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# Assessment the Functional Properties of Different Parts of Graviola and Its Application in A Bakery Product

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**Abstract**

The aim of this paper is to identify active compounds in graviola leaves (L), peels (PI), and pulp (P) to be used in the production of functional pies. The methodology included in this paper is based on determining the three active components of the methanolic extracts colorimetrically and quantitatively. The methanolic extracts were tested for percent cell viability and median lethal dose (IC50) of liver cancer cells (HePG-2). The results showed that the methanolic extracts exhibited yields of 13, 16.68, and 71.13 for L, PI, and P. The antioxidant activity of P against 2,2-diphenyl-1-Picryllhydrazyl (DPPH) was 79.16%. The HP-LC results identified that oleuropein, benzoic, and catechin were the major phenolic compounds. Luteolin, 7-glycerol, and quercetin were the major identified flavonoids. Isorhamnetin was identified as the major isoflavone in three parts. The methanolic extracts of three parts showed that IC50 was 10.52, 15.52, and 19.00 µg/ml. The pulp had 473.33, 50, and 56.5 mg/100g of vitamin C, B6, and B12, respectively. Sensory evaluation of the pie showed a higher significance ($p \leq 0.05$) overall acceptability of the sample substituted 50% juice with kneading water: filling custard milk. The accepted pie had 15.13 g/100 gm protein, 505.75 Kcal/100 gm energy, 84.05 Ca, 1.58 Zn, and 85.67 Mg ppm. Results suggest graviola are a rich source of healthy components and are recommended for developing functional pies that may help in the complementary treatment of liver cancer.

***Keywords:*** Graviola, active compounds, cancer cell lines, cytotoxicity, sensory evaluation, chemical analysis

## 1. Introduction

Custard apple *Annona squamosa* L. in the Annonaceae family is a fruit grown in tropical regions in different countries around the world, including Egypt **[1]**. In Egypt, this fruit is called Keshta even if, in the English community, it is called sugar apple. Due to recent exposure to its medical benefits, this plant (*A. squamosa*) is now being grown for commercial purposes all over the world **[2]**. Consumption of fresh fruit extracts or beverages is increasing around the world as they are thought to contribute to a balanced diet and are an excellent source of antioxidants, vitamins (C, A, B2, and B6), and minerals (potassium, calcium, phosphorus, magnesium, and iron) **[3].** Plant byproducts such as fruit or vegetable pomace, bran/husk/seed coat, seeds, peel, and leaves contain phytochemicals and can be used as novel food ingredients **[4]**. Traditional pharmaceutical uses have employed extracts from various parts of the Annona squamosa plant in various countries to treat a variety of illnesses, including dysentery, epilepsy, bleeding, fever, and tumors **[5].** Phytochemical studies found that the custard apple contains many phenol-based compounds, for instance,

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proanthocyanins, including 18 unique phenolic substances, primarily flavonoids or alkaloids. Glycosides, phytosterols, sugars, oils, saponins, tannins, alkaloids, phenols, flavonoids, peptides, and other acetogenin compounds all contribute to these functions **[4]. [6]** reported that catechin and gallic acid were the main phenolic compounds in the sugar apple peel. Phytochemicals like acetogenins and flavonoids are highlighted in the fruit of graviola like acetogenins and flavonoids. Its compounds lead to cytotoxic, anti-malarial, anti-diabetic, and immune suppressive activities. There are reported medicinal advantages of using graviola against human tumors and various disease factors. The capacity of culture *in vitro* and preclinical models to specifically target disease with little to no impact on healthy cell viability was explored. Graviola extracts contained over 212 phytochemicals. Specific bioactive ingredients were found to have powerful anti-cancer, antioxidant, anti-inflammatory, and antimicrobial properties. Other health benefits of plants include various classes of acetogenic agents, alkaloids, flavonoids, sterols, and others **[7]**. Cancer is the second cause of death in the world. More than 10 million new patients were diagnosed with about 12% of the global death rate. The number of new cancer cases is expected to grow by about 70 % over the next 20 years and is estimated to reach over 15 million new types diagnosed annually by 2020 **[8].**

