International Journal of Current Research in Medical Sciences

ISSN: 2454-5716

www.ijcrims.com

Coden: IJCRPP(USA)

Research Article

Antibacterial activity of *Hugonia mystax* L. (Linaceae) against the selected human pathogenic bacteria

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Abstract

This present study was aimed to assess the antibacterial activity of different solvent extracts of *Hugonia mystax* leaf *(L)* was evaluated for a Antibacterial activity hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts showed significant activity against various human pathogens. Gram positive bacteria as *Streptococcus mutans, Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus*, gram negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Schigella flexneri*.

Keywords: *Hugonia mystax*, Antibacterial activity, *S.mutans, B. subtilis, S.aureus, M. luteus, E.coli, K. pneumoniae, P. vulgaris* and *Schigella flexneri*

Introduction

The genus *Hugonia* L. of family Linaceae comprise about 40 species in the world; of which *Hugonia mystax* L. was reported from India (Santapau *et al*.,1983, Pullaiah *et al*.,1997). This plant Hugonia mystax is locally known as Modirakanni and kaarthotti. Ethnobotanically, the fruits are used by the tribals of Kalakad Mundanthurai for the treatment of Rheumatism (Sutha *et al.*,2009) Humans have constant contact with a large number of different bacteria that either temporarily or permanently inhabits in his body. These relations established are various and very complex, ranging from those positive to extremely negative. Frequently the bacterium which lives in man's body has the ability to infect the person. The knowledge and use of a wide spectrum of medicinal plants have been documented scientifically and thus it has led to the development of drugs to combat various infectious diseases impeding human life and activity. Antimicrobial, especially antibiotic drug resistance is a great challenge to public health despite the existence of a variety of antibiotics in the present scenario (Pfaller and Diekema., 2012).

Plants as potential antibacterial agents, the healing potential of plants has been known for thousands of years. Plants and their medicinal uses were passed down from generation to generation in various parts of the world and had significantly contributed to the development of different traditional systems of medicine. Even today, the

World Health Organization (WHO) has estimated that approximately 80-85% of the global population rely on traditional herbal medicines as part of standard health care (Foster *et al.*, 2005). medicinal properties of plants can be associated with secondary metabolite compounds (Hartmann., 2008).

In vitro experiments clearly proved that plants produce a vast number of secondary metabolites that have antibacterial activity (Van Etten *et al*., 1994; Iwu *et al*., 1999; Cowan., 1999; Rios and Recio., 2005; Cos *et al*., 2006). Usually, three molecule families append to have remarkable antimicrobial activity and they are alkaloids, phenolic and terpenes. The polyphenols and phenolics are one of the biggest group of active principles that have exhibited antimicrobial activity. Important subclasses in this group of compounds include phenols, phenolic acids, Quinone's, flavones, flavonoids, flavonols, tannins and coumarins (Geissman, 1963; Stern *et al*., 1996; Cowan., 1999). an important pest to humans, causing allergic responses that include local skin reaction and systemic reaction such as angioedema, and urticaria (Peng *et al*., 1999).

Materials and Methods

In the present study, samplings were carried out at different places of Salem district, Tamilnadu, India. Bulk samples (leaves) were collected, air-dried and shade dried at room temperature, after drying, each sample was separately ground to a fine powder. Samples of the aerial part of plant leaf was extracted with five different organic solvents (hexane, diethyl ether, dichloromethane, ethyl acetate and methanol with ascending order of polarity) in a sequential manner, in order to produce crude extracts containing a wide range of active compounds. About 200g of powder plant material were placed in a dry 2000ml glass jar and then 1000 ml of hexane (Merck) was added and allowed to macerate overnight.

The next day the mixture was vigorously stirred for 10 min and allowed to settle for 10 min. The supernatant liquid was filtered through a Whatmann no. 1 filter paper to remove any solid plant materials. The residual plant material was extracted one more time using 1000 ml of the same solvent. The two filtrates were combined and the solvent was condensed under reduced pressure 22–26 mmHg at 45°C to yield the respective solvent extract using rotary vacuum evaporator (SUPERFIT, PBU -6 Model. The residual plant material was then sequentially extracted with other solvents, using the same procedure described for hexane, to obtain diethyl ether, dichloromethane, ethyl acetate and methanol extracts respectively. The final extracts were collected in sterilized borosil glass vials weighed and then dried in desiccators and stored in refrigerator at 4°C for further experimentation.

