

PHARMACOGNOSTICAL STUDY AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF *SOPHORA INTERRUPTA BEDD*

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ABSTRACT

Sophora interrupta Bedd, was a common woody perennial herb native to India which belongs to Leguminosae family. The present study deals about anti-bacterial activity of *Sophora interrupta*. The antibacterial activity of benzene extract of *Sophora interrupta bedd*, was carried out by disc diffusion technique. The root extract was subjected to preliminary phytochemical screening. The benzene extract of *Sophora interrupta* of different concentrations about 0.25, 0.5, 0.75, 1.0, 1.25, 1.50mg/ml was made dissolved in DMSO. These solutions were sterilized using filtration sterilization technique, these dilutions were used to test the antibacterial activity of 3 different strains viz, *Bacillus subtilis*, *Pseudomonas aureus* and *Pseudomonas syringe*. The obtained results were compared with standard drug Ciprofloxacin. The

minimum inhibitory concentration was determined for the concerned microorganism. From the experimental results it can be concluded that root extract of *Sophora interrupta Bedd.*, has very good Antibacterial activity.

KEYWORDS: Antibacterial activity, Benzene extract, Ciprofloxacin, Disc diffusion technique, *Sophora interrupta*.

INTRODUCTION

Many higher plants accumulate extractable organic substances in quantities sufficient to be economically useful as pharmaceuticals/antibiotics. Species of higher plants are much less surveyed for antibacterial activity.^[1] Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. It is estimated that only one percent of 2, 65,000 flowering plants on earth have been studied exhaustively for their chemical composition and medicinal value.^[2]

In many developing countries traditional medicine is one of the primary health care systems.^[3] India is well known for Ayurveda, which is one of important traditional medicine practiced. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented.^[4] Plants grown in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular. Alternatives to available antibiotics for disease management are increasingly felt due to the increase in the resistance of bacterial isolates. This has necessitated the requirement of second and third line drug.^[5]

Antibacterial active principles isolated from higher approaches to contain antibiotic resistance and the management of disease. It is believed that plant based drugs cause less or no side effect when compared with synthetic antibiotics.^[6] Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of the active principle and further leading to drug development.

The major drawbacks of synthetic antimicrobials include:

➤ **Adverse effects like**

Hypersensitivity,

Immune suppression

Allergic reaction.

➤ Incidence of resistance to antibiotics

By considering the drawbacks of synthetic antimicrobials the attempts were made to evaluate the potentiality of the plant extract *Sophora interrupta* against standard micro-organisms. *Sophora interrupta* belongs to the family Fabaceae. There are more than hundreds of species belongs to this family which have various pharmacological activities such as anti-cancer, anti-inflammatory, antispasmodic, antibacterial. From the preliminary phytochemical studies it was identified that it has constituents like alkaloids, flavonoids, glycosides, phenols, carbohydrates and proteins.^[7]

Table 1: Taxonomic status of *Sophora interrupta*^[8]

Domain	Eukaryota
Kingdom	Plantae
Sub-kingdom	Viridiaeplantae
Phylum	Tracheophyta
Class	Spermatopsida
Sub-class	Magnoliidae
Super Order	Rosanae
Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Tribe	Sophoreae
Genus	Sophora
Species	interrupta

MATERIALS

The Roots of plant (*sophora interrupta* fabaceae) were collected from surroundings of Tirumala hill, Tirupati, Andhra Pradesh, India. chemicals and reagents like Benzene, Dimethyl Sulfoxide, Hydrochloric acid, Nutrient Agar Medium, Iodine solution, Choral hydrate, Phloroglucinol, Potassium hydroxide, Picric acid, Lead acetate.