Antimetabolites, DNA-interacting agents, anti-tubulin agents, hormones, and molecular targeting agents are the most commonly utilized chemotherapy medications; they all kill malignant cells or restrict their ability to proliferate. adverse effects of most cytotoxic medications such as losing hair, bone marrow elimination, resistance to drugs, gastric harm, neurological malfunction, and heart toxicity are, nonetheless, since they operate on cancer and normal cells **[9]**. Therefore, there is an urgent need to develop new anti-cancer drugs with efficacy, selectivity, and few or no side effects. Natural substances, particularly phytochemicals, have been employed to assist people maintain their health Since the beginning of medical science **[10]**. Phytotherapy (also called herbalism) has provided cures for diseases, including cancer, at this time **[11]**. Dietary phytochemicals offer many intrinsic benefits over artificial substances due to their demonstrated protection, affordable price, and oral availability **[12]**. Graviola has selective growth inhibition against diverse cancer cells such as lung, breast, prostate, pancreatic, colon, hepatocellular, and human lymphoma cell lines. Active substances such as natural phenols with anti-oxidant activity. sector, it is essential for promoting health in the food sector **[13]**.

Pie can be described as a savory fermented product made mainly from wheat flour, yeast, and other ingredients through several processes, including mixing, kneading, proofing, shaping, and cooking. Pies filled with meat, eggs, cheese, or a mix of vegetables and meat (pot pie). Filled pies include various pies like a two-crust pie, which has the filling fully enclosed in the dough **[14].**

Bakery products are considered the better vehicles for conveying functional components, so, the present study focused on identifying the active compounds of the various parts and using the functional juice to produce healthy pies. Additionally, we explored the effects of graviola parts, especially in graviola fruit, on cell cancer to detect its antitumor activity, which may make it a source for developing functional pies that may help in the complementary treatment of liver cancer.

## 2. Material and Methods

### 2.1 Materials

The graviola and leaves were purchased from a farm in Almansuria, Giza, Egypt. Wheat flour 72% extra. rate was obtained from Al-Salam-Company for Milling and Baking, Al-Salam City, Giza, Egypt. Cells for the liver ($HEPG_2$) were obtained from the Cancer Biology Dep., National Cancer Institute, Cairo University, Egypt. Cane sugar, instant dry yeast, table salt, fresh milk, skim milk**,** custard powder, and margarine type as a fern (%fat up to80%) were obtained from the local market, Giza, Egypt. Instant dry yeast ingredients as mentioned on the label where *Saccharomyces cerevisiae* was obtained from Bake Land Company, Industrial Zone C-1, 10th. Ramadan City, Egypt. Folin-Ciocalteu reagent, 2,2-diphenyl-1-Picrylhydrazyl (DPPH), Gallic acid, Catechin, Pyrogallol, Chlorogenic acid, Coumaric acid, Ferulic acid, Cinnamic acid, Benzoic acid, Rutina, Isorhamnetin, Genistein, and Kaempferol were obtained from Sigma-Aldrich Chemical Co., St. Louis, USA. All the other chemicals were analytical reagent grade.

### 2.2. Methods

#### 2.2.1. Preparation of different parts of graviola

The fruit and leaves were washed with tap water. Juice, leaves, and peels were collected and separated manually; the previously mentioned parts were stored in sealed containers and kept at - 20 ± 2°C for further use. The graviola parts (leaves,

peels, and fruit plup) was dried in the air oven at 40 °C until dry, ground, and sifted through an 80-mesh Cycrotex sample mill before being stored until use.

**2.2.2. Preparation of graviola extract from various parts**

The extraction process was performed on the previously mentioned parts of the graviola, according to **Rajesh *et al* [15].** The powdered materials were weighed and soaked with petroleum ether for 30 minutes, then the extract was removed, and the powder was dried and steeped in 5% (w/v) methanol and ethyl acetate in a pear-shaped funnel. The mixture was agitated at regular intervals for 24 h. The filtrate was removed, and a new solvent stream was added to the processed powder. The filtrate was concentrated in a water bath at 40°C and dried at 40°C in an oven. The dried extract was powdered and then packed in an airtight container.

