

EVALUATION OF ANTI BACTERIAL ACTIVITY OF FRUIT RIND EXTRACT OF *GARCINIA MANGOSTANA LINN* ON ENTERIC PATHOGENS-AN IN VITRO STUDY

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ABSTRACT

The aim of the present study was to evaluate the anti bacterial activity of fruit rind extract of *Garcinia mangostana Linn* on selected enteric pathogens. *Garcinia mangostana*, known simply as mangosteen has a diverse pharmacological and phytochemical importance and is a potent medicinal plant in the traditional Indian medicinal systems. Antibacterial activity of ethanolic and aqueous fruit rind extract of *Garcinia mangostana* was screened against *Salmonella typhi*, [Gram negative bacilli-GNB], *Shigella dysenteriae*[GNB], *E.coli*[GNB], *Klebsiella pneumoniae*[GNB], *Vibrio cholerae*[GNB], *Pseudomonas aeruginosa*[GNB] and *Staphylococcus aureus*,[Gram positive cocci] using agar well diffusion technique. The results of this study showed that both the extracts at different concentrations exhibited anti bacterial activity against the bacterial species tested. The ethanolic extract showed higher degree of activity than aqueous extract compared with the standards.

Key Words: *Garcinia mangostana Linn*, Agar well diffusion, Mac Farland's standard, Zone of inhibition, MIC, MBC.

INTRODUCTION

Enteric or diarrhoeal infections are major public health problems in developing countries. Enteric bacteria comprised of *Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli*, *Pseudomonas* sp., *Vibrio cholerae*, and *S. aureus*, which are major etiologic agents of sporadic and epidemic diarrhoea both in children and in adults.¹ Resistance to antibacterial agents has important implications for morbidity, mortality and health care in the community. So it makes necessary to discover new classes of compounds to treat infections. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, alkaloids, and flavonoids, which are reported to have in vitro antibacterial properties.^{2, 3} currently many studies, are being conducted to know these herbs in depth. Screening of medicinal plants for antimicrobial activities are important for finding potential new compounds for therapeutic use. There have been numerous reports of the use of traditional plants and natural products for the treatment of enteric diseases.

Garcinia mangostana, colloquially known simply as mangosteen, is a tropical evergreen tree believed to have originated in the Sunda Islands and the Moluccas of Indonesia. The pleasant taste (sweet and slightly acidic) and medicinal qualities of the reddish purple mangosteen fruit has led to its common name as "Queen of Fruits".^{4,5} Numerous studies have shown that mangosteen has high concentrations of xanthenes, a class of polyphenolic compounds. Researches have identified a total of over 40 Xanthenes from the hull, rind and the pulp of mangosteen fruits.^{6,7} The major xanthenes are alpha-mangostin, beta-mangostin, gamma-mangostin, and methoxy-beta-mangostin, and the most abundant is alpha-mangostin. Calcium, phosphorus, iron, thiamine, riboflavin, niacin, and ascorbic acid are found in mangosteen.⁸⁻¹⁰ Xanthenes have antibacterial,^{11,12} antifungal,¹³ antioxidant,¹⁴, antitumor,^{15,16} antiplatelet aggregation, immuno stimulatory,¹⁷ anti-inflammatory¹⁸ and vasorelaxant activities. The rind is used as an astringent medicine for diarrhoea and dysentery. It has been found very useful in chronic diarrhoea in children.¹⁹ The value of the rind lies in the yellow resin which may act as a muculant to the intestines. They have an astringent effect on mucosal tissue and can reduce secretions, thus reducing diarrhea.

Modern day science has recently begun to appreciate the incredible, nutrient-rich value of mangosteen and its wide-reaching, health-promoting properties. The present study was to evaluate the antibacterial activity of ethanolic & aqueous fruit rind extract of *Garcinia mangostana Linn* on selected enteric pathogens.

MATERIALS AND METHODS

Plant material

The ethanolic and aqueous fruit rind extract of *Garcinia mangostana Linn* was obtained from Green Chem Herbal Extract & Formulations, Bangalore.

Test microorganisms

Bacterial strains used were *Salmonella typhi*, [Gram negative bacilli-GNB], *Shigella dysenteriae*[GNB], *E.coli*[GNB], *Klebsiella pneumoniae*[GNB], *Vibrio cholerae*[GNB], *Pseudomonas aeruginosa*[GNB], and *Staphylococcus aureus*,[Gram positive cocci]. The organisms were obtained from department of Microbiology, Saveetha Dental College and maintained in nutrient agar slope at 4°C.

