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The effect of leaves extracts of *Clitoria ternatea* Linn against the fish pathogens

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ABSTRACT

Objective: To investigate the antimicrobial activity of *Clitoria ternatea(C. ternatea)* against the fish pathogens viz., *Pseudomonas aeruginosa(P. aeruginosa), Escherichia coli(E. coli), Klebsiella pneumonia(K. pneumonia), Bacillus subtilis(B. subtilis), Aeromonas formican(A. formicans)s, Aeromonas hydrophila(A. hydrophila)* and *Streptococcus agalactiae(S. agalactiae)*isolated from diseased Tilapia (*Oreochromis niloticus*). **Methods:** The extracts of *C. ternatea* was tested against *P. aeruginosa, E. coli, K. pneumonia, B. subtilis, A. formicans, A. hydrophila* and *S. agalactiae* by the agar well diffusion method. **Results:** Different extracts of *C. ternatea* showed inhibitory effects against *P. aeruginosa, E. coli, K. pneumonia, B. subtilis, A. formicans, A. hydrophila* and *S. agalactiae*. Ethyl acetate extracts of *C. ternatea* showed maximum of zone of inhibition against *A. formicans* (18 mm) , *A. hydrophilia* (19 mm), *B. subtilis* (19 mm) and *P. aeruginosa* (21 mm) next to that ethanol extract of *C. ternatea* showed *A. formicans* (18 mm) and *E. coli* (14 mm) followed by Acetone extract showed maximum zone of inhibition *S. agalactiae* (19 mm) and *K. pneumonia* (17 mm). **Conclusions:** The antimicrobial activities of all the four plant extracts are comparable and their potential as alternative in the treatment of infectious by these microorganisms was present in the fish. Susceptibility testing is conducted on isolates using drugs selected on the basis of their importance to human medicine and use in fish production.

1. Introduction

Medicinal plants are gifts of God, to cure infinite number of diseases among the human beings and other living organism[1]. India throughout its long history has accumulated a rich body of experiential facts of the use of medicinal plants for the treatment of various diseases. Chemical studies of Indian medicinal plants offer a valuable material base for the discovery and development of new drugs of natural origin. Systematic screening of them may result in the discovery of novel effective compounds[2].

The wealth of the medicinal plants in India especially South India has led us to an escalating curiosity in the exploration of ethnomedicinal plants as potential source of new antimicrobial agents. The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents[3]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. The antimicrobial properties of secondary metabolites have been recognized long ago and they have been scientifically established. Many efforts have been made to ascertain new antimicrobial compounds from plants. Recently several workers have reported antibacterial activities medicinal plants[4-15]. Due to over population, there is constant and urgent call for to ascertain new antimicrobial compounds with diverse chemical structures

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and novel mechanism of action for few and re-emerging transmittable diseases[16-22]. Consequently researchers are focusing their attention more and more to herbal medicine pursue for new direction to develop superior drugs against microbial infections. This revival of interest in plant derived drugs is mainly due to current wide spread belief that "herbal medicines" is safe and more trustworthy and steady than expensive synthetic drugs may of which have adverse side effects.

Clitoria ternatea L. (butterfly pea in English) belongs to the family Fabaceae and subfamily Papilionaceae is an herbaceous perennial legume valued for its forage and medicinal importance. The plant has been adopted in the traditional Indian system of medicine (folk medicine) due to its multiple pharmaceutical applications. The active constituents include lactones, aparajitin, taraxerol, phenol glycoside, alkaloid, phydroxycinnamic acid polypeptide, hexacosanol, anthoxanthin, kaempferol, clitorin, stigmast-4-ene 3, 6-dionie, cyanine chloride, palmitic, stearic, oleic, linolcic, linolenic acids, tannins, resins, finotin etc. It has been recommended as a rejuvenating brain tonic having anxiolytic, anti-depressant, anticonvulsant, and anti-stress properties and is believed to promote memory and intelligence and Anti-inflammatory, analges antipyretic activities of the plant were attributed to its flavonoid content[23]. The whole plants and seed extract are useful in stomaitis piles, sterility in female, hematemesis, insomnia, epilepsy, psychosis, leucorrhea and polyurea. The seeds are purgative, cathartic, and useful in visceralgia[24]. There are reports on Callus induction and antimicrobial activity of seed and callus extracts of *Clitoria ternatea L(C. ternatea)*. There is no report on application leaves extracts of Clitoria ternatea against the fish pathogen. In continuation of our research program, the present study we are aimed to study the anti-bacterial properties of *C. ternatea L* leaves extract against the fish pathogens.

2. Materials and methods

2.1. Collection of plant materials

C. ternatea L were collected from the Botanic Garden attached to Muthayammal College of Arts and Sciences, Rasipuram, Namakkal (India) and authenticated at the Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, India.

2.2. Preparation of crude extract

Leaves samples of *C. ternatea L* were air and shade dried for two weeks and pulverized to powder using mortar. The dried and powered leaves materials (50 g) were extracted successively with 200 mL of petroleum ether, Ethyl acetate, Ethanol, Acetone and double distilled water by using Soxhlet extractor for 48 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40 ℃ using Rotary evaporator. The residues obtained were stored in a freezer -70 ℃ until further tests[25]. These extracts were dissolved in dimethyl sulphoxide (100 mg/mL) to make the final concentrations.