**2.2.3. Extraction yield of graviola parts**

The yield of the different parts of graviola fruit was calculated according to **Onoja *et al* [16]** as follows:

$$\% \text{ Extraction yield (w/w)} = (\text{weight beaker with extract} - \text{weight baker}) / (\text{weight material}) \times 100$$

**2.2.4. Preparation of gravolia filling custard**

The filling custard was prepared by dissolving custard powder in cold milk, then the mixture was heated until gel consistency took place. The milk was replaced by graviola juice at 25%, 50%, and 75%.

**2.2.5. Preparation of pies with graviola**

The graviola pies were prepared by following the standard recipe and baking conditions using the method described by **Pongianta [17]**. Different ingredients in Formula presented in Table (1) were mixed and kneaded to form a similar dough. The pie has used the juice as an alternative to the kneading water at a replacement rate of 25%, 50%, and 75%. The dough is rested for 30 min, then divided into 50 gm pieces. Every piece is filled with about 15 gm of custard made with milk, and milk is replaced with juice (25%, 50%, or 75%). The fermentation temperature is about 36°C in a fermenter for 20 minutes with a relative humidity of 85%. Then bake at 260°C until completely set. Samples were cooled at room temperature for about 6 hrs. before instrumental and sensory analyses.

**Table (1):** Formula of graviola pies

| Ingredients | Samples | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Cont.** | **25/25** | **25/50** | **25/75** | **50/25** | **50/50** | **50/75** | **75/25** | **75/50** | **75/75** |
| **Wheat flour** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |
| **cane sugar** | **4** | **4** | **4** | **4** | **4** | **4** | **4** | **4** | **4** | **4** |
| **margarine** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** |
| **Table salt** | **1.65** | **1.65** | **1.65** | **1.65** | **1.65** | **1.65** | **1.65** | **1.65** | **1.65** | **1.65** |
| **Skim milk** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** | **5** |
| **Instant dry** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **yeast** | | | | | | | | | | |
| **% Water: juice for dough(W/j)** | | | | | | | | | | |
| **%** | **62.5 W** | **W/J 75/25** | | | **W/J 50/50** | | | **W/J 25/75** | | |
| **Proportion of filling custard with (milk: Juice)** | | | | | | | | | | |
| **%** | **100 M** | **M/J** | | | | | | | | |
| | | **75/25** | **50/50** | **25/75** | **75/25** | **50/50** | **25/75** | **75/25** | **50/50** | **25/75** |

**2.2.6. Sensory evaluation**

A panel of ten experts from the staff of the Food Technology Research Institute- Agricultural Research Center, Giza, Egypt, evaluated the sensory properties of graviola pies according to **Watts *et al* [18]**. The panelists gave scores for appearance, color, odor, taste, texture, and overall acceptability (OAA) of the graviola pies, on a hedonic scale from one (dislike extremely) to nine (like extremely).

**2.2.7. Proximate analysis**

Chemical composition was performed on juice and pie samples. The contents of moisture, protein, fat, crude fiber, and ash were determined according to the **AOAC [19]**. The determination of Available carbohydrates was calculated by difference.

$$\text{Available carbohydrates(on dry wieght basis)} = 100 - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{ash} + \%\ \text{fiber}) \quad (2)$$

Energy (Kcal/ 100g) was calculated by applying the conversion values for the equation

$$\text{Total energy (K.cal/100g)} = 4(\%\ \text{avialable}\ carbohydrates + \%\ \text{protein}) + \%\ fat \times 9 \quad (3)$$

**2.2.8. Determination of Total soluble solids (TSS)**

Total soluble solids were determined by mixing 1 g of fresh fruit with 1 mL of distilled water, then using the refractometer (ATAGO® Pocket PAL-Japan). TSS was measured in °Brix using the procedure described by **Latimer [20].**

**2.2.9. Determination of Total Sugars**

The total soluble sugars of graviola juice were determined according to **Dubois *et al* [21]**. By using a Perkin Elmer Lambda 11 Spectrophotometer: (Perkin Elmer, Massachusetts, USA).

