



IN VITRO ANTIBACTERIAL ACTIVITY OF ACETONE EXTRACT OF *PARTHENIUM HYSTEROPHORUS* AND *HIBISCUS ROSA-SINENSIS* LEAVES

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ABSTRACT

In present study antibacterial activity of two different plants viz., *Parthenium hysterophorus* and *Hibiscus Rosa-sinensis* was evaluated against four different test bacteria. The acetone extracts of leaf of *Parthenium hysterophorus* and *Hibiscus Rosa-sinensis* was screened for the antibacterial activity were measured by agar well diffusion assay against *Pseudomonas aeruginosa* NCIM 5029, *Staphylococcus aureus* NCIM 5021, *E. Coil* NCIM 2563 and *Bacillus subtilis* NCIM 2063. Varying degree of antibacterial activity was recorded. The highest antibacterial activity is being depicted by *Parthenium hysterophorus* followed by *Hibiscus Rosa-sinensis* against all bacteria. *E.Coil* and *Bacillus subtilis* were most susceptible when compared to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It is a significance to exploit novel antibacterial drugs from these medicinal plants.

Keywords: Antibacterial activity, *Hibiscus Rosa-sinensis*, *Parthenium hysterophorus*, Agar diffusion assay.

INTRODUCTION

Herbal medicine involves the use of plants for medicinal purposes. The term "Herb" includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and bark. There can be little doubt that the use of plants for healing purposes is the most ancient form of medicine known. The quest for plants with medicinal properties continues to receive attention as scientists are in need of plants, particularly of ethno botanical significance for a complete range of biological activities, which ranges from antibiotic to anticancerous. Several plants and herb species used traditionally have potential antimicrobial and antiviral properties^{1,2}.

Plants based drugs are less toxic and have acceptable side effect. It is therefore essential to bring the use of these natural remedies into an existing frame work of rational scientific use of medicine based on the strong traditional knowledge. A rational approach is been developed to use medicinal plants as a lead for the discovery of active molecule, which act as one of the largest reservoirs for many remedies. Many of the plants that were discovered by ancient civilizations are still in use today. The world health organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs³.

Several phytochemical surveys have been published, including the random sampling approach which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys were the alkaloids and steroidal sapogenins, however other diverse groups of naturally occurring phytocomponents such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils etc., have also been reported⁴.

These plants have a history of use for the treatment of various ailments. The present study intends to study about the antibacterial activity of the acetone extracts of *Hibiscus Rosa-sinensis* and *Parthenium hysterophorus* leaves against selected microbes.

MATERIALS AND METHODS

Plant Material

The plants were collected from Indira Park and Public Gardens, Nampally, Hyderabad. The identification was confirmed by Head Department of Botany, Osmania University. The fresh leaves were separated from the plant and washed thoroughly for 2-3 times with running tap water and then with sterile water followed by shade drying. The separated leaves were powdered in a mixer and fine powder was collected by passing through sieve no: 40. The fine powder is used for extraction.

Test microorganisms

Human pathogenic bacteria such as *Pseudomonas aeruginosa* NCIM 5029, *Staphylococcus aureus* NCIM 5021, *Escherichia Coil* NCIM 2563 and *Bacillus subtilis* NCIM 2063, were collected from Microbiology Lab, Bhaskar medical college, Moinabad. All the test bacterial species were maintained on nutrient agar media.

Chemicals

DMSO, acetone was obtained from Sigma aldrich. Other chemicals used in these experiments were also of medicinal grade.

Preparation of Inoculum

The gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C.



Preparation of solvent extractions

Approximately 20g of the shade dried powder of plant materials were filled separately in the thimble and extracted with 150 ml acetone using a Soxhlet extractor for 48 hrs. The extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract were weighed and preserved at 4°C in airtight bottles until further use. 15 mg of each solvent residue were dissolved in 1ml of DMSO as a solvent and were used as the test extracts for antibacterial assay.