In vitro antimicrobial evaluation of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol crude extracts were carried out against 8 bacterial strains, which includes 4 Gram-positive bacteria (*Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus and Streptococcus mutans*) and 4 Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Schigella flexneri*). The bacterial strains were obtained from the Institute of Basic Medical Sciences (IBMS), University of Madras, Taramani Campus, Chennai, India. An inoculums of each bacterial strain was suspended in 5 ml of nutrient broth and incubated for 24 h at 37°C. A loopful bacteria was taken from the stock cultures and dissolved in 0.1 ml of saline.

Simultaneously paper discs dipped with pure respective organic solvents were used as positive controls. The Petri plates were then pre-incubated for 3 h at 5°C to permit maximum diffusion of the extracts into the media. Cefalexin and Gentamycine (10µg/ml) was used as negative control against gram positive and gram negative bacteria respectively (Hailu Tadeg *et. al.*, 2005; Karman *et al*., 2002) were used as reference standards. After the incubated period, the zone of inhibition (mm) was measured with a scale and the data were tabulated.

Results

Hexane, diethyl ether, dichloromethane, ethyl acetate, and methanol extracts of *H. mystax* leaf was tested for its antibacterial activity against the selected gram positive bacteria such as *S. mutans, B. subtilis, S. aureus* and *M, luteus* and also against some of the selected gram negative

bacteria such as *E. coli, K. pneumoniae, P. vulgaris* and *S. flexneri*. The data pertaining to the above experiments clearly reveals that the different extracts produced varying bacterial growth inhibitory activity against the selected bacteria and the values obtained are shown in table 1.

Table 1: Antibacterial activity (zone of inhibition) of different solvent extracts of *Hugonia mystax* (leaf) against the selected human pathogenic bacteria.

Solvents tested	Gram positive bacteria					Gram negative bacteria				
	Control *	*Sm*	*Bs*	*Sa*	*Ml*	*Control* **	*Ec*	*Kp*	*Pv*	*Sf*
Hexane	25	-	5	-	-	28	-	-	-	-
Diethyl ether		-	-	7	-		12	-	7	5
Dichloromethane		8	14	11	14		9	8	9	8
Ethyl acetate		10	16	10	12		12	10	5	12
Methanol		18	20	16	24		17	16	12	18

C*= Positive Control ,Cefalexin.Negitive Control C ****Gentamycin** ***Sm*** *=Streptococcus mutans;* ***Bs*** *= Bacillus subtilis;* ***Sa*** *= Staphylococcus aureus;* ***Ml*** *= Micrococcus luteus;* ***Ec*** *= Escherichia coli;* **Kp** *= Klebsiella50neumonia;* ***Pv*** *= Proteus vulgaris;* ***Sf*** *= Schigellaflexneri*

Among the gram positive bacteria, hexane inhibited the growth of *B. subtilis* with 5mm zone of inhibition. Whereas, Diethyl ether showed remarkable activity against *S. aureus* (7mm zone of inhibition). Similarly, *B,subtilis* and *M. luteus* showed more susceptibility to the dichloromethane extract of *H. mystax* followed by, *S. aureus* and *S. mutans* with 14mm,14mm, 11mm and 8mm respectively. Further, the ethyl acetate extract showed 16mm, 12mm, 10mm and 10mm zone of inhibition against *B. subtilis, M. luteus, S. aureus* and *S. mutans* respectively. Eventually, methanol extract showed remarkable antibacterial activity against the selected bacteria than the other four extracts. It was found that 24mm, 20mm, 18mm and 16mm zone of inhibition was observed with the *M. luteus, B. subtilis, S. mutans* and *S. aureus* respectively.