**2.2.10. Determination of minerals**

The mineral content (Ca, K, Mg, P, Zn, Mn, Cu) of graviola juice and pie samples were determined by using Perkin Elmer (Model 3300, USA) atomic absorption spectroscopy (AA-6801), as described by **AOAC [19].**

**2.2.11. Determination of total phenolic (TPC) and total flavonoid content (TFC)**

The TPC of graviola parts was determined according to **Singleton and Rossi [22]** using the Folin-Ciocalteu reagent. Samples were measured at 650 nm, and the concentration was expressed as milligrams of gallic acid equivalents (GAE)/g. The TFC of graviola parts was determined according to the procedure described by **Zhishen *et al* [23]**. The extract (250 µL, the concentration of 1–10 mg/mL depending on solvent used) was mixed with distilled water (1.25 mL) and sodium nitrite solution (5%, 75 µL). After 6 min of incubation, aluminum chloride (10%, 150 µL) was added to the blend, followed by sodium hydroxide (1 M,500 µL). They were immediately diluted in distilled water (2.5

mL). The absorbance was measured at 510 nm. TFC was expressed as mg (+) catechin equivalents (EC) per gram of extract.

**2.2.12. Determination of antioxidant activities**

The AOA of graviola parts and pies was evaluated by measuring free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) scavenging capacity, according to **Blois [24]**. The absorbance was measured against pure methanol at 515 nm, and the AOA was calculated as percent discoloration from the following equation:

$$\% \text{ Inhibition} = [1 - (A1/A0)] \times 100 \quad (4)$$

where A0 is the absorbance of the pure methanol control at the beginning of the reaction (t = 0) and A1 is the absorbance of the sample extract at the end of the reaction (t = 30 min). All chemical tests were averaged from three replicates.

**2.2.13. High-Performance Liquid chromatograph (HPLC)**

The phenolic compounds (PC) were determined by an HPLC method according to **Khalil *et al* [25]**. Phenolic compounds were separated on Hewlett Packard, series 1050, USA, equipped with column $C_{18}$ hyper sail BDS with a particle size of 5 mm. The PCs were detected using a UV detector at 280 nm, and the results were expressed as g/100 gm of the sample.

**2.2.14. Determination of vitamin B complex**

The vitamins B1 (thiamine); B2 (riboflavin); B12; B9 (folic acid); and B6 (pyridoxine) of graviola pulp were determined as described in the **AOAC [19]**. Hewlett Packard, series 1050, USA, equipped with a column $C_{18}$ hyper sail BDS with a particle size of 5 mm.

**2.2.15. Determination of vitamin C**

The *L*-ascorbic acid content of fruit pulp was determined according to the method described in the **AOAC [19]**. Hewlett Packard, series 1050, USA, equipped with a column $C_{18}$ hyper sail BDS with a particle size of 5 mm. The L-ascorbic acid was separated on a $C_{18}$ column using ion-pairing chromatography. Where quantification by UV absorption at 247 nm means measuring the peak area of the external standard and sample and the concentration of the external standard.

**2.2.16. Determination of Vitamin A (Retinol)**

The vitamin A (retinol) content of fruit pulp was measured by HPLC, using the method of **AOAC [19]**. Hewlett Packard, series 1050, USA, equipped with a column $C_{18}$ hyper sail BDS with a particle size of 5 mm. The samples were saponified with alcoholic potassium hydroxide (2N), extracted with diethyl ether or alcohol, and the liquid extracted with petroleum ether (B.p. 40–60°C). The un-saponified fraction was taken up in a suitable amount of n-heptane, and retinol acetate was added as an internal standard. The solvent (n-heptane) solution was centrifuged, and 15 ul of it was injected into HPLC equipped with a Kieselgedl-column. All trans retinol and retinol acetates were eluted with n-heptane isopropanol in the mobile phase. All trans retinol and vitamin A acetate peaks in the area were determined with spectrophotometric detection at 325nm. The amount of vitamin A in the sample was calculated.

