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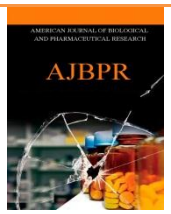

ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL STUDIES ON *GARCINIA MANGOSTANA*

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ABSTRACT

The discovery of active biocompounds is a matter of urgency as most of the pathogens are getting resistance against number of drugs. Ethanolic extract of *Garcinia mangostana* peel analysed for its antioxidant and antimicrobial properties reveals higher amount of polyphenols (3.717 µg/ml) and flavonoids (2.98 µg/ml) compared to the selected fruit juices. Moreover against all the selected microbial strains *Candida albicans, Candida tropicalis, Candida cruzi, Candida parapsolis, E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Proteus mirabilis*, significant activity is observed at both the concentrations 5000µg/ml and 10000µg/ml**.** Even against the multi-drug resistant *E.Coli* (18mm) and *Candida albicans* (13mm) remarkable inhibitory activity is recorded. Against *E.Coli* (18mm), *Candida tropicalis* (15mm) and *Candida parapsolis* (17mm) comparable activity is obtained with that of the control at the lower concentration itself.

INTRODUCTION

Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. Plants are good source of more economically important compounds such as phenolic compounds, nitrogen containing compounds, vitamins and minerals which have anti-oxidant, anti-inflammatory, anti-tumor, anti-mutagenic, anti- carcinogenic and diuretic activities [1,2]. Out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their phytochemical and antimicrobial activities [3-9].

Plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [10].

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According to WHO (World Health organization) 80% of the developed countries, use traditional medicine [11] obtained from medicinal plants. It is cheaper than modern medicine [12].Today, a number of pathogens have developed resistance to multiple antibiotics (Multiple Drug Resistance), due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The limited life span of antibiotics has rendered a necessity to search for antioxidant and antimicrobial potential agents [13-16].

Garcinia mangostana commonly known as mangosteen has been a part of the traditional medicine of various Asian countries for a very long time; Different parts of the fruit and plant are used in the treatment of various diseases and disorders [17-19]. Scientific Research has revealed that Mangosteen which includes its peel, contains a class of naturally occurring polyphenol compounds known as xanthonoids like alpha mangostin and gamma mangostin [20-25]. Xanthones and their derivatives have been shown to have several benefits, including anti-

inflammatory, antioxidant, antitumoral, antiallergy, antibacterial, antifungal, antidiabetic, anticancer, antiplasmodial, and antiviral activities [21,23,26-28]. Also it can boost immunity and some studies have demonstrated that juice containing mangosteen peel extracts may reduce blood levels of C-reactive protein, a biomarker of inflammation. Xanthones are an effective remedy against various cardiovascular diseases and have healing properties which heal cells damaged by free radicals, slow down aging and ward off degenerative diseases and physical and mental deterioration [29].

Facing the increase in public demand for naturally safe foods and products, the present work focuses on antioxidant activity of the mangosteen peel in terms of polyphenols and flavonoids as well as antimicrobial activity of the ethanolic extract of mangosteen peel.

EXPERIMENTAL

Chemicals required

Fruit samples were purchased commercially. Quercetin, gallic acid and Folin-Ciocalteu reagent used were purchased from Sigma Aldrich Pvt.Ltd. All other chemicals sodium carbonate, methanol, ethanol, potassium acetate and aluminum chloride used were of analytical grade.

Extraction from Mangosteen

Peel of mangosteen was collected from the field. The mangosteen peel was air-dried at room temperature for four months. They are powdered using mortar and pestle. The finely powdered peel of mangosteen was extracted from ethanol using soxhlet apparatus by repeating for about 10 cycles. The excess ethanol was drained off by vacuum evaporator. Further the extracts were dried and stored below 15^{o}C.

Fruit juices (black grapes, brown grapes, green grapes, red pomegranate and white pomegranate) were extracted by squeezer, further filtered and the juice as such was used freshly for analysis.

Antioxidant Activity of Mangosteen

Natural sources exhibit antioxidant activity mainly because of its polyphenols and flavonoid content, since they impart effective free radical quenching thereby inhibiting the oxidation chain. Polyphenol and flavonoid content of the ethanolic extract of mangosteen peel was studied based on standard methods and compared with some fruit juices (black grapes, brown grapes, green grapes, red pomegranate and white pomegranate).

