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REGULAR ARTICLE

IN VITRO EVALUATION OF ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF THE PLANT *Coccinia grandis* (L.) VOIGT. (FAMILY- Cucurbitaceae)

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SUMMARY

Coccinia grandis, family-Cucurbitaceae, a perennial tendril climber plant, possessing significant antidiabetic property. Other plants in the family Cucurbitaceae possessing diuretic, aphrodisiac, bitter stomachic, purgative and emetic properties. But antimicrobial activity of *C. grandis* has not been evaluated extensively. We have paid more importance on antimicrobial activities against various fungal strains and different gram positive and gram negative bacteria. Aqueous and ethanolic extract were used to evaluate antifungal and antibacterial activities. Antifungal activity was observed on *Candida albicans* and *Aspergillus niger* and antibacterial activity was observed on gram positive bacteria like *Bacillus subtilis UC564, Bacillus pumilus-8241, Enterococcus faecalis ATCC-29212, Bacillus licheniformis, Staphylococcus aureus ATCC6571, Streptococcus faecalis-52* and gram negative bacteria like *Shigella boydii-Type12, Shigella flexneri E03429, Shigella dysenteriae-3 , Pseudomonas aeruginosa , Escherichia coli-K88, Salmonella typhi-62 , Salmonella choleraesuis-36 , Shigella boydii-8, Shigella flexneri NICED, Shigella sonnei E08869.*

Key words: *Coccinia grandis*, MIC, antifungal and antibacterial activities

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1. Introduction

Coccinia grandis, family- Cucurbitaceae is a climber or trailer type plant generally distributed throughout the tropical countries of Asia and Africa [1]. Leaves and fruits of the plant are consumed as vegetables in tropical countries of Asia like India, Bangladesh, Pakistan etc. [1]. Since long before the leaves are consumed to control hyperglycemia as indigenous system of medicine [2, 3, 4, 5]. It is also noted by Unani system of medicine that the plant is used in ring worm, psoriasis, scabies etc. [6]. Unani system of medicine also report that various preparation of the plant parts were used for ulcer [7].

The plant is used for gonorrhoea [8]. It can prevent renal stone formation [2]. Besides it can also control plasma lipid concentration [3]. Another species, *Coccinia indica* also possess analgesic and hepatoprotective property [9]. Aqueous extract of *Coccinia indica* after oral consumption can reduce fasting blood sugar of guinea pig [10]. The object of the present study is to examine antifungal activities and antibacterial activities against some fungi and gram positive and gram negative bacterial strains.

2. Method

Coccinia grandis leaves were collected from various parts of Hooghly District, West Bengal, India, in the month of August – September, 2008. The plant was identified by Botanical Survey of India, Shibpur, West Bengal, India. The leaves were shade dried and comminuted by grinder and extracted successively with solvents of increasing order of polarity i.e. Petroleum spirit, Chloroform, Ethyl acetate, Ethanol and Water. Petroleum spirit is generally utilized

to remove fatty or waxy materials and pigments present in the leaves [9]. The extracts other than the aqueous one were dried under reduced pressure at temperature below 40°C by using Eyela Rotary Evaporator (Japan) [11]. Aqueous extract was dried by Rotating vacuum evaporator. All solvents (Analytical grade) were purchased from Merck (Mumbai) and all the media ingredients were Hi Media (Mumbai).

Sources of fungal strains

Seven fungal strains were tested for antifungal properties of the leaf extracts. *Candida albicans*-II, *Candida tropicalis, Aspergillus niger, Saccharomyces cerevisiae* were collected from Advanced Medicare and Research Institute, Kolkata, India. *Candida tropicalis II* and *Cryptococcas neoformans* were collected from Dr. S. Bhattacharya, NRS Medical College and Hospital, Kolkata, India. *Candida albicans* ATCC 10231 was collected from Central Drug Laboratory, Kolkata, India.