**2.2.17. Measurement of potential cytotoxicity by sulphorhodamine-B (SRB) assay**

The potential cytotoxicity of different fruit parts was tested using the method of **Skehan *et al* [26]** in the Cancer Biology Department, National Cancer Institute, Cairo University, Egypt. The cells for liver (HEPG2) cancer were plated in a 96-multiwall plate (104 cells per well) for 24 hours before treatment samples to allow attachment of the cells to the wall of the plate. Sample plates received 0, 6.25, 12.5, 25, and 50.0 µg/ml of graviola fruit parts extract

methanol-containing medium, which was added to the cell monolayer. All the respective extracts were dissolved in DMSO (Dimethyl Sulphoxide) to prepare a (10 mg/ml) stock solution for culture studies. The control cultures were treated with DMSO. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Triplicate wells were developed per individual dose (concentration). Monolayer cells were incubated with samples prepared for 48 hours at 37 °C in an atmosphere of 5% $CO_2$. After 48 hrs, cells were fixed, washed, and stained with sulphorhodamine-B stain. The excess stain was washed with 1% acetic acid, and the attached stain was recovered with Tris EDTA buffer. The color intensity was measured by an ELISA reader. The relationship between the surviving fraction and the concentrations of the samples tested was plotted to obtain the survival curve of each tumor cell line after the cytotoxicity of the specified compound and IC50 (the dose of the tested samples that reduces the survival rate to 50%) were evaluated.

### 2.2.18. Statistical Analysis

The obtained results are statistically analyzed using CoStat, version 3.03 for PC, as described by **Ott [27]**. The tests used were ANOVA tests and descriptive statistics tests. An effect of treatment was assumed to be statistically significant at P≤ 0.05.

## 3. Results and Discussion

### 3.1. Active compounds of graviola L, PI, and P

Table (2) shows the extract yield (%), total phenolic (TPC), total flavonoids (TFC), and antioxidant activity (AOA) of the graviola leaves (L), peels (PI), and pulp (P). The pulp had the highest ($P\leq$ 0.05) extract yield (%), followed by the peels. The fruit pulp had the highest extract yield when extracted with methanol solvent (71.13%). The fruit pulp extract had the highest ($P\leq$ 0.05) TPC (378.15 0.00 mg GAE equivalent/g). The flavonoid content ranged from 38.41 ± 0.31 to 89.55 ± 0.8 mg CE equivalent/g dry sample. The leaf extract with methanol solvent exhibited the highest flavonoid content (89.55 µg CE equivalent/g), followed by the fruit pulp extract (75.68 ± 0.5 CE equivalent/g). The pulp had the highest ($P\leq$ 0.05) antioxidant activity (AOA%), which was extracted with methanol solvent (79.17%). Also, Table (2) observes that the methanol solvent used is superior to ethyl acetate for extraction. Those results agreed with those of **Nandhakumar and Indumathi [28]**, who reported that methanol extract has a higher antioxidant activity than aqueous extract, resulting from the increased presence of phenolic and flavonoid compounds. **Tiencheu *et al* [29]** reported that Annona fruits are a source of phenolic compounds. For example, *Annona muricata L.* has 624.2–941.4 mg of GAE mg-1 pulp and *Annona squamosa* 583.45 µg catechin $mL^{-1}$.

**Table (2):** Extract yield, TPC, TFC, and AOA of parts graviola

| Parts | Extract yield (%) | TPC | TFC | AOA* |
|---|---|---|---|---|
| L-MeOH | 13.04±0.72$^{c}$ | 260.15 ± 1.55$^{b}$ | 89.55 ± 0.8 $^{a}$ | 64.85±1.45$^{b}$ |
| L-EtOAC | 1.1±0.06$^{d}$ | 79.87 ± 0.38$^{e}$ | 66.74 ± 1.03$^{c}$ | |
| PI-MeOH | 16.73±0.92$^{b}$ | 208.25 ± 2.35$^{c}$ | 45.41 ± 0.31$^{e}$ | 77.44±4.98$^{a}$ |
| PI-EtOAC | 0.86±0.06$^{d}$ | 86.39 ± 0.00$^{d}$ | 38.81 ± 0.41$^{f}$ | |

| | | | | |
|---|---|---|---|---|
| P-MeOH | 71.37±3.91[a] | 378.15 ± 0.00[a] | 75.68 ± 0.5 [b] | 79.17±1.00 [a] |
| P-EtOAC | 0.61±0.03[d] | 87.67 ± 0.495[d] | 62.63 ± 0.47 [d] | |

TPC: total phenolic expressed as milligrams of Gallic acid equivalents (GAE), TFC: Total flavonoids expressed as mg catechin equivalents/ g extract) (CE), AOA*: antioxidant activity—calculated as percent discoloration of 2,2-diphenyl-1-picrylhydrazyl (DPPH), L: leaves, PI: Peel, P: pulp, MeOH: methanol solvent, EtOAc: ethyl acetate.

