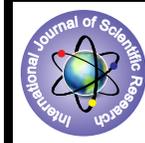


Radical Scavenging Activity and Invitro Anticarcinogenic Potential of Dried and Powdered Mangosteen (Garcinia Mangostana) Pericarp



Home Science

KEYWORDS :antioxidant, radical scavenging activity, phytochemicals, anticarcinogenic, free radicals

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ABSTRACT

Mangosteen is an emerging category of novel functional foods that has strong antioxidant strength that has the ability to reduce diseases. The pericarp of mangosteen, which is regarded as a waste product is rich in antioxidants. The volatile bioactive compounds in the pericarp were identified using Gas Chromatography-Mass Spectrometry(GC-MS). Certain phytochemicals having antioxidant and anticarcinogenic effects in the pericarp of mangosteen were identified and quantified. The antioxidant potential of methanolic extract of dried and powdered mangosteen pericarp was determined by DPPH (1,1 diphenyl- 2-picryl hydrazyl) assay. It was found that the ability of mangosteen pericarp to scavenge DPPH radical, a stable free radical was comparable to that of Vitamin C, which is a potent antioxidant. The damage to cells caused by free radicals, especially to DNA play an important role in the development of cancers and other health conditions. The antioxidants in the pericarp of mangosteen have the potential to prevent free radical damage

INTRODUCTION

Several researchers have found that the pericarp of mangosteen is rich in certain antioxidants that makes it an effective natural agent in curing various ailments. The main function of antioxidants is to prevent free radical damage to tissues by preventing their formation, scavenging and promoting their disintegration (Young & Woodside, 2001). Many studies have proven that plant based antioxidants play an important role in reducing the risk for chronic diseases including cancer and heart disease.

Phytochemicals are naturally occurring biochemical substances in plants that help to give plants their characteristic colour, flavour, smell and texture. Phytochemicals are antioxidants and the study of it will continue to be a promising and active research area in the near future (Boyer & Liu, 2004). This study aimed at assessing the antioxidant and anticarcinogenic potential of mangosteen pericarp. Table 1 shows the different phytochemicals present in the pericarp of mangosteen.

Table 1
Phytochemicals in mangosteen pericarp

PHYTOCHEMICALS	SUPPORTING LITERATURE
Triterpenoids	Among the terpenoids, triterpenoids have recently emerged as a unique group of phytochemicals with multifunctional anticancer activities as demonstrated by promising results in preclinical studies (Bishayee et al., 2011).
Phenols	Phenols are phytochemical compounds that function in nutrient uptake (Maobe et al., 2012)
Tannins	Tannins are one of the major phytochemicals found in many higher plants (Ukoha et al., 2011)
Saponins	Saponins are phytochemicals with emerging potential for curing clinical depression (Abbas , Rauf & Mahmood , 2015)
Flavonoids	Flavonoids are phytochemical compounds that provide protection against ultraviolet radiation, pathogens and herbivores (Moabe et al., 2012)
Anthocyanin	Anthocyanins are water soluble, flavonoid pigments responsible for attractive pigments in fruits and vegetables and flavonoids are the most abundant polyphenols (Joshi & Goyal, 2011)
Xanthones	Mangosteen has been noted to be an abundant source of a class of polyphenols known as xanthones (Akao et al., 2008).

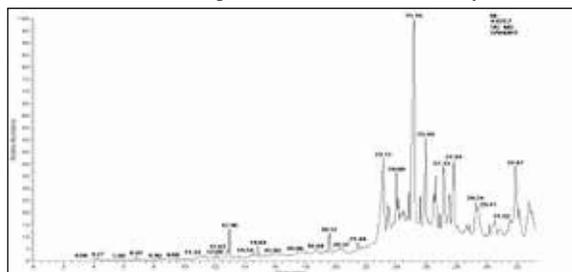
Objectives of the study

- 1) To analyze the presence of bioactive components present in dried and powdered mangosteen pericarp using the Gas Chromatography Mass Spectrometry (GCMS) method.
- 2) To quantify specific phytochemicals in the dried and powdered mangosteen pericarp such as terpenoids, phenols, tannins, saponins and flavonoids
- 3) To study the Radical Scavenging Activity of dried and powdered mangosteen pericarp using DPPH assay.
- 4) To study the anticarcinogenic potential of mangosteen pericarp powder against MCF-7breast cancer cell line using the MTT Assay