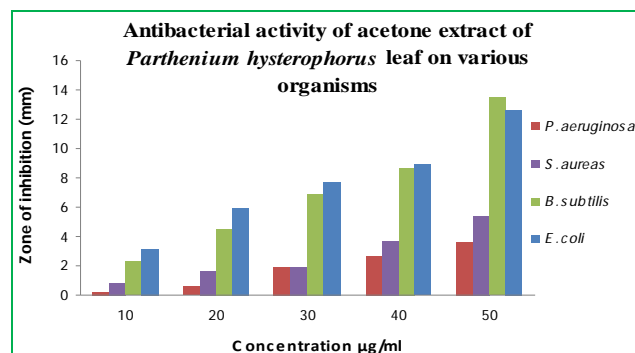
Antibacterial assay

Antibacterial activity of acetone extract of *Parthenium hysterophorus* and *Hibiscus rosa-sinensis* were determined by Well-diffusion method using nutrient agar medium. Well were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 10⁶ CFU/ml of bacteria were spread on the solid plates separately with a sterile swab moistened with the bacterial suspension. Then 50 µl of acetone extracts of different concentration i.e., 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml of *Parthenium hysterophorus* and *Hibiscus rosa-sinensis* were poured separately in the wells of the inoculated plates. The treatments also included 50 µl of solvents served as control. The plates were incubated for 24 hrs at 37°C and zone of inhibition if any around the wells was measured in mm⁵.

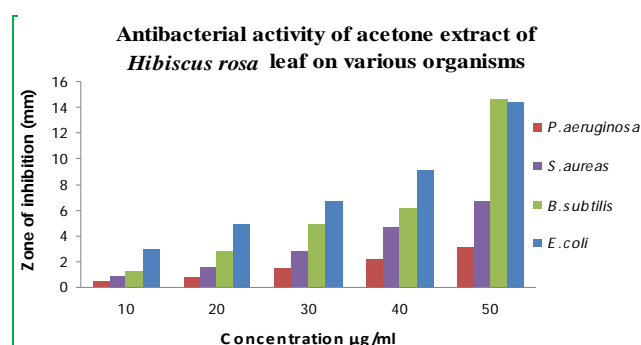
RESULTS

To search for traditionally used medicinal plants with potent antibacterial properties against gram negative and gram positive bacteria, two medicinal plants viz., *Parthenium hysterophorus* and *Hibiscus rosa-sinensis* were screened. The acetone extracts of both *Parthenium*

hysterophorus and *Hibiscus rosa-sinensis* were very effective against *Bacillus subtilis* and *Escherichia coli*. The results showed unique characters of the plants in inhibiting bacterial growth. The results of anti bacterial activity by Agar diffusion method of both the plant extracts against selected bacteria as shown in table 1 & 2 and graphically represented in graph 1 & 2.



Graph 1: In-vitro antibacterial activity of acetone crude extract of *Parthenium hysterophorus* leaves.



Graph 2: In-vitro antibacterial activity of acetone crude extract of *Hibiscus rosa-sinensis* leaves.

Table 1: In-vitro antibacterial activity of acetone crude extract of *Parthenium hysterophorus* leaves.

S.No	Conc. µg/ml	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>B.subtilis</i>	<i>S.aureas</i>
1	10	3.1±0.3	0.2±0.1	2.3±0.5	0.8±0.4
2	20	5.9±0.05	0.6±0.1	4.5±0.1	1.6±0.2
3	30	7.7±0.2	1.9±0.2	6.9±0.3	1.9±0.9
4	40	8.9±0.4	2.7±0.3	8.7±0.2	3.7±0.4
5	50	12.6±0.6	3.6±0.2	13.5±0.2	5.4±0.8

Table 2: In-vitro antibacterial activity of acetone crude extract of *Hibiscus rosa-sinensis* leaves.

S.No	Conc. µg/ml	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>B.subtilis</i>	<i>S.aureas</i>
1	10	2.9±3.4	0.4±0.2	1.3±2.7	0.9±0.9
2	20	4.9±5.8	0.8±0.7	2.8±4.7	1.6±1.6
3	30	6.7±7.9	1.5±2.1	4.9±7.2	2.8±2.0
4	40	9.1±9.3	2.2±4.0	6.1±8.7	4.7±1.0
5	50	14.4±13.3	3.1±5.6	14.6±14.3	6.7±4.0

DISCUSSION

The acetone extracts of leaves of *Parthenium hysterophorus* and *Hibiscus rosa-sinensis* presented a better inhibitory effect on the test organisms. *Escherichia coli* and *Bacillus subtilis* were most susceptible when

compared to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The active substance causing the inhibitory effect which could have been higher in the leaves. The use of acetone as extracting solvents proved to be more efficient in extracting the active compounds.



CONCLUSION

Plants are important source of potentially useful for the development of new chemotherapeutic agents. The plant extracts showed the highest activity against *E.coli* and *Bacillus subtilis* indicating that these plants are good source of antibacterial agents for the treatment of certain bacterial diseases. It was also found that the antibacterial activity of extracts increased with increasing concentration. However, further experimental and research work on these plants and their extracts are needed to specify the pharmacological implication. It is a significance to exploit novel antibacterial drugs from these medicinal plants.

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