Case Report

Antibacterial and Antifungal Activity of *Coccinia grandis* Leaves' Extracts against Fish Pathogens

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ABSTRACT

Coccinia grandis is used in India and other parts of the world as a medicinal plant. In the present study an attempt was made to identify the presence of phytochemicals in the Aqueous, Ethanol, Methanol and Petroleum ether extracts of leaves of *C. grandis.* The qualitative Preliminary phytochemical present of study reveals that alkaloids, phenol, flavonoids, steroids, terpenoids, saponins, tannins, protein, carbohydrates, glycosides, oils, gums, resins and amino acid by adopting standard methods. In this study antibacterial and antifungal activity of leaves extracts of *C. grandis* were tested against five pathogenic bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus, Psedomonas aurginia, Escherichia coli* and *Vibrio anguillarum* by agar well diffusion method. *C. grandis* were tested against four pathogenic fungal strains such as *Candida albicans, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus.* The ethanol leaves extracts of *C. grandis* demonstrated higher antibacterial activities against *Vibrio anguillarum* (31±1.00 mm) in 500 µl concentration. A higher antifungal effect was observed with the ethanol leaves extracts with an inhibitory halo of *Aspergillus niger* (30.33±1.53 mm) in 500 µl concentration.

Key words: *Coccinia grandis,* Phytochemical screening, Antibacterial activity, Antifungal activity.

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INTRODUCTION

Fish farming is a growing field in the aquaculture ecosystem which can be done in salt as well as fresh water environment.[1] Fishes are considered as a good source of food with highest nutritive value for enhancing the health of animal and human needs. Fish is a significant diet for a large percentage of the people living in the world and earn more income. Fish diet represents the chief source of creature protein for a billion people in 58 countries worldwide.[2] Fish practices can enhance the aquaculture field for the past 20 years which increases the fish productivity worldwide with predominant level.[3] Fish is among the most essential sources of protein and Vitamin A and D containing diet so that humans have to include with their supplements.

The fish production heavily goes to retrogressive stage due to the unknown harmful diseases and water quality that makes significant losses in many aqua based industries and small companies this obstacle have to cleared in future for making abundance level of productivity.[4,5] The infection of single fish in cultured condition may spoil the entire pool with notable constraints, that potentiality provide bad environment for upcoming fish lets.[6-8]

Fish culture basically needs following parameters such as selection of fish, antibacterial resistance, conversion of feed assimilation, marketing, consumption of local area, fast growth etc these things enhances economic efficacy of fish farming. In animal husbandry continuous usage of antibacterial drugs can cause polluted environment in water body which results highest antibacterial resistant strains that increases mortality of fish and pathogenic fishes in culture pond.[9-13]

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Fish diseases can cause the highest death rate, sluggish growth of fish and drop of feed conversion rates and they diminish the commercial value of fish.[14] Henceforth, there is an invariable requirement for improvements in prevention of diseases among farmed fish, to keep away from such production losses. The fish diseases emerged from infection of following foreign agents like bacteria, parasites, fungi and viruses which causes dermatitis. Researchers have the interest over bacterial diseases because of their potentiality and causes increased death rate in untreated conditions. Symptoms of bacterial skin diseases 1. Spotted reddened lesions, 2. Sores, or inflammation on the body, 3. Reddening of the base of the fins and dulling or darken of skin color. Most common bacterial diseases are caused by both gram positive and gram negative such as *Streptococcus, Aeromonas hydrophila, Flavobacterium columnare, Vibrio* and *Pseudomonas, Streptococcus*.[15]

The ancient time diseases were cured by a number of different kinds of therapeutic methods like siddha, Ayurveda and Unani but all medicinal methods completely depend on medicinal plants for treating diseases and till now seventy percent of village people have been taking plants as a medicine. Recently innovation of plant based drugs have gained greater attention than allopathy pills which has enormous advantage likewise cheapest rate, abundant nature, highest productivity, without side effects.[16] Traditional plants are a chief source in the medicinal world, these are having infinite number of phytochemical agents that act as wonderful medicine for untreatable diseases. Medicinal plants are expected sources of compounds that can be used in opposition to numerous diseases today.[17] Herbal medicine is the foundation of about 75%-80% of the entire population and the key part of conventional therapy involves the utilization of plant extract and their dynamic constituents.

