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ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF LEAVES OF *HUGONIA MYSTAX*

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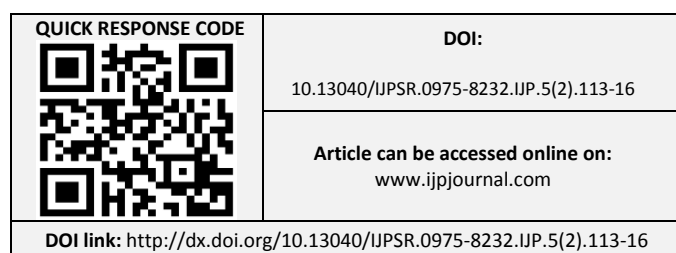
ABSTRACT: The objective of this research is to evaluate the preliminary phytochemical analysis and anti-bacterial activity from the medicinal plant of *Hugonia mystax* of Dindigul district, Tamil Nadu. The leaves of plant *Hugonia mystax* was subjected to soxlet extraction process by using solvent ethanol. Preliminary phytochemical analysis of ethanol extract of *Hugonia mystax* leaves showed the presence of carbohydrate, flavonoids, phenolic groups, saponins, steroids, tannins and terpenoids. *In-vitro* antibacterial activity was performed by plate hole diffusion method in MH agar medium. The bacteria used for antibacterial study were *Bacillus licheniformis* (NCIM 2468), *Brevibacterium luteum* (ATCC 15830), *Escherichia coli* (ATCC 11775), *Flavobacterium devorans* (NCIM 2581), *Klebsiella pneumonia* (ATCC 11229), *Micrococcus flavus* (NCIM 2984), *Micrococcus luteus* (NCIM 1207). Ethanol extract of *Hugonia mystax* exhibited best result of maximum zone of inhibition observed against gram positive as well as gram negative bacteria. The anti-bacterial activity revealed the medicinal potential of *Hugonia mystax* to develop a drug against human ailments.

INTRODUCTION: Medicinal plants are expensive gift from nature to humans. Even today, the world health organization (WHO) has estimated that approximately 80-85% of the global population relies on traditional herbal medicines as part of standard health care¹. Plants have provided a good source of antibacterial activity against microbial infections. The micro-organisms have developed resistance to many antibiotics because of indiscriminate use of anti-bacterial drugs that create a big problem in the treatment of infectious diseases. The present using anti biotics are synthetic drugs, expensive and inadequate for the treatment of diseases and also these are causing different side effects to people².

With the increase in resistance of many microorganisms to the currently used anti biotics and the high cost of production of synthetic compounds, in addition to many side effects, there is a need to look for the alternatives.

According to literature review revealed less work of this plant. Hence in the present study aimed to evaluate the preliminary phytochemical screening and *in-vitro* antibacterial activity of ethanol extract of *Hugonia mystax*. This investigation may reveal the basis for new antibacterial medicine which would increase the commercial value of the medicinal plants. For this purpose *Hugonia mystax* was selected for the screening of antibacterial activity.

The genus *Hugonia* Linn. of family Linaceae comprise about 40 species in the world; of which two species namely *Hugonia mystax* Linn. and *Hugonia ferruginea* are found in India^{3,4}. *Hugonia mystax* locally known as modirakanni is used for reducing inflammation and as an antitode to snake



bites. In the form of powder, it is administered internally as an anthelmintic and febrifuge. *Hugonia mystax* are useful in fever, verminosis and vitiated conditions of veta. Biological activities such as analgesic, anti-inflammatory, ulcerogenic, diabetic were reported⁵.



FIG. 1: HUGONIA MYSTAX PLANT

Hugonia mystax is a rambling scandent shrub with yellow tomentose twigs and branchlets horizontal provided with a pair of strong hooks. Leaves are simple, alternate, elliptic-obovate glabrous and penninerved⁶. Anti-microbials of plant origin has enormous therapeutic potential.

Sepals -5, petals -5, hypogynous, contorted fugacious. Stamens-10, connate at the base into a short tube with glandular swellings between the petals.

