PHYTOCHEMICAL PROFILING OF MANGOSTEEN FRUIT, 
GARCINIA MANGOSTANA

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ABSTRACT

The present study deals with phytochemical profiling of epicarp and endocarp extracts of mangosteen fruit, *Garcinia mangostana*. Fresh fruits were procured from market, identified and authenticated and methanol, chloroform, hexane and ethyl acetate extracts were prepared for both epicarp and endocarp of the fruit. The extracts were standardized against hepatocellular carcinoma (HeP-G2) cells and the extracts (Chloroform extract of epicarp and hexane extract of endocarp) showing best anti-proliferative activity was selected for qualitative and quantitative phytochemical screening. Qualitative analysis revealed the presence of phenols, flavonoids and triterpenoids in both the extracts, while the quantitative phytochemical analysis of HPLC and GC revealed that phenol content was higher in chloroform extract of epicarp than hexane extract of endocarp and flavonoids and triterpenoid content were higher in hexane extract of endocarp. The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. Likewise, GC-MS spectral analysis showed 160 aromatic compounds in epicarp and 105 compounds in endocarp. The results are discussed in the light of previous literature.

KEY WORDS: *Garcinia mangostana*, phytochemical profiling, epicarp and endocarp.

INTRODUCTION

Renewed scientific interest in herbs and herbal products for health care has started in the last two decades. This shift from synthetic chemical agents to plant-based products is primarily due to more frequent untoward effects seen with the former treatment.¹ Medicinal plant derived drug research has made a significant progress in anticancer therapies. Nature has
bestowed our country with an enormous number of medicinal plants and therefore India has often referred to as the medicinal garden of the world. In the armory of modern medicine, the components of synthetic drugs or the medicinally accepted plants are evaluated for their efficacy against certain diseases thus forming a valuable source of therapeutic agents.\(^2\)\(^{25}\)

Many components of medicinal plants or dietary plants have been identified as possessing potential chemo-preventive properties capable of inhibiting, retarding or reversing the multistage process.\(^26\)\(^{28}\) The important advantages claimed for therapeutic use of medicinal plants in various ailments due to their safety besides being economical, effective and their easy availability.\(^29\)\(^{31}\) Therefore, scientific validation of such medicinal plants is needed in order to find out their possible use in cancer prevention as opined by Krishnaveni and Mirunalini (2011).\(^1\) Of the various types of cancers, Hepatocellular Carcinoma (HCC) is the most frequent cause of all liver cancers and constitutes 90% of cancers of liver globally as stated by EASL-EORTC (2012).\(^42\) The authors also added that approximately 7.5 lakhs of new cases of HCC per year occurs globally which makes HCC as the 5th common cause of cancers effecting human. The mortality in HCC is very high; death due to HCC is about 7 lakhs annually and has been estimated to be 3rd common cause of death due to cancers effecting human.\(^32\)\(^{42}\)

The treatment for HCC depends on the site and stage of the disease, surgical methods, radiotherapy, chemotherapy and hormonal therapy etc. However, side effects may occur during this treatment such as fatigue, diminished appetite, diarrhea, destroying of bone marrow cells, nausea, vomiting, hair loss, weakness, skin rashes etc. Hence these can be avoided when the treatment is done by chemo preventive methods especially by the extracts of plant parts such as fruits, leaves, barks, vegetables, seeds etc., which are rich in antioxidants, whose chemical components like vitamins such as (C, A, E, carotenoids) flavonoids such as (flavones, flavonones, catechins and anthocyanins) and polyphenols such as (ellagic acid, gallic acid and tannins) and xanthones possess remarkable antioxidant activity that help in lowering the incidence of diseases such as cancer, arthritis, arteriosclerosis, heart diseases, inflammation, brain dysfunction and ageing process. They help our body to kill bacteria and fight free radicals that damage healthy cells and DNA. Numerous investigations have indicated that free radicals cause oxidative damage to lipids, proteins, and nucleic acids.\(^43\)\(^{48}\)
Hence search for an effective chemo preventive agent has led to the identification of various naturally occurring compounds like xanthones from mangosteen (Garcinia mangostana L. Clusiaceae) fruit which is known to possess number of pharmacologic properties such as antioxidant, antitumor, antiallergic, anti-inflammatory, antibacterial, neuroprotective, antifungal, and antiviral activities.\[^{49-72}\]

