ABSTRACT
The antioxidant and anticancer potential of mangosteen fruit *Garcinia mangostana* have been unraveled in the present study. Extraction of the epicarp and endocarp of the fruit with methanol, chloroform, hexane and ethyl acetate revealed that methanol gave maximum yield of extract. However, standardization of the solvents by MTT assay showed that only chloroform extract of epicarp and hexane extract of endocarp had significant effect on HePG-2 cells, thus revealing that the hexane extract has profound effect on HePG-2 cells. *In toto*, it can be concluded that both the epicarp and endocarp of *Garcinia mangostana* have antioxidant and anticancer potential. Moreover, identification of active phytoconstituents in the extracts will pave a way for using this fruit as a natural cytotoxic agent against various cancers.

KEY WORDS: DPPH assay, ORAC assay, MTT and cell cycle analysis.

INTRODUCTION
Cancer is a multi-factorial, multi-faceted and multi-mechanistic disease requiring a multidimensional approach for its treatment, control and prevention. Cancer remains a major public health burden in developed as well as developing countries.\(^1\) 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.\(^2\) 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.[5-15]

Chemoprevention is a rapidly growing area of oncology which focuses towards the cancer preventive strategy of natural or synthetic interventions.[16-19] Chemoprevention also deals with the chemotherapy of pre-cancerous lesions, which are called pre-invasive neoplasia, dysplasia or intra-epithelial neoplasia depending on the organ system as stated by Kelloff et al.[20] Likewise, Ito et al. [21] have pointed out that chemoprevention by synthetic agents can produce toxic side effects, which have limited their extensive use. There is an urgent need for new active and well-tolerated treatments to improve survival among patients with advanced HCC (palliative setting) and to increase long lasting remission after curative treatments (adjuvant setting). Studies, especially in China, on the prevention and treatment using herbal medicine against HCC have been accumulated during the past decades.[2, 22-37]

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.[33] In this context it should be kept in mind that herbal compounds could have an curative effect on all phases of HCC, including initiation, promotion and progression as opined by Liu[38], Shiota et al.[39], Yoysungnoen et al.[40], Lin et al.[41], Treasure[42], Ruan et al.[43], Chen et al.[44], Tsuchiya et al.[45], Janani et al.[29], Janani et al.[30], Li et al.[46], El-Sayeed et al.[34], Prabhu et al.[47], Asadi-Samani et al.[48] and Ashok and Sivakumari.[49]

Although a large number of plants and formulations have been investigated, the studies were mostly unsatisfactory. For instance, the therapeutic values, in most of these studies, were assessed against a few chemicals-induced subclinical levels of liver damages in rodents. The reasons that make us arrive at such a conclusion are lack of standardization of the herbal drugs, limited number of randomized placebo controlled clinical trials, and paucity of traditional toxicologic evaluations.[50-61, 29, 62-64, 36]

Nevertheless, this wide group of natural molecules represents a promising class as anticancer drugs, since their multiple targets in cancer cells, with limited toxic effect on normal cells. Phytochemicals can prove their therapeutic efficacy in mono-treatments or in association with classical chemotherapeutic drugs. In the latter case, a double positive effect can be expected: (i) phytochemicals can synergize with cytotoxic drugs, increasing their efficacy and
lowering the toxic side effects on normal cells (ii) combined treatment can delay resistance onset. Despite this encouraging preamble and the abundant literature describing the molecular mechanisms triggered by phytochemicals to inhibit cell growth and induce apoptosis in cancer cells, only few of them entered clinical trials.[65-77]

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.[78-103, 36, 104,105]

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,[106,107,58, 108-111], 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 15th century Chinese record Yinyai 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.[112,113]

The major bioactive compounds found in mangosteens are phenolic acids,[114-117], prenylated xanthone derivatives, anthocyanins, and procyanidins.[118, 110, 119, 120] 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 *et al*.[121,122], Lodovici *et al*. [123], Robbins [124], Shahidi and Naczk [125] and Zadernowski *et al*. [126] The major anthocyanin in mangosteen was cyanidin-3-sophoroside.[118]

Mangosteen peel contains xanthonoids, such as mangostin, and other phytochemicals.[127, 128, 79, 129, 83, 130-134, 110,135-138, 38] Research on the phytochemistry of the plant without human clinical study, however, is inadequate to assure the safety or efficacy of its use as a supplement as opined by Greim and Reuter [139], Gross and Crown [140] and American Cancer Society.[141]
Likewise, the other species of *Garcinia* such as *Garcinia dilcis* have been traditionally used by the people of Thailand; the leaf and seed being used for the treatment of lymphatitis, parotitis and goitre, the stem and bark being used as an anti-inflammatory agent, while the fruit juice is being used as an expectorant.\cite{142} Deachathai *et al.* \cite{143} have reported that the ripe fruit of *Garcinia dulcis* contained at least 22 known compounds and Hemshekhar *et al.* \cite{144} identified two new compounds in the same fruit *viz.*, Dulcisflavan and Dulcisxanthone B. Moreover, Abu Bakar *et al.* \cite{142} have determined the anticancer potential of 80% aqueous methanol extract of *Garcinia dulcis* towards the proliferation of liver carcinoma (HeP-G2) cell line in vitro and investigated their possible mechanism of action.