Methodology

The extracts were prepared in the following concentrations in sterile water. 2mg/ml, 3mg/ml and 4mg/ml, so that 50µl of extract of different concentrations delivers 100µg, 150µg and 200 µg respectively. .

Assay for antibacterial activity using agar well diffusion method

The screening of antibacterial activity of plant extracts was carried out using the agar well diffusion method. The bacterial strains were inoculated into tubes of nutrient broth and incubated at 37°C overnight. Each of the cultures were then adjusted to 0.5 McFarland turbidity standard.^{20,21} Lawn culture of the test organisms were made on the Muller Hinton agar [MHA-Hi media M1084] plates using sterile cotton swab and the plates were dried for 15 minutes. A sterile cork borer was then used to make wells (6mm diameter) for different concentrations of the extracts on each of the plates containing cultures of the different bacterial strains. 50µl of the varying concentrations (100,150, 200µg) of the extracts were introduced into the wells with the help of micropipettes. The culture plates were allowed to stand on the working bench for 30 min for pre-diffusion and were then incubated in upright position at 37°C for 24 h. After 24 hrs, antibacterial activity was determined by measurement of diameter of zones of inhibition (mm). Standard antibiotic discs of amoxicillin (30mcg/disc) and Ciprofloxacin (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

Determination of minimum inhibitory concentration

Macro broth dilution or tube dilution method was done to determine the Minimum inhibitory concentration (MIC) of the extracts.^{22,23} A series of two fold dilution of each extract ranging from 8mg/ml to

0.125mg/ml was made in Muller Hinton broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1998). 100µl of standard inoculum of the bacterial strains matched to 0.5 Mc Farland's standard were seeded into each dilution. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium and the inoculum). The tubes were incubated at 37°C for 24 hours and checked for turbidity. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that showed no visible growth

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the extracts was carried by pipetting 100ul of broth from the tube that showed no growth in MIC determination and streaked on to MHA plates and incubated for 24 h at 37°C. The least concentration of the extract with no visible growth after incubation was taken as the minimum bactericidal concentration.

RESULT AND DISCUSSION

The antibacterial activity of the extracts (Ethanolic and Aqueous) at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the table 1 and Fig: 1 & 2. The minimum inhibitory concentration [MIC] and minimum bactericidal

concentration [MBC] were also determined for the extracts and the results are given in table 2 and Fig: 3

Both the extracts at different concentration exhibited antibacterial activity against all bacterial strains tested. Ethanolic extract exhibited comparably a high degree of activity than the aqueous extract. The ethanolic extract was more effective against *Shigella dysenteriae*, *Vibrio cholerae* and *Staphylococcus aureus* with a zone of inhibition of 25mm, 24mm and 24 mm diameter (at conc 200 µg.) respectively and was least effective against *Pseudomonas aeruginosa* with zone of inhibition of 11mm (at conc. 200 µg.) Among the other bacterial species studied *E.coli* and *Salmonella typhi* showed a zone of inhibition of 20mm diameter (at conc. 200 µg.) and *Klebsiella pneumoniae* showed inhibition zone of 17mm diameter (at conc. 200 µg.).

The MIC and MBC values of ethanolic extract were found to be low compared to aqueous extract. The ethanolic extract was found to have Low MIC and MBC values of 1mg/ml & 1mg/ml for both *Shigella dysenteriae* and *Vibrio cholerae* and for *Staphylococcus aureus* it was 1mg/ml and 2mg/ml. With *Pseudomonas aeruginosa* ethanolic extract showed a higher MIC and MBC value of 4mg/ml & 4mg/ml and for *E.coli*, *Salmonella typhi* and *Klebsiella pneumoniae* it was 2mg/ml & 2mg/ml, 2mg/ml & 2mg/ml and 1mg/ml & 2mg/ml respectively. The lower MIC and MBC value is an indication of high effectiveness of the extract whereas higher MIC and MBC indicates the less effectiveness of the extract.

Table 1: Anti bacterial activity of fruit rind extract of *Garcinia mangostana* Linn

Extract	Conc [µg]	Zone of inhibition [in mm diameter]						
		B1	B2	B3	B4	B5	B6	B7
Ethanolic	100	10	15	14	10	10	7	12
	150	15	20	21	15	13	8	19
	200	20	25	24	20	17	11	24
Aqueous	100	9	8	11	8	8	-	10
	150	11	12	13	11	11	7	16
	200	14	15	16	16	14	9	20
Ciprofloxacin	30mcg/disc	24	21	22	22	23	23	24
Amoxycillin	30mcg/disc	25	23	20	24	25	25	22

B1- *E.coli*, B2- *Shigella dysenteriae*, B3-*Vibrio cholerae*, B4- *Salmonella typhi*, B5- *Klebsiella pneumoniae*, B6- *Pseudomonas aeruginosa*, B7- *Staphylococcus aureus*

