Antimicrobial Properties of the Leaves of *Saraca indica*

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Acetone, chloroform, methanol, petroleum ether and water extracts of leaves of *Saraca indica* (family-Caesalpiniaceae) were investigated for their antibacterial activity against standard strains of *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* by well diffusion method. Acetone, chloroform, methanol and petroleum ether extracts showed significant antibacterial activity against only Gram positive bacteria. Water extract is found active against *Bacillus subtilis* and *Bacillus megaterium*. Fresh leaves juice and acetone extracts showed highest antimicrobial activity against Gram positive microorganisms. Results were compared with standard antibiotics Amoxicillin-Am30, Ciprofloxacin-Cf 30 , Cotrimaxazole-Co25, Gentamicin-G30 and Tetracycline-T30. The antibacterial activities of the Acetone, chloroform, methanol and petroleum ether extracts are discussed according to their phytochemical components. It is concluded that the *Saraca indica* may serve as valuable source of compounds with therapeutic potential.

**Key words:** Antibacterial activity, *Saraca indica*, antibiotics, Acetone, Chloroform, Methanol and Petroleum ether extracts.

Herbal medicine has such an extraordinary influence that numerous alternative medicine therapies treat their patients with Herbal remedies, Unani and Ayurveda. Approximately 25 percent of all prescription drugs are derived from trees, shrubs or herbs. Nature has bestowed our country with an enormous wealth of medicinal plants therefore India has often been referred to as the medicinal garden of the world. So stand the medicinal plants *Saraca asoca* as one of the foremost plants utilized from antiquity till to date (Pradhan,P. et al., 2009).

The diverse culture of our country is a rich source of traditional medicine, many of which are of plant origin. Scientific data of such plant derivatives could be of clinical use. Through out the world in current research scenario, the development of a new drug entity with promising efficacy and less toxicity for treatment of various dreadful diseases requires huge investment and longer period of clinical trials. Drugs from natural plants are considered to be a promising source to serve purposes. Among the diversity of most natural products, medicinal plants are still the most favorable for the scientist to explore a new drug. *Saraca indica* is the one of the most interesting medicinal plant as all part of this plant contain significant & beneficial chemical constituents, which helps in treating various diseases and disorders (Shelar,D.B. et al., 2010).

Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs (Hidayathula S.et al., 2011). The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries.

* To whom all correspondence should be addressed. E-mail: chandekarc@yahoo.com
(Shihabudeen HMS et al., 2010). Several top selling drugs of modern times such as Quinine, Artemisinin, Shikonin, etc. are obtained from plants (Hidayathula S. et al., 2011). Most of the phytochemicals, secondary metabolites of plants, are physiologically active (Shihabudeen HMS et al., 2010). The plants are known to provide a rich source of botanical, anthelmintic, antibacterials, and insecticides (Acharya S. et al., 2011).

Plants are used as medicine since time immemorial. Drugs from plant sources are being used by about 80% of the world population. Herbal medicines have stood the test of time for their safety, efficacy, acceptability and lesser side effects (Kamboj, V. P., et al., 2000, Sannomiya, M., et al., 2007).

*Saraca indica* belongs to the family Caesalpiniaceae. It is found in India, China, Ceylon and Malaysia. It occurs almost throughout India up to an altitude of 750 m in the central and in the eastern Himalayas and Khasi, Garo and Lushai hills, wild in Chittagong, Bihar, Orissa, Konkan, Deccan, Mysore. It has become quite scarce in several localities and is reported to be threatened in North Eastern Region of India (Shilpakala Sainath R. et al., 2009).


**MATERIAL AND METHODS**

**Selection of medicinal plant for this study**

*Saraca indica*

**Family**

Caesalpiniaceae

**Parts used**

Leaf

**Traditional uses**

The plant is useful in dyspepsia, fever, burning sensation, colic, ulcers, menorrhagia, leucorrhoea, pimples etc. The bark, used for 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 stomachaalgia (Srivastava G. N. et al., 1988).

**Chemical constituents**

It mainly contains glycosidic principles, non-phenolic, sapogenetic glycoside, sterols and aliphatic alcohols. catechin, (-) epicatechol and leucocyanidin has been isolated from pods and wood contains quercetin. In the powdered bark ash of Ashoka minerals like silica, sodium, potassium, phosphate, magnesium, iron, calcium, strontium and aluminium have been found (N. Indrani, K. et al., 1984, D. J. Kaur, et al., 1980, T. B. Middelkoop, et al., 1985). The flowers contain fatty acids and gallic acid; apigenin-7-O-beta-D-glucoside, cyanidin-3, 5-diglucoside, kaempferol 3-O-beta-D-glucose, pelargonidin-3, 5-diglucoside, quercetin and its 3-O-beta-D-glucoside and sitosterol. The bark yields alkanes, esters and primary alcohols. It gave n-octacosanol, tannin, catechin, (+)-catechol, (-)-epicatechin, (-)-epicatechol, leucoycyanidin, leucopelargonidin, procyandin derivatives, methyl- and ethylcholesterol derivatives. Quercetin and its 3-O-rhamnoside, kaempferol-3-O-α-L-rhamnoside, amyrin, ceryl alcohol and β-sitosterol have been isolated from leaves and stems (M. Behari, et al., 1977). It also contains flavonoids and sterols (L. Vijai., et al., 1976, M. Behari., et al., 1977).

