A Preliminary screening of the Medicinal Plant *Couroupita guianensis* for its Antimicrobial Potential against Clinical and Fish-borne pathogens

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ABSTRACT

In the present investigation the methanol extract of the flowers of the medicinal plant *Couroupita guianensis* was evaluated for its antimicrobial efficacy and phytochemical contents. The results of the antimicrobial activity showed effective inhibitory activity against *Staphylococcus aureus*, *Plesiomonas shigelloides*, *Vibrio mimicus*, and *Proteus vulgaris*. Moderate antimicrobial activity was recorded against *E.coli, Salmonella typhi* and *Klebsiella pneumoniae*. Preliminary phytochemical analysis of the flower extract of this plant showed the presence of phytoconstituents such as carbohydrates, protein, alkaloids, terpenoids, phenolic compounds, reducing sugar and triterpenoids. The alkaloids present in the crude flower extracts of this plant *Couroupita guianensis* is profoundly expected for its potential antimicrobial activity.

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Introduction

Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh et al., 2003). Therefore investigation on some active principles from traditional medicinal plants has become more important (Suba et al., 2004). The world health organization (WHO, 1980) has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs (Upathaya and Pandy, 1984). *Couroupita guianensis* (Aubl) Family Lecythidaceae commonly known as Cannon ball tree, locally known as “Kailashpati” is found throughout India in plains. It is widely cultivated for its large showy flowers and reddish brown woody capsular fruits up to 20 cm in diameter. It is grown in Indian gardens as an ornamental tree. It is native to South India and Malaysia and commonly known as Nagalinga pushpam in Tamil. Traditionally, the leaves of this plant have been used in the treatment of skin disease (Satyavathi et al., 1976). Native Amazonian people used the infusion or tea obtained from leaves, flower and bark of *C. guianensis* to treat hypertension, tumours, pain and inflammatory processes (Sanz et al., 2009). In Orissa decoction of flowers has been used to boost the immune system to fight number of disease (Kokat, 1988 and Boyum, 1968). The flower extracts of this plant had been screened for larvicidal activity against vector (Desal et al., 2003) and immunomodulatory activity (Pradhan et al., 2009). In the present investigation, the flower extracts of the medicinal plant *Couroupita guianensis* was evaluated for in-vitro antimicrobial activity and of phytochemical components.

Plant Material:

The flower of *Couroupita guianensis* was collected from Kumarar koil temple located in Illanji nearby to Tenkasi, Tirunelveli District, Tamilnadu during the month of September 2012 and was brought about to the laboratory. A voucher specimen of this plant was maintained in Department of Zoology laboratory, SPK College, Alwarkurichi, Tirunelveli (Dt), Tamilnadu, India.

Material and Methods

Preparation of Extract

Fresh flowers (3kg) of *Couroupita guianensis* were used in the extraction. The solvent methanol was used for the maceration of flower for seven days at room temperature. The extract was then filtered using filter paper and was followed by filtration using Whatmann No I filter paper. The filtrate was condensed using rotary vacuum evaporator under reduced pressure. The crude extract obtained was about 150 g.

Microbes and Media:

The bacterial isolates such as *E.coli* (NCIM 2065), *Salmonella typhi*, *Klebsiella pneumoniae* (NCIM 2719), *Vibrio cholerae*, *Staphylococcus aureus* (NCIM 5021), *Pseudomonas aeruginosa* (NCIM 2036), were obtained from NCIM, Pune. *Vibrio parahaemolyticus*, *Plesiomonas shigelloides*, *Vibrio mimicus*, *Vibrio harveyi* were isolated from the disease affected fishes collected locally. The bacterial cultures were maintained in nutrient agar slants at 4°C.

Antimicrobial activity:

The extracts were screened for their antimicrobial activity in-vitro by agar disc diffusion method (Bauer, 1966). The clinical pathogens such as *E.coli, Salmonella typhi, Klebsiella pneumoniae, Vibrio cholerae, Staphylococcus aureus*, *Pseudomonas aeruginosa* and fish-borne pathogens such as *Vibrio parahaemolyticus, Plesiomonas shigelloides, Vibrio mimicus* and *Vibrio harveyi* were inoculated in nutrient broth and kept for incubation at 37 °C for 24 h and was used as the seed culture. For screening Muller-Hinton agar was prepared and seeded with 0.5 ml of respective bacterial pathogens. Then discs impregnated with different concentrations (25µl, 50µl, 75µl) of methanol extract of *Couroupita guianensis* was placed on the media and kept for incubation at 37 °C for 24 h. Next day the
plates were observed to measure the zone of inhibition. Streptomycin (10µg) was used as positive control for this study.