However, there is no systematic evidence that has been reported till date for the *in vitro* anti-proliferative effect of *Garcinia mangostana* epicarp and endocarp extracts against hepatocellular carcinoma. Therefore, the present study was designed with an aim to explore and evaluate the antioxidant and anticancer potential of *Garcinia mangostana* epicarp and endocarp extracts against hepatocellular carcinoma (HepG-2) cell line.

### MATERIALS AND METHODS

#### Collection and Identification of Fruit

Fresh Mangosteen fruits *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 Tambaram, Chennai, India, as *Garcinia mangostana* (Clusiaceae) with voucher specimen no: PARC/2015/214.

#### 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.[145] The morphological changes of crude extract treated HepG2 cell lines were assessed by using light microscopy. The IC 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 *Garcinia mangostana*. Based on the above observations, chloroform extract of epicarp and hexane extract of endocarp were selected for further study.

Antioxidant Potential
The antioxidant potential of the extracts was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity by the modified method of McCune and Johns[146] and ORAC assay according to the method of Huang *et al.*[147,148] using diagnostic reagent kit by Sigma St. Louis, USA, according to the manufacturer’s protocol.

Cell Cycle Analysis (Flow Cytometry)
The cell cycle phase’s distribution and measurement was assessed by flow cytometry using the method of Pozarowski and Darzynkiewicz.[149]

Apoptotic Morphology Analysis (Dot Plot Analysis by Dual Staining)
According to the method of Koopman *et al.*[150], apoptotic morphology analysis was done using apoptosis detection kit (Sigma St. Louis, USA) according to the manufacturer’s protocol.

Nuclear Staining and Apoptotic Morphology (DAPI Staining Method)
The flow cytometry results were confirmed by nuclear staining by using DAPI stain and viewing the apoptotic morphology of the cells under a fluorescence microscope, using filters appropriate for DAPI stain by the method of Spector *et al.*[151]

Determination of DNA Damage by Comet Assay (Single Cell Gel Electrophoresis)
DNA damage in the cell suspension was done following the methodology of Singh *et al.*[152]

Application of CASP Software
The comets were analyzed using the CASP software. The images were used to estimate the DNA content of individual nuclei and to evaluate the degree of DNA damage representing
the fraction of total DNA in the tail. Cells were assigned to five classes: 0 (<7% of the DNA in the tail undamaged), 1 (7 to 15%), 2 (15 to 22%), 3 (22 to 30%), 4 (>50%, maximally damaged) accordingly.\[153\]

DNA Fragmentation Study
DNA fragmentation study was carried out by DNA extraction and agarose gel electrophoresis according to the method of Bortner.\[154\]

Apoptotic Protein Expression Study
SDS-PAGE Analysis
SDS-PAGE was performed according to the method of Laemelli.\[155\]

Western Blot Analysis
Western blot analysis was performed according to the method described by Towbin et al.\[156\]

Statistical Analysis
The data was subjected to statistical analysis and the Mean ± SE for six individual observations are given in appropriate tables. The significance of the sample mean was tested by student’s-‘t’ test and the differences were considered as significant at p<0.01 level.

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%).

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. 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 1). The other endocarp extracts such as chloroform, ethyl acetate, and methanol did not show significant effect against HepG-2 cells (Fig 2).

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. 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 (5, 25, 50µM), 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.

**Antioxidant Potential**

**DPPH Assay**

The per cent inhibition of DPPH of epicarp and endocarp extracts of mangosteen along with L-Ascorbic acid (standard) is presented in Table 1. Depicted that the maximum inhibition of DPPH occurred at 12.5 µM concentration itself in all the above mentioned three groups. Likewise, the inhibition was high in both the extracts then that of the standard. The results indicate that mangosteen epicarp and endocarp have antioxidant potential and can be used as a drug for combating various ailments.

**ORAC Assay**

The net relative fluorescence unit of ORAC assay of chloroform extract of epicarp and hexane extract of endocarp of mangosteen along with Trolox (standard) presented in Table 2 and showed the maximum net relative fluorescence unit at 25 µM concentration of hexane extract of endocarp when compared to the standard and chloroform extract of epicarp. On the other hand, the maximum net relative fluorescence unit for chloroform extract of epicarp was observed at 50 µM concentration only. However, the activity was less in both epicarp and endocarp, when compared with that of the standard. The results indicate that mangosteen endocarp has antioxidant potential and can be used as a drug for combating various ailments.
Cell Cycle Analysis: The effect of epicarp and endocarp extracts of mangosteen on cell cycle distribution was determined using flow cytometry analysis and the results showed that chloroform extract of epicarp induced G0/G1 arrest of HepG-2 cells in dose-dependent manner (Fig 3, 4 and 5). The hexane extracts of endocarp at various doses also had similar effects that induced G0-G1 cell cycle arrest. The result suggests that both epicarp and endocarp extracts significantly induced HepG-2 cell arrest in G0/G1 phase.