Leaves 3.8 - 6.3 by 2.5 - 3.8 cm, elliptic-obovate, obtuse or subacute, entire, reticulately veined, the veins conspicuous on surfaces, glabrous, base tapering, and petioles 1.5 mm long hairy, stipules lanceolate - subulate.

Anti-bacterial activity was carried out by taking the ethanol extract of leaves of *Hugonia mystax* at different concentration and their activity was recorded by estimating the zone of inhibition as produced by plate hole diffusion method on Mueller - Hinton agar media.

MATERIALS AND METHODS:

Collection and Identification: Fresh leaves of *Hugonia Mystax* free from diseases were collected from Sirumalai hills (Eastern Ghats) Dindigul district. It was identified by Botanical survey of India, Coimbatore, Tamil Nadu, India. The reference number was BSI/SRC/5/23/10-11/Tech-1522.

The leaves were cleaned with 2-3 times running water and were rinsed with sterile distilled water. The remaining water was wiped with the help of clean cloth. Then the leaves were air dried in shade under the newspaper at room temperature in a well ventilated room. The drying was carried out for two weeks with proper checking at regular interval.

Preparation of Extract: After the leaves were dried, they were grinded to a fine powder using grinding machine. The reduced powder mass was then passed through the sieve of mesh size 40. The sieved powder was kept in air tight container, sealed in order to prevent contamination and stored at a room temperature in a dark place until use⁷.

Dried powder was filled in soxlet apparatus and ethanol was used as solvent for extraction. The powdered plant material (100 gm) was taken in a thimble and kept the thimble on round bottom flask. Added 300 ml of ethanol in round bottom flask, then the condenser was fixed on upper part of the thimble and heated about 60 °C in the heating mantle. The soxhlet apparatus was run for 12 hours until the solvent in the siphon tube became colourless.

The extract was concentrated to dryness using rotary vaccum evaporator. The ethanolic extract was evaporated at a temperature of 30 °C. The gummy concentrate was kept in glass vials and was covered with aluminium foil and stored in the refrigerator at temperature of 4 °C until use.

Phytochemical Analysis:

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACT OF LEAVES OF HUGONIA MYSTAX⁸⁻¹⁰

Constituent	Ethanol extract
Alkaloids	-
Amino acid	-
Anthroquinones	-
Carbohydrate	+
Catechin	-
Coumarin	-
Flavonoid	+
Gum, oil and Resins	-
Phenolic groups	+
Proteins	-
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

Test Organisms: The test microorganisms used for this research were *Bacillus lichenformis* (NCIM 2468), *Brevibacterium luteum* (ATCC 15830), *Escherichia coli* (ATCC 11775), *Klebsiella pneumonia* (ATCC 11229), *Micrococcus flavus* (NCIM 2984), *Micrococcus luteus* (NCIM 1207), *Flavobacterium devorans* (NCIM 2581)

Anti-bacterial Activity:

Plate Hole Diffusion Method: The plate hole diffusion assay was used to determine the growth inhibition of bacteria by plant extract^{11, 12, 13}. Bacteria were maintained at 4 °C on nutrient agar plates before use. Nutrient agar was prepared and 25 ml of each was poured into sterile universals.

The universals with the broth were inoculated with different species of bacteria and incubated at 37 °C overnight.

A total of 25 ml of Mueller Hinton (MH) agar held at 40 °C was poured into sterile universals maintained at 40 °C in a water bath. Each universal was inoculated with 0.2 ml of different bacterial species mixed well, transferred into sterile petri dishes and allow to set. Using a sterile cork-borer 6 mm diameter, four holes per plate were made into the set agar containing the bacterial culture. Plant extract was poured into the wells, the plates were kept in incubator overnight and the zone of inhibition was then recorded if greater than 6 mm.