Table 2: MIC and MBC values of Ethanolic and Aqueous extracts

Extract		MIC/MBC values [mg/ml]						
		B1	B2	B3	B4	B5	B6	B7
Ethanolic	MIC	2	1	1	2	2	4	1
	MBC	2	1	1	2	4	4	2
Aqueous	MIC	4	2	2	4	4	8	4
	MBC	4	4	2	4	8	8	4

B1- *E.coli*, B2- *Shigella dysenteriae*, B3-*Vibrio cholerae*, B4- *Salmonella typhi*, B5- *Klebsiella pneumoniae*, B6- *Pseudomonas aeruginosa*, B7- *Staphylococcus aureus*.

Prevalence of drug resistance to various antibiotics among pathogenic bacteria has paved way for the search of new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk

medicine for the treatment of infectious diseases produced by common pathogens.

The present study was to evaluate the antibacterial activity of fruit rind extract of *Garcinia mangostana* Linn against enteric pathogens. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. It was evident that antimicrobial activity

was more apparent in ethanol than aqueous extracts. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

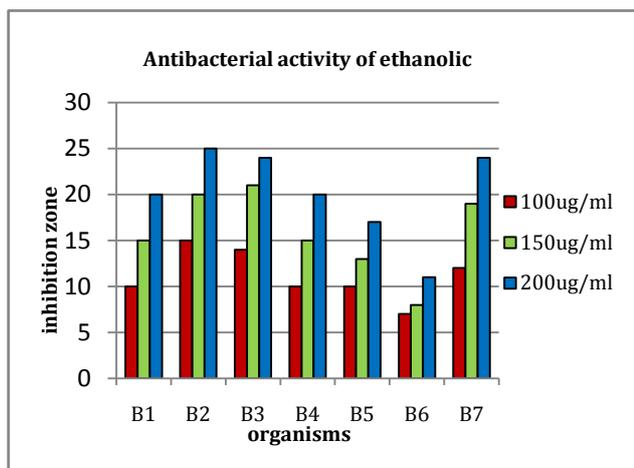


Fig 1: Graph showing antibacterial activity of ethanolic extract

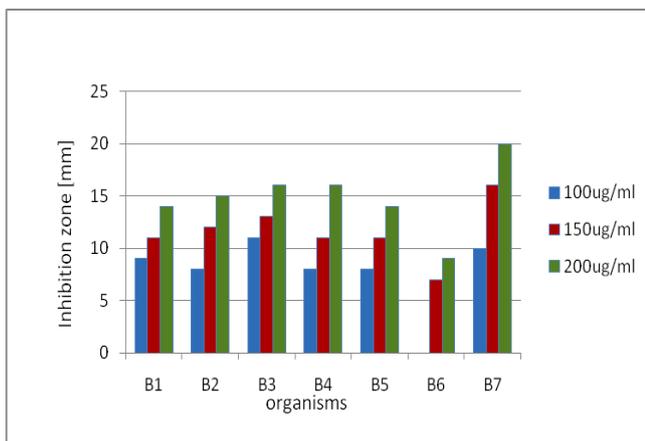


Fig 2: Graph showing antibacterial activity of aqueous extract

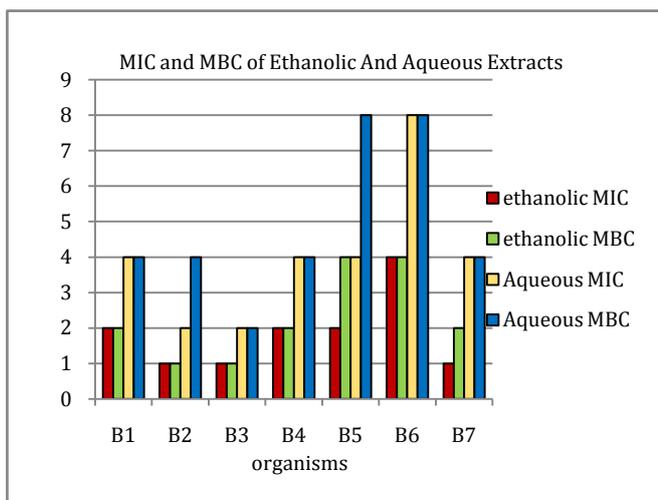


Fig 3: Graph showing MIC and MBC values of Ethanolic and Aqueous extracts

CONCLUSION

This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. It is clear from the results that, the extract acts as a good source of antimicrobial agent against various bacterial pathogens tested and exhibited broad spectrum of antibacterial activity.

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