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Antioxidant and anticancer activities of pericarp of *Garcinia mangostana* L.

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Abstract
Natural products play an important role in protection against reactive oxygen species to maintain a state of well-being health, including the prevention of cancer progression. *Garcinia mangostana* has a rich history dates back in its antioxidant activity and cytotoxicity of xanthones on some cancer cells. Food sources have variety of phytonutrients, as antioxidants have health-protecting qualities required for healthy survival. The phytocompounds in herbs to inhibit the rate of cancer growth and they are harmless as well as safe to use. *Garcinia mangostana* fruits are roughly 4-8 cm in size with a thick, brittle, deep purple spherical outer pericarp. The edible snow white endocarp consists of 4 to 8-segmented wedge-shaped arils. *G. mangostana* fruit and its pericarp possess potential medicinal properties in the ancient treatment strategies. The bioactive substances present in it are utilized in the Ayurvedic treatment of chronic infections and other ailments. People in Southeast Asia have used dried mangosteen pericarp as an antiseptic, an analgesic, an anti-inflammatory, anti-parasitic, antipyretic and as a treatment for skin rashes since ancient times. Although the fruit pulp has several utilisations, the present study was to evaluate the antioxidant activity of methanol extract of Mangosteen pericarp and its anticancer activity against breast cancer cells (MCF7).
The antioxidant activity was carried out by DPPH radical and $ABTS^{\bullet+}$ radical cation scavenging assay methods as well as phosphomolybdenum reduction and Fe^{3+} reducing power assay methods. Anticancer activity was done in MCF-7 cancer cell line by MTT assay method. The maximum DPPH radical scavenging activity was 78.58±0.89% at 300 µg/mL concentration The results showed that the methanol extract of pericarp of *G. mangostana*, effectively inhibit free radicals and the antioxidant activity increases with increasing concentration of the extract. The maximum MCF7 cell death by *G. mangostana* pericarp extract was 67.05±1.28% at 500 µg/mL concentration and the cell death increases with increasing concentration of the extract.

Keywords: *Garcinia mangostana*, DPPH, $ABTS^{\bullet+}$, MCF-7, MTT assay

Introduction
Garcinia mangostana or Mangosteen is a tropical tree from the family Clusiaceae. The tree is native to Thailand and cultivated for centuries in Southeast Asia rainforests [1]. Mangosteen is a smooth, conical tree growing up to 10 meters high, outer bark smooth, dark brown, inner bark yellowish, branches nearly horizontal. The leaves are opposite, thick, leathery, 15 to 25 centimeters long, 6 to 11 centimeters wide, lanceolate, base tapering, apex acuminate, upper surface glossy, under surface dull, lighter color. The petioles are short and thick and about 1 centimeter long. The fruits are berry, dark purple, globose, 5 to 7 centimeters in diameter, smooth; rind firm, spongy, thick, resinous; seeds 4 to 8, dark brown, flattened, each surrounded by white or pinkish-white, juicy, sweet, edible pulp. The flowers are 5 centimeters in diameter, 4-parted, bisexual, and borne singly or in pairs at the ends of the branchlets. The seeds are large, flattened- and embedded in snowy-white or pinkish delicious pulp, which is botanically called the aril. Pericarps of the fruit have been used in folk medicine for the treatment of many human illnesses such as skin and wound infections, and inflammatory diseases [2]. In Cambodia, the bark and the rind of the fruit are used for diarrhoea and dysentery as astringents. Xanthones in *G. mangostana* rind had been proven to have strong antioxidant activity [3]. Besides antioxidant activities, it has antitumour, antiallergy, antibacterial, antiviral, and antifungal activities [4]. The pericarp of *G. mangostana* contains the highest level of xanthones and is used in medicine for the treatment of abdominal pain, suppuration, and chronic ulcer.
Medicinal plants have great importance for the production nutraceuticals and medicines due to enriched quality of phytochemicals produced by them as the byproduct of secondary metabolism. Antioxidants play an important role in the body defense system against reactive oxygen species (ROS) as they combine with reactive oxygen species and neutralize their toxic

effect. Reactive oxygen species (ROS) such as hydroxyl radical, superoxide anion and hydrogen peroxide and other exogenous factors are generally the cause of several fatal diseases such as coronary heart disease, stroke, rheumatoid arthritis, diabetes and cancer [5]. Hence, plants possessing antioxidant activity could be a potential lead for curing any of the above ailments.