METHODS

MACROSCOPICAL AND MICROSCOPICAL STUDY

The macroscopic, microscopy of plant was studied, the cross sections were prepared. The microscopic analysis of root powder was also done. Initially a small quantity of powder drug or thin cross section was taken in to watch glass and few drops of chloral hydrate solution was added and mix it thoroughly and drain off excess of reagent with filter paper. This was continued by staining process by using phloroglucinol and hydrochloric acid in the ratio 1:1 and mounted on glass slide. Then add half drop of glycerin water and mix the contents

properly and place the cover slip carefully to mount the slide. Finally observe the slide under low power magnification.^[9]

PHYSICOCHEMICAL ANALYSIS

Air dried plant material was used for quantitative determination of ash and extractive values. The residue remaining after incineration is ash value. Priorly thin porcelain dish or tarred silica crucible was weighed. The powder of about 2gms was taken and made incinerated in muffle furnace at temperature of 450⁰C or above. Then the sample was removed from muffle furnace and made cool in desiccators and finally the obtained ash was weighed and calculated.

Extractive values may be studied on wet weight or dry weight basis using maceration or percolation or continuous extraction process (soxhlet extraction) was determined by weighing about 5g of powdered drug of *Sophora interrupta* was added then which is transferred to 250ml conical flask and 100ml of desired solvent. The above conical flask was set aside for 24 hrs with frequent shaking and continued by filtering. 25ml of filtrate was collected and transferred into porcelain dish and evaporated to dryness on water bath. The residue was dried in an oven and subjected for cooling in desiccator and finally the percentage of extractive value on wet weight or dry weight basis was calculated. Fluorescence analysis of extract was carried out under ultra violet radiation.^[10]

EXTRACTION

The roots were collected and shade dried for 5days. Extraction of active constituent was done by using benzene in soxhlet apparatus.

The root powdered drug of *Sophora interrupta* of about 50gms was placed inside thimble made from thick filter paper and cotton, which is loaded in to the main chamber of soxhlet extractor. Then the soxhlet extractor is placed onto the flask containing the solvent benzene. The soxhlet is then equipped with a condenser. The solvent is heated to reflux over 4 days and finally the obtained compound was concentrated in the distillation flask.^[11]

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was carried out by using standard procedure.^[12] The benzene extract was tested for the presence of phytoconstituents viz. Flavanoids, alkaloids, glycosides, saponins and carbohydrates.

➤ **TEST FOR CARBOHYDRATES**

a) **MOLISCH TEST**

To 1 mg powder, two drops of alcoholic solution of alpha-naphthol were added. The mixture was shaken and 1 ml of conc. Sulphuric acid was added slowly along the sides of the test tube, the test tube was cooled in ice water and allowed to stand. A violet coloured ring at the junction indicates the presence of carbohydrates.^[13]

b) **BENEDICTS TEST**

To 1 mg of the powder drug 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 minutes. A red green or yellow coloured precipitate indicates the presence of sugar.^[14]

➤ **TEST FOR PROTEINS**

BIURET TEST

To the 2 ml of test solution, add 2 ml of Biuret reagent, violet colour indicates presence of proteins.

➤ **TEST FOR ALKALOIDS**

DRAGONDROFFS TEST

To the 2 ml of test solution add 2 ml of Dragondroff's reagent (potassium bismuth iodide solution). Reddish brown precipitate indicates the presence of alkaloids.

➤ **TEST FOR TANNINS**

FERRIC CHLORIDE TEST

Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

➤ **TEST FOR STEROIDS**

SALKOWSKI TEST

Treat the extract with few drops of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of tri terpenoids.

➤ **TEST FOR FLAVANOIDS**

SHINODA TEST

To the test solution add few magnesium turnings and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

➤ **TEST FOR SAPONINS**

FROTH FORMATION TEST

Place 2 ml solution of drug in water in a test tube, shake well, stable froth (foam) is formed.