Coccinia grandis L., belongs to the family Cucurbitaceae and which is dispersed in tropical Asia, Africa and is generally found in Pakistan, India and Sri Lanka. This plant is commonly known as Kovai in Tamil. Each and every part of this plant is typically used for various medicinal purposes, following diseases that have been treated by kovai plants. They are as follows scabies and other itchy skin eruptions, skin diseases, bronchitis, psoriasis, smallpox, and ulcers, bronchial catarrh, diabetes, wounds, pyelitis, cystitis, gonorrhoeae, strangury, snake bite, urinary gravel and calculi inflammation, asthma and cough. *Coccinia grandis* leaves are supported the following activity; antibacterial,[18] antitussive,[19] cerebral related oxidative stress,[20] inflammatory disorders,[21] free radical scavenging activity and fruits are reported for hepatoprotective,[22] antihyperlipidemic.[23]

The *Coccinia grandis* plant contains various secondary metabolites such as Phenols, saponins, steroids, alkaloids, carbohydrate, resins, tannins, flavonoids and fatty acids. These phytochemical compounds enhance the potentiality against ailments. The single active phytochemical compound isolation provides a valuable drug for treating diseases. Phenolic compounds are commonly well-known for their antimicrobial activities.[24] Apart from antimicrobial activity, the plant could be used for preparing plant oils, food preservation, therapeutic applications, drug designing, cosmetics etc.[25]

There are a number of previous research proven Antimicrobial activities of *C. grandis* leaf and fruit extracts against numerous bacterial and fungal strains.[26,27] Microbes have developed a battle against many antibiotics and this has twisted a vast medical problem in the treatment of infectious diseases.[28] Antimicrobials of plant origin have enormous therapeutic potential. The medicinal effects of plant materials due to the mixture of secondary metabolites that naturally present in the plant. The plant screening is done for understanding their biological activity that helps chemotaxonomic investigation or ethnobotanical knowledge for specific diseases. More than hundreds of plants are used as traditional medicine for the treatment of bacterial infections and other infectious diseases.[29,30]

The aim of the present study is to identify the *C. grandis* extracts against fish pathogens which causes bacterial diseases in freshwater fishes. The investigation beings with phytochemical screening studies subjected to GC-MS analysis. The antibacterial and antifungal activities of successive extracts of *C. grandis* against fish pathogens have been reported here.

MATERIALS AND METHODS

Collection of Plant material

C. grandis leaves were collected from Kannanur, Tiruchirappalli District, Tamil Nadu and subsequently they were authenticated at St. Josheph's College (Autonomous), Tiruchirappalli, Tamil Nadu. The air-dried leaves of *C. grandis* (150g each) were utilized for aqueous, ethanol, methanol and petroleum ether extraction by using Soxhlet apparatus for 24 to 48 hr using 800 ml of solvent. The separated leaves' extracts were then filtered by using Whatman No. 1 filter paper. Later, each extract was transferred to airtight bottles, labelled and stored at 4°C until further analysis was performed.

Preliminary phytochemical analysis

Preliminary phytochemical screening was performed in all the four extracts, individually, to identify the phyto-chemical constituent's *viz.,* alkaloids, phenols, flavonoids, steroids, terpenoids, saponins, tannins, proteins, carbohydrates, glycosides, gums, oils, resins and amino acids by adopting standard protocols.[31]

Micro-organisms used

The micro-organisms used in this study were obtained from K.A.P. Viswanathan Medical College, Tiruchirappalli District, Tamil Nadu. The bacterial strains used in the study were *Bacillus subtilis*, *Staphylococcus aureus, Psedomonas aurginia, Escherichia coli* and *Vibrio anguillarum*. The fungal strains used for the study were *Candida albicans, Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*. All these microbial isolates were sub cultured and utilized for assessment against leaves' extracts activities, individually. Chloramphenicol and Nystatin were used in the present investigation as positive control for antibacterial and antifungal activities, respectively.

Minimum inhibitory concentration (MIC)

In vitro antibacterial activity

The leaves' extracts of aqueous, ethanol, methanol and petroleum ether were evaluated for their antibacterial activity against the chosen pathogenic bacteria. The agar diffusion method was performed using Muller-Hinton agar (Hi-Media) medium. Suspension of each micro-organism was prepared and applied to plates with serially diluted compounds to be tested and incubated for 24 h at 37°C. The compounds were tested at concentrations of 100, 250 and 500 µl. Chloramphenicol was used as a reference standard. The zone of inhibition appearing around the discs were measured and recorded in millimeter diameter.[32]

In vitro antifungal activity

The leaves' extracts were evaluated for their *in vitro* antifungal activities against the selected pathogenic fungi using agar diffusion method with Saburoud's dextrose agar (Hi-Media). Suspensions of each fungus were prepared and applied to agar plates with serially diluted compounds to be tested. The compounds were tested at three concentrations such as 100, 250 and 500 µl. Nystatin was used as a reference standard. The plates were incubated at 26°C for 72 h and MIC was determined and recorded.[33]

Statistical analysis

All these experiments were performed in triplicates. The inhibition zone data were expressed as mean ± standard division values and the same are presented in the form of Tables.

