IJP (2018), Vol. 5, Issue 2 **(Research Article)**

Received on 06 October 2017; received in revised form, 14 November 2017; accepted, 18 November 2017; published 01 February 2018

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

B. Vasuki * and R. Sambathkumar

Department of Pharmaceutical Chemistry, J. K. K. Nattraja College of Pharmacy, Komarapalayam, Namakkal - 638183, Tamil Nadu, India.

Keywords:

Hugonia mystax, Ethanol extract, Anti-bacterial, Zone of inhibition

Correspondence to Author:
B. Vasuki

Department of Pharmaceutical Chemistry, J. K. K. Nattraja College of Pharmacy, Komarapalayam, Namakkal - 638183, Tamil Nadu, India.

E-mail: Vasukithiyagu211@gmail.com

ABSTRACT: The objective of this research is to evaluate the preliminary phytochemical analysis and antibacterial activity from the medicinal plant of *Hugonia mystax* of Dindigul district, Tamil Nadu. The leaves of plant *Hugonia mystax* was subjected to Soxhlet 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 the plate hole diffusion method in MH agar medium. The bacteria used for the antibacterial study were *Bacillus lichenformis* (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 the best result of the 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 an 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 care [1]. 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.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.5(2).113-16

The article can be accessed online on www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(2).113-16

The present using antibiotics are synthetic drugs, expensive and inadequate for the treatment of diseases and also these are causing different side effects to people [2]. With the increase in resistance of many microorganisms to the currently used antibiotics 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 i*n-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 comprises 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* is useful in fever, verminosis and vitiated conditions of veta. Biological activities such as analgesic, anti-inflammatory, ulcerogenic, diabetic were reported[5].

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 penninerved [6]. Anti-microbials of plant origin has enormous therapeutic potential. Sepals -5, petals -5, hypogynous, contorted fugaciously. 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.

The antibacterial 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 the clean cloth. Then the leaves were air dried in the 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 ground 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 an airtight container, sealed to prevent contamination and stored at room temperature in a dark place until use [7].

The dried powder was filled in a Soxhlet apparatus, and ethanol was used as a 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 a round bottom flask, then the condenser was fixed on the upper part of the thimble and heated about 60 °C in the heating mantle. The Soxhlet apparatus was run for 12 h until the solvent in the siphon tube became colorless. The extract was concentrated to dryness using rotary vacuum 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 a temperature of 4 °C until use.

Phytochemical Analysis:

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACT OF LEAVES OF *HUGONIA MYSTAX* [8-10]

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. The plant extract was poured into the wells, the plates were kept in an incubator overnight, and the zone of inhibition was then recorded if greater than 6 mm.

RESULTS: The seven bacterias of both 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 antibacterial activity. It was estimated that if a zone of inhibition is obtained by 250, 500, 1000 of the test solution. Streptomycin 10 µg/ml was used as a standard drug. The results were shown in the table.

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

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

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 *Escherichia coli* and *Flavobacterium devorans* at 1000 µg/ml; 19 mm against *Klebseilla pneumonia* at 1000 µg/ml; 15, 16 and 18 mm against *Escherichia coli, Klebseilla pneumonia* and *Flavobacterium devorans* at 500 µg/ml; 12, 11 and 14 mm against *Escherichia 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 ethanolic 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 antibacterial activity is due to the presence of various classes of phytochemicals such as carbohydrates, flavonoids, phenolic groups, saponins, steroids, tannins, terpenoids [14].

Increase in concentration of the plant extract showed a gradual increase in the zone of inhibition. Researchers have already shown that gram-positive bacteria are more susceptible to 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 structure [15].

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 the alternative system of medicine.

ACKNOWLEDGEMENT: The authors wish to express our thanks to J. K. K. Nattraja College of Pharmacy, Komarapalayam, Namakkal District, Tamil Nadu for providing the facility to conduct this research work and also express gratitude to all of the Department members.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Jayakumar J, Elumalai K and Backiyaraj: Anti-bacterial activity of *H. mystax* L. (Linaceae) against the selected human pathogenic bacteria. International Journal of Current Research in Medical Sciences 2015; 1(3): 48-52.
2. Rajananda T, Mallikarjuna T and Ganga B: Phytochemical analysis and antibacterial activity of the *Hugonia mystax* aerial parts. International Journal of Green Pharmacy 2016; 10(1): 42-46.
3. Santapau H: A Dictionary of flowering plants in India. Council of Scientific and Industrial Research, New Delhi, 1983: 103.
4. Pullaiah T: Flora of Andhra Pradesh. Scientific Publishers & Distributors, Edition 1st, 1997.
5. Sutha S, Mohan VR, Kumaresan S, Murugan C and Athiperumalsami T: Ethnomedicinal plants used by the tribals of kalakad Mundanthurai Tiger Reserve (KMTR), Western Ghats, Tamil Nadu for the treatment of rheumatism. Indian Journal of Traditional Knowledge 2009; 9(3): 502-509.
6. Devendra S, Brijendra B and Mahendra Kumar CB: *In-vivo* anti-oxidant and hepatoprotective effects of *Hugonia mystax* in paracetamol-induced hepatotoxicity in rats. Asian Journal of Pharmaceutical and Clinical Research 2015; 8(5): 291-294.
7. Anandakumar S, Prakash M and Karmegam N: *In-vivo* anti tumor and anti-oxidant activity of *Hugonia mystax* L. (Linaceae). International Journal of Current Research in Biosciences and Plant Biology 2014; 1(3): 67-74.
8. Harborne JB: Phytochemical methods. A guide to modern techniques of plant analysis, Chapman and Hall Publishers & Distributors, Edition 3rd, 2008.
9. Gokhale SB: A Textbook of Pharmacognosy. Nirali Prakashan Publisher & Distributors, Edition 13th, 2002.
10. Evans WC: Pharmacognosy. Reed Elsevier Publishers & Distributors, Edition 15th, 2006.
11. Saravanakumar A, Venkateshwaran K, Vanitha J and Ganesh M: Evaluation of antibacterial activity, phenol and flavonoid contents of *Thespesia populnea* flower extracts. Pakistan Journal of Pharmaceutical Sciences 2009; 22(3): 282-286.
12. Vimalavady A and Kadavul K: Phytochemical screening and anti-microbial activity of the leaves of *Hugonia mystax* Linn. (linaceae). Indian Journal of Natural Products and Resources 2012; 3(2): 161-165.
13. Mahesh B and Satish S: Anti-microbial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Sciences 2008; 4(5): 839-843.
14. Taleb-contini SH, Salvador MJ, Watanabe EE, Ito IY and Oliveura DCR: Antimicrobial activity of flavonoids and steroids isolated from two chromolaena species. Brazillian Journal of Pharmaceutical Sciences 2003; 39(4): 403-408.
15. Bhandari PS, Bhandari R and Sah BK: Antibacterial activity of methanolic extract of *Manfifera indica* (Bark) and *Osyris lanceolata* (Leaves) from the western region of Nepal. International Journal of Pharmacognosy 2017; 4(6): 200-207.

How to cite this article:
Vasuki B and Sambathkumar R: Antibacterial activity of ethanol extract of leaves of *Hugonia mystax*. Int J Pharmacognosy 2018; 5(2): 113-16. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(2).113-16.