➤ **TEST FOR GLYCOSIDES**

BORNTRAGERS TEST

Boil the test material with 1ml of sulphuric acid in a test tube for five minutes and filter while hot. Cool the filtrate and shake with equal volume of chloroform. Separate the lower layer of chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

EVALUATION OF ANTIBACTERIAL ACTIVITY

The antibacterial activity of benzene extract of *Sophora interrupta bedd*, was carried out by disc diffusion technique. The test organisms used for evaluation of antibacterial activity are mutant strain of *Bacillus subtilis*, *Pseudomonas aureus* and *Pseudomonas syringe*. These cultures were maintained on nutrient agar by sub culturing them on fresh slants after every 4 weeks. Temperature for incubation was at 30⁰c for 24 hrs.

The benzene extract of *Sophora interrupta* of different concentrations about 0.25, 0.5, 0.75, 1.0, 1.25, 1.50mg/ml was made dissolved in DMSO. These solutions were sterilized using filtration sterilization technique (membrane filter # 0.45μ), these dilutions were used to test the antibacterial activity of 3 different strains viz, *Bacillus subtilis*, *Pseudomonas aureus* and *Pseudomonas syringe*. The obtained results were compared with standard drug Ciprofloxacin. The minimum inhibitory concentration was determined for the concerned microorganism.^[15]

RESULTS AND DISCUSSION

MACROSCOPIC CHARACTERS

The roots are woody, tuberous, perennial, about 4 to 8 cm in diameter, light brownish in colour with characteristic odour and highly bitter in taste. Fractured surface is fibrous, inner tuber is whitish cream coloured. The tuber showed many rootlets.



Figure No. 1: Twig of *Sophora interrupta*



Roots of *Sophora interrupta*

MICROSCOPIC CHARACTERS

Cork cells are arranged in 12-14 layers with lignified suberised rectangular shaped cells. Cortex composed of several layers of loosely arranged thin walled parenchymatous cells. In cortex the cells are arranged without any intercellular spaces. Calcium oxalate crystals are seen in this region. Vascular bundles are radially arranged, xylem and phloem constitutes xylem bundles, phloem bundles respectively and present alternatively on different radii. Uniseriate medullary rays are seen in between the vascular bundles, they are formed of radially arranged thin walled parenchymatous cells from centre to cortex (Figure no.2).

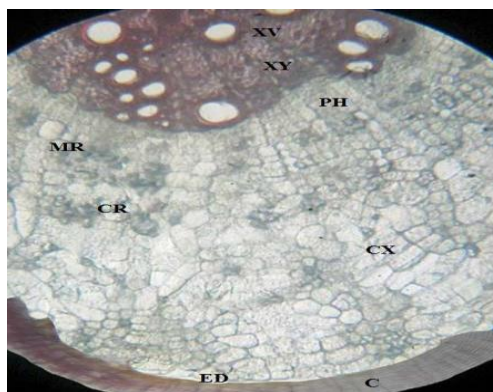


Figure No. 2: T.S of *Sophora interrupta* root

POWDER CHARACTERS OF *Sophora interrupta* ROOT

Powder of root material showed the presence of xylem vessels with annular and scalariform thickenings, cork cells, starch grains and calcium oxalate crystals. These powder characteristics can be used for diagnostic purpose of crude drug. (Figure no. 3). Quantitative analysis of root powder was also done and the results were shown in the table 1.

