ISSN(O): 2456-6683
[Impact Factor: 6.834]
Publication Date: 30/11/2023

IJRCS

DOIs:10.2017/IJRCS/202311025 --:-- Research Paper / Article / Review

Phytochemical Assessment and Biological Features of Fruit Pericarp Extract of *Garcinia mangostana* L.: Antioxidant and Anticancer (Human Neuroblastoma IMR-32 Cells)

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Abstract: *The objective of the study was to reveal Garcinia mangostana L. fruit pericarp extract's (GME) phytochemical and biological features (antioxidant and anticancer properties). Abundant primary and secondary metabolites, including carbohydrates, flavonoids, tannins, alkaloids, quinines, terpenoids, glycosides, anthraquinone glycosides, phenols, triterpenoids, and cardiac glycosides, were discovered to be present in the GME. About 17.09 ± 0.24 mg gallic acid equivalent of phenolics, 21.91 ± 1.02 mg of catechin equivalent of flavonoids, and 14.39 ± 0.22 mg tannic acid equivalent of tannins are present in one gram of GME. In the DPPH and ABTS tests, the GME demonstrated high antioxidant potential; its EC_{50} value, or the concentration of the compound at which 50% of free radicals are scavenged, was determined to be 148.93 ± 3.87 and 157.80 ± 4.08 µg/mL in the DPPH and ABTS assays, respectively. The MTT study was used to assess the anticancer activity of GME, and the results showed dose-dependent anticancer activity of GME in human neuroblastoma IMR-32 cells. After a 24-hour period, the concentration of GME needed to inhibit 50% of cell viability was found to be 243.77 ± 10.24 µg/mL, which is known as the IC_{50} value (concentration required to inhibit 50% of cell viability). Because of its possible antioxidant activity, GME may be helpful in the treatment of oxidative-mediated illnesses. In the field of biomedicine, GME may be helpful in treating cancer since it exhibits possible anticancer activity against human neuroblastoma IMR-32 cells.*

Key words: *Garcinia mangostana L., Phytochemical profile, Antioxidant activity, Human neuroblastoma, Anticancer activity.*

1. INTRODUCTION :

One of the most common causes of death globally and one of the worst diseases affecting humans is cancer. One of the deadliest illnesses, cancer presents numerous health risks to people in both developed and developing nations. The adverse effects of current chemotherapy and treatments are excruciating. Therefore, research into novel complementary and alternative therapies with anticancer potential is imperative. It has been noted that novel medications with minimal or no negative effects are often derived from ethnomedicinal plants. Due to the fact that traditional medicine has long recognized the intrinsic ability of phytochemicals to treat illness, they have always been sought after. Additionally, it has been demonstrated that a number of plants are sources of essential chemicals that are useful in the treatment of cancer. For example, there are highly effective cancer chemotherapy medications that come from natural sources. They include plant-derived substances like the vinca alkaloids vinblastine and vincristine, which were extracted from *Catharanthus roseus*; Taxol, which was derived from *Taxus brevifolia*, and its analogue, docetaxel; etoposide and teniposide, which were obtained semisynthetically from epipodophyllotoxin, an epimer of podophyllotoxin, which was extracted from the roots of Podophyllum species; and camptothecin, which was extracted from the bark of *Camptotheca acuminata* and served as a precursor to the semisynthetic medications topotecan and irinotecan (Khazir et al., 2014).

In the present study, fruit pericarp extract of *Garcinia mangostana* L. was obtained by cold maceration technique. Subsequently, the phytochemical profile of *G. mangostana* L. extract (GME) was examined both

quantitatively and qualitatively. Using the DPPH and ABTS assay, the antioxidant activity of GME was revealed. Using MTT and micro-morphological analyses, the anticancer activity of GME was shown in human neuroblastoma IMR-32 cells in an *in-vitro* setting.

2. LITERATURE REVIEW :

Since various bioactive components of plants are responsible for certain particular physiological processes in the human body, plant extracts have been employed for thousands of years. Edible fruits, flowers, seeds, and leaves are rich in phytochemicals that have been linked to a host of health advantages, including anti-inflammatory, anti-cancer, hypoglycemic, anti-obesity, and protective effects on the neurological, hepatic, cardiovascular, and gastrointestinal systems. Their chemical components are more physiologically compatible with the human body because they are found in live plants. To demonstrate the effectiveness of plant extracts against a range of illnesses, various instances of research using animal models are also covered. Overall, the data indicates that more research on people is necessary to confirm a true cause-and-effect relationship, even if recommendations regarding the likely benefits of plant extract use have been made. New uses will appear as technology advances and our knowledge of these compounds' mechanisms of action grows (Majeed & Bhat, 2022).