Fig 1: Fruits of *Garcinia mangostana*

Materials and Methods

Preparation of extract

G. mangostana were purchased from Kodaikanal, Dindigul, Tamil Nadu, India. Then, its Pericarps were removed, cut into small pieces by blender and soaked in methanol for 72 h. Then the supernatant was filtered through filter paper and condensed by using rotary evaporator at 50 °C. Finally brownish-yellow coloured sticky residue was obtained.

Antioxidant activity

DPPH˙ radical scavenging activity

The radical scavenging activity was carried out by the scavenging of stable DPPH (1, 1- diphenyl-2-picrylhydrazyl) free radical assay method [6]. Pericarp extract of *G. mangostana* with different concentrations (50-300 µg/mL) was taken and mixed with 1 mL of DPPH (0.1 mM) solution in methanol and was incubated in dark at room temperature for 30 min. one mL methanol mixed with 1 mL DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm using the UV-Vis Spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of DPPH radical inhibition was calculated using the formula

$$\% \text{ of DPPH radical inhibition} = \left(\frac{\text{Control–Sample}}{\text{Control}} \right) \times 100$$

$ABTS^{\bullet+}$ radical cation scavenging activity

The antioxidant capacity was determined by $ABTS^{\bullet+}$ radical cation scavenging activity method [7]. $ABTS^{\bullet+}$ radical cation was obtained by reacting 7mM of ABTS with 2.45mM of potassium persulfate and the mixture was left to stand for 12-16 h in dark at room temperature before use. The $ABTS^{\bullet+}$ radical cation solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) and brought up to the absorbance of 0.70±0.02 at 734 nm. Various concentrations (5-30 µg/mL) of pericarp extract of *G. mangostana* were mixed with 500 µL of diluted $ABTS^{\bullet+}$ solution. After 5 min, the absorbance was measured at 734 nm. Ascorbic acid was used as the reference standard. The $ABTS^{\bullet+}$ radical-scavenging activity was calculated as:

$$\% \text{ of } ABTS^{\bullet+} \text{ radical cation inhibition} = \left(\frac{\text{Control–Sample}}{\text{Control}} \right) \times 100$$

Phosphomolybdenum reduction activity

The reduction activity was carried out to evaluate the antioxidant capacity of pericarp extract of *G. mangostana* [8]. Different concentrations of pericarp extract (20-120 µg/mL) was blended with 1 mL of reagent solution consisting of ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The mixture was incubated in water bath for 90 minutes at 95°C.The increasing absorbance of the coloured complex was read at 695 nm using UV-Vis spectrophotometer. Ascorbic acid has been used as the standard reference. The percentage of the phosphomolybdenum reduction was calculated using the formula

$$\% \text{ of phosphomolybdenum reduction} = \left(\frac{\text{Sample–Control}}{\text{Sample}} \right) \times 100$$

Ferric (Fe^{3+}) reducing power activity

The reducing power activity was evaluated to find out the reduction capacity of pericarp extract of *G. mangostana* [9]. One mL of varying concentration of pericarp extract (20-120 µg/mL) was combined with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% (w/v) potassium ferricyanide [$K_3Fe(CN)_6$] solution. The reaction mixture was incubated at 50°C for 30 minutes in water bath. Five hundred µL of 10% (w/v) trichloroacetic acid was added to each mixture. Then to this, 100 µL of freshly prepared 0.1% (w/v) $FeCl_3$ solution was added. The increasing absorbance was read at 700 nm using a UV-Vis Spectrophotometer. Ascorbic acid has been used as the standard reference. The percentage of Fe^{3+} reduction was calculated using the formula

$$\% \text{ of } Fe^{3+} \text{ reduction} = \left(\frac{\text{Sample–Control}}{\text{Sample}} \right) \times 100$$

Anticancer activity

Chemicals and reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) Invitrogen, USA. Acridine orange and other fine chemicals were obtained from Sigma, Aldrich, USA.

Cell culture

MCF7 cells were obtained from NCCS (National Centre for Cell Science, Pune) and cultured in Rose-well Park Memorial Institute (RPMI) medium, supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin (250 U/mL), gentamicin (100 µg/mL) and amphotericin B (1mg/mL) obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2. Cells were allowed to grow on colonies for over 24 h before use.

