

## Research Article

# Anti-bacterial Investigation on Different Root Extracts of *Mimosa rubicaulis* Lam.

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### ABSTRACT

The current study was to investigate the presence of Anti-Bacterial activity on the different root extracts of *Mimosa rubicaulis* Lam.. The extracts obtained using continuous hot percolation method and Anti-Bacterial activity tested by using cup plate method. The present investigation reveals that all the three extracts namely ethylacetate, methanol and water extracts of concentration 1000 mcg/ml showing Anti-Bacterial activity against both gram + ve and gram -ve organisms. Among all the three extracts root methanolic extract showing more anti-bacterial activity. The study shown that the all the three root extracts of *Mimosa rubicaulis* Lam. Possess Anti-Bacterial activity.

**Key words:** *Mimosa rubicaulis* Lam., Anti-Bacterial activity, Continuous Hot Percolation, Cup plate method.

### INTRODUCTION

In the present day so many Anti-Bacterial agents were available. But most of the bacteria were got resistance to the most of the products. Traditionally *Mimosa rubicaulis* Lam. Had higher value in treating of various diseases like laxative, leucoderma, leprosy, chronic diarrhea, Rheumatism, Anti diabetic, Treatment of snake bites, Anti-fungal agent, Used for cuts & wounds (had wound healing property). As we know that generally natural origin compounds had less side effects and less toxic when compared with synthetic compounds.

In the early 1800s, an emerging fascination with such molecules give rise to the field of organic chemistry, so named for its emphasis on the chemistry of living things. Later discrete organic compounds were discovered and were known as 'natural products' or 'secondary metabolites'. In particular, higher plants have been the source of medicinal agents since the earliest times and today they continue to play a dominant role in the primary health care of about 80% of the world's

population<sup>1</sup> and it is not surprising to find that in many countries of the world there is a well-established system of traditional medicine, whose remedies are still being compiles. Natural products and medicinal agents derived there from are also an essential feature in the healthcare systems of the remaining 20% of the population residing mainly in developed countries, with more than 50% of all drugs in clinical use having a natural product origin<sup>2</sup>. The recent application of genetic approaches towards the study of biosynthesis has created a new and excited avenue for the discovery of new bioactive molecules.

The traditional use of the medicinal plants played a vital role in sustaining disease free human existence on this planet and can be traced back over five millennia to written documents of the early civilizations in China and India.

Although we have drugs of mineral and animal origin from Nature and synthetic substances, plants are the almost exclusive source of drugs for the majority of



Figure I: Plant images (*Mimosa rubicaulis* Lam.)

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**Table II: Preliminary phytochemical investigation:** The extracts prepared were tested for the type of chemical constituents present by known qualitative tests. The results are given as follows.

S.No.	Name of the test	Ethyl acetate extract	Methanol extract	Water extract
1	Liebermann burchard (for terpenes& steroid)	-	-	-
2	Salkowski (for forterpenes& steroid)	-	-	-
3	Mayer's (for alkaloids)	+	+	+
4	Molisch (for carbohydrates)	+	+	+
5	Fehling's (for carbohydrates)	+	+	+
6	Baljet's (for cardiac glycosides)	+	+	+
7	Legal's (for cardiac glycosides)	+	+	+
8	Test for phenolics (FeCl <sub>3</sub> test)	+	+	+
9	Shinoda (for flavonoids)	+	+	+

(+):Presence (-):Absence.

the world's population. In spite of overwhelming influence of modern medicine and tremendous advances made in the population of synthetic drugs, traditional medicament referred to now a day as herbal drugs in different places in literature, have retained their place in therapy. Their effectiveness, low cost and comparative freedom from serious toxic effects make these medicaments not only popular but also an accepted mode of treating disease even in developed countries.

**Table I: Details of the Soxhlet Extraction**

Plant material	Solvent used	Volume of the solvent	Weight of the extract	% Yield
Root (250gm)	Ethyl acetate	500 ml	4.20 gm	1.68
	Methanol	500 ml	5.10 gm	2.04
		500 ml	3.70 gm	1.48
	Water			

The isolation of alkaloids such as morphine, strychnine, quinine etc. marked a new era in the use of medicinal plants. In spite of extensive research on plant products the potential of higher plants as sources of new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fractions submitted to biological or pharmacological screening is even smaller. In many cases the isolated plant constituents do not explain the rationale of using these plants in traditional medicine for particular ailments. Chemical reinvestigation and pharmacological screening of extracts may throw some light on this aspect. In any case freedom from toxic effects is a must for any herbal medicines that are commercially available. The process that leads the plant to pharmacologically active pure constituent is very long and tedious and requires a multidisciplinary collaboration of botanists, Phytochemists and Pharmacologists. This approach involves the following steps<sup>3</sup>.