RESULTS

Phytochemical screening

The aqueous extract shows the presence of Flavonoids, Phenols, Terpenoids, Carbohydrates, Proteins, Tannins, oils, resins and amino acids. Ethanolic extracts have significant changes over the Alkaloids, Steroids, Saponins, Flavonoids, Phenols, Proteins, Terpenoids, oils, resins and amino acids. Methanolic extract contains following compound such as Alkaloids, Steroids, Saponins,

Table 1: Phytochemical screening of the leaves' extracts of *C. grandis*.

S. No.	Plant constituent	Aqueous	Ethanol	Methanol	Petroleum ether
1	Alkaloids	-	+	+	+
2	Phenols	+	+	+	+
3	Flavonoids	+	+	+	+
4	Steroids	-	+	+	+
5	Terpenoids	+	+	-	+
6	Saponins	-	+	+	-
7	Carbohydrates	+	-	-	-
8	Proteins	+	+	+	-
9	Tannins	+	-	-	-
10	Oils	+	+	-	-
11	Resins	+	+	+	+
12	Glycosides	-	-	-	-
13	Gums	-	-	-	-
14	Amino acids	+	+	+	+

(+) = Denotes presence of compound, (-) = Denotes absence of compound.

Flavonoids, Phenols, Proteins, Resins and Steroids whereas Petroleum ether extract indicates the presence of Steroids, Alkaloids, Flavonoids, Phenols, Terpenoids, resins and amino acids. Among the four samples ethanol extract shows highest phytochemical content when compared to other extracts.

Antibacterial activity

The *C. grandis* aqueous, ethanol, methanol and petroleum ether extracts were analyzed against five bacterial pathogens such as *B. subtilis*, *S. aureus, P. aurginia, E. coli* and *V. anguillarum* and the results are provided in Tables 2-5 and Figure 1, respectively. The results confirmed that the whole of the four different leaves' extracts of *C. grandis* had good control over all the five pathogenic organisms tested and on par or in many cases higher than the control values. The antibacterial activity has been proven the highest antibacterial activity was observed with ethanol leaves' extracts of *C. grandis* against *V. anguillarum* (31±1.00mm) in 500 µl concentration (Table 3).

Antifungal activity

The four different extracts of *C. grandis* (aqueous, ethanol, methanol and petroleum ether) were

Table 2: Magnitude of zone of inhibition observed by using three different concentrations of aqueous leaves' extracts of *C. grandis* and a known antibiotic (control) against five bacterial pathogens.

Organisms	Zone of inhibition (mm) Mean ± SD			
	100µl	250 µl	500 µl	Control
B. subtilis	12.33±0.57	10±2.00	17.33±1.52	8.67±0.58
S. aureus	20±2.64	22.67±1.52	24.33±1.52	24.33±4.04
P. aurginia	10.67±1.52	15±1.00	17.67±1.52	30±2.00
E. coli	11±1.00	8.33±0.58	18±2.00	25.33±2.30
V. anguillarum	22±1.00	20±2.00	28.33±2.08	2.67±1.16

Mean ± Standard Division values were obtained from triplicate observations.

Table 3: Degree of zone of inhibition recorded by using three different concentrations of ethanol leaves' extracts of *C. grandis* and a known antibiotic (control) against five bacterial pathogens.

Organisms	Zone of inhibition (mm) Mean ± SD			
	100µl	250 µl	500 µl	Control
B. subtilis	20.33±1.52	21.67±0.58	27±1.00	21.33±1.16
S. aureus	18±2.00	22.33±2.08	25.67±4.50	21.67±2.08
P. aurginia	8.33±1.52	10.67±1.16	16.67±2.08	28±2.00
E. coli	12.67±1.16	11.67±1.52	20.33±0.58	22.67±2.30
V. anguillarum	16.33±1.52	16.67±2.08	31±1.00	8±1.00

Mean ± Standard Division values were obtained from triplicate observations.

Table 4: Propensity of zone of inhibition recorded by using three different concentrations of methanol leaves' extracts of *C. grandis* and a known antibiotic (control) against five bacterial pathogens.

Organisms	Zone of inhibition (mm) Mean ± SD			
	100µl	250 µl	500 µl	Control
B. subtilis	20±2.00	26.33±1.52	29.67±1.52	24.33±0.58
S. aureus	16±2.00	18.67±2.30	24.33±4.04	24±0.00
P. aurginia	11±1.00	15±1.00	17.33±1.52	22±2.00
E. coli	10.67±1.16	9±1.00	16.33±1.52	20.33±0.58
V. anguillarum	20.33±1.52	16.67±1.16	30±2.00	11±1.73

Mean ± Standard Division values were obtained from triplicate observations.