Cell growth inhibition studies by MTT assay

The cell viability was measured by the conventional MTT reduction assay method [10]. MCF7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (1.625–500 µg/mL) of methanol pericarp extract of *G. mangostana* were added and incubated for 48 h. After treatment, the cells

were incubated with MTT (10 µL, 5 mg/mL) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer. The viability of cells was calculated by the following equation

$$\text{Cell viability (\%)} = \left[\frac{\text{Mean OD}}{\text{Control OD}}\right] \times 100$$

Results and Discussion

Antioxidant activity

DPPH radical scavenging assay

The DPPH assay showed that the capacity of methanol extract of pericarp of *G. mangostana* to reduce purple colored 1,1-diphenyl-2-picryl hydrazyl (DPPH) to yellow colored 1,1-diphenyl-2-picryl hydrazine [11]. Decrease in absorbance of the reaction mixture indicates increase in radical scavenging activity. The maximum DPPH radical scavenging activity was 78.58±0.89% at 300 µg/mL concentration and the IC_{50} was 182.05 µg/mL concentration (Table 1; Fig 2). It was compared with the standard ascorbic acid (IC_{50}=11.98 µg/mL concentration). The scavenging activity increases with increasing concentration of the extract. The DPPH free radical reduced to non radical by the hydrogen donating ability of pericarp extract of *G. mangostana*.

Table 1: DPPH radical scavenging activity of methanol extract of pericarp of *G. mangostana*

S. No	Concentration (µg/mL)	% of inhibition
1	50	25.95±0.28
2	100	35.32±0.79
3	150	43.97±0.36
4	200	54.93±1.04
5	250	64.03±0.14
6	300	78.58±0.89

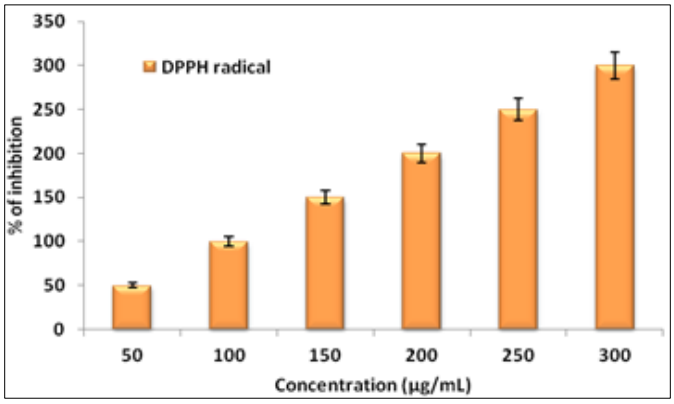

Fig 2: DPPH radical scavenging activity of methanol extract of pericarp of *G. mangostana*

ABTS$^{\bullet+}$ radical cation scavenging activity

ABTS$^{\bullet+}$ radical cation is produced by the reaction between ABTS and potassium persulfate and has absorption at 734 nm. The addition of antioxidants reduces the pre-formed blue-green coloured ABTS$^{\bullet+}$ radical cation to ABTS, depending on the antioxidant activity and the concentration of the antioxidant present in the extract. Antioxidant compounds quench the ABTS$^{\bullet+}$ radical cation and changed it to colourless, which is proportional to amount of antioxidants [12]. The maximum ABTS$^{\bullet+}$ radical cation scavenging activity was 86.58±1.90% at 30 µg/mL concentration and the IC_{50} was 10.52 µg/mL concentration (Table 2; Fig 3). It was compared with the standard ascorbic acid (IC_{50} = 4.21 µg/mL concentration).

Table 2: ABTS$^{\bullet+}$ radical cation scavenging activity of methanol extract of pericarp of *G. mangostana*

S. No	Concentration (µg/mL)	% of inhibition
1	5	26.46±1.96
2	10	47.52±0.78
3	15	56.97±0.20
4	20	72.58±0.64
5	25	75.80±0.57
6	30	86.58±1.90

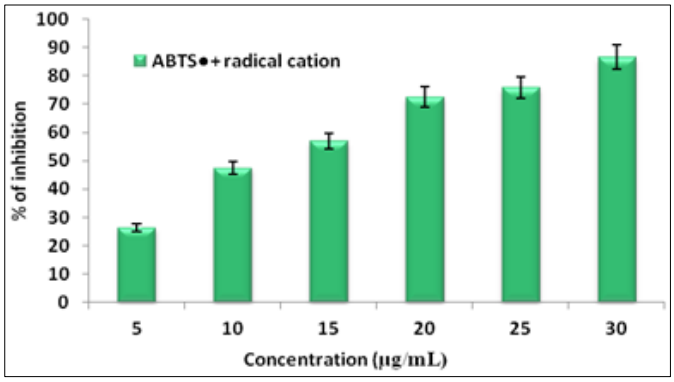

Fig 3: ABTS$^{\bullet+}$ radical cation scavenging activity of methanol extract of pericarp of *G. mangostana*