Media preparation

Czapek Dox Liquid media (Hi Media Lab Pvt. Ltd., Mumbai) is prepared. Fungal strains were transferred aseptically into Czapek Dox Liquid media and incubated at 28°C for 7 days. Now slant is prepared with same liquid media with 2% agar and adding different concentration of extracts. After the slant is prepared well, above fungal strains are inoculated aseptically from fungal broth to Czapek Dox Liquid media and incubated at 28°C for 7 days and growth inhibition is observed[12]. Here Fluconazole is used as reference antifungal drug.

Bacterial culture

Gram positive bacterial strains like *Bacillus subtilis UC564*, *Bacillus pumilus-8241, Enterococcus faecalis ATCC-29212, Bacillus licheniformis, Staphyloccus aureus ATCC6571, Streptococcus faecalis-52* and gram negative bacterial strains like *Shigella boydii-Type12, Shigella flexneri E03429, Shigella dysenteriae-3, Pseudomonas aeruginosa , Escherichia coli-K88, Salmonella typhi-62 , Salmonella choleraesuis-36 , Shigella boydii-8, Shigella flexneri NICED, Shigella sonnei E08869* were selected for screening and growth inhibition was observed in some pathogenic strains.

Sources of bacterial strains

Staphyloccus aureus ATCC6571, Streptococcus faecalis-52, Pseudomonas aeruginosa and *Escherichia coli-K88* were collected from Central Drug Laboratory, Kolkata, India. *Enterococcus faecalis ATCC-29212, Shigella boydii-Type12, Shigella boydii-8, Shigella flexneri E03429, Shigella flexneri NICED, Shigella sonnei E08869* were collected from National Institute of Cholera and Enteric Diseases, Kolkata, India. *Bacillus subtilis UC564* was collected from Upjohn Laboratory, USA. *Bacillus pumilus-8241* was collected from Dr. S.P. Lapage, London. *Bacillus leicheniformis* was collected from Dr. A. Ghosh, London. *Shigella dysenteriae-3*was collected from Dr. K. Patricia Carpenter, London. *Salmonella typhi-62, Salmonella choleraesuis-36* were collected from Dr. Joan Taylor, Salmonella Reference Laboratory, London. All strains were preserved in freeze-dried state and at 4°C in stab slant agar [13].

Media preparation

Nutrient agar media (pH 7-7.4) plates were prepared containing various concentrations of different extracts. Now organisms from Nutrients broth media were inoculated with sterile loop in laminar chamber into respective plates containing nutrient agar media and various concentrations of desired extracts.

Determination of zone of Inhibition

Here inoculum is spread over nutrient agar media with sterile glass spreader. Small circular paper disks (6 mm diameter) impregnated with known amount of extracts are placed upon the surface of the inoculated plates [14]. Zone of inhibition is measured by using divider and ruler [15, 16]. Each experiment was repeated three times and the mean diameter of zone of inhibition was measured [17].

Statistical analysis

The data of all the parameters were statistically analysed (Statistical software used- Minitab 14-State College, PA, USA) and zone of inhibition diameter values are expressed as *Mean diameter* ± *SEM* (n=5), value of '*p*' is also calculated and mentioned below table 2 and 3. (*When value of 'p' is 0.01 to 0.05 the result is statistically significant, 0.01 to 0.001 the result is statistically very significant*).

3. Result

Among the seven fungal strains ethanolic extract showed remarkable antifungal activities against *Candida albicans* and *Aspergillus niger*. *Aspergillus niger* and *Aspergillus fumigatus* both are responsible for Aspergillosis where pulmonary allergy, bronchopulmonary aspergillosis and pulmonary aspergilloma occurs. Aqueous extract showed significant antifungal activities against *Aspergillus niger*. Aqueous extract is more sensitive for both strain of *Candida albicans* (oral and vaginal candidiasis, moniliasis etc.) and Ethanolic extract is more sensitive for *Aspergillus niger* (Aspergillosis) and both strains of *Candida albicans.* MIC ranges have been represented in the Table 1.

Table1: Antifungal property of *Coccinia grandis* leaf extract (MIC in µg/ml.)