Table 5: Extent of zone of inhibition recorded by using three different concentrations of petroleum ether leaves' extracts of *C. grandis* and a known antibiotic (control) against five bacterial pathogens.

Organisms	Zone of inhibition (mm) Mean ± SD			
	100µl	250 µl	500 µl	Control
B. subtilis	16.67±1.52	12.33±0.58	20.33±1.52	11.33±1.16
S. aureus	13.67±1.52	18.33±2.88	20.67±0.33	24.67±4.16
P. aurginia	9±1.00	13.67±3.21	12.67±2.30	20.67±1.15
E. coli	15.67±0.58	11.33±1.16	19.67±1.52	22±2.00
V. anguillarum	16±1.00	12±1.16	24±4.00	20±0.00

Mean ± Standard Division values were obtained from triplicate observations.

Table 6: Degree of zone of inhibition recorded by using three different concentrations of aqueous leaves' extracts of *C. grandis* and a known antibiotic (control) against four fungal pathogens.

Organisms	Zone of inhibition (cm) Mean ± SD			
	100µl	250 µl	500 µl	Control
C.albicans	12±1.00	14.33±1.52	17.33±1.52	9.67±0.58
A. niger	12±1.00	24.67±2.52	27.67±1.52	31±1.00
A. flavus	10±1.00	20.67±0.58	26.67±1.52	24±1.00
A. fumigatus	10±1.00	11.67±0.58	14.33±1.52	10.33±0.58

Mean ± Standard Division values were obtained from triplicate observations.

Table 7: Propensity of zone of inhibition recorded by using three different concentrations of ethanol leaves' extracts of *C. grandis* and a known antibiotic (control) against four fungal pathogens.

Organisms	Zone of inhibition (cm) Mean ± SD			
	100µl	250 µl	500 µl	Control
C.albicans	10.67±0.58	14.33±1.16	17.67±1.52	9.33±0.58
A. niger	18±1.00	27.67±1.52	30.33±1.53	31.33±1.16
A. flavus	10.67±0.58	17±1.00	20.67±1.52	24±1.00
A. fumigatus	17±1.00	15.33±1.16	19.67±1.52	10.33±0.58

Mean ± Standard Division values were obtained from triplicate observations.

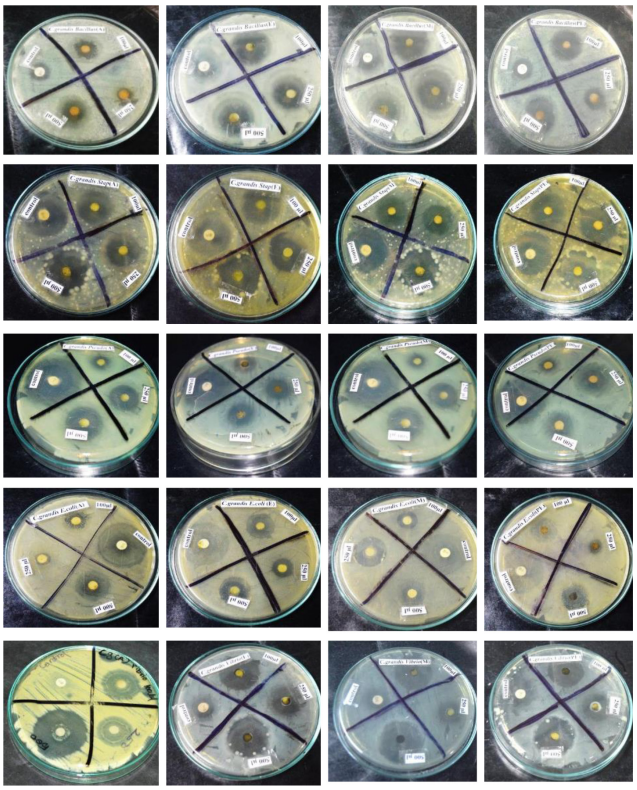

Figure 1: To present in the antibacterial activity in plants to given positive and negative results for the disc diffusion assay. (A) Aqueous extract, (E) Ethanol extract (M) Methanol extract (PE) Petroleum ether leaves' extracts of *C. grandis* and a known antibiotic (control) against five bacterial pathogens.

Table 8: Extent of zone of inhibition recorded by using three different concentrations of methanol leaves' extracts of *C. grandis* and a known antibiotic (control) against four fungal pathogens.