**Identification and Preservation of Plant materials**

Fresh plant leaves were collected from the Nagpur area of India. The taxonomic identities of this plant was determined by the expertise of the Post Graduate Department of Botany of Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. Specimen was labeled, numbered and noted with date of collection, the locally and their medicinal uses and their approximate dosages of administration were recorded. Plant leaves were washed with 70% alcohol and then rinsed with sterilized distilled water, air dried and stored in airtight bottles at 4°C for further use.

**Preparation of crude extracts (Fresh juice)**

Plant leaves were collected from around
Nagpur region in the month of August-September. Leaves were cleaned under running potable water and were washed with 70% alcohol and then rinsed with sterilized distilled water, cut into pieces and grounded in pestle and mortar (made up of dolerite stone) till homogenized mass was obtained. Homogenized mass was squeezed in 400 mesh nylon cloth (pore size 37 micron) to obtain crude extract. Crude extract was kept in sterilized glass bottle. All crude extract were prepared fresh and used before 2 hours. Cold extracts were prepared using individual fresh plant leaves.

**Aqueous extraction**

Ten grams of dried powder was extracted in 100 ml distilled water for 6 h at slow heat. Every 2 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 15 min. The supernatant was collected. This process was repeated twice and after 6 h, the supernatant was concentrated to make the final volume one-fourth of the original volume (Shahidi Bonjar GH 2004). It was then autoclaved at 121 ºC and 15 lbs pressure and then stored at 4ºC.

**Solvent extraction**

Ten grams of dried powder was extracted with 100 ml of each solvent (acetone, chloroform, methanol and petroleum ether) and flasks were kept on a rotary shaker at 190-220 rpm for 24h. Thereafter, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Shahidi Bonjar, G.H. 2004). It was stored at 4ºC in airtight bottles for further studies.

**Bacterial cultures**

The microbial strains are identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The studied bacterial strains were Bacillus cereus NCIM2155, Bacillus subtilis NCIM2063, Bacillus megaterium NCIM2087, Escherichia coli NCIM2931, Proteus vulgaris NCIM2857 and Pseudomonas aeruginosa NCIM5029. Staphylococcus aureus MTCC96 this strain was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. They were sub-cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4ºC, fresh inoculums were taken for test.

**Media**

- Hi-Sensitivity test broth (M 486) and Hi-sensitivity test agar (M 485) were procured from Hi-media Mumbai, India. The media were prepared according to the instructions given.

**Screening for the antimicrobial potential of the plant leaves extracts**

The antimicrobial activity of different solvent extracts was evaluated by agar well diffusion (Perez C, et al. 1990 & Parekh, J, et al., 2007) using Hi-sensitivity test agar (M 485).

**Preparation of inoculum**

A loopful of culture was inoculated from the stock slant culture in 5 ml of Hi-sensitivity test broth and broth was incubated at 35±0.5ºC in incubator for 18-20 hrs. After incubation a loopful of actively growing culture was inoculated into 10 ml of Hi-sensitivity broth. Broth was incubated at 35±0.5ºC for 6-8 hours. This culture was used for the inoculation of Hi-sensitivity test agar plates.

**Preparation of Hi-sensitivity test agar medium**

Hi-sensitivity test agar medium was prepared as per instructions of manufacturer. Required amount of agar medium was melted and 25 ml of molten medium was distributed in test tubes (25x150 mm). Medium was autoclaved at 15 lb. for 20 min. After autoclaving, medium was maintained at 45-50ºC in constant temperature water bath.

**Inoculation of medium with test organism**

0.5 ml of 6-8 hours old test organism is transferred to petridish of 100mm size (Sterilized in oven at 180ºC for 1 hr.) using sterile micropipette. Hi-sensitivity test agar medium maintained at 45-50ºC was poured and mixed properly to ensure uniform distribution of organism with medium. Seeded plates are allowed to set at room temperature.

**Preparation of agar well for fresh leaves juice**

10 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made. A 100 µl crude extract (fresh leaves juice) was transferred by micropipette per well. Plates were immediately kept at 4ºC in refrigerator for 1 hr. for the diffusion of extract and then shifted to 35±0.5ºC in incubator. Zone of inhibition was measured after 24 hrs. of incubation by zone scale.

**Preparation of agar wells for different solvent extracts**

5 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made.
A 50 ul solvent extract was transferred by micropipette per well. Plates were immediately kept at 4°C in refrigerator for 1 hr. and then shifted to 35°C±0.5°C in incubator. Zone of inhibition was measured after 24 hrs. of incubation. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter is obtained.

RESULTS

All the microorganisms responded differently to the various plant extracts and standard antibiotics. All the plant extracts and antibiotics tested showed some antimicrobial activity (Table-1). The fresh leaves juice was found to be active against only Gram positive bacteria. When we compared the activity of aqueous extract with fresh leaves juice, the fresh leaves juice is more active. The aqueous extract found to be active against Bacillus subtilis and Bacillus megaterium (Fig-3 & Fig-2).