1. Collection of plant material and proper botanical identification.

2. Drying of plant material and extraction with appropriate solvent.

3. Pharmacological screening.

Nearly all 'wonder drugs' in use today are derived from natural products of about 120 plant derived drugs commonly in use in one or more countries, 74% were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine<sup>1</sup>. Well known examples include the cardiac glycosides from *Digitalis purpurea*, the antihypertensive agent and tranquillizer reserpine from *Rauwolfia serpentina*, the antimalarial agent quinine from *Cinchona* spp and the analgesics, codeine and morphine from *Papaver somniferum*. Secondary metabolites isolated from medicinal plants have also served as precursors or models for the preparation of effective agents through semisynthesis or lead-based total synthesis. Examples include in anticancer agents, etoposide a semisynthetic derivative of epipodophyllotoxin isolated from *Podophyllum* spp<sup>4,5</sup> and anticholinergic drugs modeled on the Belladonna alkaloids isolated from *Atropa belladonna*.

The pioneering studies of the active constituents of *Catharanthus roseus*<sup>6</sup> and discovery of antileukemic agents, vincristine and vinblastine provided convincing evidence that plants could be sources of novel, potential cancer chemotherapeutic agents. It has been discovered taxol<sup>7</sup> from *Taxus brevifolia*, now approved for the treatment of ovarian, breast cancer and camptothecin, which has been converted through semi synthesis to several analogues that are currently showing promise in advanced clinical trials<sup>8</sup>. The role played by plants in providing novel agents having potential in the treatment and prevention of many diseases such as cancer, AIDS and malaria has been reviewed<sup>9</sup>.

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antibacterial substances from other sources including plants. Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens and therefore

**Table III: Results of zone of inhibition of Anti-bacterial activity of the different Root extracts**

Extract	dose (µg/ml)	Zone of inhibition# (diameter in mm)		
		gram (+)ve		gram (-)ve
		<i>B.s.</i>	<i>B.p</i>	<i>E.c</i>
Ethyl acetate extract	1000	10	10	10
Methanol extract	1000	11	19	22
Aqueous extract	1000	10	7	9
<b>Standard:</b> Streptomycin	25	16	17	15
<b>Vehicle:</b> DMSO	-	-	-	-

*B.s*=*Bacillus subtilis*; *B.p*=*Bacillus pumilis*; *E.c*=*Escherichia coli*; - =No activity.

#Values are the average of triplicate; Includes the cup diameter (6mm).

considered as potential source for different classes of antibacterial substances.

Plants used in traditional medicine contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases. The substances that can either inhibit the growth of micro-organisms or kill them are considered as candidates for developing new drugs for treatment of various infectious diseases. The plants used in traditional system of medicine are well known as remedies for diseases in the rural areas of developing countries. Herbal medicines have been used in developing countries as an alternative to Allopathic medicines. Our extensive review of literature has revealed a variety of medicinal plants possessing Anti-Inflammatory and Hepatoprotective activity which also exhibited good antibacterial properties. On the basis of these reports the plant extracts were subjected for antibacterial activity against various bacteria's.

#### Plant material collection

The plant material of *Mimosa rubicaulis* Lam. (figure I) was collected from the kotappakonda forest area, Kotappakonda, Narasaraopet, Guntur (District), A.P. India, in July 2009 and identity was confirmed by Dr. M.Venkaiiah, Associate professor, Dept of Botany, University College of Science and Technology, Andhra University, Vishakapatnam.

After collection it was shade dried at room temperature.