Apoptotic Morphology Analysis by DAPI stain: A significant nuclear morphological changes were observed in the fluorescent microscopy. A significant increase in the percentage of apoptotic cells (p < 0.01) was observed as duplicated in Plate 2.

Determination of DNA Damage by Comet Assay: Comet assay results indicated that DNA fragmentation was much higher in 25µM in both the epicarp and endocarp extract treated cells when compared to 5µM concentration of the extract and fragmented DNA migrating out of the nucleus appeared as comet like tail in HepG-2 treated cells in a dose-dependent manner. But in control cells there was no fragmentation as shown in the Plate 3.

DNA Fragmentation Study: The respective extracts of both epicarp and endocarp at 5µM and 25 µM concentrations induced DNA fragmentation. Lots of small DNA fragments were observed from 100 bp to 500 bp, which indicates that HepG-2 cells underwent apoptosis which were induced by the extracts (Plate 4).

Apoptotic Protein Expression Study
Cyclin D Expression: Both epicarp and endocarp extracts (5 µM and 25 µM) significantly decreased the expression of cell cycle regulator protein Cyclin D (Plate 5a) in a dose-dependent manner in HepG-2 cells.

p53 Expression: Likewise, both epicarp and endocarp extracts (5 µM and 25 µM) significantly increased the levels of p53 protein in a dose-dependent manner in HepG-2 cells (Plate 5b)

Caspase-3 Expression
The effector caspase-3 significantly increased in a dose-dependent manner when treated with epicarp and endocarp extracts (5µM and 25µM) in HepG-2 cells. In this study, the active fragment (17 kDa) levels were found to be significantly elevated when compared to the
control (Plate 5d). Result revealed that epicarp and endocarp extracts activated the cleavage of caspase-3 leading to the induction of apoptosis in HepG-2 cells (Plate5c)

![Graph showing cell viability of various epicarp extracts of *Garcinia mangostana* against HepG2 cells](image1)

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

![Graph showing cell viability of various endocarp extracts of *Garcinia mangostana* against HepG2 cells](image2)

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

![Plate 1 showing cell morphology of HepG2 cells when treated with chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*](image3)

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

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>Standard (L-Ascorbic Acid)</th>
<th>Epicarp extract</th>
<th>Endocarp extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>0.178± 0.008</td>
<td>0.253 ± 0.017*  (+42.59)</td>
<td>0.203±0.006NS (+13.98)</td>
</tr>
<tr>
<td>25</td>
<td>0.079± 0.006</td>
<td>0.178 ± 0.009*  (+126.11)</td>
<td>0.153±0.019* (+94.48)</td>
</tr>
<tr>
<td>50</td>
<td>0.054 ± 0.005</td>
<td>0.087± 0.003*  (+60.31)</td>
<td>0.082 ± 0.004* (+51.69)</td>
</tr>
</tbody>
</table>

Values are mean ± SE of six individual observations. Values in parantheses are per cent change over standard. + denotes per cent increase over standard. *Values are significant at p<0.01.

Table 2: ORAC Assay of chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>Standard (Trolox)</th>
<th>Epicarp extract</th>
<th>Endocarp extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>90.40±0.618</td>
<td>46.47 ± 1.543*  (-48.60)</td>
<td>58.59±0.988* (-35.19)</td>
</tr>
<tr>
<td>25</td>
<td>84.60±0.599</td>
<td>59.91 ±1.228*  (-29.18)</td>
<td>71.05±1.287* (-16.02)</td>
</tr>
<tr>
<td>50</td>
<td>77.26±1.587</td>
<td>65.92± 1.058*  (-14.68)</td>
<td>65.68 ± 1.506* (-14.99)</td>
</tr>
</tbody>
</table>

Values are mean ± SE of six individual observations. Values in parantheses are per cent change over standard. - denotes per cent decrease over standard. *Values are significant at p<0.01.