RESULTS OF THE STUDY

Volatile bioactive constituents identified in mangosteen pericarp using GC-MS based on the polarity

Gas Chromatography coupled with Mass Spectrometry (GC-MS) gives a clear insight of the bioactive constituents present in the sample (Doshi et al., 2015). In this study, the GC-MS technique was used to analyse the bioactive constituents in dried and powdered mangosteen pericarp. The spectrum of the peaks were visualized when the compounds of a mixture were mainly separated according to their polarities. The interpretation of GC-MS was elucidated using National Institute Standard and Technique (NIST) database. The particular compounds present in the sample were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database library (NIST-MS, 1998). The spectrum of the unknown components was compared with the spectrum of the known components stored in NIST library.



GC-MS based on the polarity

The various bioactive volatile constituents in the crude mangosteen pericarp sample separated by the gas chromatography technique showed peaks at different locations on the chromatogram. In figure 1, the x axis represents the retention time and the y-axis represents relative abundance. Retention time of the compound is the period following injection that is required for a compound to pass through the column. The area under a gas chromatograph peak is proportional to the amount of compound eluted. Eleven bioactive volatile components were identified in the crude mangosteen pericarp powder. The compound name, molecular formula, molecular mass and CAS number are presented in the table 2. A CAS Registry Number (often referred to as CAS RNs or CAS Numbers) is usually used to provide a unique, unmistakable identifier for chemical substances. Table 2 presents the compounds that were identified by comparing the spectrum of the unknown components with that of the spectrum of the known components stored in NIST library.

Table 2
Compounds identified in the crude sample (dried and powdered mangosteen pericarp) through Gas Chromatography- Mass Spectrometry

Serial No.	Peak	Compound name	Formula	Molecular Mass	CAS no.	Antibacterial/ antioxidant / anti-carcinogenic activity
1	12.96	Benzenamine	C14H23N	205	613-29-6	Nil
2	14.84	4-ethoxymethyl-6-ethyl-2-hydroxy-nicotinitrile	C11H14N2O2	206	613-29-6	Nil
3	19.57	3-aminobutyric acid	C14H27NO4	273		Nil
4	23.12	2-Benzothiazolamine	C8H8N2O5	180	5464-79-9	Nil
5	24	4,25 Secoobscurinervan-4-ol	C25H32N2O5	440	54658-05-8	Nil
6	25.16	13,27 cycloursan 3-ol acetate	C32H52O2	468	100324-79-6	Antibacterial, antioxidant and anti-carcinogenic activity
7	25.94	4-(3-Buten-1-ynyl)2-methyldecahydro-4quinilol	C14H21NO	219	38569-29-8	Nil
8	27.13	13,27 cycloursan 3-ol acetate	C32H52O2	468	100324-79-6	Antibacterial, antioxidant and anti-carcinogenic activity
9	27.81	12-Azabicyclo(9.2.2)pentadeca-1(14),11(5)-dien-13-one	C14H21NO	219		Nil
10	29.24	Pyrrrolidine	C18H32BN	273		Nil

11	31.87	14-Norursane-3,12-diol	C29H50O2	430		Antibacterial, antioxidant and anti-carcinogenic activity
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According to table 2, the predominant volatile bioactive components in crude mangosteen pericarp are: Benzenamine ; 4-ethoxymethyl-6-ethyl-2-hydroxy-nicotinitrile; 3-aminobutyric acid; 2-Benzothiazolamine; 4,25 Secoobscurinervan-4-ol ; 1 ; 4-(3-Buten-1-ynyl)2-methyldecahydro-4quinilol;13,27 cycloursan 3-ol acetate ; 12-Azabicyclo(9.2.2)pentadeca-1(14),11(5)-dien-13-one ; Pyrrolidine and 14-Norursane-3,12-diol. At 25.16 retention time, the highest peak was obtained.