Preliminary Phytochemical screening:
The crude methanol extract of Couroupita guianensis was subjected to various biochemical tests as per the standard procedure of Harbone, (1973).

Results and Discussion:
The results of the present investigation showed the antimicrobial potential of the methanol extracts of the flowers of the medicinal plant Couroupita guianensis against both the clinical and fish-borne pathogens as well. The antimicrobial activity was found to be effective against the clinical pathogens viz., Staphylococcus aureus (NCIM 5021), Proteus vulgaris (NCIM 2027) and the fish-borne pathogens viz., Plesiomonas shigelloides and Vibrio mimicus. Moderate antimicrobial activity was recorded against E.coli (NCIM 2065), Salmonella typhi and Klebsiella pneumoniae (NCIM 2019). The crude methanol extract of the flowers of Couroupita guianensis was tested positive for the presence of carbohydrates protein, alkaloids, terpenoids, phenolic compounds, reducing sugar and triterpenoids. It was tested negative for aminoacids, flavonoids, tannin, Phlobatinins, aromatic acids and xanthoproteins.

The alkaloids present in the flower extract are expected to be the major reason for its antimicrobial potential against the clinical and fish-borne pathogens (Table 1). Aquaculture has been a growing activity these days worldwide because each people in the world like to eat fishes for its nutritive values. On the other hand disease outbreaks are the common setback in aquaculture industry due to fish-borne pathogens. The bacterial infections are considered major cause of mortality in aquaculture (Naylor and Burke, 2005). Plesiomonas shigelloides and Vibrio mimicus are the major pathogens causing major outbreaks in aquaculture industry. These days the aquaculture farms are being treated with commercial antibiotics to compete the outbreak of fish-borne diseases. But the antibiotics usage in aquaculture poses a serious threat of the development of multiresistant bacterial pathogens. An alternative strategy is needed to manage the fish-borne multiresistant pathogen. So, compounds isolated from the flower extracts of the plant Couroupita guianensis can be used for aquaculture disease management as a safe drug to inhibit the fish-borne pathogens viz., Vibrio alginolyticus and Plesiomonas shigelloides. Krishnan et al., (2003) reported that the aqueous extract of Azadirachta indica effectively controlled Aeromonas hydrophila infection.

In aquaculture the common problem is the usage of potassium permanganate, hydrogen peroxide and Chloramine salts to manage with the outbreaks of fish diseases. However, they affect the aquaculture environment and young fingerlings in the hatchery. As an alternative to control the disease outbreaks the flower extract of this plant Couroupita guianensis can be used. The report of Shangliang et al., (1990) showed the antimicrobial activity of 5 Chinese herb extracts against 13 bacterial and 2 viral fish pathogens. The plant extracts can be applied as an alternative to prevent columnaris, mainly in hatchery. As reported (Castro et al., 2008) the extract is natural, their hazardous potential is lower than the commercial antibiotics used in aquaculture industry.

Moreover, in clinical fields also there is a need for novel antibiotics to compete the newly evolving multiresistant bacterial pathogens. The crude methanol extract of the medicinal plant Couroupita guianensis inhibited the activity of the clinical strains Staphylococcus aureus and Pseudomonas aeruginosa. In wound infection, Staphylococcus aureus is the predominant pathogen which delays the healing of burns and wounds. As the extract showed potential activity against Staphylococcus aureus (NCIM 5021). It can be utilized for the preparation of wound healing ointments. The phytochemical analysis of the flower extracts showed the presence of phytoconstituents such as carbohydrate, protein, alkaloids, terpenoids, phenolic compounds, reducing sugar and triterpenoids.

Conclusion
In this present study the antimicrobial activity of the flower of the medicinal plant Couroupita guianensis was tested against human clinical and fish-borne bacterial pathogens. The antimicrobial activity was found to be effective against clinical pathogens such as Pseudomonas aeruginosa, Staphylococcus aureus and the fish-borne pathogens such as Vibrio mimicus and Vibrio harveyi. Further study is needed to isolate specific compounds with potential antibiotic activity against the clinical and fish-borne pathogens to develop a novel drug.

Acknowledgement:
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References:

Table: 1 Shows the result of phytochemical analysis of methanol extracts of flower extracts of the medicinal plant

<table>
<thead>
<tr>
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<tr>
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</tr>
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<td>3</td>
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<tr>
<td>4</td>
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