Estimation of polyphenols in the extracts

Polyphenols were estimated following the procedure of Mcdonald*et al*. [30]. Exactly 0.5 ml of 1:10 gml^{-1} extract was added to 4 ml sodium carbonate (1M) and 5 ml Folin-Ciocalteu reagent (1:10 dilution with distilled water). The solution was allowed to stand for 15 minutes and absorbance was measured at 750 nm by using Double Beam Spectrophotometer (HITACHI). A calibration plot was drawn using the values obtained in the following concentrations of Gallic acid 125 - 830 µg/ml.

Estimation of flavonoids in the extracts

Total flavonoids were estimated by aluminium chloride colorimetric method following the procedure of Chang *et al*. [31]. Exactly 0.5 ml of 1:10 gml^{-1} extract was added to 4.3 ml methanol. To the above solution 0.1 ml of 10% $AlCl_3$ and 0.1 ml of 1 M potassium acetate were added. Absorbance at 428 nm was measured by using Double Beam Spectrophotometer (HITACHI) after allowing to stand for 30 minutes. A calibration plot was drawn using the values obtained in various concentrations of quercetin 20 - 200 µg/ml.

Antimicrobial Activity of Mangosteen

Tested microorganisms

Antibacterial activity of the ethanolic extract of mangosteen was tested against four different bacterial strains *E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus mirabilis*. Similarly the inhibitory action against four different fungal strains *Candida albicans, Candida tropicalis, Candida cruzi and Candida parapsolis* was also analysed.

Antimicrobial assay

The microbial strains were tested at two different concentrations of the extract (5000µg/ml and 10000µg/ml). About 0.01ml of extract should be poured into Petri dishes on a flat horizontal surface to a depth of 4mm (25 ml in an 85mm circular dish, 60 ml in a 135 mm circular dish). The poured plates were stored at 4^{0}C and used within one week of preparation. Before inoculation, plates should be dried with lipids jar so that there were no droplets of moisture on the agar surface. The pH of the medium should be checked at the time of preparation and should be 7.2 to 7.4.

At least four morphologically similar colonies from an agar medium were touched with a wire loop and the growth was transferred to a test tube containing 0.01 ml of sterile suitable broth. The tubes were incubated for 2 hours at 35 to 37^{0}C to produce a bacterial suspension of moderate turbidity. Plates were inoculated within 15 minutes of preparation of the suspension so that the density does not change. After the inoculums have dried, single discs were applied with forceps, a sharp needle or a dispenser and pressed gently to ensure even contact with the medium. When fastidious organisms were to be tested touch multiple colonies with a loop and cross streak the appropriate plate for uniform distribution. It was repeated for each antimicrobial agents *E.Coli, Klebsiella pneumonia, P.aeruginosa and Proteus mirabilis* to be used, placing the impregnated discs in their respectively labeled segments. After 24 hours, the diameters of the inhibition zones were

measured to the nearest millimeter with vernier calipers (preferably) or a thin transparent millimeter scale. For fungal strains same method was followed but the period of time was 48 hours.

RESULTS AND DISCUSSION

Antioxidant Activity of Mangosteen

Natural sources contain antioxidants like vitamin C, vitamin E, selenium and carotenoids. Recent studies have reported that flavonoids and polyphenols found in fruits and vegetables account for its antioxidant activity. In the present study, the antioxidant nature of mangosteen has been analysed in terms of polyphenols and flavonoids. Polyphenols provide much of the flavor, colour and taste to fruits, vegetables, seeds and other parts of the plants. The concentration was measured by using calibration plot plotted with gallic acid as standard at a concentration range of 125 – 830 µg/ml. The calibration plot obtained was found to be linear (Figure 1) with a very good correlation coefficient of r = 0.89. The respective concentration of polyphenols in the peel with respect to its absorbance value 1080.49 was found to be 3.717 µg/ml, which was relatively higher than all the other selected fruit juices (Table 1).