Fig. 3: Cell arrest at G0-G1 phase of HeP-G2 cells induced by chloroform extract of epicarp of *Garcinia mangostana*
Fig. 4: Cell arrest at G0-G1 phase of HeP-G2 cells induced by hexane extract of endocarp of *Garcinia mangostana*

Plate2: Changes in apoptotic morphology in HeP-G2 cells induced by chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*

Fig. 5: Apoptosis of HeP-G2 cells induced by chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*
Plate 3: DNA damage and comet formation in HeP-G2 cells induced by chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*

Plate 4: DNA fragmentation in HeP-G2 cells induced by chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*

Plate 5: Expression of apoptotic proteins in HeP-G2 cells induced by chloroform extract of epicarp and hexane extract of endocarp of *Garcinia mangostana*
DISCUSSION
India is one of the 12 mega biodiversity centers having 45,000 plant species; its diversity is unmatched due to the 16 different agro-climatic zones, 10 vegetative zones and 15 biotic provinces. The country has a rich floral diversity of flowering plants (15,000 - 18,000), fungi (23,000), algae (25,000), lichens (1,600), bryophytes (1,800) and microorganisms (30 million). Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. Traditional preparation comprises medicinal plants, minerals and organic matters etc. Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. The ancient record is evidencing their use by Indian, Chinese, Egyptian, Greek, Roman and Syrian dates back to about 5000 years as stated by Zeeshan Hasan et al.[157]

According to Chopra et al. [158], nearly 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments, which also forms a rich source of knowledge. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments.[159] In India around 20,000 medicinal plant species have been recorded recently [160], but more than 500 traditional communities use about 800 plant species for curing different diseases.[161] Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the world contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.[157]

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 *Garcinia* fruit extracts on various cancer cells have been recorded by Matsumoto et al. [162], Moongkarndi et al. [58], Akao et al. [163], Magadula and Sulaimani [164], Abu Bakar et al. [142] and Silva et al. [165]. 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 *Garcinia* species as suggested by Abu Bakar et al. [142]. In addition to that, mangostin (a type of xanthone compound) that is found in
ripe fruit of *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 *et al.*[73]

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 *Garcinia mangostana* with SKBR3 human breast cancer cell lines by Moongkarndi *et al.* [58], with DLD-1 human colon cancer cells by Akao *et al.* [163] and with B16-F10 melanoma cells by Cunha *et al.* [166], in *Garcinia dulcis* with Hep-G2 cell line by Abu Bakar *et al.* [142] and in *Garcinia xanthochymus* with Hepa-1c1c7 murine hepatoma cells by Silva *et al.* [165] Sun *et al.* [66] 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 *et al.* [67] stated that rowanberry, raspberry, lingonberry, cloudberry, arctic bramble and strawberry also showed potent activity against HeLa cell line.

Antioxidants are radical which acts by donating hydrogen atoms to the radical compounds. Radicals derived from antioxidants such as phenol with a molecular structure are stable chemical species and can stop the chain reaction of oxidation.[167,168] Natural antioxidants protect the body against damage caused by reactive oxygen species, which inhibits the occurrence of the degenerative diseases and is able to inhibit lipid peroxidation in foods. Increased interest for natural antioxidants generally have a hydroxyl group in its molecular structure.[169]

*Garcinia* species is one of the most important plants that have a potential source of bioactive chemical compounds. *Garcinia* species are widely used by many people for food and traditional medicine. *Garcinia* is found to contain mostly xanton, benzophenone and triterpene which is known to possess antibacterial, antioxidant and anticancer potential. Antioxidants found in these showed higher activity, than with the known antioxidant.[170] Arazo *et al.* [91] reported that the peel extract of yellow mangosteen *Garcinia tinctoria* exhibited a higher scavenging activity of DPPH radical compared with the pulp extract.
In the present investigation, the antioxidant potential of both the epicarp and endocarp extracts were determined by DPPH and ORAC assay and the data revealed that the extract has antioxidant potential, which is directly proportional to the concentration. Likewise, standard also showed similar activity and this may be due to phyto-constituents present in the extracts. Similar results were also reported by Moongkarndi et al. [58], Elya et al. [168], Ogunmoyole et al. [171], Lim et al. [172], Lin et al. [173], Sumarny et al. [174], Abu Bakar et al. [142] and Fayaz and Ramachandran. [175]

Suksamrarn et al. [176] and Pedraza-Chaverri et al. [177] stated that xanthone compound isolated from mangosteen have remarkable biological activities such as antioxidant, antitumor, anti-inflammatory, anti-allergy and antimicrobial activities. Limei et al. [170] stated that Garcinia is mostly contains xanton, benxophenone and triterpene which is found to have antibacterial, antioxidant and anti-cancer effects in nature and antioxidant levels found in these fruits showed higher activity than the known antioxidant entities. An increasing interest in determining the antioxidant activities exhibited by phenolic acids and their derivatives has been recorded by several workers. [121, 122, 178-181, 115, 182, 124, 183, 116, 184, 172, 185, 186, 119, 187-189, 35, 190, 191, 142]

