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BIO-EFFICACY OF THE LEAVES EXTRACTS OF *HYPTIS SUAVEOLENS* (L.) POIT AGAINST THE FISH PATHOGENS

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ABSTRACT

The objective of the present study is to investigate the bioefficacy of the leaves extracts of *Hyptis suaveolens* (L.) Poit against the fish pathogens isolated from diseased Tilapia (*Oreochromis niloticus*). The petroleum ether, ethanol, ethyl acetate, chloroform and aqueous extracts of *H. suaveolens* were tested against viz., *Aeromonas formicans, Aeromonas hydrophila, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* by the agar well diffusion method. Different extracts of *H. suaveolens* showed the zone of inhibition against the isolated pathogens. The results of ethanolic extracts of *H. suaveolens* demonstrated broad spectrum of activities that inhibited the growth with the maximum zone of inhibition 21 mm for *B. subtilis* (100 µg/mL), 21 mm for *P. aeruginosa* (75 µg/mL), 17 mm for *K. pneumonia* (100 µg/mL) and 21 mm for *E. coli* (75 µg/mL). The results of the ethyl acetate extracts of *H. suaveolens* illustrated the widest spectrum activities with the maximum zone of inhibition 23 mm for *A. formicans* (75 µg/mL), 16 mm for *A. hydrophilia* (100 µg/mL), 13 mm for *B. subtilis* (75 µg/mL) and 21 mm for *P. aeruginosa* (100 µg/mL).The double distilled water extracts of *H. suaveolens* showed zero percent of inhibition against the pathogens viz., *B. subtilis, K. pneumonia* and *P. aeruginosa*. The result of the present study gives more opportunity to screen the phytoconstituents which will be very functional to combat the various diseases caused by pathogenic bacteria**.**

Key words: Bio-efficacy, *Hyptis suaveolens*, Fish Pathogens

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg and Newman, 2001). Natural products either as pure compounds or standardized plant extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Parekh and Chanda, 2007). Over the past few decades, there has been much interest in natural materials as source of new antibacterial agents. Different extracts from traditional medicinal plants have been tested. Many reports show the effectiveness of traditional herbs against microorganisms. As a result, plants have become one of the bases of modern medicine (Evans et al. 2002). Overall, 50% of modern clinical drugs

are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Baker et al. 1995).

Antibiotics are associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions (Ahmad et al. 1998). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Monroe and Polk, 2000), there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2000). Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by large number of researchers in different parts of the world. Much work has been done on ethno medicinal plants in India (Joshi et al. 2009; Dey et al. 2010; Sumathi et al. 2011). Interest in a large number of traditional natural products has also increased (Taylor et al. 1996).

Hyptis suaveolens (L) Poit commonly known as 'Wilayati tulsi' belongs to the family Lamiaceae. The plant has been measured as an obnoxious weed, distributed throughout the tropics and subtropics. The whole plant is used in traditional medicine to treat various diseases. The leaves of *H. suaveolens* have been utilized as a stimulant, carminative, sudorific, galactogogue and as a cure for parasitic cutaneous diseases. Crude leaf extract is also used as a relief to colic and stomachache. Leaves and twigs are considered to be anti-spasmodic and used in anti-rheumatic and anti-suporific baths, anti-cancer, anti-inflammatory, anti-fertility agents (Olivier-Bever, 1986; Kirtikar and Basu, 1991) and also applied as an anti-septic in burns, wounds, and various skin complaints. The decoction of the roots is highly valued as appetizer and is reported to contain urosolic acid, a natural HIV-integrase inhibitor (Chatterjee and Pakrashi, 1997). Fumes of the dried leaves are also used to repel mosquitoes and control insect pests of stored grains. *H. suaveolens* is used traditionally for the treatment of respiratory tract infections, colds, pain fever, cramps and skin diseases (Mabberley, 1990; Iwu, 1993). The phytoconstituents isolated from the plant are hyptadienic acid, suaveolic acid, suaveolol, methyl suaveolate, Beta sitisterol, Oleanolic acid, ursolic acid, rosamarinic acid, dehydroabietionol, 3 Beta hydroxyl lup 12-en-28oic acid, 3 betat hydroxyl lup-20(29)-en-29-oic acid and essential oil. Essential oils isolated from aerial parts of this plant have showed antifungal (Pandey et al. 1982; Singh et al. 1992; Zollo Amvam et al. 1998; Sharma et al. 2004; Mandal et al. 2007; Tonzibo et al. 2009), antibacterial (Iwu et al. 1990; Asekun et al. 1999; Mandal et al. 2007; Shenoy et al. 2009; Sathish et al. 2010) activities. With this knowledge, the present study was designed to screen the bio-efficacy of petroleum ether, ethanol, ethyl acetate, chloroform and aqueous leaves extracts of *Hyptis suaveolens* against the fish pathogens.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

Hyptis suaveolens were collected from the natural habitats of Nagercoil, Kanyakumari District, Tamil Nadu (India) and authenticated by Dr. M. Johnson, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, India.