TABLE 2: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF LEAVES OF *HUGONIA MYSTAX* AGAINST VARIOUS MICRO-ORGANISMS

S. no.	Microorganism	Zone of inhibition(mm)			
		1000 µg/ml	500 µg/ml	250 µg/ml	Streptomycin 10 µg/ml
1	<i>Bacillus lichenformis</i>	24	22	21	25
2	<i>Brevibacterium luteum</i>	20	18	15	24
3	<i>Micrococcus flavus</i>	21	17	15	23
4	<i>Micrococcus luteus</i>	22	17	12	23
5	<i>Escherichiae coli</i>	20	15	12	24
6	<i>Klebsiella pneumonia</i>	19	16	11	21
7	<i>Flavobacterium devorans</i>	20	18	14	22

RESULTS: The seven bacterias of both the strains were used for antibacterial screening. Various concentrations of ethanolic extracts of *Hugonia mystax* were used (1000 µg/ml; 500 µg/ml; 250 µg/ml) to test the anti-bacterial activity. It was estimated that if a zone of inhibition is obtained by 250, 500, 1000 of test solution. Streptomycin 10 µg/ml was used as a standard drug. The results were shown in the table.

Ethanol extract of *Hugonia mystax*, the zone of inhibition recorded range between 11 - 24 mm against gram positive and gram negative bacteria. Maximum zone of inhibition was recorded as 24 mm against *Bacillus lichenformis* at 1000 µg/ml; 22 mm against *Micrococcus luteus* at 1000 µg/ml; 21 mm, 20 mm against *Micrococcus flavus* and *Brevibacterium leuteum* at 1000 µg/ml; 22 mm, 18 mm against *Bacillus lichenformis* and *Brevibacterium leuteum* at 500 µg/ml, 17 mm against *Micrococcus flavus*, *Micrococcus luteus* at 500 µg/ml; 21 mm against *Bacillus lichenformis* at 250 µg/ml; 15 mm against *Brevibacterium leuteum* and *Micrococcus flavus* at 250 µg/ml; 12 mm against *Micrococcus luteus* at 250 µg/ml.

For gram negative bacteria 20 mm against *Escherichiae coli* and *Flavobacterium devorans* at 1000 µg/ml; 19 mm against *Klebseilla pneumonia* at 1000 µg/ml; 15, 16 and 18 mm against *Escherichiae coli*, *Klebseilla pneumonia* and *Flavobacterium devorans* at 500 µg/ml; 12, 11 and 14 mm against *Escherichiae coli*, *Klebseilla pneumonia* and *Flavobacterium devorans* at 250 µg/ml. Plant showing significant activity may be due to the presence of carbohydrate, flavonoids, phenolic groups, saponins, steroids, tannins, terpenoids. Among the various microorganisms the ethnolic extract of *Hugonia mystax* was more active against *Bacillus lichenformis*.

DISCUSSION: In our study, the maximum zone of inhibition against gram positive bacteria such as *Bacillus lichenformis*, *Brevibacterium leuteum*, *Micrococcus flavus*, *Micrococcus luteum*. In literature, it has been indicated that the anti-bacterial activity is due to the presence of various classes of phytochemicals such as carbohydrates, flavonoids, phenolic groups, saponins, steroids, tannins, terpenoids¹⁴.

Increase in concentration of the plant extract showed gradual increase in the zone of inhibition. Researchers have already shown that gram positive bacteria are more susceptible towards plant extracts as compared to gram negative bacteria. These differences may be attributed to the fact that the cell wall in gram positive bacteria is of single layer whereas cell wall in gram negative bacteria is multilayered structure¹⁵.

CONCLUSION: Ethanol extract of *Hugonia mystax* have exhibited antibacterial activity against gram positive as well as gram negative bacteria. The presence of phytochemicals such as carbohydrates, flavonoids, phenolic groups, saponins, steroids, tannins and terpenoids were major constituents in *Hugonia mystax* may acknowledge the medicinal property of this plant. This antibacterial activity would support the folk therapy of bacterial infections and it can be used in alternative system of medicine.

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CONFLICT OF INTEREST: Nil

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