The purple mangosteen (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. It grows mainly in Southeast Asia, and also in tropical countries such as Colombia, Sri Lanka, in the state of Kerala in India and in Puerto Rico and Hawaii\[^{73-78}\], where the tree has been introduced. Highly valued for its juicy, delicate texture and slightly sweet and sour flavour, mangosteen has been cultivated in Java, Sumatra, Mainland Southeast Asia, and the southern Philippines since ancient times. The 15\(^{\text{th}}\) century Chinese record Yingyai Shenglan described mangosteen as mang-chi-shih (derived from Japanese manggis), a native plant of Java of white flesh with delectable sweet and sour taste.\[^{79-80}\]

The major bioactive compounds found in mangosteens are phenolic acids.\[^{81-87}\] Ten phenolic acids were identified in mangosteen fruit and of these, protocatechuic acid was the major phenolic acid in the peel and rind, while p-hydroxybenzoic acid was the predominant phenolic acid in the aril as reported by Rice-Evans \textit{et al.} (1996, 1997)\[^{88-89}\], Lodovici \textit{et al.} (2001)\[^{90}\], Robbins (2003)\[^{91}\] and Zadernowski \textit{et al.} (2007).\[^{92}\] Mangosteen peel contains xanthonoids, such as mangostin, and other phytochemicals.\[^{93-108}\]

Moreover, screening of phytochemicals present in a medicinal plant or fruit will throw more light on the beneficial effects of it and the means by which it controls or cures a disease.

In view of this, it was thought that it would be worthwhile to explore the qualitative and quantitative phytochemicals present in epicarp and endocarp of mangosteen, \textit{Garcinia mangostana} by various methods.

**MATERIALS AND METHODS**

\textbf{Collection and Identification of Fruit:} Fresh Mangosteen fruits \textit{Garcinia mangostana} were purchased from Hosur fruit market, Tamil Nadu, India, and were authentically identified by Prof. P. Jayaraman, Institute of Herbal Science, Plant Anatomy Research Centre, West
Preparation of Fruit Extracts
Fresh fruits were washed and the epicarp and endocarp regions were separated. Then they were shade-dried up to fifteen days and powdered by maceration method. The dry powders (50 g each) of epicarp and endocarp were extracted with methanol, chloroform, hexane and ethyl acetate. Dried powder was soaked separately at room temperature (1:5 w/v) for 72 h. The extract was filtered using Whatmann filter paper and the filtrate was concentrated at 45 to 55°C under reducing pressure using vacuum rotary evaporator. The yield of extracts was quantified and concentrated crude extracts were further subjected to biological activity.

Human Hepatocellular Carcinoma (HepG2) Cell Line
Human Hepatocellular Carcinoma (HepG2) cell line used for the present study was procured from National Centre for Cell Science (NCCS), Pune, India.

Standardization of Crude Extracts by MTT Assay
Standardization of crude extracts against human hepatocellular carcinoma (HepG2) cell line was done by cell viability assay or MTT assay as described by Mosmann (1983). The morphological changes of crude extract treated HepG2 cell lines were assessed by using light microscopy. The IC\textsubscript{50} concentration of various epicarp and endocarp extracts was determined; the values being 50 µM concentration of chloroform extract of epicarp and 5.25 µM concentration of hexane extract of epicarp of \textit{Garcinia mangostana}. Based on the above observations, chloroform extract of epicarp and hexane extract of endocarp were selected for further study.

Qualitative Phytochemical Analysis
Chloroform extract of epicarp and hexane extract of endocarp of \textit{Garcinia mangostana} were subjected to preliminary phytochemical screening for its phytoconstituents according to Kokate (1988) method.

Quantitative Phytochemical Analysis
Qualitative phytochemical analysis revealed that only flavonoids, phenols and triterpenoids were present in both epicarp and endocarp. So, quantitative estimation was carried out only
for these three of phytochemicals. Flavonoids and phenols were analyzed by HPLC (Emil-Perkin, USA) and triterpenoids were estimated by GC (Thermo Scientific).