### 3.2. Identified in the methanolic extract of graviola L. fruit parts using HPLC

The PC has enabled the identification and quantification of 33 phenolic compounds. Table (3) represents their respective concentrations in the graviola fruit parts. Benzoic, Oleuropein, Catechin of the leaves dried powdered, Benzoic, Chlorogenic of the peels dried powdered, and Benzoic and Gallic of the pulp fruit dried powdered were the major phenolics identified at 85.24 ± 0.03, 67.36 ± 0.44, 50.45 ± 0.02, 58.04 ± 0.18, 80.99 ± 0.01, 25.76 ± 0.25 and 29.2 ± 3.04 mg/100g, respectively. The major flavonoids identified were as follows: 8.99 ± 5.77, 5.67 ± 0.01, 4.14± 0.00, 4.09 ±5.77, 8.83 ± 0.24, and 5.81 ±0.01 mg/100g Luteolin 7- glucose, Quercitrin of the leaves dried powdered, Luteolin 7-glucose, Rutin of the peels dried powdered, and Quercitrin, Hesperidin of the pulp fruit dried powdered, respectively. Isorhamnetin was the major isoflavonoid identified at 46.71 ± 0.03, 18.09 ±0.5, and 30.47 ± 0.01 of the dried powdered leaves, peels, and pulp fruit, respectively. **Jiménez *et al* [30]** determined that phenolic compounds (PC) were extracted from the pulp of ripe soursop fruits (Annona muricata L., Annonaceae). The PC was separated into two parts by solid phase extraction, which confirmed that the predominant compounds were cinnamic acid and coumaric acid, along with many secondary compounds that may have health benefits due to their antioxidant properties. **Yang *et al* [31]** demonstrated the phytochemical compounds in graviola leaf extract compared to its acetogenins and flavonoid fractions. Comparative quantification of flavonoids revealed a 7-fold enrichment of rutin and a 3-fold enrichment of quercetin-3-glucoside, with concentrations of rutin, Q-3-G, quercetin, and kaempferol in the graviola leaf extract being 16.7, 1.16, 1.25, and 0.08 g/mg, respectively, while concentrations in the graviola flavonoid leaf extract being 122.8, 3.53, and 1.43, respectively.

**Table (3):** Quantifications of some phenolic, flavonoid, and isoflavonoid compounds identified in the methanolic extract of graviola parts using HPLC

| Peak No. | Phenolic Compound | RT (min) | *Conc. mg± SD/100g of parts for Custard apple | | |
|---|---|---|---|---|---|
| | | | Leaves (L) | Peels (PI) | Pulp(P) |
| | | Phenolic acids | | | |
| 1 | Coumarin | 11.779 | 0.5 ± 0.02 | 1.14 ± 0.04 | 0.23 ± 0.01 |
| 2 | Ellagic | 11.534 | 1.66 ± 0.06 | 2.58 ± 0.00 | 0.51 ± 0.01 |
| 3 | Oleuropein | 11.195 | 67.36 ± 0.44 | 6.51 ± 0.025 | 9.93 ± 0.05 |
| 4 | Salicylic | 10.917 | 2.6 ± 0.1 | 3.91 ± 0.07 | 0.59 ± 0.01 |
| 5 | Ferulic acid | 10.026 | 2.19 ± 0.01 | 5.06 ± 0.05 | 0.75 ± 0.02 |
| 6 | Caffeine | 8.482 | 1.15 ± 0.05 | 2.13 ± 0.01 | 0.63 ± 0.01 |
| 7 | Caffeic | 8.372 | 3.22 ± 0.19 | 1.65 ± 0.00 | 1.09 ± 0.11 |
| 8 | Vanillic | 8.303 | 3.99 ± 0.015 | 1.69 ± 0.00 | 2.16 ± 0.05 |
| 9 | P-OH-Benzoic | 7.77 | 9.05 ± 0.15 | 5.37 ± 0.00 | 1.20 ± 0.1 |
| 10 | Benzoic | 7.767 | 85.24 ± 0.03 | 58.04 ± 0.18 | 25.76 ± 0.25 |
| 11 | Chlorogenic | 7.701 | 13.43 ± 0.02 | 80.99 ± 0.01 | 1.61 ± 0.08 |
| 12 | Catechin | 7.122 | 50.45 ± 0.02 | 13.67 ± 0.01 | 20.41 ± 0.11 |
| 13 | 4-Amino-benzoic | 6.801 | 3.30 ± 0.05 | 1.76 ± 0.01 | 2.45 ± 0.15 |
| 14 | Catechol | 6.727 | 1.03 ± 0.00 | 4.86 ± 0.05 | 6.11 ± 0.2 |
| 15 | 3-OH-Tyrosol | 6.277 | 2.86 ± 0.05 | 1.29 ± 0.05 | 6.56 ± 0.15 |
| 16 | Gallic | 5.347 | 6.43 ± 0.05 | 1.29 ± 0.01 | 29.2 ± 3.04 |
| 17 | Pyrogallol | 4.823 | 8.01 ± 0.00 | 28.8 ± 0.00 | 1.63 ±0.11 |