Phosphomolybdenum reduction assay

The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of bluish green phosphate/Mo(V) complex with the maximum absorption at 695 nm [13]. This assay was performed for plant polyphenols have the capacity to reduce the transition metal ions and these ions are involved in Fenton reaction. The maximum phosphomolybdenum reduction was 66.39±0.38% at 120 µg/mL concentration and the RC_{50} was 43.08 µg/mL concentration (Table 3; Fig 4). It was compared with the standard ascorbic acid (RC_{50} = 6.34 µg/mL concentration). The result obtained was confirmed the methanol extract of pericarp of *G. mangostana* has significant reduction of transition metal ions. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes and act as primary and secondary antioxidants.

Table 3: Phosphomolybdenum reduction activity of methanol extract of pericarp of *G. mangostana*

S. No	Concentration (µg/mL)	% of reduction
1	20	35.88±0.83
2	40	46.43±1.67
3	60	55.66±0.47
4	80	60.23±0.67
5	100	61.87±3.73
6	120	66.39±0.38

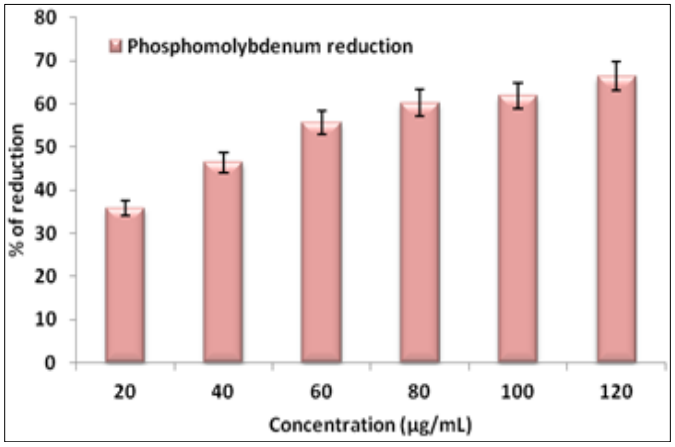

Fig 4: Phosphomolybdenum reduction activity of methanol extract of pericarp of *G. mangostana*

Ferric (Fe^{3+}) reducing power assay

Ferric ion (Fe^{3+}) reducing power activity determines the electron donating ability of an antioxidant. The reducing power of methanol pericarp extract of *G. mangostana* from Fe^{3+} to Fe^{2+} was measured and the reduction ability increases with increasing concentration of the extract (Table 4; Fig 5) due to the formation of ferro-ferric complex [14]. The maximum Fe^{3+} reduction was 87.69±0.32% at 120 µg/mL concentration and the RC_{50} was 21.20 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50}= 7.72 µg/mL concentration). The reducing properties are generally associated with the presence of reductones, such as flavonoids and phenolic compounds, which have been shown to exert an antioxidant action by breaking down the free radical chain by donating electrons. Reductones are directly reacting with peroxides and with certain precursors of peroxides, thus preventing peroxide formation. In this assay, the yellow colour of the test solution changes to various shades of green and blue green colour depending on the reducing power of the test sample.

Table 4: Fe^{3+} reducing power activity of methanol extract of pericarp of *G. mangostana*

S. No	Concentration (µg/mL)	% of reduction
1	20	47.17±0.55
2	40	68.92±0.77
3	60	77.54±0.59
4	80	82.06±1.08
5	100	86.05±1.03
6	120	87.69±0.32

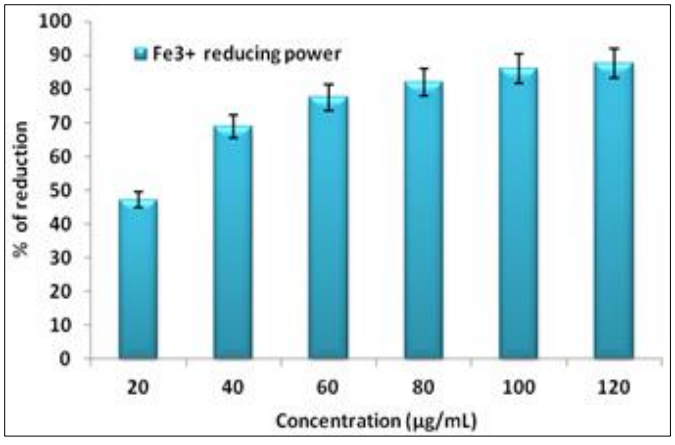

Fig 5: Fe^{3+} reducing power activity of methanol extract of pericarp of *G. mangostana*