According to Polterait, [192] that the phenolic compounds such as flavonoids, tannins are the major group compounds that act as a primary antioxidant of free radical scavengers. It has been demonstrated by Yu et al. [193], Chaivisuthangkura et al. [120] and Obolskiy et al. [137] that there are many xanthones in the mangosteen shell. All xanthones bear a phenolic –OH and they can be considered as the phenolics. The results of Lin et al. [173] suggested a high level of total phenolics content in mangosteen shell extract. The authors are of the opinion that obviously, these phenolic xanthones can be responsible for the antioxidant ability of mangosteen shell. The authors further used a typical xanthone, γ-mangostin, as a reference compound for the following discussion. It is well known that hydroxyl radical (OH) can be generated via, Fenton reaction as depicted in Equation 1:

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{HO}^- + \text{OH}^- + \text{Fe}^{3+} \quad \text{(Eq. 1)}$$

As the most reactive ROS, hydroxyl radical can easily attack DNA to bring about various classes of oxidative lesions from base, nucleoside, nucleotide, oligonucleotide and DNA fragment. In addition, malondialdehyde (MDA) will also be yielded. Previous reports of Li et al. [194] depicted that MDA could reflect the protective percentages as well. In their study, the
protective percentages of mangosteen shell increased in a dose-dependent manner, which means that mangosteen shell can more effectively protect against hydroxyl-induced DNA oxidative damage than a standard antioxidant BHA. Previous works have demonstrated that there are two approaches for natural phenolic antioxidant to protect DNA oxidative damage: one is to fast repair the deoxynucleotide radical cations damaged by free radicals, one is to scavenge ROS (especially OH radicals) prior to DNA damage. To explore whether the protective effect of mangosteen shell is associated to ROS scavenging, Lin et al. further determined its OH radical-scavenging ability by deoxyribose degradation assay.

As illustrated in Eq. 1, the generation of OH radical relies on the catalysis of transition metals (especially Fe and Cu). Lin et al. then explored the metal-chelating ability of mangosteen shell. The dose-response curves indicated an effective metal-chelating ability of mangosteen shell. The IC 50 values of their experiments also suggested that mangosteen shell had the stronger metal-chelating ability than positive controls Trolox and BHA. Now it is clear that metal-chelating may be one approach for mangosteen shell to scavenge OH radical. For example, γ-mangostin naturally occurring in mangosteen shell, may bind metal ions via., the proposed mechanism of reaction for γ-mangostin to bind Cu2+ and Fe2+ to the OH radicals present in γ-mangostin as proposed by Lin et al.

To verify whether mangosteen shell can directly scavenge free radicals, Lin et al. further investigated the radical-scavenging effects on DPPH and ABTS+ which don’t require metal catalysis. The DPPH assay confirmed that mangosteen shell could efficiently eliminate DPPH radical. The previous studies of Bondet et al. and Li et al. suggested that DPPH may be scavenged by an antioxidant through donation of hydrogen atom (H) to form a stable DPPH-H molecule. On the basis of previous reports of Dimitrios and Vassiliki and Khanduja and Bhardwaj, γ-mangostin might scavenge DPPH via., the mechanism of homolysis of ortho-dihydroxy moiety by donation of H atom to form a stable DPPH-H molecule and resulting in an ortho-benzoquinone form. It has been demonstrated by Zhang et al. that ortho-dihydroxyl groups in benzene ring play a critical role in the antioxidant ability of phenolic antioxidants. Hence, in γ-mangostin molecule, ortho-dihydroxyl groups were thought to homolysis to produce H and γ-mangostin radical (I). H then combined with DPPH to generate DPPH-H molecule and the γ-mangostin radical might transform into (II), which could be further extracted H by excess DPPH to form the stable quinone form (III).
Further the findings of Lin et al.\textsuperscript{[173]} also showed that mangosteen shell could also scavenge ABTS$^+$ in a dose-dependent manner. Hence, mangosteen shell was considered as an effective radical scavenger on ABTS$^+$ as well. Unlike DPPH radical, ABTS$^+$ radical cation however needs only an electron (e) to neutralize the positive charge. Therefore, ABTS$^+$ scavenging is an electron (e) transfer process as suggested by Aliaga and Lissi.\textsuperscript{[201]} For example, $\gamma$-mangostin scavenged ABTS$^+$ possibly via, the following mechanism: At first, $\gamma$-mangostin produced electron (e) and H$^+$ cation. The electron (e) was then donated to ABTS$^+$ to form stable ABTS molecule. Meanwhile, $\gamma$-mangostin molecule was changed to $\gamma$-mangostin radical (I), which could also be further converted to (II), even (III) in excess ABTS+. The electron (e) transfer mechanism of ABTS assay was also supported by the Cu and Fe-reducing power assays, in which mangosteen shell exhibited a good dose response. The IC$_{50}$ values also suggested that mangosteen shell could successfully reduce Cu$^{2+}$ to Cu$^{+}$, and Fe$^{3+}$ to Fe$^{2+}$. As we know, reductive reaction is actually accepting electron (e) process, so it agrees with the findings of ABTS assay above.