| | | | | | |
|---|---|---|---|---|---|
| | | | Flavonoids | | |
| 1 | Rosmarinic | 10.82 | 1.68 ± 0.00 | 1.64 ± 0.01 | 1.48 ± 0.02 |
| 2 | Rutin | 10.971 | 1.49 ± 0.01 | 4.09 ± 5.77 | 1.65 ± 0.02 |
| 3 | Apigenin 7- glucose | 11.527 | 5.47 ± 0.01 | 3.07 ± 5.77 | 1.54 ± 0.02 |
| 4 | Quercitrin | 11.909 | 5.67 ± 0.01 | 3.33 ± 0.01 | 8.83 ± 0.24 |
| 5 | Naringin | 13.096 | 0.42 ± 0.01 | 0.23 ± 5.77 | 0.69 ± 0.01 |
| 6 | Naringenin | 13.112 | 1.98 ± 0.01 | 1.11 ± 0.00 | 2.66 ± 0.01 |
| 7 | Quercetin | 13.253 | 0.35 ± 5.77 | 0.76 ± 5.77 | 0.63 ± 0.02 |
| 8 | kaempferol 3-2-p-coumaryl-glucose | 13.809 | 4.12 ± 0.00 | 1.2 ± 0.00 | 1.09 ± 0.01 |
| 9 | Kaempferol | 14.599 | 2.38 ± 0.00 | 1.8 ±0.00 | 1.18 ± 0.01 |
| 10 | Apigenin | 14.886 | 1.83 ± 0.00 | 3.95 ± 0.00 | 3.41 ± 0.11 |
| 11 | Luteolin 7- glucose | 10.795 | 8.99 ± 5.77 | 4.14 ± 0.00 | 3.75 ± 0.05 |
| 12 | Hesperidin | 10.753 | 5.21 ± 5.13 | 1.30 ± 1.53 | 5.81 ± 0.01 |
| | | | Isoflavones | | |
| 1 | Isorhamnetin | 1.951 | 46.71 ± 0.03 | 18.09 ±0.5 | 30.47 ± 0.01 |
| 2 | Biochanin | 9.562 | 2.06 ± 0.00 | 3.41 ± 0.00 | 1.78 ± 0.05 |
| 3 | Daidzein | 3.166 | 6.78 ± 0.00 | 1.2 ± 0.01 | 4.78 ± 0.05 |
| 4 | Genistein | 4.017 | 0.81 ± 0.00 | 1.19 ±5.77 | 0.78 ± 0.04 |

*Average concentration of three HPLC determinations ± SD, RT; retention time in minutes.