Fungus strains	*Candida albicans ATCC 10231*	*Candida albicans* -II	*Candida tropicalis*	*Candida tropicalis - II*	*Asperagillus niger*	*Cryptococcas neoformans*	*Saccharomyces cerevisiae*
MIC of Fluconazole (µg/ml)	15	15	20	20	15	15	20
MIC of Aqueous extract(µg/ml)	1000	1250	4500	4250	4500	>5000	4750
MIC of Ethanol extract(µg /ml)	750	750	4750	>5000	1000	4500	4250

Twenty six bacterial strains were targeted for screening of antibacterial properties. Among those, sixteen bacteria are sensitive to the leaf extracts. Aqueous extract showed more significant antibacterial activity in comparison to ethanol extract. Various bacterial strains produced different zone-diameter (mm.) in their respective MIC in comparison with Chloramphenicol (reference drug). MIC values and zone of inhibition has been represented in the Table 2, 3. Comparative zone of inhibition (diameter in mm.) with concentration (µg /ml) of leaf extract for *Shigella dysenteriae-3* and *Shigella flexneri E03429* has been represented in Figure-1 and Figure-2 respectively.

Table-2: Zone of Inhibition (diameter in mm.) of *Coccinia grandis* leaf extract and Chloramphenicol (reference drug) on Gram + ve organisms- Concentration- µg /ml

organisms (Gram+ve bacteria)	Aqueous extract		Ethanol extract		Chloramphenicol	
	MIC (µg /ml)	Zone of inhibition diameter in(mm.)	MIC (µg /ml)	Zone of inhibition in diameter (mm.)	MIC (µg /ml)	Zone of inhibition in diameter (mm.)
Bacillus subtilisUC564	1250	8±0.080	1250	7.9±0.058	15	6.4±0.058
Bacillus pumilus-8241	1250	7.8±0.058	1500	6.6±0.066	17.5	6.7±0.066
Enterococcus faecalis ATCC-29212	1500	6.6±0.037	1750	6.7±0.073	20	6.3±0.044
Bacillus licheniformis	1500	7±0.067	1250	7.7±0.080	27.5	7.2±0.037
Staphyloccus aureus ATCC6571	1250	8±0.058	1750	7.8±0.060	22.5	7.5±0.060
Streptococcus faecalis-52	1500	6.7 ±0.080	-	-	20	6.6±0.05

***'-'** indicates no growth inhibition. Zone of inhibition diameter values are Mean diameter ± SEM (n=5), value of 'p' < 0.05 i.e. statistically significant.* (Statistical software used- Minitab 14-State College, PA, USA)

Table-3: Zone of Inhibition (diameter in mm.) of *Coccinia grandis* leaf extract and Chloramphenicol (reference drug) on Gram – ve organisms- Concentration- µg /ml

organisms (Gram –ve bacteria)	Aqueous extract		Ethanol extract		Chloramphenicol	
	MIC (µg /ml)	Zone of inhibition in diameter (mm.)	MIC (µg /ml)	Zone of inhibition in diameter (mm.)	MIC (µg /ml)	Zone of inhibition in diameter (mm.)
Shigella boydii-Type12	1250	7.4±0.086	1500	7.5±0.107	22.5	6.6±0.107
Shigella flexneri E03429	1000	7.9 ±0.086	1250	7.8±0.092	15	6.3±0.107
Shigella dysenteriae-3	1250	8.1±0.144	1500	7.9±0.144	17.5	6.5±0.074
Pseudomonas aeruginosa	1250	6.7±0.092	1500	6.7±0.092	-	-
Escherichia coli-K88	1000	6..9±0.144	1500	7.7±0.092	25	6.4±0.058
Salmonella typhi-62	1500	6.6±0.092	1750	6.7±0.070	20	7±0.05
Salmonella choleraesuis-36	1250	6.7±0.092	1500	7±0.092	22.5	6.5±0.05
Shigella boydii-8	1250	7.6±0.092	1250	8±0.092	17.5	6.7±0.05
Shigella flexneri NICED	750	7.8±0.070	1000	7..8±0.106	20	6.4±0.058
Shigella sonnei E08869	1000	7.5±0.106	1250	7.4±0.092	22.5	6.3±0.058

***'-'** indicates no growth inhibition. Zone of inhibition diameter values are Mean diameter ± SEM (n=5), value of 'p' < 0.05 i.e. statistically significant.* (Statistical software used- Minitab 14-State College, PA, USA)