This work examines and suggests biological uses of fruit pericarp or hull extract of *Garcinia mangostana* L. Mangosteen, or *G. mangostana* L., is a member of the Clusiaceae family. Southeast Asia is home to the tropical fruit known as mangosteen (*G. mangostana* L.), a member of the Clusiaceae family. There are currently about 400 species of Garcinia known to science, ranging from shrubs to evergreen trees. Though it was recently cultivated in many other tropical regions, *G. mangostana* is natively found in Malaysia, Indonesia, Thailand, Myanmar, Sri Lanka, India, and the Philippines. In Southeast Asia, it is among the most well-known fruits. Its instant visual and taste appeal has earned it the title of "Queen of Tropical Fruits." The edible pulp of the mangosteen fruit is white, soft, juicy, and has a slightly acidic and sweet flavor. Its aroma is pleasant. The fruit is dark purple or reddish. Bioactive substances comprising phenolics and flavonoids have been found in a variety of mangosteen tissues, including the pericarp, seeds, leaves, and plantlets (Osman & Milan, 2006).

3. MATERIALS AND METHODS :

3.1 Chemicals and reagents

Distilled water, pyridine, sodium chloride, quercetin, isopropyl alcohol, potassium persulfate, conc. sulfuric acid, Folin-Ciocalteau, ethanol, Whatman® grade 1 filter paper, gallic acid, potassium chlorate, hydrochloric acid, sodium hydroxide, ferric chloride, aluminum chloride, silver nitrate, chloroform, glacial acetic acid, copper sulfate solution, Mayer’s reagent, lead acetate, Molisch’s reagent, and other reagents used in the experiments were from Merck, India. Dimethyl sulfoxide (DMSO), 2,2-diphenylpicrylhydrazyl (DPPH), phosphate-buffered saline of pH 7.4 (PBS), ascorbic acid, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were acquired from Sigma-Aldrich, India. The plasticware was bought from Tarsons Products, India.

3.2 *G. mangostana* L. fruits collection and extraction

The *Garcinia mangostana* L. fruits have been collected in India's Andra Pradesh state at the agricultural market in Guntur. To remove any dust particles, the fruits were cleaned with distilled water, and the pericarp was obtained with a sterile knife and allowed to air dry. The pericarp was ground into a fine powder using an electrical chopper. To prepare *G. mangostana* L. pericarp fruit extract, approximately 250 g of dry fine powder was added to 1000 mL of ethanol, and the extract was obtained by cold maceration technique (Sankeshwari et al., 2018). Following a maceration period of three days, the filtrate was then filtered through a Whatman® grade 1 filter paper and lyophilized at -40 °C to concentrate it. The obtained *G. mangostana* L. fruit extract (GME) was vacuum-dried and stored at 4 °C for further utilization.

3.3 Qualitative phytochemical profile of fruit pericarp extract of *G. mangostana* L.

3.3.1 Carbohydrates screening

Screening for carbohydrates was done using Hossain et al., (2022). In test tubes, 1 mL (10 mg/mL) of GME was cooked for a few minutes in a water bath after adding a few drops of Benedict's reagent. The appearance of a reddish-brown precipitate in the tube indicates the presence of carbohydrates in the GME.

3.3.2 Flavonoids screening

Hossain et al., (2022) methodology was followed in the flavonoid screening process. 0.5 g of GME was extensively mixed with petroleum ether (lipid layer) to remove the fatty components. It was filtered after dissolving the defatted residue in 20 mL of 80% ethanol. The following experiments employed the filtrate: Three mL of the filtrate and four mL of 1% aluminium chloride in methanol were mixed in a test tube. The extracts contain chalcones, flavones, and flavonols, as shown by the production of a yellow color. A portion of the aqueous filtrate from GME was mixed with 5 mL of diluted ammonia solution, and then concentrated sulfuric acid was added. The production of yellow color in the GME indicates the presence of flavonoids.

3.3.3 Saponins screening

Minor adjustments were made to the Hossain et al., (2022) approach in order to carry out the saponins screening. After adding two mL of distilled water to 20 mg of GME, the mixture was thoroughly shaken lengthwise in a graduated cylinder for fifteen minutes. By formation of a 1 cm layer of foam, the GME saponin content is demonstrated.

3.3.4 Tannins screening

Hossain et al., (2022) method was slightly modified to perform the tannin screening. 1 mL of the GME (10 mg) and 2 mL of 5% ferric chloride were combined in tubes. The development of greenish-black or dark blue in the tube indicates the presence of tannins in the GME. The GME was filtered after being dissolved in 10 mL of distilled water. Ferric chloride aqueous solution was also added at a rate of around 1%. Strong green, purple, blue, or black hues that appear in the tube are a sign that tannins are present in the GME.