In conclusion, Lin et al.\textsuperscript{[173]} have stated that mangosteen shell can effectively protect against hydroxyl-induced DNA oxidative damage and the protective effect can be attributed to the xanthones. One approach for xanthones to protect against hydroxyl-induced DNA oxidative damage may be ROS scavenging, which may be mediated via, metal-chelating, and direct radical-scavenging which is through donating hydrogen atom (H) and electron (e). However, both donating hydrogen atom (H) and electron (e) can result in the oxidation of xanthones to stable quinone form. In the present study also a combination of similar mechanism such as metal-chelating ability $\gamma$-mangostin and radical scavenging ability by oxidation of various xanthones into stable quinone forms that might have operated, thus resulting in the increased antioxidant potential of the epicarp and endocarp of \textit{Garcinia mangostana}, thus finding support from the above authors.

Cell cycle analysis by flow cytometry showed that both chloroform epicarp extract and hexane endocarp extracts significantly induced Hep-G2 cell arrest in G$_0$/G$_1$ phase itself in a dose-dependent manner and the similar results were also observed in the \textit{Garcinia dulcis} as reported by Abu Bakar et al.\textsuperscript{[142]} against the Hep-G2 cell lines. Li et al.\textsuperscript{[202]} and Abu Bakar et al.\textsuperscript{[142]} stated that the causes of inhibition in transition of cells towards S phase and the increase in the proportion of cells in sub-G$_1$ phase and an obvious accumulation of cells in sub G$_1$ phase indicates the reflection of apoptosis induction. A gradual decrease in G$_0$/G$_1$
phase, S phase and G2/M phase was observed by the above authors from 24 hrs to 72 hrs when compared to control and they opine that this condition might be due to the inhibition of DNA replication affected by the inability of the cells to replicate damaged DNA caused by the sample extracts, resulting in condensation of chromatin and nuclear fragmentation. In our study also, a similar mechanism might have operated, leading to the arrest of cell growth as opined by the above authors.

Kaufmann and Earnshaw\(^{[203]}\) stated that apoptosis is a physiological process of killing cells and is an important process to eliminate tumours. The apoptosis process can be characterized by membrane bleeding, shrinkage of cells and nuclear volume, chromatin condensation, DNA fragmentation and formation of membrane bound vesicles which can be triggered by multiple independent pathways by within or outside of the cell. Wang \textit{et al.}\(^{[204]}\) observed a significant increase in the sub G1 peak with a concomitant decrease in G1 phase, indicating induction of apoptosis, during the treatment of SK-MEL-28 cells with \(\alpha\)-mangostin. In our study also we observed apoptosis of Hep-G2 cells during treatment with epicarp and endocarp extracts of \textit{Garcinia mangostana}, by DNA fragmentation, comet assay, dot plot, nuclear staining and protein expression studies.

According to Abu Bakar \textit{et al.}\(^{[205,142]}\), the Annexin V-FITC apoptosis detection kit is the combination of fluorescein isothiocyanate (FITC) and Annexin V with propidium iodide to distinguish living cells in early and late apoptosis. The authors are also of the opinion that Annexin is a group of homologous proteins which bind phospholipids in the presence of calcium. During early apoptosis, phosphatidylserine which is usually located in the inner membrane of cells is transported into the outer portion of the membrane which can be detected by its strong affinity for Annexin V-FITC, whereas the dead cells can be detected by the binding of propidium iodide to the cellular DNA in cell.\(^{[205,142]}\) In our study also, we observed apoptosis in Hep-G2 cells, which resulted in 9- to 52-fold increase in Annexin-V and PI staining when compared to control. Abu Bakar \textit{et al.}\(^{[142]}\) also recorded similar results in Hep-G2 cells, when treated with \textit{Garcinia dulcis}, thus supporting our findings.

Likewise, the DNA damage by comet assay also depicted that DNA fragmentation was observed in all the concentrations in both the extracts; the damage being directly proportional to the concentration of the extracts. The induction of apoptosis was confirmed through activation of Caspase by Abu Bakar \textit{et al.}\(^{[142]}\) in Hep-G2 cells treated with \textit{Garcinia dulcis}. According to Silva \textit{et al.}\(^{[165]}\), flavonoids having antioxidant potential and these anti-radicals
are capable of quenching free radicals, which can promote DNA damage and mutations. Moreover, Leung et al. [206], stated that the Comet assay technique helps to identify the presence of breaks in single strands of DNA and is used to verify the chemopreventive potential against certain DNA damage.