Figure-1: Representing comparative zone of inhibition (diameter in mm.) with concentration (µg /ml) of leaf extract for *Shigella dysenteriae-3*

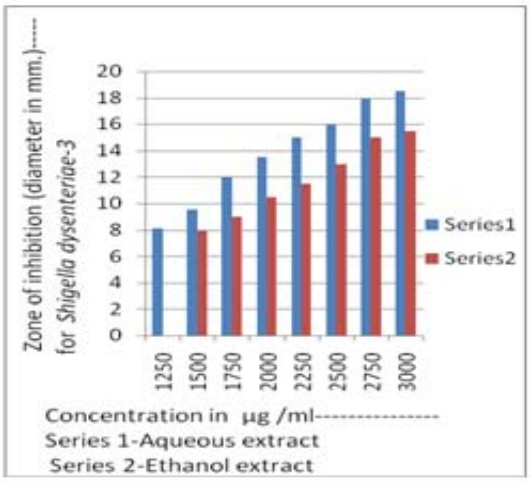
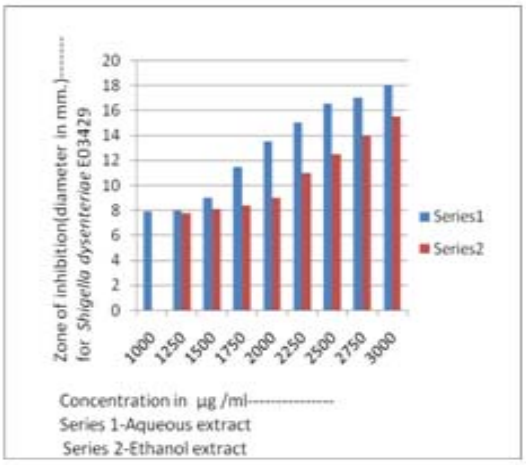


Figure-2: Representing comparative zone of inhibition (diameter in mm.) with concentration (µg /ml) of leaf extract for *Shigella flexneri* E03429



4. Discussion

Antifungal activities

Except *Saccharomyces cerevisiae*, all other fungal strains considered here are pathogenic for human being. Hence Ethanol extract is more significant for producing antifungal activities. So, this observation points out that non-polar fractions in the extract possess higher level of antifungal properties.

Candida tropicalis, Cryptococcas neoformans and *Saccharomyces cerevisiae* are comparatively less sensitive to both aqueous and ethanol extract and MIC for them is more than 4000 µg/ml. Aqueous extract is more sensitive for both strain of *Candida albicans* (oral and vaginal candidiasis, moniliasis etc.) and Ethanol extract is more sensitive for both strains of *Candida albicans* and *Aspergillus niger* (Aspergillosis)[18].

Antibacterial activities

Chemical properties and pharmacological features of phytochemicals collected from herbal extracts of one plant differ from one solvent to another [19]. Aqueous extract showed more significant antibacterial activity in comparison to ethanol extract. Other solvents like Chloroform, Ethyl acetate, Petroleum spirit extracts also showed antibacterial activity but less prominent. From the Figure 1 and Figure 2 it is also evident that degree of antibacterial activity is more in case of aqueous extract. This observation elucidates that polar moiety of the extract is more responsible for antibacterial properties. *P. aeruginosa* which is resistant to different antibiotics, also inhibited by the extract. Such results are very interesting, because control of this bacterium is very difficult by therapeutic means [20].

5. Conclusion

Remarkable antibacterial activities has been observed in case of *Shigella flexneri NICED, Shigella flexneri E03429, Shigella dysenteriae-3, Escherichia coli-K88, Salmonella choleraesuis-36,Bacilius subtilis UC-564* etc. It is evident from the plots that aqueous extract possess more predominant antibacterial property in comparison to ethanolic extract. *Coccinia indica* plant contains alkaloids, flavonoids, terpenoids, thymol and phenolic compounds which are categorized as antimicrobial agents [14]. Sufficient scope is there for future research on rational drug design from isolated chemicals of *Coccinia grandis*.

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