3.3.5 Alkaloids screening

To do the alkaloid screening, a few minor modifications were applied to the Hossain et al., (2022) methodology. Two mL of conc. HCl and 2 mL (10 mg/mL) of the GME were combined. Mayer's reagent was then added in a few drops. A green or white precipitate that forms in the GME is a sign that alkaloids are present.

3.3.6 Quinines screening

Subtle modifications were implemented to the Hossain et al., (2022) methodology to facilitate the quinine screening process. To one millilitre of GME (10 mg), an alcoholic potassium hydroxide solution was added. Quinines are present in the GME as evidenced by the colour shift from red to blue. 1 mL of GME (10 mg) was combined with 1 mL of concentrated H_2SO4. The creation of a crimson tint in the tube indicates the presence of quinones in the GME.

3.3.7 Terpenoids screening

To conduct the terpenoid screening, a few minor modifications were made to the Hossain et al., (2022) methodology. Saturated H_2SO_4 (3 mL), chloroform (2 mL), and 1 mL of solvent extract of GME (10 mg/mL) were mixed together. The presence of terpenoids in the GME was indicated by the appearance of a reddish-brown coating at the tube interface.

3.3.8 Glycosides screening

Hossain et al., (2022) method was slightly modified to perform the glycoside screening. A solution of ferric chloride and a few drops of glacial acetic acid were applied to the GME (10 mg). After vigorous shaking thoroughly mix the tube, conc. H_2SO_4 was added. Two layers are visible in the tube: the top acetic acid layer and the bottom reddish-brown layer. Consequently, the GME's existence of glycosides demonstrates the solution.

3.3.9 Anthraquinone glycosides screening

To do the anthraquinone screening, a few minor modifications were applied to the Hossain et al., (2022) methodology. 10 mg of GME was combined with 5 mL of chloroform and filtered. The filtrate was then vigorously stirred with an equivalent volume of ammonia solution (10%). The appearance of a red or pink-violet hue in the ammoniacal layer indicates the presence of anthraquinones in the GME.

3.3.10 Phenols screening

Subtle modifications were implemented to the Hossain et al., (2022) methodology to facilitate the phenols screening process. A quantity of 25 mg of GME was well combined with 5 mL of distilled water, and then a few drops of 5% $FeCl_3$ solution were added. The development of blue, green, and violet hues indicates the presence of phenols in the GME.

3.3.11 Steroids screening

To conduct the steroid screening, a few minor modifications were made to the Hossain et al., (2022) methodology. Strong sulfuric acid was applied to chloroform GME after they had been heated, cooled, and treated with acidic anhydride. The brief emergence of a greenish tinge indicates the presence of steroids in the GME.

3.3.12 Triterpenoids screening

A few small adjustments were made to the Hossain et al., (2022) methodology in order to do the triterpenoid screening. The GME was combined with a few drops of acetic anhydride, boiled, and then cooled. Conc. H_2SO_4 was applied along the test tube walls. The formation of a brown ring, with a deep red colour in the lower half and a green colour in the top part, at the junction of two layers indicates the existence of triterpenoids in the GME.

3.3.13 Cardiac glycosides screening

Hossain et al., (2022) methodology was slightly modified in order to perform the cardiac glycoside screening. Two mL of the GME solution (10 mg/mL), two millilitres of sodium nitroprusside in pyridine, and a few drops of mild hydrochloric acid were combined in a test tube. The GME's ability to develop a pink to blood-red colour indicates the presence of cardiac glycosides.

3.4 Quantitative phytochemical profile of fruit pericarp extract of *G. mangostana* L.

3.4.1 Quantification of total phenolics

The Folin-Ciocalteu test was used to reveal the total phenolics of the GME (Zarena & Sankar, 2012). A volume of 1 mL of GME (different concentrations made in distilled water) was quickly combined with 0.5 mL of the Folin-Ciocalteau component and a volume of 1 mL of sodium carbonate (7.5%) after being diluted three times with filtered water. The obtained mixture was incubated for 45 minutes at room temperature in the dark, and a microplate reader was used to measure the transmission density at 765 nm (Synergy H1, BioTek, USA). Gallic acid was quantified as the standard phenolic compound, and the results were expressed as milligrams of gallic acid equivalents (mg GAE/g) per gram of GME.

3.4.2 Quantification of total flavonoids

The aluminum chloride colorimetric assay was used to quantify the total flavonoids in GME (Zarena & Sankar, 2012). Briefly, 1 mL of GME (different concentrations made in distilled water) was combined with 140 µL of 5% sodium nitrite and incubated for 15 minutes at room temperature. The mixture was combined with 1 mL of 1M sodium hydroxide, 0.30 mL of 10% aluminum chloride, and 2.5 mL of distilled water. The next experimental assortment was incubated for 15 minutes at room temperature, and the microplate reader was used to measure the transmission density at 415 nm (Synergy H1, BioTek, USA). Quercetin served as the reference flavonoid, and the assay's result was expressed as mg of quercetin equivalents per g of GME (mg QUE/g).

