* (approx. 10^7 CFU/ml) and poured into sterile petriplates. In each plate, four wells of 6 mm in diameter were punched using a sterile borer and the plates were allowed to dry for 5 min. In one well 50 μ l of ethanolic extract was poured. In other wells, 50 μ l 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 μ g/ml and 200 μ g/ml concentrations¹⁴. The concentration of chloramphenicol was 100 μ g/ml¹⁵. After holding the plates 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 °C for 24 hrs. Then they were examined for inhibition of the bacterial growth around the wells. The diameters of the zones of inhibition in each case were measured¹⁴.

RESULTS

We have performed phytochemical screening of ethanolic and methanolic extracts of *Saraca indica* leaves. For the purpose of screening, different phytoconstituents were studied. Using standard methods, we tested for alkaloids, amino acids, carbohydrates, flavonoids, glycosides, saponins, tannins, proteins, steroids and triterpenoids. In case of ethanolic extract, alkaloids, flavonoids, glycosides, saponins and tannins were present, whereas flavonoids, glycosides, saponins and steroids were detected in the methanolic extract (Table 1).

While the zone of inhibition was the largest when ethanolic extract (100 μ g/ml and 200 μ g/ml) was used against *Escherichia coli*, it was least in case of *Staphylococcus aureus*. The methanolic extract (100 μ g/ml and 200 μ g/ml) produced maximum zone of inhibition against *Staphylococcus aureus* and the minimum zone was found in case of *Bacillus subtilis*. Chloramphenicol was found to produce 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.

DISCUSSION

Like Setharam et al⁹, we found that ethanolic extract of the plant was active against *Escherichia coli*. Unlike Setharam et al⁹, we observed that ethanolic extract of the plant was also effective against *Staphylococcus aureus*. Like Pal et al¹⁰, we also saw that the methanolic extract was active against *Bacillus subtilis*. Moreover, we found that the methanolic 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 (100 μ g/ml), both the extracts showed antibacterial activity. Those extracts at 200 μ g/ml produced good antibacterial activity against *Escherichia coli* and *Staphylococcus 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^{6,7}. From our result, it may be mentioned that methanolic extract was relatively more potent against *Staphylococcus aureus*. This 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* which was appeared to be due to the involvement of alkaloids and tannins. Those phytoconstituents were present in this extract only. In addition, the methanolic extract was effective against different strains of bacteria which may 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 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.

In conclusion, the extracts studied here have displayed variable antibacterial activities most probably due to the differences in the phytochemical constituents extracted by two different solvents used in our study. Systematic

screening of plants (using crude plant extract instead of taking pure compounds) may lead to the discovery of novel active compounds.

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Table 1- Qualitative analysis of various extracts of *Saraca indica* leaves.

| Phytoconstituents | Ethanollic extract | Methanollic 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) | | | | | |
|------------------------------|--|-----------|---------------------|-----------|------------------|------|
| | Ethanollic Extract | | Methanollic Extract | | Chloramphe-nicol | DMSO |
| | 100 µg/ml | 200 µg/ml | 100 µg/ml | 200 µg/ml | 100 µg/ml | |
| <i>Staphylococcus aureus</i> | 12.6 | 14.3 | 19.3 | 23.3 | 36 | 0 |
| <i>Bacillus subtilis</i> | 13.2 | 16.6 | 12.3 | 15.3 | 24 | 0 |
| <i>Escherichia coli</i> | 13.3 | 18.3 | 13.5 | 15.6 | 24.5 | 0 |

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