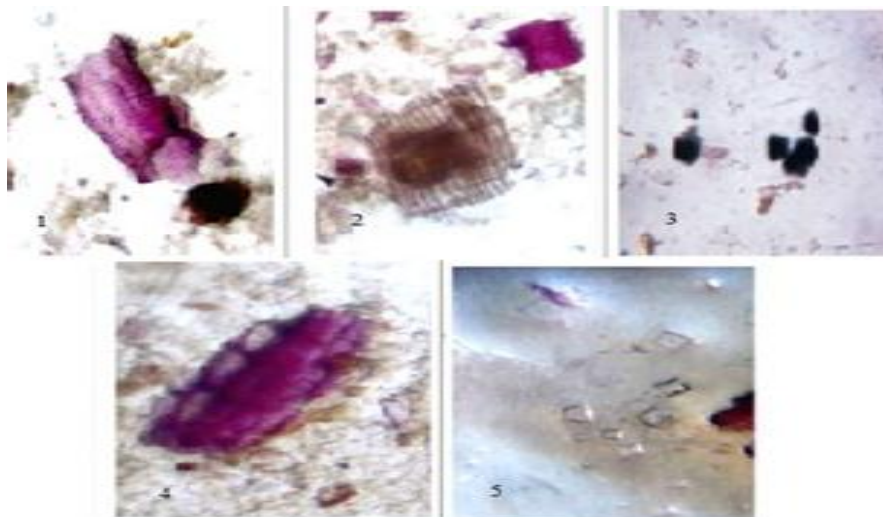


Figure No. 3: Powder characteristics of *Sophora interrupta* root

PHYSICOCHEMICAL ANALYSIS

Air dried root material was made into fine powder and used for quantitative determination of physicochemical values. Total, acid insoluble and water soluble ash (table 2) was determined in triplicate and its mean + S.D. was calculated (table 3). Alcohol and water extractive values were determined as per WHO recommendations while benzene soluble extractive was determined due to the medicinal attributes of the extract. Water extractive was found to be very high when compared to the other extractable matter in the powder.

Table 2: Extractive values

S. NO	PARAMETERS	VALUE(% W/W)
1	Water soluble	17.6 ± 0.08
2	Alcohol soluble	12.8 ± 0.12
3	Benzene soluble	16.9 ± 0.09

Table 3: Ash values

S. NO	PARAMETERS	VALUE (%W/W)
1	Total ash	3.95 ± 0.12
2	Acid insoluble	0.6 ± 0.04
3	Water soluble	7.6 ± 0.152
4	Sulphated ash	3.56 ± 0.13

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical analysis revealed the presence of flavanoids, alkaloids, glycosides, saponins and carbohydrates.

Table 4: Preliminary phytochemical screening

S. No	Test	Benzene
1	Carbohydrates	--
2	Amino acid	--
3	Protein	+
4	Alkaloids	+
5	Tannins	--
6	Steroids	+
7	Flavonoids	+
8	Saponins	--
9	Glycosides	--

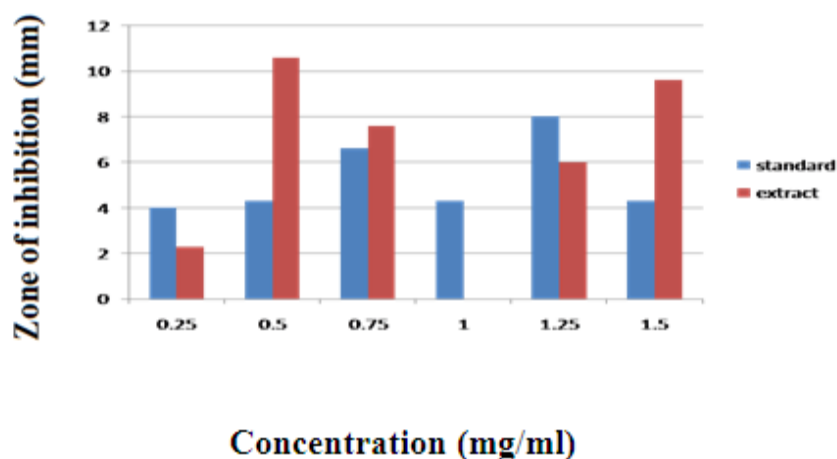
Table 5: MIC of benzene extract of *Sophora interrupta*