Flavonoid a powerful antioxidant, have strong anti-inflammatory properties. It has been reported to increase the energy levels, reduce blood pressure and decrease Parkinson's symptoms. Flavonoid concentration recorded (as quercetin equivalent) by using the calibration plot (r = 0.91) with respect to its absorbance value 961.79 (Figure 2) was 2.98 µg/ml, which was also found to be greater than the other selected fruit juices (Table 1).

Antimicrobial Activity of Mangosteen

The antimicrobial activity of the mangosteen peel extract at two different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. Significant inhibitory activity has been recorded at the two different concentrations 5000µg/ml and 10000µg/ml against the 8 tested microorganisms (Figure 3). Lower concentration was found to be sufficient to obtain efficient activity against *E.coli and Pseudomonas aeruginosa* (Table 2). With increase in concentration the activity was found to get reduced. Inhibition diameter for *E.coli* at 5000µg/ml concentration showed a value of 18mm against the control (22mm) whereas at 10000µg/ml concentration the value observed was 11mm. Similarly, against *Pseudomonas aeruginosa*, it was 16mm at 5000µg/ml and 13mm at 10000µg/ml against the control of 28mm. *Proteus mirabilis* gave the same value (15mm) for both the concentrations. The result indicated that 5000µg/ml concentration was enough for obtaining significant activity against all the three bacterial species. But for *Klebsiella pneumonia*, higher concentration was required to obtain its optimum activity. Zone of inhibition diameter was found to be enhancing with increase in extract concentration. The observed zone of inhibition at lower concentration was 13mm whereas for higher concentration it was 17mm against the control of 23mm.

The extract exerted considerable activity against all the fungal species also even against multidrug resistant *Candida albicans*. At 5000µg/ml concentration, the zone of inhibition against *C.albicans* was 10mm and with increase in concentration to 10000µg/ml an increase in its activity was noted with 13mm diameter against the control flucanazole (30mm). Further increase in activity could be expected by advocating higher concentration of the extract.

Pronounced but same activity was exhibited against *Candida tropicalis* at both 5000 and 10000µg/ml concentrations (15mm against the control which showed zone of inhibition of 18mm diameter). Against *Candida cruzi* strain, significant activity (12mm against 30mm for control) was observed but remained constant at both the concentrations whereas for *Candida parapsolis* a decrease in inhibitory action was recorded (17mm to 15mm) with increase in concentration from 5000µg/ml to 10000µg/ml (Table 3). So the concentration requirement could be limited within 5000µg/ml for its optimum activity against the three strains. Higher concentration of polyphenols and flavonoids recorded in the ethanolic extract of mangosteen peel well correlates with its observed antimicrobial activity. The peel has been reported to have naturally occurring polyphenol compounds known as xanthonoids like alpha mangosteen and gamma mangosteen [20]. The bioactive compounds impart pronounced activity against *E.coli* (18mm against 22mm for the control), *Candida tropicalis (15mm against 18mm)* and *Candida parapsolis* (17mm against 20mm) comparable with that of their control even at the lower concentration of the extract (5000µg/ml).

Figure 1. Calibration plot for polyphenol estimation

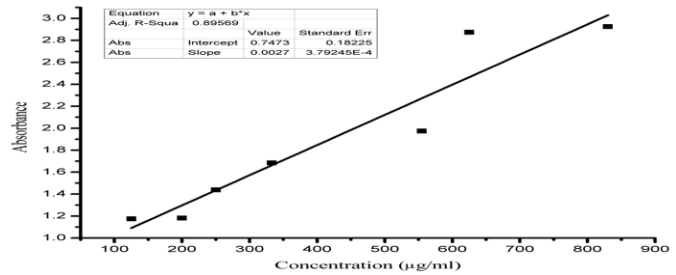

Figure 2. Calibration plot for flavonoid estimation

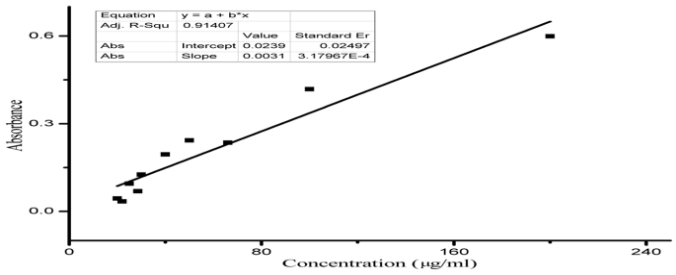

Figure 3. Antimicrobial activity of mangosteen peel extract a. *E.coli, b. Klebsiella pneumonia, c. Pseudomonas aeruginosa, d. Proteus mirabilis* e. *Candida albicans, f. Candida tropicalis, g. Candida cruzi and h. Candida parapsolis*