3.4.3 Quantification of total tannins

Calorimetric analysis was used to determine the total tannin content of GME using the methods described by Palacios et al., (2021) with some modifications. Briefly, 4 mL of GME (different concentrations made in distilled water), 6 mL of 0.1 M $FeC1_3$ in 0.1 N HC1, and 6 mL of 0.008 M $K_3Fe(CN)$ were mixed together and incubated at room temperature under dark for 20 minutes. Using a multimode reader, the optical compactness of the samples was then measured at 720 nm (Synergy H1, BioTek, USA). Tannic acid served as the study's reference tannin compound, and the results were expressed as milligrams of tannic acid equivalents (mg TAE/g) per gram of GME.

3.5 Antioxidant activity of fruit pericarp extract of *G. mangostana* L.

3.5.1 DPPH assay

The assay was performed as per the methodology of Krishnamoorthy et al., (2023) with minor modifications. Briefly, a fresh 0.1 mM DPPH solution in 100% methanol was made and used. This mixture, with different doses of GME (different concentrations up to 100 µg/mL), was combined with 4 mL of the sample in 40% methanol. The mixture was then left to react in the dark for 30 minutes. Using a spectrophotometer (Synergy H1, BioTek, USA), the activity was measured at 517 nm against a blank. As a control, the reaction combination without GME was employed, and ascorbic acid was measured as the standard. The amount of GME needed to cause the optical density of the DPPH free radical

solution to drop by 50% is referred to as the IC_{50} value of GME. Using the following formula, the percentage of DPPH free radical scavenging activity was computed.

The percentage of DPPH radical inhibition was calculated as follows:

$$\text{GME's DPPH free radical scavenging activity (\%)} = \frac{A_t}{A_c} \times 100$$

In the equation, the terms A_t and A_c denote the absorbance of the test and control, respectively.

3.5.2 ABTS assay

The assay was performed as per the methodology of Krishnamoorthy et al., (2023) with minor modifications. In short, different doses of GME (different concentrations up to 100 µg/mL) were mixed with 3 mL of ABTS (7 mM) and potassium persulfate (2.4 mM) solution of 0.7 optical density. The mixture was then incubated at room temperature for 10 min under dim conditions. Using a spectrophotometer (Synergy H1, BioTek, USA), the activity was measured at 730 nm against a blank. As a control, the reaction combination without GME was employed, and ascorbic acid was measured as the standard. The amount of GME needed to cause the optical density of the ABTS free radical solution to drop by 50% is referred to as the IC_{50} value of GME. Using the following formula, the percentage of ABTS free radical scavenging activity was computed.

The percentage of ABTS free radical inhibition was calculated as follows:

$$\text{GME's ABTS free radical scavenging activity (\%)} = \frac{A_t}{A_c} \times 100$$

In the equation, the terms A_t and A_c denote the absorbance of the test and control, respectively.

3.6 Anticancer activity of fruit pericarp extract of *G. mangostana* L.

The National Centre for Cell Science (NCCS), Pune, India, provided human neuroblastoma IMR-32 cells. DMEM medium was used for growing cells. The incubation process took place at 37 °C and 5% CO_2, and all media contained 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were grown in 75 cm^2 flasks using DMEM complete media that was substituted every alternate day. Cells with a confluency of 85 to 90% were used for the experiments. MTT assay and bright-field inverted microscopy (observe the morphology) were used to assess the anticancer efficacy of GME on human neuroblastoma IMR-32 cells.

3.6.1 MTT assay

Yu et al., (2009) technique of MTT was to evaluate the impact of GME on the growth of human neuroblastoma IMR-32 cells. Briefly, in 96-well plates, the cells were seeded as 10,000 cells per well and allowed to adhere for 8 hrs. Cells in 96-well plates were exposed to GME (increasing dose of up to 400 µg/mL) in DMEM without FBS (total volume adjusted to 150 µL/well with DMEM devoid of FBS) for a 24-hour incubation period at 37 °C in a CO_2 incubator. The standard anticancer agent was cisplatin. The cells in the control group had just 100 µL of DMEM without any FBS. After the 24-hour exposure period, DMEM was removed, and 20 µL of MTT solution (5 mg/mL in physiological saline) was added. The mixture was once more incubated for three hours at 37 °C and 5% CO_2 in a humid incubator. After that, the formed formazan crystals were dissolved by adding 100 µL of dimethyl sulfoxide after the MTT solution was withdrawn. Using a microplate reader (Synergy H1, BioTek, USA), the optical density (OD) of the resulting MTT was determined at 570 nm. The inhibitory effects of GME on the cells were compared to the control group using the cell viability percentage.