**GC-MS Spectral Analysis**

GC-MS spectral analysis was carried out to determine the presence of aromatic compounds in the extracts. The model of the GC-MS used for mass spectral identification was an Agilent 7890 interfaced to a 240 mass selective detector with ion trap.

**Identification of Components in GC-MS**

Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

**RESULTS**

**Yield of Extracts**

The yield of epicarp extracts of *Garcinia mangostana* was maximum in methanol (3.5%), followed by chloroform (1.5%), hexane (1%) and ethyl acetate (1%). On the other hand, the endocarp extracts showed maximum yield in methanol (1.5%), followed by ethyl acetate (1.5%), chloroform (1%) and hexane (1%). The color of extracts was dark brown; the consistency was paste (Table 1).

**Cell Viability Assay (MTT Assay)**

The MTT results showed a profound loss of cell viability in chloroform extract of epicarp at the dose of 100 µM about 25% viability loss (Fig. 1). In addition, dose calculation revealed that 50 µM chloroform extract of epicarp killed 50% HepG-2 cells at 48 h. The other epicarp extracts such as ethyl acetate, hexane and methanol did not show significant effect against HepG-2 cells. On the other hand, the cell viability loss was significantly higher in hexane extract of endocarp. Interestingly, the MTT results showed that below 50% cell viability loss at the dose of 10µM in 48 h. The 10 µM hexane extract of endocarp exactly reduced cell viability at 34% against HeP-G2 cells and that of 5.25 µM hexane extract of endocarp reduced the cell viability at 50% against HeP-G2 cells (Fig. 2). The other endocarp extracts such as chloroform, ethyl acetate, and methanol did not show significant effect against HepG-2 cells.
The MTT results showed chloroform extract of epicarp at 50 µM had 50% viability loss against HepG-2 cells. Furthermore, hexane extract of endocarp at 5.25 µM had 50% viability loss against HepG-2 cells. Based on the above results, 5 µM, 25 µM and 50 µM concentrations were selected for further experiments. When these two extracts were compared, only hexane extract exhibited profound effect on HepG-2 cells when tested by MTT assay (Fig. 3). Treatment of HepG-2 cells with these two extracts even at low doses induced morphological changes in the HepG-2 cells, which had similar effect on cells morphology. It was observed that most of the cells became round in shape and were not attached to substratum after treatment with the extracts, which was in dose-dependent manner (Plate 1). From the above results we can assume that these extracts might have affected cell cycle and induced apoptosis pathways.

**Qualitative Phytochemical Analysis**

The phytochemical characteristics of *Garcinia mangostana* chloroform extract of epicarp and hexane extract of endocarp revealed the presence of phenols, flavonoids and triterpenoids in both the extracts were summarized in Table 2. Carbohydrates, proteins, tannins, steriods, saponins and cardiac glycosides were absent in both the extracts.

**Quantitative Phytochemical Analysis**

The quantitative phytochemical analysis of HPLC and GC revealed that phenol content was higher in chloroform extract of epicarp than hexane extract of endocarp. In contrary, flavonoids and triterpenoids content were higher in hexane extract of endocarp. The results are given in the Table 3 and the HPLC peaks are shown in the Fig. 4A, B, C and D.

**FT-IR Spectral Analysis**

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FT-IR peak values and functional groups were presented in Table 4 and Fig. 5, Table 5 and Fig. 6 and Table 5 and Fig 7 and, Table 7 and Fig. 8. The chloroform extract of epicarp revealed the presence of alkyl halides, phenols, alcohols, carboxylic acids, amines, amides, acyl chlorides, alkynes, ketones, aromatic rings, nitro compounds and esters. Likewise, hexane extract of endocarp also showed the presence of alkyl halides, phenols, alcohols, aromatic rings, carboxylic acids, nitro compounds, free hydroxyl groups, amines, amides and ethers.
GC-MS Spectral Analysis
The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*. The active principles with their retention time (RT) as shown in the mass spectras with the respective library match and the chlorophyll extract of epicarp revealed 160 aromatic compounds with their respective RT, peak name and peak area and hexane extract of endocarp also showed 105 compounds with their respective RT, peak name and peak area and the phytoconstituents were matched with NIST/NBS spectral database. The mass spectra and their mass peaks are given in Fig. 9 and Fig. 10 for epicarp and endocarp, respectively.