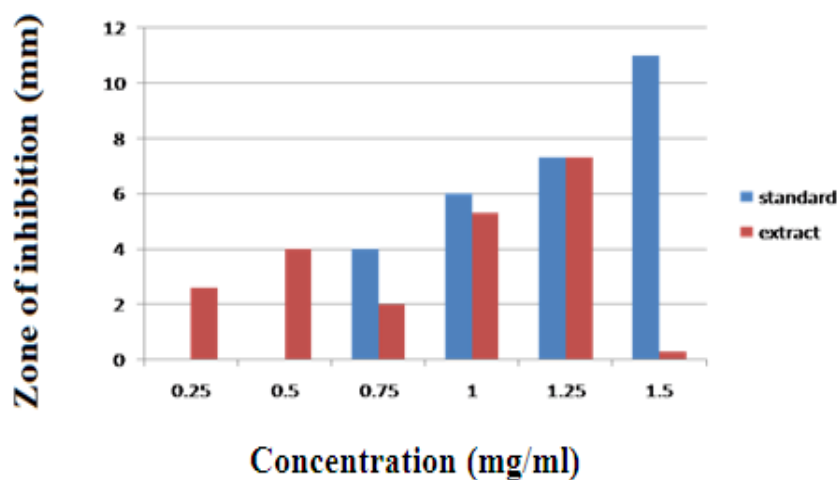
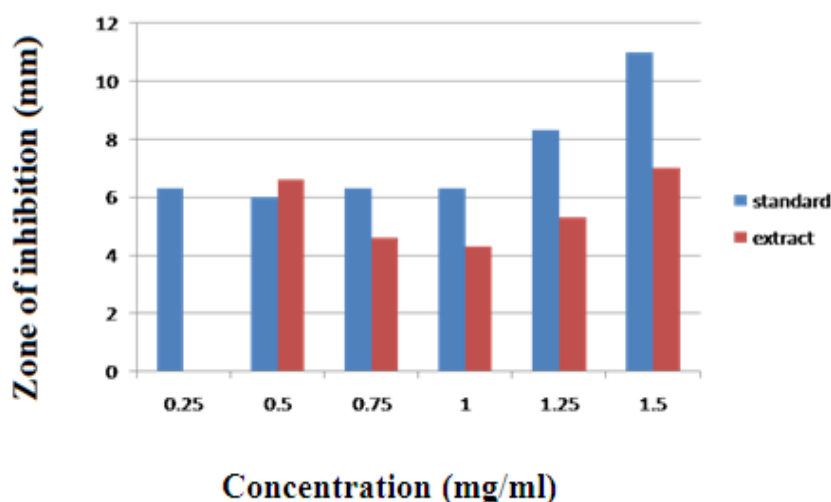
S.No	MICRO ORGANISM	ZONE OF INHIBITION(mm)											
		0.25mg/ml		0.5mg/ml		0.75mg/ml		1mg/ml		1.25mg/ml		1.5mg/ml	
		S1	E1	S2	E2	S3	E3	S4	E4	S5	E5	S6	E6
1	G+ <i>B. subtilis</i>	4	2.3	4.3	10.6	6.6	7.6	4.3	0	8	6	4.3	9.6
2	G- <i>P. syringe</i>	0	2.6	0	4	4	2	6	5.3	7.3	7.3	11	0.3
3	G+ <i>P. aureus</i>	6.3	0	6	6.6	6.3	4.6	6.3	4.3	8.3	5.3	11	7

Note: S: Standard; E: Extract.

EVALUATION OF ANTIBACTERIAL ACTIVITY

Bacillus subtilis



Pseudomonas syringe*Pseudomonas aureus***CONCLUSION**

Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents.

Plant based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials need to occur. Antimicrobials of plants origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases

while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

The active constituents of roots of *Sophora interrupta* Bedd., is extracted by using solvent benzene and the antibacterial activity is evaluated by Disc diffusion method. It showed a great significant antibacterial activity in concentrations of 0.5 to 1.5 mg/ml than standard concentrations of Ciprofloxacin.

Thus from the study it can be concluded that root extract of *Sophora interrupta* Bedd., has very good Antibacterial activity.

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