Among the gram negative bacteria, hexane extract of *H. mystax* did not show any response to the bacteria *P. vulgaris, S. flexneri, K. pneumoniae* and *E. coli*.Whereas, Diethyl ether showed remarkable antibacterial activity against *E. coli* (12mm) followed by *P. vulgaris* (7mm) and *S. flexneri(5mm) respectively*. But the same extract had no influence over the growth of *K. pneumonia*. In addition *E. coli* and *P. vulgaris* showed more susceptibility to the dichloromethane extract of *A. monophylla* followed by *S. flexneri* and *K. pneumonia* with 9mm, 9mm, 8mm and 8mm respectively. Likewise, Ethyl acetate extract showed significant antibacterial activity against *E. coli, S. flexneri, K. pneumoniae* and *S. flexneri* with 12mm, 12mm, 10mm and 5mm zone of inhibition respectively. Eventually, methanol extract showed significant antibacterial activity against the selected gram negative bacteria than the other four extracts. It was found that 18mm and 17mm zone of inhibition was observed against *S. flexneri* and *E. coli*. 16mm zone of inhibition was observed with the *K. pneumoniae* and 12mm zone of inhibition was noted with *P. vulgaris* respectively.

Discussion

It is estimated that there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). A relatively small percentage (1 to 10%) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, 1996). Hippocrates (in the late fifth century B.C.) mentioned 300 to 400 medicinal plants (Schultes., 1978). In the first century A.D., Dioscorides wrote De Materia Medica, a medicinal plant catalog, which became the prototype for modern pharmacopoeias. The Bible offers descriptions of approximately 30 healing plants.

In the last decades, there has been particular interest in the use of abundant naturally occurring antimicrobials. Antimicrobial agents are chemical compounds derived from herbs, shrubs and or whole plants. Antimicrobial plant compounds or extracts from different studies is limited because of the differences in the methodologies used and different definitions of minimum inhibitory concentration (Burt, 2004). Basically, there are two ways to control or inhibit the growth of microorganisms, i.e. through physical or chemical agents, where choice is made on the basis of the situation. Heat, pasteurization, freezing, radiation and filtration are regarded as physical agents, whereas a wide variety of antimicrobial substances and drugs are categorized as chemical agents.

Antibiotic and antimicrobial agents are two different terms. An antibiotic is a product produced by microorganisms to inhibit the growth of other microorganisms whereas antimicrobial agent encompassed any compound either derived from nature or synthetically produced that can be applied clinically in the treatment of bacterial infection. In more specific, antimicrobial agents are categorized based on the spectrum of action, namely narrow and broad spectrum. Narrow spectrum antimicrobial agents can only inhibit the growth of either Gram positive or Gram negative bacteria, whereas a broad spectrum antimicrobial agent can inhibit both Gram positive and negative bacteria. Nevertheless, most of the antibiotics have no longer effective to control bacterial diseases due to the occurrence of antibiotic resistance. Therefore, scientists around the world were struggling to find for alternative, preferably from the natural resources. There have been many studies reported in plants, especially medicinal plants as potential antimicrobial.

As the results from different studies need to be comparable, we examined the activity of plant extracts of *H.mystax* and by disc diffusion methods against the selected gram-positive and gram-negative bacteria and the results obtained from the present investigations are discussed in the light of recent research hereunder.

Among the gram positive bacteria, *H. mystax* methanol extract showed remarkable antibacterial activity against the selected bacteria than the other four extracts. It was found that 24mm, 20mm, 18mm and 16mm zone of inhibition was observed with the *M. luteus, B. subtilis, S. mutans* and *S. aureus* respectively. Eventually, methanol extract showed significant antibacterial activity against the selected gram negative bacteria than the other four extracts. It was found that 18mm and 17mm zone of inhibition was observed against *S. flexneri* and *E. coli.* 16mm zone of inhibition was observed with the *K. pneumoniae* and 12mm zone of inhibition was noted with *P. vulgaris* respectively.

This present study envisages and offers a new scope in the pharmacology in general and bacteria control in particular. Thereby, it provides a better scope in the near future by replacing the chemical drugs through phytochemicals.

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