Organisms	Zone of inhibition (cm) Mean ± SD			
	100µl	250 µl	500 µl	Control
C. albicans	13.67±1.52	16±1.00	20.67±1.52	12±1.00
A. niger	18.33±1.52	25±2.00	24.33±0.58	30±2.00
A. flavus	15.67±0.58	20±2.00	20.33±1.52	21.67±1.52
A. fumigatus	10.33±1.52	17.33±1.52	23±1.00	11.67±0.58

Mean ± Standard Division values were obtained from triplicate observations.

investigated against five fungal pathogens such as *C. albicans, A. niger, A. flavus* and *A. fumigates* the results are presented in Tables 6-9 and Figure 2. In general, the performance of antifungal activities of all the four leave's extracts of *C. grandis* has significant activity and particularly at 500 µl, was found to be higher than the control values. A highest antifungal activity was observed with the ethanol extracts of *C. grandis* against *A. niger* (30.33±1.53mm) at 500 µl concentration (Table 7).

Table 9: Magnitude of zone of inhibition observed by using three different concentrations of petroleum ether leaves' extracts of *C. grandis* and a known antibiotic (control) against four fungal pathogens.

Organisms	Zone of inhibition (cm) Mean ± SD			
	100µl	250 µl	500 µl	Control
C.albicans	7.67±1.16	11±1.00	14.33±0.58	10±1.00
A. niger	11.67±1.53	16±1.00	20.67±1.53	29.33±1.16
A. flavus	11.67±0.58	14±1.00	15.67±1.52	20.67±0.58
A. fumigatus	10.33±0.58	11±1.00	12.33±1.52	9.33±0.58

Mean ± Standard Division values were obtained from triplicate observations.

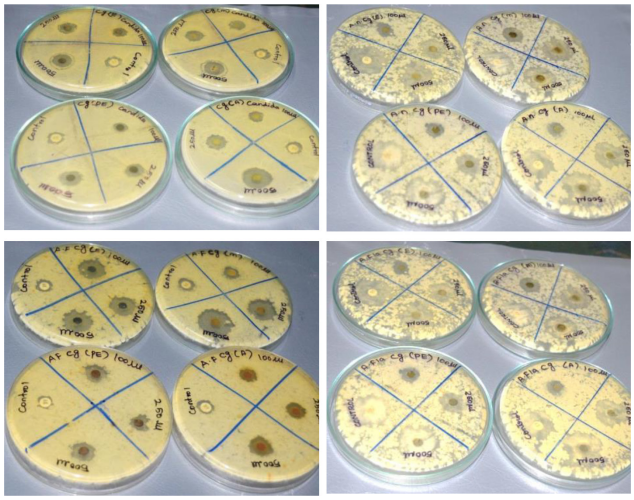

Figure 2: To present in the antifungal activity in plants to given positive and negative results for the disc diffusion assay. (A) Aqueous extract, (E) Ethanol extract (M) Methanol extract (PE) Petroleum ether leaves' extracts of *C. grandis* and a known antibiotic (control) against four fungal pathogens.

Further, it is obvious from the results, all the four types of extracts of *C. grandis* acted well against both five different strains of bacteria and fungi particularly at 500 µl concentrations Table (2-9). However, ethanol extracts of *C. grandis* performed well against the chosen bacterial and fungal pathogens when compared to the other three extracts. This might be due to the presence of various phyto-chemical constituents present in them (Methanol extracts) when compared to the other extracts (Table 1). Further studies like GC-MS are required to identify the active ingredients from the fractions obtained in all the four types of leaves' extracts.

DISCUSSION

Some of the phytochemical constituents are known to possess various biological activities. Some examples include alkaloids, flavonoids, terpenoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds.[34] Experimental screening

method is vital for setting up the safety and understanding the efficacy of traditional and herbal products.[35] Previous studies on antibacterial and antifungal activity of leaves and stem of *C. grandis*[36,18,37] have also detected the significant activity of methanol and ethyl acetate extracts against different bacteria and fungi and providing support to the fact that methanol is a better solvent for extraction and isolation of phytochemicals which having highest antimicrobial activity. The present study also proved the fact and revealed the moderate activity of water extract agreeing with earlier reports that use of organic solvents is always better.[38]

CONCLUSION

In conclusion, the results of this investigation revealed that methanol extracts possess antimicrobial activity against selected bacterial and fungal strains. The activities against variety of micro-organisms of these three extracts encourage developing a novel broad spectrum antimicrobial formulation in future. Now our research will be directed to develop a broad spectrum antimicrobial herbal formulation with these plants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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