PREPARATION OF CRUDE EXTRACT

Leaf samples of *Hyptis suaveolens* were air and shade dried for fifteen days and pulverized to powder using mortar and pestle. The dried and powdered leaves (50 g) were extracted successively with 200 mL of petroleum ether, ethanol, ethyl acetate, chloroform and aqueous by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40 ℃ using Rotary evaporator.

ISOLATION AND IDENTIFICATION OF FISH PATHOGENS

Aeromonas formicans (A. formicans), Aeromonas hydrophilia (A. hydrophilia), Bacillus subtilis (*B. subtilis*), *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*K. pneumonia*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were isolated from diseased Tilapia (Hardi and Uddin, 2004). The bacteria were identified and confirmed by the morphological and biochemical properties using the conventional procedure described by Mukherjee, 2004 and Roggers et al. 1990. Stock cultures of *B. subtilis, E. coli, K. pneumonia, A. formicans, A. hydrophilia* and *P. aeruginosa* were grown in nutrient broth at 30° C and were sub-cultured and maintained in nutrient broth at 4°C.

EVALUATION OF ANTIBACTERIAL ACTIVITIES

The crude extracts were used for bioassay against both Gram negative and Gram positive bacteria. Inoculum was prepared from the 24 hours old culture of bacterial isolates in nutrient broth. Nutrient agar plates were prepared and the inocula were seeded by spread plate method. The agar well diffusion method was used for the antibacterial evaluations. Wells of 6 mm diameter were punched into the sterile medium with the test organisms and the wells were filled with 25, 50, 75 and 100 µL of plant extracts. The plates were incubated at 37^0C for 18-24 h. Antibacterial activity was evaluated by measuring the inhibition zone in millimeter and the observations were tabulated. All the experiments were performed with triplicates and the mean values were taken. Both positive and negative controls were determined. For negative control, the solvents (distilled water and ethanol) were used to determine their effect on test organisms. While the common antibiotic tetracycline discs were used to compare the effectiveness of the plant extracts with that of the antibiotics.

RESULTS

A total of five extracts viz., petroleum ether, chloroform, ethanol, ethyl acetate and double distilled water were examined against the isolated fish pathogens. The anti-bacterial activity of the leaves extracts of *H. suaveolens* were illustrated in Table 1. The results of ethanolic extracts of *H. suaveolens* demonstrated broad spectrum of activities that inhibited the growth with the maximum zone of inhibition 21 mm for *B. subtilis* (100 µg/mL), 21 mm for *P. aeruginosa* (75 µg/mL), 17 mm for *K. pneumonia* (100 µg/mL) and 21 mm for *E. coli* (75 µg/mL). The results of the ethyl acetate extracts of *Hyptis suaveolens* illustrated the widest spectrum activities with the maximum zone of inhibition 23 mm for *A. formicans* (75 µg/mL), 16 mm for *A. hydrophilia* (100 µg/mL), 13 mm for *B. subtilis* (75 µg/mL) and 21 mm for *P. aeruginosa* (100 µg/mL). The double distilled water extracts of *H. suaveolens* showed zero percent of inhibition against the pathogens viz., *B. subtilis, K. pneumonia* and *P. aeruginosa*. The negative control (solvents alone) failed to show the inhibition.

Table - 1 ***Antimicrobial activities of leaves extracts Hyptis suaveolens against the Fish Pathogens (mm).***

Name of the Pathogens	Zone of inhibition in diameter (mm)																								AMOXI
	Ethyl acetate extract in µg/mL					Ethanol extract in µg/mL					Chloroform extract in µg/mL					Petroleum Ether extract in µl µg/mL					Water extract in µg/mL				
	25	50	75	100	NC	25	50	75	100	NC	25	50	75	100	NC	25	50	75	100	NC	25	50	75	100	NC	
E. coli	03	06	08	11	00	06	12	**21**	**21**	00	04	07	12	17	00	07	13	16	19	00	03	07	11	13	00	26
K. pneumonia	00	05	08	09	00	05	10	14	**17**	00	03	06	08	11	00	02	06	08	14	00	00	00	00	00	00	17
B. subtilis	00	00	04	09	00	11	13	16	**21**	00	00	06	09	12	00	04	07	11	16	00	08	12	16	19	00	21
P. aeruginosa	08	12	15	**21**	00	09	15	**21**	**21**	00	02	06	08	13	00	03	05	08	12	00	05	09	12	16	00	21
A. formicans	09	15	**23**	**23**	00	08	14	17	**23**	00	03	05	09	12	00	05	08	13	18	00	00	00	00	00	00	00
A. hydrophilia	06	11	14	**17**	00	03	06	09	11	00	02	05	08	11	00	04	09	12	15	00	00	00	00	00	00	00