Table 1: Yield of various solvent extracts of epicarp and endocarp of *Garcinia mangostana*

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Epicarp</th>
<th>Endocarp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yield (%)</td>
<td>Colour</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.5</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.5</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Hexane</td>
<td>1</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1</td>
<td>Dark brown</td>
</tr>
</tbody>
</table>

Fig. 1: Cell viability of various epicarp extracts of *Garcinia mangostana* against HepG2 cells
Fig. 2: Cell viability of various endocarp extracts of *Garcinia mangostana* against HepG2 cells

Fig. 3: Comparison of cell viability of best epicarp and endocarp extracts of *Garcinia mangostana* against HepG2 cells

Plate 1: Cell morphology of Hep-G2 cells when treated with chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*
Table 2: Qualitative phytochemicals of chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Epicarp</th>
<th>Endocarp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates presence of compounds
- indicates absence of compounds

Fig. 4A: HPLC quantification of flavonoids present in chloroform extract of epicarp of *Garcinia mangostana*

Fig. 4B: HPLC quantification of flavonoids present in hexane extract of endocarp of *Garcinia mangostana*
Table 3: Quantitative phytochemicals of chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Epicarp (mg/g)</th>
<th>Endocarp (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>220.10</td>
<td>25.20</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>35.10</td>
<td>119.80</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>0.56</td>
<td>1.20</td>
</tr>
</tbody>
</table>

Fig. 4C: HPLC quantification of phenols present in chloroform extract of epicarp of *Garcinia mangostana*

Fig. 4D: HPLC quantification of phenols present in hexane extract of endocarp of *Garcinia mangostana*

Table 4: FT-IR spectral peak values and functional groups (4000 to 400 cm\(^{-1}\)) obtained for chloroform extract of epicarp of *Garcinia mangostana*

<table>
<thead>
<tr>
<th>Epicarp Extract</th>
<th>Peak Values</th>
<th>Functional Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td></td>
<td></td>
</tr>
<tr>
<td>413.01</td>
<td>C-I stretch, R-I, Alkyl Halides</td>
<td></td>
</tr>
<tr>
<td>444.29</td>
<td>C-I stretch, R-I, Alkyl Halides</td>
<td></td>
</tr>
<tr>
<td>Peak values</td>
<td>Functional group</td>
<td>Epicarp Extract</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>413.01</td>
<td>C-I stretch, R-I Alkyl Halides</td>
<td>Chloroform</td>
</tr>
<tr>
<td>461.87</td>
<td>C-I stretch, R-I Alkyl Halides</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5: FT-IR spectra of various functional groups (4000 to 400 cm$^{-1}$) obtained for chloroform extract of epicarp of *Garcinia mangostana*.

Table 5: FT-IR spectral peak values and functional groups (2000 to 400 cm$^{-1}$) obtained for chloroform extract of epicarp of *Garcinia mangostana*.
<table>
<thead>
<tr>
<th>Wavenumbers (cm⁻¹)</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>423.27</td>
<td>C-I stretch, R-I Alkyl Halides</td>
</tr>
<tr>
<td>1853.10</td>
<td>C=O stretch Acyl Chlorides</td>
</tr>
<tr>
<td>688.66</td>
<td>Alkynes</td>
</tr>
<tr>
<td>1033.14</td>
<td>C-F stretch Alkyl Halides</td>
</tr>
<tr>
<td>1053.53</td>
<td>C-O stretch Alcohols</td>
</tr>
<tr>
<td>795.41</td>
<td>C-Cl stretch Alkyl Halides</td>
</tr>
<tr>
<td>1521.02</td>
<td>N-H bend Amides</td>
</tr>
<tr>
<td>1015.51</td>
<td>C-F stretch Alkyl Halides</td>
</tr>
<tr>
<td>1716.38</td>
<td>C=O stretch Ketones</td>
</tr>
<tr>
<td>1559.14</td>
<td>C=C stretch Aromatic Ring</td>
</tr>
<tr>
<td>1457.33</td>
<td>N–O asymmetric stretch Nitro Compounds</td>
</tr>
<tr>
<td>1411.41</td>
<td>C–C stretch (in–ring) Aromatics</td>
</tr>
<tr>
<td>1540.66</td>
<td>N-H Bend Amines</td>
</tr>
<tr>
<td>1473.26</td>
<td>N-H Bend Amines</td>
</tr>
<tr>
<td>1261.73</td>
<td>C-O Stretch Esters</td>
</tr>
</tbody>
</table>