Gupta et al. [207] stated that Caspase-3 was responsible for DNA fragmentation and morphological changes associated with apoptosis whereas Caspase-2 and Caspase-9 were used as an early biomarkers of apoptosis which act as downstream targets of cytochrome C release from the mitochondria which will then activates Caspase-3 and finding was in agreement with the previous study of Johnson et al. [94]. In our study also, a similar mechanism viz., the activation of Caspase and/or presence of flavonoids that are anti-radicals might have quenched free radicals, thus promoting DNA damage and mutations might have operated leading to apoptosis of Hep-G2 cells.

Apoptotic protein expression in the present study showed that both epicarp and endocarp extracts of *Garcinia mangostana*, significantly decreased the expression of Cyclin D, while significantly increased p53 and Caspase-3 expression in all the concentrations in both epicarp and endocarp extracts. Similar reports were also documented by Matsumoto et al. [208], Nakagawa et al. [209] and Akao et al. [163].

Caspase-3 is the most prevalent Caspase within cells and responsible for most of apoptotic effects and upon activation, it was able to induce Poly ADP Ribose Polymerase (PARP) cleavage and DNA break and finally leads to apoptosis. The exposure to the flesh of *Garcinia dulcis* towards Hep-G2 cancer cell line led to the activation of Caspase-3. This activation confirms that one of the initiator Caspases (Caspase-2, Caspase-8, and Caspase-9) was first activated which eventually could activate the executioner Caspase (Caspase-3, Caspase-6, and Caspase-7) which in this case was Caspase-3 and thus led to the induction of apoptosis. [210] This finding was in agreement with the study of Johnson et al. [94], where α-mangostin was able to suppress cell viability and colony formation which caused cell cycle arrest upon activation of Caspase-3 towards PC3 and 22Rv1 (human prostate) cancer cell line. Induction of apoptosis was observed by Cortese [210] in COLO205 (human colorectal adenocarcinoma) when exposed to 48 mg of α-mangostin and 6.40 mg of γ-mangostin per gram extract, where the apoptosis induction happened at Caspase-3 and Caspase-8 pathway.
Kurose et al. [97] evaluated α-mangostin in the human breast cancer cell line MDA-MB231 observing evidence of apoptosis by the findings of significantly elevated DNA level and Caspase 3, 8, and 9 levels. Significant cytochrome c release was also observed in α-mangostin-treated cells, suggesting α-mangostin induced MDA-MB231 cell apoptosis through the mitochondrial pathway. α-mangostin treatment also induced cell cycle arrest through upregulation of the Cyclin-dependent kinase (CDK) inhibitor p21cip1 and cell cycle checkpoint regulator. [97]

In a study by Matsumoto et al. [208], it was demonstrated that α-mangostin activated Caspase-9 and Caspase-3 but not Caspase-8 in HL60 cells, indicating that α-mangostin may mediate the mitochondrial pathway in the apoptotic process. The authors also pointed out that parameters of mitochondrial dysfunctions such as swelling, loss of membrane potential, decrease in intracellular ATP, ROS accumulation, and cytochrome c/AIF release, were observed within 1 or 2 h after the treatment, indicating that α-mangostin preferentially targets mitochondria in the early phase. Interestingly, replacement of hydroxyl group by methoxy group remarkably decreased the potency to cause mitochondrial dysfunction. It was also shown that the cytotoxicity was correlated with the decrease in the mitochondrial membrane potential. Furthermore, Matsumoto et al. [211] demonstrated that α-mangostin induced a cell cycle arrest at G1/S and the subsequent apoptosis via., the intrinsic pathway in DLD-1 cells, while a cell cycle arrest by γ-mangostin was at S phase. Likewise, Nakagawa et al. [209] also demonstrated that α-mangostin-induced apoptosis was mediated by a Caspase-independent pathway via., mitochondria with the release of Endo-G, which is a known 30-kD nuclease residing in mitochondria, is able to induce nucleosomal DNA fragmentation. In the present investigation also, a similar mechanism of induction of mitochondrial pathway and Caspase-independent pathway by α-mangostin might have caused apoptosis in Hep-G2 cells when treated with epicarp and endocarp extracts of mangosteen.

Bava et al. [212] have opined that phytochemicals are known to exert a considerable effect on the efficacy of anti-cancer agents, depending on their concentrations, by modulating the intracellular signaling pathways. Likewise, the enhanced efficacy of α-mangostin with other anti-cancer agents was also shown by the studies of Akao et al. [163] *Garcinia mangostana* xanthones are gaining more and more interest due to their pharmacological properties such as analgesic, antioxidant, anti-inflammatory, anti-cancer, anti-allergy and antimicrobial, cardioprotective, neuroprotective and immunomodulation properties as opined by Peres et
Rajput et al.\textsuperscript{(214)} stated that the cytotoxicity of the xanthones extract, $\alpha$-mangostin and $\gamma$-mangostin was comparable to that of cisplatin and the xanthones extract was almost two times more cytotoxic to colon cancer cells than the normal cells and it also indicates the higher selectivity towards the colon cancer cells. Similarly, Matsumoto et al.\textsuperscript{(162,208)} have pointed that executioner of Caspase-3 and Caspase-7, activation of the initiator Caspase-9, induction of DNA fragmentation and chromatin condensation and loss of mitochondrial potential plays a major role in the mitochondrial pathway of apoptosis in mediating cytotoxicity potential of xanthone compound as potential anticancer candidates.