3.6.2 Morphological observation of cells

Briefly, at a density of 1.5×10^4 cells per well, cells were plated in 24-well cell culture plates and left to settle for 8 hours at 37 °C in an incubator with humidified CO_2. Subsequently, cells were incubated in 500 µL of DMEM without bovine fetal serum for 24 hours after exposure to GME at varying doses (up to 400 µg/mL). The standard anticancer agent was cisplatin. The cells in the control group had 500 µL of DMEM without any fetal serum. After that, cells were

examined under a bright-field inverted microscope, and pictures were taken to evaluate their morphology (Manimekalai et al., 2016).

3.7 Statistical analysis

Three separate, independent runs of the study's experiments were conducted. The mean ± standard deviation of the triplicate research was expressed using the acquired results from each experiment. One-way ANOVA was used to analyze the statistical data, and Tukey's test was used to determine significance between test samples. Significant data was reported at p-value ≤ 0.05.

4. RESULTS AND DISCUSSION :

4.1 Phytochemical profile of fruit pericarp extract of *G. mangostana* L.

The current study assessed the *G. mangostana* L. fruit extract's (GME) qualitative phytochemical profile, which included primary and secondary metabolites such as carbohydrates, flavonoids, saponins, tannins, alkaloids, quinines, terpenoids, glycosides, anthraquinone glycosides, phenols, steroids, triterpenoids, and cardiac glycosides (**Table 1**). The results showed that GME has a wide variety of primary and secondary metabolites, including carbohydrates, flavonoids, tannins, alkaloids, quinines, terpenoids, glycosides, anthraquinone glycosides, phenols, triterpenoids, and cardiac glycosides. However, the results showed that saponins and steroids were absent. The results showed that the GME contained a wide range of primary and secondary metabolites, which may have advantages in the field of biology. In support of our study, Ovalle-Magallanes et al., (2017) have reviewed the phytochemical profile and confirmed the biopotential role of various metabolites.

Table 1: Qualitative phytochemical profile of *G. mangostana* L. fruit extract

S. No	Phytochemical compound	Result
1	Carbohydrates	Present
2	Flavonoids	Present
3	Saponins	Absent
4	Tannins	Present
5	Alkaloids	Present
6	Quinines	Present
7	Terpenoids	Present
8	Glycosides	Present
9	Anthraquinone glycosides	Present
10	Phenols	Present
11	Steroids	Absent
12	Triterpenoids	Present
13	Cardiac glycosides	Present

The Folin–Ciocalteu, aluminum chloride, and ferric chloride techniques were used to estimate the total phenolics, total flavonoids, and total tannins, respectively, in order to establish the quantitative phytochemical profile of GME. The current study investigated the dose-dependent concentrations of phenolics, tannins, and flavonoids. As shown in **Figure 1**, the results reveal that these phenolics, tannins and flavonoids chemicals' levels surged with the amount of GME. The study's GME contains higher concentrations of flavonoids, and followed by phenolics and tannins. Quantity of 17.09 ± 0.24 mg GAE of phenolics, 21.91 ± 1.02 mg CE of flavonoids, and 14.39 ± 0.22 mg TAE of tannins per gram of GME. As reported in the study, the high phenolic, flavonoid, and tannins content of the GME indicates that it may have uses in functional biology (Luna-Guevara et al., 2018; Oluwole et al., 2022).