Fig. 6: FT-IR spectra for various functional groups (2000 to 400 cm⁻¹) obtained for chloroform extract of epicarp of *Garcinia mangostana*
Table 6: FT-IR spectral peak values and functional groups (4000 to 400 cm\(^{-1}\)) obtained for hexane extract of endocarp of \textit{Garcinia mangostana}

<table>
<thead>
<tr>
<th>Endocarp Extract</th>
<th>Peak values</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>403.97</td>
<td>C-I stretch, R-I Alkyl Halides</td>
</tr>
<tr>
<td></td>
<td>429.79</td>
<td>C-I stretch, R-I Alkyl Halides</td>
</tr>
<tr>
<td></td>
<td>451.65</td>
<td>C-I stretch, R-I Alkyl Halides</td>
</tr>
<tr>
<td></td>
<td>3415.57</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>3467.52</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>3357.29</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>3236.88</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>3290.26</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>3382.90</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>3325.93</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>3178.23</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>7511.46</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
<tr>
<td></td>
<td>3154.80</td>
<td>Hydrogen-bonded O-H Stretch \textbf{Phenols and Alcohols}</td>
</tr>
</tbody>
</table>

Fig. 7: FT-IR spectra for various functional groups (4000 to 400 cm\(^{-1}\)) obtained for hexane extract of endocarp of \textit{Garcinia mangostana}
Table 7: FT-IR spectral peak values and functional groups (2000 to 400 cm\(^{-1}\)) obtained for hexane extract of endocarp of *Garcinia mangostana*

<table>
<thead>
<tr>
<th>Endocarp Extract</th>
<th>Peak values</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hexane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3126.39</td>
<td>Hydrogen-bonded O-H Stretch <strong>Phenols and Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>2961.99</td>
<td>Hydrogen-bonded O-H Stretch <strong>Phenols and Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>3530.64</td>
<td>Hydrogen-bonded O-H Stretch <strong>Phenols and Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>3012.87</td>
<td>Benzene C=C-H Asymmetric Stretch <strong>Aromatic Rings</strong></td>
<td></td>
</tr>
<tr>
<td>1652.04</td>
<td>C=O Stretch <strong>Carboxylic Acids</strong></td>
<td></td>
</tr>
<tr>
<td>3562.39</td>
<td>Hydrogen-bonded O-H Stretch <strong>Phenols and Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>3667.24</td>
<td>Hydrogen-bonded O-H Stretch <strong>Phenols and Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>3596.58</td>
<td>Hydrogen-bonded O-H Stretch <strong>Phenols and Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>3807.60</td>
<td>Hydrogen-bonded O-H Stretch <strong>Phenols and Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>3616.02</td>
<td>Hydrogen-bonded O-H Stretch <strong>Phenols and Alcohols</strong></td>
<td></td>
</tr>
<tr>
<td>3627.55</td>
<td>O–H stretch <strong>Free Hydroxyl, Alcohols, Phenols</strong></td>
<td></td>
</tr>
<tr>
<td>1515.95</td>
<td>N–O asymmetric stretch <strong>Nitro Compounds</strong></td>
<td></td>
</tr>
<tr>
<td>1411.16</td>
<td>C–C stretch (in–ring) <strong>Aromatics</strong></td>
<td></td>
</tr>
<tr>
<td>1455.60</td>
<td>N-H Bend <strong>Amines</strong></td>
<td></td>
</tr>
<tr>
<td>2343.37</td>
<td>N-H Stretch <strong>Amides similar to Amines</strong></td>
<td></td>
</tr>
<tr>
<td>1309.74</td>
<td>C-O Stretch <strong>Ethers</strong></td>
<td></td>
</tr>
<tr>
<td>2317.86</td>
<td>N-H Stretch <strong>Amides similar to Amines</strong></td>
<td></td>
</tr>
<tr>
<td>1282.43</td>
<td>C-O Stretch <strong>Ethers</strong></td>
<td></td>
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</tbody>
</table>