DISCUSSION

Although a variety of solvents have been employed in extraction and bio-activity, it is still uncertain what kind of solvent is the most effective and suitable for extraction. The zone of inhibition against the pathogens is varied due to the active principles present in the crude extract. A few workers tried using different solvents for screening the antibacterial activity of plant extracts and made evaluations. Paulraj et al. (2011) indicated that acetone was the best solution for extracting the effective antimicrobial compounds from the epidermal glands of *Christella parasitica*. Nair and Sumitra (2007) and Renisheya et al. (2011) reported the usage of ethanol as a solvent for the preparation of plant extract for antibacterial studies. Johnson et al. (2011) determined the anti-bacterial efficacy of chloroform, ethanol, ethyl acetate and water extracts of *Mentha arvensis* against *S. typhi, S. pyogenes, P. vulgaris* and *B. subtilis.* Irudayaraj et al. (2010) used five different extracts (Petroleum Ether, Benzene, Chloroform, Ethanol and Distilled Water) of the spike-moss *Selaginella inaequalifolia* to examine the antibacterial activities against the selected pathogens. Of which petroleum ether extract showed the maximum zone of inhibition. Anpin Raja et al., (2011) used methanolic extract of *C. peltata* to observe strong antibacterial activities. It has been shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both Gram positive and Gram negative bacteria (Sastry and Rao, 1994). In the present study also we observed the ethanolic extracts of *H. suaveolens* showed maximum degree of zone of inhibition (5 out of 6) against the isolated fish pathogens with varied diameter (Table 1).

Bacterial infection causes high rate of mortality in human population and aquaculture organisms (Kandhasamy and Arunachalam, 2008). For example, *B. cereus* is responsible for causing food borne diseases (Shan et al. 2007). *E. coli* causes mainly four types of clinical syndromes namely urinary tract infections, diarrhoea or gastroenteritis, pyogenic infections and septicemia. *K. pneumoniae* causes destructive changes to human lungs inflammation and hemorrhage with cell death (necrosis) that sometimes produces a thick, bloody, mucoid sputum. *P. aeruginosa* is a nosocomial pathogen, it infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections. *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness (Kandhasamy and Arunachalam, 2008). *A. hydrophila* causes internal, sometimes fatal hemorrhaging in fish and amphibians. When infected with *A. hydrophila*, fish develop ulcers, tail rot, fin rot and hemorrhagic septicaemia. Hemorrhagic septicaemia causes lesions that lead to scale shedding, hemorrhages in the gills and anal area, ulcers, exophthalmia and abdominal swelling. It causes gastroenteritis in humans. Mandal et al. (2007) observed less activity in the ethanolic extracts of *H. suaveolens* than that of steam distillation and petroleum ether extract against both bacterial and fungal strains. In contrary to their observation, we observed highest degree of activity against the fish bacterial pathogens. A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of monoterpene constituents which exert membrane-damaging effects to microbial strains (Sikkema et al. 1994) and also stimulates leakage of cellular potassium ions which provides evidence of a lethal action related to cytoplasmic membrane damage (Cox et al. 1998). The results of the present study also directly coincided with the previous observations. Many plants have been reported for antimicrobial properties across the world (Iwu et al. 1990; Asekun et al. 1999; Mandal et al. 2007; Shenoy et al. 2009; Sathish et al. 2010). The results of the present study confirm and supplement the previous observations and suggest that the extracts of *H. suaveolens* may be applied as a controlling agent against the selected pathogens viz., *E. coli, K. pneumonia, B. subtilis, P. aeruginosa, A. formicans* and *A. hydrophilia*.

CONCLUSION

It is hoped that this study would lead to the establishment of some active compounds that could be used to formulate new and more potent

antibacterial drugs of natural origin. Studies are in progress to identify the bioactive compound and to evaluate the mechanisms of action of *H. suaveolens* extracts on some organisms associated with human diseases.

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