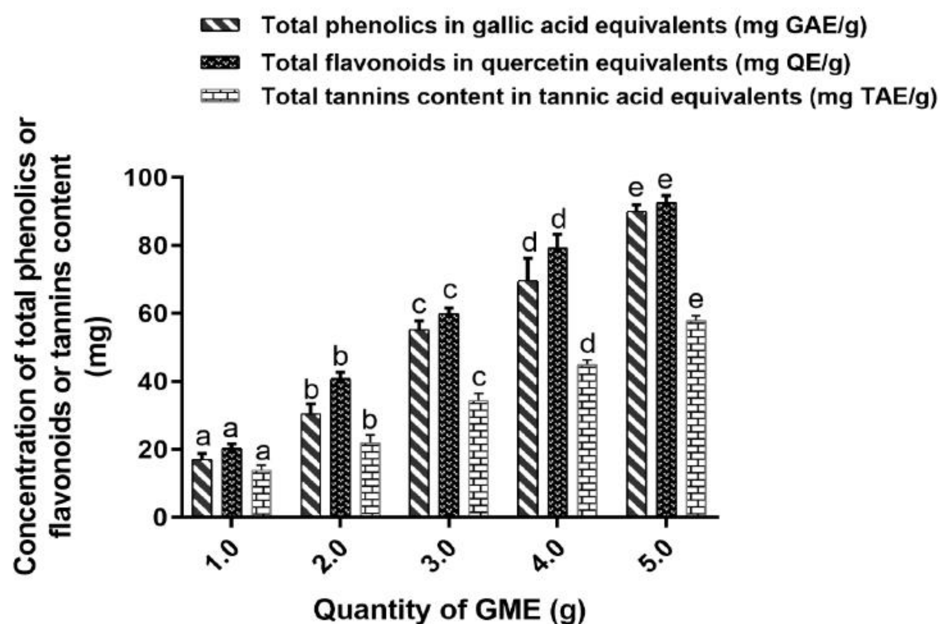

Figure 1: Total phenolics, flavonoids, and tannins in *G. mangostana* L. fruit extract (GME) were determined in a dose-dependent manner. Tukey's test was used to determine the significance between test samples. Significant data was reported at *p*-value ≤ 0.05. Bar charts with different alphabets show statistical significance for that particular study group.

4.2 Antioxidant activity of fruit pericarp extract of *G. mangostana* L.

The antioxidant activity of the GME was assessed in this study using the DPPH and ABTS free radical scavenging tests. The findings of the dose-dependent analysis of GME's antioxidant activity are shown in **Figures 2** and **3**. The antioxidant assays, DPPH and ABTS showed that the GME exhibited dose-dependent free radical scavenging activity. In the DPPH assay related to the ABT test in the study, the GME offered higher antioxidant potential. The concentration of GME at which 50% of the free radicals are scavenged, or the EC_{50} value, was determined to be 148.93 ± 3.87 and 157.80 ± 4.08 µg/mL in the DPPH and ABTS assays, respectively. The results of the study showed that because GME was high in secondary metabolites, it had antioxidant action. According to Yu et al., (2009), the GME may be very beneficial in biomedicine for treating a variety of oxidative stress-mediated illnesses (Xu et al., 2017).

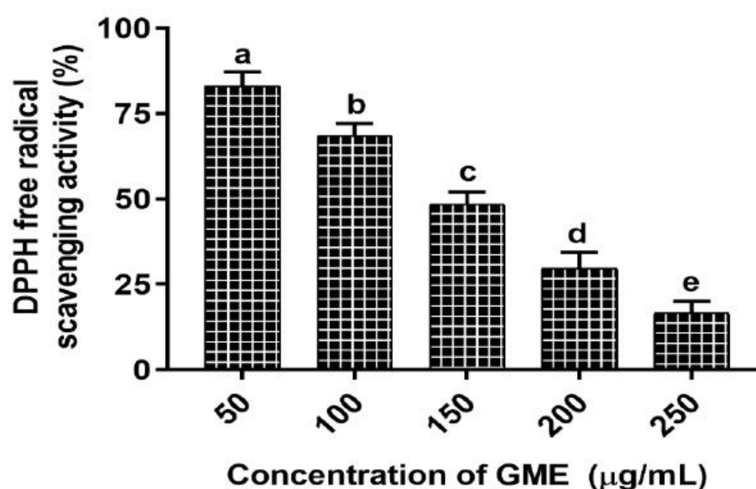

Figure 2: Dose-dependent DPPH free radical scavenging ability of *G. mangostana* L. extract (GME). The data were compared between the test samples using Tukey's test at a $p \leq 0.05$ significance level. Bar charts with different alphabets show statistical significance for that particular study group.

ISSN(O): 2456-6683
[Impact Factor: 6.834]
Publication Date: 30/11/2023

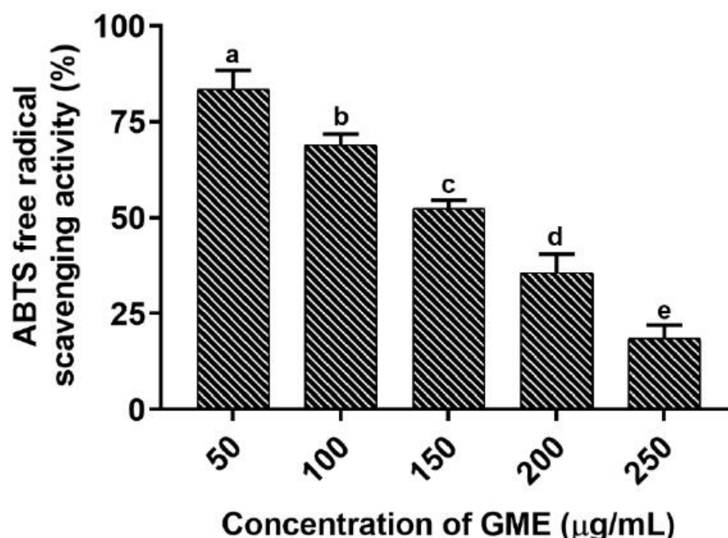

Figure 3: Dose-dependent ABTS free radical scavenging ability of *G. mangostana* L. extract (GME). The data were compared between the test samples using Tukey's test at a $p \leq 0.05$ significance level. Bar charts with different alphabets show statistical significance for that particular study group.