Fig. 8: FT-IR spectra for various functional groups (2000 to 400 cm\(^{-1}\)) obtained for hexane extract of endocarp of *Garcinia mangostana*
DISCUSSION

All species of mangosteen contain abundant bioactive substances such as mangostin, tannins, xanthenes, anthocyanins, flavones, phenolic compounds, phytosterols and so on. Moreover, the ripe fruit of *Garcinia dulcis* contained at least 22 known compounds (Deachathai *et al.*, 2005) and two new compounds *viz.*, Dulcisflavan and Dulcisxanthone B (Hemshekhar *et al.*, 2011). A new compound taraxerol was identified by mass spectra and isolated in *Garcinia* species by Mawa and Said (2012). Similarly, apart from flavonoids and xanthenes, phloroglucinols have been identified by Ritthiwigrom *et al.* (2013) in *Garcinia cowa*. Likewise, Ramachandran (2014) and Fayaz and Ramachandran (2015) have screened and reported the presence of terpenoids, alkaloids, saponins, flavanoids, glycosides, carbohydrates, phenolic, tannins and phytosterols, garcinol, anthocyanins and hydroxycitric acid in *Garcinia indica*.

The presence of these bioactive molecules make it to possess a wide range of biological activities, such as antioxidant activity, antibacterial activity, anti-
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inflammatory activity\cite{124,61,62,63,64,65,67,46}, antitumor activity\cite{125,71,72,48}, cytotoxic activities\cite{127,128,129,62,70,71}, neurodegenerative disorder\cite{74} and HIV infection.\cite{128}

In the present study, both epicarp and endocarp had a profound effect on the inhibition of cell viability of Hep-G2 cells. Similar observations on antiproliferative effects of \textit{Garcinia} fruit extracts on various cancer cells have been recorded by Matsumoto \textit{et al}. (2013)\cite{67}, Moongkarndi \textit{et al}. (2004)\cite{75}, Akao \textit{et al}. (2008)\cite{130}, Magadula and Sulaimani (2010)\cite{131}, Abu Bakar \textit{et al}. (2015)\cite{132} and Silva \textit{et al}. (2015).\cite{133} The inhibition of Hep-G2 cells can be partially explained by the presence of phenolic phytochemicals such as anthocyanins, phenolic acids, carotenoids, and flavonoids as well as xanthone compounds that are mainly distributed in \textit{Garcinia} species as suggested by Abu Bakar \textit{et al}. (2015).\cite{133} In addition to that, mangostin (a type of xanthone compound) that is found in ripe fruit of \textit{Garcinia} species is known to contain \( \alpha \)-mangostin and \( \gamma \)-mangostin. Consumption of mangosteen pericarp extract (81% \( \alpha \)-mangostin and 16% of \( \gamma \)-mangostin) in the ratio of 0.25% and 0.5% extract to food dosage in daily diet is known to inhibit tumour growth in HCT 116 (human colorectal carcinoma) and reduce blood vessels in tumour towards Athymic NCr nu/nu mice by Aisha \textit{et al}. (2011).\cite{62}

MTT assay in the present investigation also revealed that the IC\(_{50}\) values of Hep-G2 cells was observed at 50 \( \mu \)M of chloroform extract of epicarp and at 5.25 \( \mu \)M of hexane extract of endocarp. Similar results were also observed in \textit{Garcinia mangostana} with SKBR3 human breast cancer cell lines by Moongkarndi \textit{et al}. (2004)\cite{75}, with DLD-1 human colon cancer cells by Akao \textit{et al}. (2008)\cite{130} and with B16-F10 melanoma cells by Cunha \textit{et al}. (2014), in \textit{Garcinia dulcis} with Hep-G2 cell line by Abu Bakar \textit{et al}. (2015)\cite{132} and in \textit{Garcinia xanthochymus} with Hepa-1c1c7 murine hepatoma cells by Silva \textit{et al}. (2015).\cite{133} Sun \textit{et al}. (2002)\cite{134} stated that some edible fruit extracts possess anticancer properties such as cranberry, lemon, apple, strawberry, red grape, banana and grape fruit against Hep-G2 cell line. Likewise McDougall \textit{et al}. (2008)\cite{135} stated that rowanberry, raspberry, lingonberry, cloudberry, arctic bramble and strawberry also showed potent activity against HeLa cell line.