As suggested by Matsumoto et al.\textsuperscript{(208)}, Moongkarndi et al.\textsuperscript{(58)}, Matsumoto et al.\textsuperscript{(211)}, Nakagawa et al.\textsuperscript{(209)} and Huang et al.\textsuperscript{(218)}, the compound $\alpha$-mangostin isolated from the pericarp has been shown to induce cell-cycle arrest and apoptosis in various types of human cancer cells. Hung et al.\textsuperscript{(219)} and Lee et al.\textsuperscript{(220)}, also stated that $\alpha$-mangostin have been additionally shown to inhibit cell invasion and migration in mammary and prostate cancer cells with the association of down regulation of MMP-2 and MMP-9. Iinuma et al.\textsuperscript{(81)} reported that the scientific data based on the principle of evidence based analysis is still remains scanty in the fields of complementary and alternative medicine that the $\alpha$-mangostin, an active compound present in the pericarp of the mangosteen fruit has been shown to induce many biological actions. Yang et al.\textsuperscript{(69)} stated that the inhibition of liver cancer cell lines can be partially explained by the presence of phenolic phytochemicals and flavonoids and as well as xanthone compound mainly present in the \textit{Garcinia species}.  

According to Settheetham\textsuperscript{(221)} ideally, new chemical entities that being evaluated as an investigational drug should have little to no toxicity and xanthones isolated from the pericarp of the mangosteen are non-genotoxic in mutagenesis studies. Li et al.\textsuperscript{(194)} stated that the mangosteen fruit has been noted as an abundant source of a class of polyphenols known as xanthenes and the diverse structure and chemical properties of xanthenes have been reported to have a many health promoting properties including anti-inflammatory, antioxidant and anti-cancer potential. Likewise, Balunas et al.\textsuperscript{(222)} stated that the inhibitory effect of 12 pure xanthones isolated from \textit{Garcinia mangostana} on microsomal aromatase in breast cancer
cells and reported that Gracinone D, Gracinone E, α-mangostin and γ-mangostin exhibited
dose dependent inhibition. Yamaguchi et al. [223] and Wadkar et al. [224] stated that daily
intake of fruits containing antioxidants and vitamin C reduces the risk of coronary heart
disease.

The two recent reports of Chao et al. [225] and Johnson et al. [94] stated that the anti-
tumorogenic effect s of α-mangostin in glioblastoma and prostate xenograft mouse models
that the intraperitoneal treatment with α-mangostin inhibited tumor growth by 50% in
glioblastoma xenograft model and this effect was associated with increased phosphorylation
of AMPK and induction of autophagy and similarly oral administration of α-mangostin to
athymic mice bearing 22Rv1 prostate cancer cells through the activation of Caspase-3 and α-
mangostin were also found to inhibit Cyclin/Cyclin-dependent kinase 4 which is involved in
cell cycle progression.

Although Garcinia mangosteen has long been served as traditional medicines, very few
authentic scientific studies in field of cancer therapy are available and recent in vitro studies
have shown that many constituents from Garcinia mangosteen have a wide range of
biological actions including antimicrobial, anti-helminth and insecticidal activities [226] and
HIV-1 protease. [88] Some studies have revealed that pericarp of Garcinia mangosteen is
source of xanthone, mangostin and tannin, etc., particularly tannin was found to be the inducer
for apoptosis on human leukemia cells. [227] Moreover, mangostin also inhibited low-density
lipoprotein oxidation. [228] In recent years, the mangosteen have becoming more popular
dietary supplement for its potential health promoting properties due to its unique chemical
library of xanthones to modulate multiple signaling pathways and it is clear that future work
is required to understand its potential for health promotion and potential drug discovery for
human beings.

The present study suggests that the fruit extracts of Garcinia mangosteen possess potent
antioxidative and anticancer property in both the epicarp and endocarp. It is suggested that
Garcinia mangosteen could be a potent source of natural antioxidants which are of great
importance as therapeutic agent in preventing or slowing down the progress of ageing, age
associated oxidative stress and related degenerative diseases. Further research is
recommended for better characterization of important constituents responsible for antioxidant
and anticancer activity.
CONCLUSION
In conclusion, the chloroform and hexane extract of *Garcinia mangostana* fruit has demonstrated promising antioxidant and anticancer properties against human hepatocellular carcinoma cells by *in vitro* method. Increasing awareness, promotion and utilization of this fruit for public benefits are highly encouraged and identification of active phytoconstituents in the extracts will serve as a natural cytotoxic agent against various cancers.

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