4.3 Anticancer activity of fruit pericarp extract of *G. mangostana* L.

In the present anticancer experiment of GME, the MTT study was followed to assess the anticancer activity of the GME on human neuroblastoma IMR-32 cancer cells in a dose-dependent manner. The results of the investigation, which are shown in **Figure 4**, showed that GME decreased neuroblastoma viability in a way that was dose-dependent. The concentration of GME needed to inhibit 50% of cell viability, or the IC_{50} value, was determined to be 243.77 ± 10.24 µg/mL for a 24-hour period in the study. Flavonoids, tannins, and phenolics found in the GME may provide anticancer effects. In support of our results, Yu et al., (2009) demonstrated the anticancer potential of *G. mangostana* fruit pericarp extract against human breast cancer cells (MCF-7) and human colon cancer cells (LOVO). Moreover, our results are consistent with previous studies showing that flavonoids, tannins, and phenolics function as pro-oxidants, potentially preventing cancer (Mahanta & Challa, 2022).

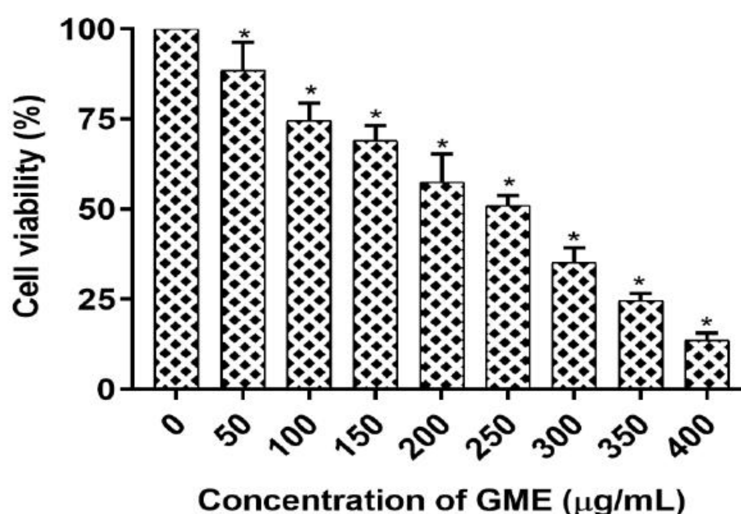

Figure 4: The anticancer effect of *G. mangostana* L. extract (GME) against human neuroblastoma IMR-32 cells in a dose-dependent manner. The data were compared using Dunnet's test (control sample with test samples) at a $p \leq 0.05$ significance level. The absence of a significant difference between the test and control samples is indicated by the asterisk (#). The asterisk (*) denotes the test sample's significance in respect to the control.

In an additional study to the MTT assay, micromorphological analyses using brightfield microscopy were utilized to validate GME's anticancer efficacy on human neuroblastoma IMR-32 cells **(Fig. 5).** The IMR-32 control cells feature a smooth monolayer and a recognizable form. The IMR-32 cells in the study that were treated with GME displayed a range of morphologies in comparison to control cells. After being exposed to GME, the IMR-32 cells showed signs of apoptotic bodies, cellular debris formation, and breakdown of the monolayer. In support of our study, Manimekalai et al., (2016) reported that GME induced anticancer activity through detrimental morphological changes and apoptosis in hepatocellular carcinoma (HePG-2) cells. In our study, a severely damaged variant of the IMR-32 cells was observed when the concentration of GME was higher (400 µg/mL) than when it was lower (250 µg/mL). The study found that GME reduced metabolic activity in the IMR-32 cells and initiated the apoptotic process, both assays (MTT and morphological observation) which resulted in anticancer action.

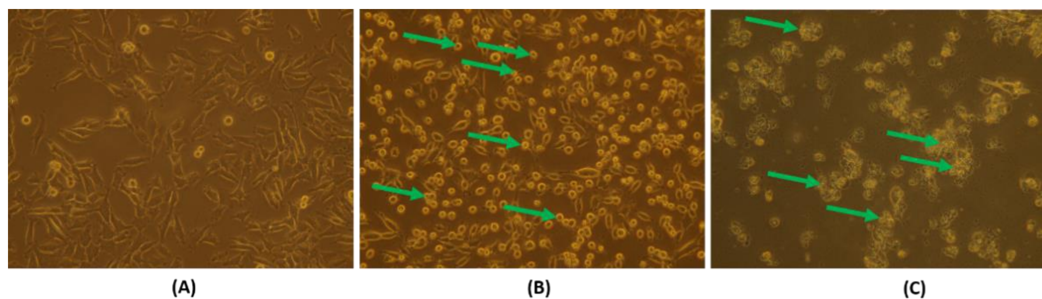

Figure 5: The effect of *G. mangostana* L. extract (GME) on the morphology of human neuroblastoma (IMR-32 cells) observed under an inverted bright-field microscope. (A) Control cells. (B) The cells were exposed to 250 µg/mL of GME. (C) The cells were exposed to 400 µg/mL of GME. The images were captured at 400x magnification. The green arrows point to damaging changes in the cell shape, like apoptosis bodies forming, cell debris leaking out, and cellular membrane rupture.