2.3. 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)*, *Pseudomonas aeruginosa(P. aeruginosa)* and *Streptococcus agalactiae(S. agalactiae*) were isolated from diseased tilapia[26-27]. The bacteria were identified and confirmed by conventional microbiology procedure[28]. Stock cultures of Aeromonas formicans, *A. hydrophilia*, *B. subtilis*, *E. coli*, *K. pneumonia*, *P. aeruginosa* and *S. agalactiae* were grown in nutrient broth at 30 ℃ and were sub-cultured and maintained in nutrient broth at 4 ℃.

2.4. 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 filled with 25, 50, 100, 200 and 400 μL of plant extracts. The plates were incubated at 37 ℃ for 18-24 h. Antibacterial activity was evaluated by measuring the inhibition zone in millimeter in diameter and tabulated. All the samples were done in triplicate. Both positive and negative controls were determined, for negative control the two solvents (distilled water and ethanol) were also used to determine their effect on test organisms. While two common antibiotics viz., Amoxicillin and tetracycline discs were also used to compare the effectiveness of the plants extracts with that of the antibiotics.

3. Results

A total of five extracts viz., petroleum ether, Ethyl acetate, Ethanol, Acetone and double distilled water were examined against the isolated fish pathogens. The anti-bacterial activity of the leaves extracts of *C. ternatea L* were illustrated in Table 1. The present study results showed that ethyl acetate extracts of *C. ternatea* gave the widest spectrum activities that inhibited the growth of all studied pathogens with the maximum zone of inhibition 18 mm for *A. formicans*, 19 mm for *A. hydrophilia*, 18 mm for *B. subtilis* and 21 mm for *P. aeruginosa*. The ethanol extracts of *C. ternatea* illustrated the highest zone of inhibition against the pathogens *A. formicans* (18 mm) and *E. coli* (14 mm). The acetone extracts demonstrated maximum zone of inhibition against *S. agalactiae* (19 mm) and *K. pneumonia* (17 mm). The double distilled water extracts of *C. ternatea* showed zero percent of inhibition against the pathogens viz., *A. formicans*, *A. hydrophilia*, *B. subtilis* and *P. aeruginosa*.

Table 1
Antimicrobial activity of leaves extracts *C. ternatea* L. against the fish pathogens (mm).

S. No.*	Zone of inhibition in diameter (mm)																								
	Ethyl acetate ether extract (μL)**					Ethanol extract (μL)**					Acetone leaf extract (μL)**					Petroleum Ether extract (μL)**					Water extract (μL)**				
	25	50	100	200	400	25	50	100	200	400	25	50	100	200	400	25	50	100	200	400	25	50	100	200	400
1	00	00	07	09	18	00	00	00	12	18	00	00	07	09	12	00	00	00	09	16	00	00	00	00	00
2	07	10	12	15	19	00	00	00	11	15	00	00	00	08	11	00	00	00	06	09	00	00	00	00	00
3	00	07	08	11	14	00	00	00	08	12	07	10	13	16	19	08	09	11	15	18	00	00	06	10	12
4	00	00	03	07	09	00	00	07	11	14	00	00	00	00	00	00	00	00	00	00	00	00	04	07	11
5	00	00	02	05	11	00	00	04	07	12	07	09	11	14	17	00	00	08	11	14	00	00	00	04	10
6	08	11	13	16	19	00	07	09	13	16	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
7	09	11	15	17	21	00	06	09	14	18	00	00	07	10	14	07	10	11	16	18	00	00	00	00	00

* 1: *A. formicans*, 2: *A. hydrophilia*, 3: *S. agalactiae*, 4: *E. coli*, 5: *K. pneumonia*, 6: *B. subtilis* and 7: *P. aeruginosa* ** Control (Solvents alone) is failed to show the zone of inhibition. μL of extract samples (100 mg/mL).

The acetone and petroleum ether extract of *C. ternatea* also showed zero percent of inhibition against the *B. subtilis*.

4. Discussion

Several workers have reported that many plants possess antimicrobial properties including the parts which include; flower, bark, stem, leaf, etc. 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 [1]. In the present study also the Acetone, Ethyle acetate, Ethanol, Petroleum ether and water extracts of *C. ternatea* showed zone of inhibition against the isolated fish pathogens with varied diameter. This work also showed that all the leaves extracts were possessed antimicrobial activity and they can be used as broad spectrum antibiotics since they were active against both Gram positive and Gram negative bacteria. Antibacterial effects of these plants on *A. formicans, A. hydrophilia, B. subtilis, E. coli, K. pneumonia, P. aeruginosa* and *S. agalactiae* showed that the plants can be used in the treatment of gastrointestinal infection and diarrhoea and skin diseases in man also[29]. Haripriya et al[30] observed that petroleum ether extracts of *S. involvens* showed higher antibacterial activity against *E. coli* and *Pseudomonas*. Similarly in the present study also, Ethyl acetate, Ethanol, Acetone and petroleum ether extracts showed the maximum zone of activity against *A. formicans, A. hydrophilia, B. subtilis, E. coli, K. pneumonia, P. aeruginosa* and *S. agalactiae*. The present study result revealed that the *C. ternatea* can be used in the treatment of boils, sores and wounds, since *P. aeruginosa* have been implicated as causative agents of these diseases[31].

It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Studies are in progress to further evaluate the mechanisms of action of *C. ternatea* extracts on some organisms associated with fish and human diseases. Ethyl acetate, ethanol, acetone and petroleum ether extracts exhibited slightly higher efficacy than water extracts, so ethyl acetate, acetone, petroleum ether and alcoholic extracts were suggested to use as natural antibiotic administration for the diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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