Two compounds that peaked (13, 27 cycloursan 3-ol acetate - molecular mass 468; 14-Norursane-3, 12-diol - molecular mass 430) were identified to be triterpenoids (refer Figure 1 & Table 2). Amongst these, the highest peak in the sample was found to be 13, 27 cycloursan 3-ol acetate that possesses antibacterial, antioxidant and anti-carcinogenic properties. This finding proves that triterpenoids were predominantly present in the sample. To elucidate the exact biochemical constituents, further isolation and characterization is required. In a study done by Walker EB (2007), the quantitative determination of the selected xanthenes were done using the HPLC-UV method in which xanthenes were identified in the retention time of 4-16 minutes. In the present study, small peaks were obtained in the retention time of 4-16 minutes. This indicates that xanthenes were present in low quantities in this sample.

B. Quantification of phytochemicals in mangosteen pericarp powder

(i) Terpenoids

Plant terpenoids play a role in many traditional herbal remedies and are under examination for antibacterial, antineoplastic and other pharmaceutical functions (Yadav M et al., 2014). In this study, the total terpenoids in mangosteen pericarp powder was estimated to be 0.39 mg/g.

(ii) Phenols

Polyphenols are micronutrients in our diet that are useful in the prevention of degenerative diseases such as cancer and cardiovascular diseases (Manach et al., 2004). In the present study, the total phenol content was estimated to be 272.95 mg/g GAE (Gallic Acid Equivalent).

(iii) Tannins

Tannins are compounds of high molecular weight that are divided into two classes: hydrolyzable and condensed tannins. In many studies, tannin extracts of mangosteen pericarp demonstrated various biological activities including antibacterial, antioxidant, anti-cancer and anti-inflammatory. Nutritionally, the rind portion is expected to contain 5.5 per cent tannins (Nadkarni & Nadkarni, 1999). The total tannins in this study was found to be 0.03 mg TAE/g (TAE-Tannic Acid Equivalent).

(iv) Saponins

Although tannins and saponins are antinutritional factors, they are also responsible for the antioxidant and antimicrobial activities (Yikal T, 2015). In this study, mangosteen pericarp powder was found to contain 0.18 g/g of the sample.

(v) Flavonoids

Flavonoids are the most diverse group of phytochemicals.

Studies have revealed a significant inverse association between flavonoid intake and myocardial infarction (Hertog, 1993). In this study, the total flavonoids were found to be 483.92 µg QE/g (QE- Quercetin Equivalent).

The phytochemicals in mangosteen pericarp powder was quantified and is represented in table 3.

Table 3
Quantification of phytochemicals in mangosteen pericarp powder

Name of the phytochemical	Quantity present
Phenols	272.95 mg/g gallic acid equivalent.
Terpenoids	0.39mg/g
Tannins	0.03 mg TAE/g (TAE-Tannic Acid Equivalent)
Saponins	0.18g /g sample
Flavonoids	483.92µg QE/g (Quercetin Equivalent)

C. Estimation of Radical Scavenging Activity (antioxidant potential) of dried and powdered mangosteen pericarp using DPPH assay

The DPPH assay is based on the reduction of DPPH, a stable free radical. When the antioxidants react with the DPPH radical, it becomes paired off in the presence of a hydrogen donor (eg, free radical scavenging antioxidant) and is converted to the reduced form. This results in decolourisation of the yellow colour with respect to the number of electrons captured (Rajesh, 2011). The absorbance of the solution is measured spectrophotometrically at 517 nm. The ability of the test sample to scavenge DPPH radical is calculated by the following equation:

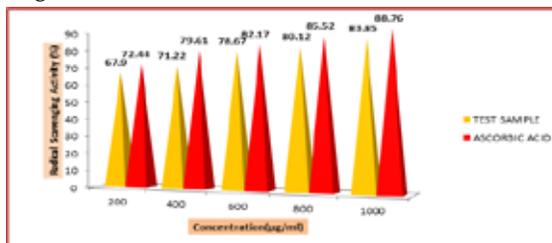
Absorbance of control represents the absorbance of DPPH radical and methanol; Absorbance of sample represents the absorbance of DPPH radical and test sample. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug (Tailor & Goyal, 2014).

The radical scavenging activity of mangosteen pericarp powder at varying concentration in comparison to Vitamin C standard is presented in table 4 and figure 2.

Table 4
Radical Scavenging Activity of dried and powdered mangosteen by DPPH Assay

Sl.No	Concentration(µg/ml)	Percentage inhibition	
		Test sample (mangosteen pericarp powder)	Ascorbic acid (standard)
1	200	67.9	72.44
2	400	71.22	79.61
3	600	78.67	82.17
4	800	80.12	85.52
5	1000	83.85	88.76

Figure 2



Radical scavenging activity of dried and powdered mangosteen pericarp (test sample) in comparison to standard ascorbic acid

From table 4 and figure 2, it is clear that the radical scavenging activity increases with increasing concentration of the test sample. The percentage inhibition of the test sample at 200, 400, 600, 800 and 1000 µg/ml was around 67, 71.22, 78.67, 80.12 and 83.85 per cent respectively. A significant observation made was that the percentage inhibition of methanolic extract of mangosteen pericarp was comparable to the percentage inhibition of the reference standard (ascorbic acid) at all concentrations.

IC50 (Inhibition Concentration 50) is the antioxidant concentration (µg/ml) which is able to inhibit 50 per cent of the activity of free radicals. In this study, the IC50 value is less than 200 µg/ml. Therefore it can be concluded that mangosteen pericarp has excellent antioxidant potential.

D. Anticancer potential of mangosteen pericarp against breast cancer

The anticarcinogenic potential of different concentrations of mangosteen pericarp powder on MCF-7 breast cancer cell line was studied using the MTT assay. The inhibition of cancer cell proliferation was found to be dose dependent. When cells were treated with various concentrations of mangosteen pericarp powder (20, 40, 80, 100 and 200µM) for 24 hours, a significant concentration-dependent growth inhibition of MCF-7 cells was detected (Table 5) .

Table 5
Per cent growth inhibition and cell viability at various concentrations of mangosteen pericarp powder

Concentration(µM)	Per cent inhibition	Per cent cell viability
Positive Control	0	0
Negative control (DMSO)	6.35	94.0
20	10.14	89.86
40	35.92	64.08
60	54.18	45.82
80	63.72	36.28
100	78.16	21.84
200	90.10	9.90

The percentage of breast cancer cell growth inhibition was directly proportional to the concentration of sample (mangosteen pericarp powder) and cell viability was inversely proportional to concentration of sample, that is, as the concentration of the sample increased, the cell viability decreased.

CONCLUSION OF THE STUDY

The findings of the present study lead to the conclusion

that the antioxidant and anticarcinogenic properties of mangosteen pericarp could be attributed to the synergistic effect of phytochemicals such as terpenoids, phenols, tannins, saponins, flavonoids, anthocyanins and xanthenes present in them. Because of the antioxidants present in the pericarp of mangosteen, various types of cancers can be prevented and controlled, thereby improving the quality of life of the cancer patients. Therefore the pericarp should never be wasted but should be utilized for promoting health because in its natural form it could be the best medicine that does not have any side effects when compared to the chemical drugs.

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