According to the study's findings, GME has a variety of primary and secondary metabolites. The flavonoids, tannins, and phenolics are abundant in the GME. Because of its possible antioxidant properties, GME may be helpful in the treatment of oxidative-mediated illnesses. In the field of biomedicine, the GME may be helpful in treating cancer since it exhibits a possible anticancer effect against human neuroblastoma cells (IMR-32).

5. CONCLUSION:

According to the study's findings, GME has a variety of primary and secondary metabolites. The flavonoids, tannins, and phenolics are abundant in the GME. Because of its possible antioxidant activity, GME may be helpful in the treatment of oxidative-mediated illnesses. In the field of biomedicine, the GME may be helpful in treating cancer since it exhibits possible anticancer activity against human neuroblastoma cells. However, the complete chemical profile of GME must be disclosed using cutting-edge analytical techniques in order to carry out functional properties.

Conflict of interest

The authors declare that no conflict of interest

Acknowledgment

The authors are thankful to Acharya Nagarjuna University for providing the facility.

REFERENCES:

1. Hossain MM, Uddin MS, Baral PK, Ferdus M, Bhowmik S. Phytochemical screening and antioxidant activity of *Ipomoea hederifolia* stems: A potential medicinal plant. Asian Journal of Natural Product Biochemistry. 2022 Aug 13;20(2).
2. Krishnamoorthy K, Jayaraman S, Krishnamoorthy R, Manoharadas S, Alshuniaber MA, Vikas B, Veeraraghavan VP. Green synthesis and evaluation of anti-microbial, antioxidant, anti-inflammatory, and anti-diabetic activities of silver nanoparticles from *Argyreia nervosa* leaf extract: an invitro study. Journal of King Saud University-Science. 2023 Oct 20:102955.

3. Luna-Guevara ML, Luna-Guevara JJ, Hernández-Carranza P, Ruíz-Espinosa H, Ochoa-Velasco CE. Phenolic compounds: A good choice against chronic degenerative diseases. Studies in natural products chemistry. 2018 Jan 1;59:79-108.
4. Mahanta SK, Challa SR. Phytochemicals as Pro-oxidants in Cancer: Therapeutic Implications. Handbook of Oxidative Stress in Cancer: Therapeutic Aspects. 2022 Sep 29:611-9.
5. Majeed T, Bhat NA. Health benefits of plant extracts. InPlant Extracts: Applications in the Food Industry 2022 Jan 1 (pp. 269-294). Academic Press.
6. Manimekalai I, Sivakumari K, Ashok K, Rajesh S. Antioxidant and anticancer potential of mangosteen fruit, *Garcinia mangostana* against hepatocellular carcinoma (HePG-2) cell line. World J Pharm Pharm Sci. 2016;5(2):253-93.
7. Oluwole O, Fernando WB, Lumanlan J, Ademuyiwa O, Jayasena V. Role of phenolic acid, tannins, stilbenes, lignans and flavonoids in human health–A review. International Journal of Food Science & Technology. 2022 Oct;57(10):6326-35.
8. Osman MB, Milan AR. Mangosteen: Garcinia mangostana L. University of Southampton, International Centre for Underutilised Crops; 2006.
9. Ovalle-Magallanes B, Eugenio-Pérez D, Pedraza-Chaverri J. Medicinal properties of mangosteen (*Garcinia mangostana* L.): A comprehensive update. Food and Chemical Toxicology. 2017 Nov 1;109:102-22.
10. Palacios CE, Nagai A, Torres P, Rodrigues JA, Salatino A. Contents of tannins of cultivars of sorghum cultivated in Brazil, as determined by four quantification methods. Food Chemistry. 2021 Feb 1;337:127970.
11. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. International journal of molecular sciences. 2017 Jan 5;18(1):96.
12. Yu L, Zhao M, Yang B, Bai W. Immunomodulatory and anticancer activities of phenolics from *Garcinia mangostana* fruit pericarp. Food Chemistry. 2009 Oct 15;116(4):969-73.
13. Zarena AS, Sankar KU. Phenolic acids, flavonoid profile and antioxidant activity in mangosteen (*Garcinia mangostana* L.) pericarp. Journal of Food Biochemistry. 2012 Oct;36(5):627-33.
14. Khazir J, Mir BA, Pilcher L, Riley DL. Role of plants in anticancer drug discovery. *Phytochemistry letters*. 2014 Feb 1;7:173-81.