Acetone, methanol and petroleum ether extracts are active against only Gram positive bacteria Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, and Bacillus megaterium. All the Gram negative microorganisms i.e. Escherchia coli, Proteus vulgaris and Pseudomonas aerogenosa are found resistant to fresh juice of leaves and acetone, methanol, chloroform and petroleum ether extracts. Seetharaman et al., and Sarojini Nayak et al., found that the ethanol extract of leaves was active against Escherchia coli and Staphylococcus aureus. Pal et al., and Sarojini Nayak et al., saw that the methanolic extract was active against Bacillus subtilis. Moreover, Sarojini Nayak et al., found that the methanolic extract was more effective in cases of Staphylococcus aureus and Escherichia coli. Rajesh Dabur et al., found that the petroleum ether extract is active against Staphylococcus aureus and Proteus vulgaris. Methanol extract is found active against Pseudomonas aerogenosa, Staphylococcus aureus and Proteus vulgaris. Chloroform extract is found inactive against Pseudomonas.

Table 1. Results of antimicrobial activities of fresh leaves juice and solvent extracts of Saraca indica and compared with standard antibiotics

<table>
<thead>
<tr>
<th>No.</th>
<th>Microorganisms</th>
<th>Leaves extracts</th>
<th>Zone of inhibition in mm</th>
<th>Standard antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FJ</td>
<td>WE</td>
<td>AE</td>
</tr>
<tr>
<td>1.</td>
<td>Escherchia coli</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Proteus vulgaris</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Pseudomonas aerogenosa</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Staphylococcus aureus</td>
<td>28</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>5.</td>
<td>Bacillus cereus</td>
<td>25</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>6.</td>
<td>Bacillus subtilis</td>
<td>28</td>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td>7.</td>
<td>Bacillus megaterium</td>
<td>23</td>
<td>10</td>
<td>24</td>
</tr>
</tbody>
</table>

Key: FJ-Fresh juice of leaves; WE-Water extract; AE-Acetone extract; ME-Methanol extract; PE-Petroleumether; Am<sup>30</sup>-Amoxycilin; Cf<sup>30</sup>-Ciprofloxacin; Co<sup>25</sup>-Cotrimaxazole; G<sup>50</sup>-Gentamicin; Tetracycline-T<sup>30</sup>. ND—Not determined; - Negative.
Antibacterial activity of different solvent extracts of leaves of *Saraca indica* (SI).
Zone of inhibition in millimetre (mm).

Activity against *Bacillus cereus*
- Acetone extract (A)-24 mm
- Chloroform extract (C)-18 mm
- Methanol extract (M)-21 mm
- Petroleum ether extract (P)-20 mm

**Fig. 1:**

Activity against *Bacillus megaterium*
- Acetone extract (A)-24 mm
- Chloroform extract (C)-18 mm
- Methanol extract (M)-15 mm
- Petroleum ether extract (P)-17 mm

**Fig. 2:**

Activity against *Bacillus cereus*
- Acetone extract (A)-32 mm
- Chloroform extract (C)-28 mm
- Methanol extract (M)-28 mm
- Petroleum ether extract (P)-28 mm

**Fig. 3:**

Activity against *Staphylococcus aureus*
- Acetone extract (A)-21 mm
- Chloroform extract (C)-14 mm
- Methanol extract (M)-18 mm
- Petroleum ether extract (P)-15 mm

**Fig. 4:**
They also found that water extract is more active against *Escherchia coli* *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus*.

The fact that the aqueous extract is more active than methanol extract implies that the antimicrobial action may be due to the synergistic action of different chemical constituents, some of which probably are lost upon extraction with solvent (Bibitha B *et al.*, 2002).

Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent (Marjorie, M.C. 1999). Acetone extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity. The demonstration of antimicrobial activity by water extracts provides the scientific basis for the use of these plants in the traditional treatment of diseases, since most traditional medicine men use water as their solvent in which the decoctions are prepared. In our study fresh leaves juice and acetone extracts showed highest antimicrobial activity against Gram positive microorganisms.

Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.*, 1996, Reuben, K.D., 2008). Natural products either extract or pure compounds provide unlimited opportunities for the development of new drugs due to the availability of chemical diversity (Cos, P., *et al.*, 2006). To overcome the problem of antibiotic resistance ethnic medicinal plants have been extensively studied as an alternative treatment for diseases due to their ability to produce a variety of compounds of known therapeutic properties (Harbone, S.B.*et al.*, 1995& Kumar, B., *et al.*, 2007) and much attention has been paid to plant extracts and their biologically active compounds (Suresh, G.*et al.*, 2010). The screening of natural products has been the source of innumerable therapeutic agents (Koroseczviv, J.I.*et al.*, 1992). Higher plants as a source for new potential drugs is still largely unexplored and only a small percentage of them has been subjected to phytochemical investigation and the fractions submitted to pharmacological screening is very low. Such screening of various natural organic compounds and identifying active agents is a need of the hour as due to successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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**REFERENCES**