Anticancer activity

The viability of MCF7 cells decreases with increase in concentration of the pericarp extract. The MTT assay was carried out by varying drug concentration, time of exposure to drug, length of assay, and cell density. The antiproliferative activity of methanol pericarp extract of *G. mangostana* was carried out by MTT assay method. Only viable cells have the ability to reduce MTT tetrazolium into a coloured formazan product. The *G. mangostana* pericarp extract showed the maximum MCF7 cell death was 67.05±1.28% at 500 µg/mL concentration and the IC_{50} was 44.99 µg/ mL concentration (Table 5; Fig 7). When the concentration of the extract is increased, it was observed that there was rapid decrease in cell-cell contact and cell proliferation [15]. The polyphenols present in the pericarp extract of *G. mangostana* inhibited the proliferation of MCF7 cells by increasing the rate of cell death, which was observed by the morphological changes of cells (Fig 6) and it was in a dose dependent manner.

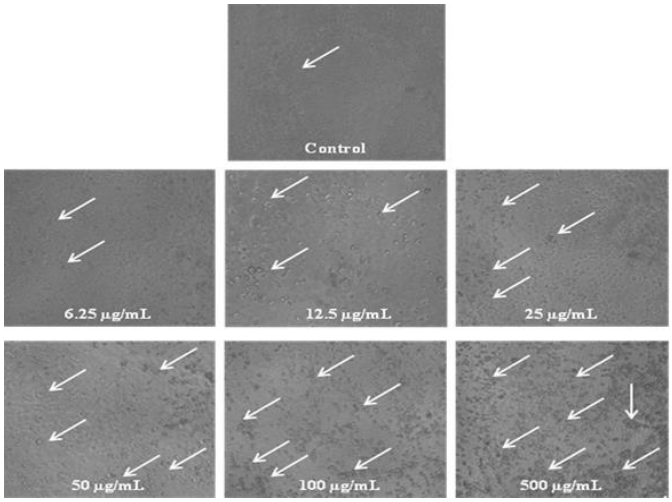

Fig 6: Morphological observation of MCF7 cell treated with methanol extract of pericarp of *G. mangostana*

Table 5: Anticancer activity of methanol extract of *G. mangostana* pericarp on MCF7 cell line

S. No	Concentration (µg/mL)	% of cell death
1	1.625	09.19±1.23
2	3.125	24.31±2.45
3	6.25	32.67±3.90
4	12.5	33.87±2.44
5	25	43.46±1.12
6	50	55.57±1.11
7	100	64.46±1.11
8	500	67.05±1.28

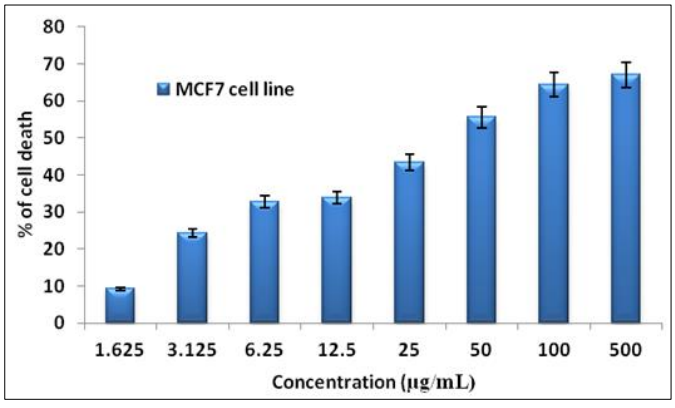

Fig 7: Anticancer activity of methanol extract of pericarp of *G. mangostana* on MCF7 cell line

Conclusion

Therefore, in this study methanol pericarp extract of *G. mangostana* was evaluated as an antioxidant by radical scavenging assay and anticancer agent by MTT assay methods. Phenolic compounds as well as flavonoids are well known as an antioxidant and an anticancer agent due to their benefits for human health, curing and preventing many diseases. Plants have been used in folk and traditional medicines and are accepted as leads for therapeutic drug development in modern medicine. The persistency search for new anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention. The antioxidant results showed that pericarp extract of *G. mangostana* have beneficial effects on human health, notably in relation to their antioxidant activity. The anticancer activity of pericarp extract in MCF7 cell showed the clear morphology changes due to the formation of apoptotic cell death. This property is due to the presence of polyphenolic compounds have the ability to scavenge free radicals originating from different oxygen and nitrogen species (ROS/RNS) and induce apoptosis in cancer cell.

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