#### Preparation of extracts

The plant material was coarsely powdered and passed through sieve no:20 and the extracted sequentially with ethylacetate, methanol and water using Soxhlet apparatus.<sup>[10]</sup> All the three extracts were filtered and allowed to evaporate to dryness. And all the extracts were transferred into clean and dried airtight vials. Details of soxhlet extraction was mentioned in table.I

#### Microorganisms

The test organisms were *Bacillus subtilis*, *Bacillus pumilis* and *E.coli*. All these organisms' cultures were obtained from National collection of Microorganisms, Pune.

All organisms were subcultured onto nutrient agar media in order to determine their viability. All the stock cultures were stored at 4°C.

#### Evaluation of Anti-Bacterial activity

##### 1. Culture media

The following media<sup>[11]</sup> were used for our antibacterial studies.

*Nutrient agar for bacteria*: Beef extract-0.3%, Sodium chloride-0.5%, Peptone-0.5%, Agar-2.0%, pH-7.2-7.4

*Sterilization*: Sterilization of the media, water, etc., was carried out by autoclaving at 15 lbs/inch<sup>2</sup> and 121°C for 20 minutes. The glassware like syringes, petri dishes, pipettes, empty test-tubes were sterilized by dry heat in an oven at a temperature of 160°C for one hour.

The sterilized medium was cooled to 40°C and poured into the Petri dishes to contain 6 mm thickness. The media was allowed to solidify at room temperature.

##### 2. Chemicals used for Anti-Bacterial Assay

Streptomycin as reference standard<sup>[12]</sup> and Di-methyl Sulphoxide (DMSO) as solvent (because of high polarity nature).

##### 3. Assay method:

All the experimentation was done in aseptic area under laminar air-flow unit. The Cylinder Plate Method or Cup Plate Method was adopted for the study<sup>[13]</sup>.

*Cylinder Plate Method or Cup Plate Method*: In cup plate method, the antibacterial substance diffuses from the cup through a solidified agar layer in a Petri dish or a plate to an extent so that the growth of added micro-organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antibacterial substance. The antibacterial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader.

The Ethyl acetate, Methanol and Aqueous extracts of *Mimosa rubicaulis* Lam. root was screened for antibacterial activity against a wide spectrum of micro-organisms and the activity was compared with appropriate reference standards (streptomycin for both gram-positive and gram-negative organisms). Micro-organisms were grown in nutrient agar medium.

Dimethyl sulphoxide (DMSO) was used as control and as drug vehicles for the plant extracts.

**Preparation of test and standard solutions:** Initially 10mg samples were weighed accurately and dissolved in 10ml DMSO to get a concentration of 1000µg/ml. The stock solution of reference standards (Streptomycin) was prepared at a concentration of 25µg/ml by using sterile water. Antibacterial activity was screened by adding 0.05 ml/50 µl stock solution to each cup by micropipette.

#### 4. Evaluation of antibacterial activity

**Determination of zone of inhibition by cup plate method:** The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of bacterial growth surrounding cylinders (cups), containing various dilutions of test compounds.

A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum was spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each extract and reference standards were added to the cups with a micropipette.

All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide which was used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded. Procedure was repeated in triplicate for accurate results.

#### 5. Measuring the zone of inhibition:

The presence of definite zone of inhibition of any size around the cup indicated **anti bacterial** activity. Zone of inhibition was measured using plastic scale. Then the values were mentioned in table-3.

#### RESULTS

The Preliminary Phyto-Chemical investigation was mentioned in table II and the zone of inhibition was mentioned in table III.

#### DISSUSSION

All the extracts at a concentration of 1000 µg per each cup exhibited antibacterial activity (Table-3) against one or the other organisms in dose dependent manner. *Mimosa rubicaulis* Lam. root extracts of different polarities have shown good antibacterial activity against Gram (+)ve and Gram (-)ve micro-organisms. Methanol extract, has exhibited inhibition against both Gram (+)ve

and Gram (-)ve. Ethyl acetate and Aqueous extracts has exhibited moderate activity against Gram (+)ve and Gram (-)ve bacteria. This study finally indicated that all the extracts of *Mimosa rubicaulis* Lam. Root extracts were found to posse's good antibacterial activity.

#### CONCLUSION

With this project on "Anti-Bacterial Investigation on the different Root Extracts of *Mimosa rubicaulis* Lam." we can conclude that Root extract of *Mimosa rubicaulis* Lam. has good Anti-Bacterial activity against Gram (+)ve and Gram(-)ve micro-organisms.

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