The occurrence of at least 24 compounds that have been identified in \textit{Garcinia dulcis} such as Dulcisflavan, Dulcisxanthone B, epicatechin by Deachathai \textit{et al}. (2005)\cite{115}, and kaempferol (kaempferol3,7-di-O-\( \alpha \)-rhamnopyranoside) by Mahabusarakam \textit{et al}. (1987)\cite{74}, where epicatechin and kaempferol are the two flavonoid compounds that belong to flavanols and
flavonols, respectively. Moreover, kaempferol is known to possess antioxidant and anticancer activity which inhibit cell proliferation of MDA-MB-453 (human breast carcinoma) as reported by Afify et al. (2011).[136] Yang et al. (2009)[137], specific phytochemicals might act additively, synergistically or antagonistically with other compounds to display anti-proliferative activity. The above authors are of the opinion that all these compounds that are present in Garcinia might have acted synergistically to inhibit the proliferation of studied cancer cell lines. In the present study also the inhibition of cell viability in Hep-G2 cells treated with epicarp and endocarp extracts of Garcinia mangostana might be due to the presence of phenolic phytocompounds, flavonoids and xanthones and their synergistic activity, which would have led to the anti-proliferation of the cancer cells, thus finding support from the above authors.

The qualitative phytochemical profiling of Garcinia mangostana fruit showed the positive results for the presence phenols, flavonoids and triterpenoids in both the extracts and quantitative HPLC analysis and GC-MS study also confirmed that the chloroform epicarp extract contained 166 compounds and hexane endocarp extract showed 105 compounds with different retention time, peak area and mass spectra. Similar phytochemical profiling studies were also carried out in various Garcinia species by Zadernowski et al. (2009)[86], Madappa and Bopaiah (2012)[138], Ogunmoyole et al. (2012)[139], Ritthiwigrom et al. (2013)[118], Widowathi et al. (2014) [140], Fayaz and Ramachandran (2015) [114] and Li and Xu (2015).[48]

The presence of phenols, flavonoids and triterpenoids in GC-MS suggests that epicarp and endocarp of Garcinia mangostana fruit is pharmacologically active, supporting the claim by traditional healers. This result obtained is comparable to the reported phytochemical components which indicate the presence of alkaloids and flavonoids in Coffea genus[141-143] and also in Coffee brivipes extract.[144] Robbins (2003)[91] has stated that phenolic acids constitute about one-third of the dietary phenols and they are present in plants in the free and bound forms. Bound-phenolics may be linked to various plant components through ester, ether, or acetal bonds as opined by Chalas et al. (2001).[145] Pino et al. (2003)[146] stated that only few studies on the composition of volatile compounds in Garcinia genus have been accomplished and also stated that there is no data in the literature concerning the possible pharmacological effects and the chemical constituents of Garcinia are available. Phenolic acid profiles of mangosteen fruits are still unknown as documented by Zadernowski (1987)[84] and Zadernowski et al. (2002, 2005).[82, 83]
Farnsworth and Bunyaphatrphatsara (1992)\textsuperscript{147} stated that the pericarp of *Garcinia mangostana* contains mangostin, tannin, xanthone, chrysanthemin, garcinone, gartanin, vitamin B1, B2, C and other bioactive substances. Different studies suggested that different types of polyphenolic compounds such as flavonoids, phenolic acids and also mangostins have multiple biological effects including antioxidant, antibacterial and antitumor activity.\textsuperscript{148-158} Moreover, Cherian and Augustin (1995)\textsuperscript{153} are also of the opinion that phytochemicals such as glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities. Presence of saponins, triterpenoids, flavonoids, tannins, steroids and alkaloid compounds have anti-inflammatory effect\textsuperscript{154}, steroids, saponins and triterpenoids indicate analgesic effects (Sayyah *et al.*, 2004)\textsuperscript{155}, and saponins have high cholestrolemic and antidiabetic properties.\textsuperscript{156}