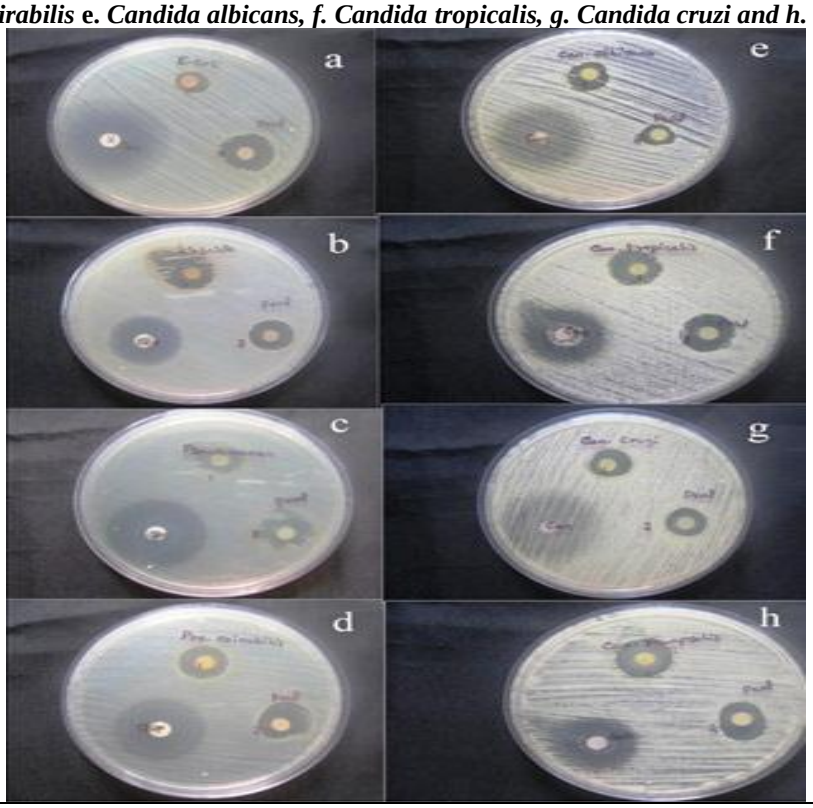

Table 1. Concentration of polyphenols and flavonoids in various fruit samples

Sample	Concentration of polyphenols (μ g/ml)	Concentration of flavonoids (μ g/ml)
Mangosteen peel	3.717	2.98
Black grapes	3.679	2.289
Brown grapes	3.325	0.432
Green grapes	2.542	0.091
Red pomegranate	3.450	1.615
White pomegranate	2.692	1.088

Table 2. Antibacterial activity of the mangosteen peel extract

Bacterial Strains	Zone of Inhibition Diameter		
	5000µg/ml	10000µg/ml	Control (Cefopers/ sulba)
E.coli	18mm	11mm	22mm
Klebsiella pneumonia	13mm	17mm	23mm
Pseudomonas aeruginosa	16mm	13mm	28mm
Proteus mirabilis	15mm	15mm	23mm

Table 3. Antifungal activity of the mangosteen peel extract

Fungal Strains	Zone of Inhibition diameter		
	5000µg/ml	10000µg/ml	Control (Flucanazole)
Candida albicans	10mm	13mm	30mm
Candida tropicalis	15mm	15mm	18mm
Candida cruzi	12mm	12mm	30mm
Candida parapsolis	17mm	15mm	20mm

CONCLUSION

The data shows the inhibition potency of the peel extract over both bacterial as well as fungal species. Significant activity is recorded against all the tested species with almost comparable activity as that of the control against *E.coli*, *Candida parapsolis* and *Candida tropicalis.* Even against the multidrug resistant *Candida albicans*, considerable activity is observed. The extract from mangosteen can be advocated for commercial use against the bacterial as well as the fungal infections.

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