Haroun *et al.* (2014)\textsuperscript{157} stated that three out of ten compounds identified in the pomegranate peels extract were also found in the methanolic extract of *Garcinia dulcis*, namely hydroxymethylfurfural or 5-hydroxymethylfurfural, n-hexadecanoic acid and octadecanoic acid and according to Hossain *et al.* (2013)\textsuperscript{158} that this compound may contribute to antimicrobial effects. Abu Bakar *et al.* (2015)\textsuperscript{132} also stated that these compounds may also contribute to antioxidant and anti-cancer properties. The inhibition of proliferation of Hep-G2 cells in the present study might be due to the presence of phenols, flavonoids and triterpenoids in the epicarp and endocarp of mangosteen, thus finding support from the above authors.

FT-IR study of the present work also revealed the possible functional group present in both the extracts with the strong absorption peaks of C-I stretch, R-I (Alkyl halides), C-I stretch, Hydrogen-bonded O-H Stretch, Phenols and alcohols, Hydrogen-bonded O-H Stretch (Carboxylic Acids), N-H symmetric, Amines, N-H Stretch (similar to amines), Amides, C=O stretch, Amides, C=O stretch, Acyl Chlorides, Alkynes, C-F stretch, Alkyl halides, C-O stretch, Alcohols, C-Cl stretch, Alkyl halides, N-H bend, Amides, C=O stretch, Ketones, C-F stretch, Alkyl halides, ring C=C stretch, Aromatic, N–O asymmetric stretch, nitro compounds, C–C stretch (in–ring), aromatics, N-H Bend, Amines, Ester (C-O stretch), Aromatic rings-Benzene (C=C-H Asymmetric Stretch), Carboxylic Acids C=O Stretch, N–O asymmetric stretch, nitro compounds, Ethers (C-O Stretch) groups. Similar results were reported by Ajayi and Adesanwo (2009)\textsuperscript{159}, who stated that the principal fatty acids of pulp and seed of *Dacryodes edulis* were oleic and palmitic acids. Oleic acid is the most widely
distributed fatty acid on nature and it’s the principal responsible of health benefits of the Mediterranean diet. Some investigations have demonstrated that oleic acid can reduce the risk to suffer breast cancer and other diseases. Other compounds detected were oleic acid isomer (3.78%); 3,4-dihydroxibenzoic acid (3.76%); eter 2-butoxy etinil (1.99%); palmitoleic acid isomer (1.99%); miristic acid (1.75%), lactic acid (1.70%) and unidentified sterol (0.72%) by the data base. The abundance of fatty acids could contribute to acidity that characterizes the fruits of *Garcinia* species, particularly *Garcinia tinctoria*.\(^{[160,161]}\)

In a chemical study of volatile constituents of *Garcinia dulcis* fruits using gas chromatography by Pino *et al.* (2003)\(^{[146]}\), it was reported that a higher amounts of fatty acids could be responsible for the acidic and pungent odour observed in the fruit. Likewise, Pedraza-Chaverri *et al.* (2008)\(^{[127]}\) and Obolskiy *et al.* (2009)\(^{[104]}\) reported high concentrations of main xanthones such as \(\alpha\)-mangostin (1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthen-9-one) and \(\gamma\)-mangostin (1,3,6,7-tetrahydroxy-2,8-bis(3-methylbut-2-enyl) xanthene-9-one), that were isolated from the fruit rind of *Garcinia mangostana*. In our study also, the results of FT-IR indicates the presence of possible functional groups, and further isolation, characterization, structural prediction and functions of specific compound might throw more light on the biological activity of mangosteen.

**CONCLUSION**

The observed results imply that the bioconstituents from the *Garcinia mangostana* fruit extract can be used as potential anticancer drug.

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