### 3.3. Cytotoxicity effect of the methanolic extract of the parts of graviola

Table (4) presents the anti-proliferation of the cells was assessed by the sulphorhodamine-B (SRB) assay for 24 h in all the fruit part extracts of the graviola. And the anti-proliferative activity was observed in HepG-2 cells when different concentrations (0., 6.2, 12.5, 25, and 50 /ml) of methanolic extract of fruit parts were used. Table 4. shows that the concentration of methanolic extract of fruit parts (leaves, peels, and pulp) at 50µg / ml recorded the highest percentage of HEPG-2 dead cells (live cells of HEPG2 at 19.75, 24.25, and 24.47), respectively. HepG-2 cells were tested for cell viability in fruit part extracts at various concentrations for 24 hours. The control cells were 100% viable, and the viability decreased significantly ($P \leq 0.05$) with the increased concentration of the fruit part extracts. The percent decrease in cell viability was proportional to the concentration of graviola extracts, three parts extracts. Increasing the concentration of methanolic extract of leaves to 50µg /ml recorded a high percentage of HEPG-2 dead cells at 80.25 (the live cell of HEPG-2 was 19.75), while the increasing concentration of methanolic extract of peels to 50µg /ml recorded a high percentage of HEPG-2 dead cells at 75.75 (the live cell of HEPG-2 was 24.25), and by increasing the concentration of methanolic extract of pulp to 50µg / ml, recorded a high percentage of HEPG-2 dead cells to 50g/ml recorded a high percentage of HEPG-2 dead cells at 75.53 (the live cell of HEPG-2 was 24.47). The highest HEPG-2 dead cell percentage was recorded by the fruit part extract of all parts of the graviola *muricata* species, which is used in natural medicine in the tropics. The shoots, leaves, roots, fruits, and seeds were associated with the existence of some phenolic compounds: chlorogenic, oleuropein, salicylic, ferulic, caffeine, and catechins; and some flavonoids, especially rosmarinic acid, rutin, quercitrin, and apigenin. Also, *A. muricata* is a rich source of acetogenin-enriched extracts, and they revealed that the acetogenin-enriched extract was more toxic than the other extracts on cancer **Yang *et al* [31]**.

**Table (4):** Percent cell viability of HePG- 2 cell when treated with various fruit parts methanol extracts of *Custard apple* L

| concentration µg/ml | Leaves | Peels | pulp |
|---|---|---|---|
| control (0) | 100 ± 0.00 [a] | 100 ± 0.00 [a] | 100 ± 0.00 [a] |
| 6.2 | 65.30 ± 0.5 [b] | 71.55 ± 0.45[b] | 70.95 ± 0.05 [b] |
| | (- 34.7) | (-28.45) | (-29.05) |
| 12.5 | 40.65 ± 0.55[c] | 63.85 ± 0.65 [c] | 64.35 ± 0.15[c] |
| | (-59.35) | (-36.15) | (-35.65) |
| 25 | 33.00 ± 0.5[d] | 37.75 ± 0.25 [d] | 38.45 ± 0.05[d] |

| | | | |
|---|---|---|---|
| | (-67) | (-62.25) | (-61.55) |
| 50 | 19.75 ± 0.25$^{e}$ | 24.25 ± 0.25$^{e}$ | 24.47 ± 0.06 $^{e}$ |
| | -80.25 | (-75.75) | (-75.53) |

Means ± S.D of Three individual observations values in parentheses are percent change over control
Donates percent decrease over control.
* Denotes those values are significant at p< 0.05

Table (5) shows cytotoxic effects with the $IC_{50}$ value of graviola methanolic extract. Data appeared that fruit part extracts of graviola showed cytotoxic effects with $IC_{50}$ values of 10.52, 15.52, and 19 µg /ml of leaves, peels and pulp methanol extracts in the HEPG-2 cell line, respectively. **Hemalatha *et al* [32]** decided that the Methanol extract showed higher activity when compared with the other four isolates. Therefore, this study revealed that the methanol extract had cytotoxic activity. The juice of the *Annona Muricata* fruit was purified using chromatographic techniques and HPLC. These isolates exhibited antiproliferative activity against human prostate cancer PC-3 cells. Apart from the leaves and fruits of the *muricata,* the stem also exhibited a cytotoxic effect against a variety of cell lines, including U937 and Histiocyte lymphoma cell lines, with $IC_{50}$ of 10.5, 18.2, and 60.9g/ml produced by ethyl acetate, hexane, and methanol extracts, respectively **Valencia *et al* [33]**.

**Table (5):** $IC_{50}$ values of HepG-2 cell when treated with fruit parts methanol extracts of *Custard apple* L.

| S. No. | Fruit parts methanol extract | $IC_{50}$ |
|---|---|---|
| 1 | Leaves | 10.52 |
| 2 | Peels | 15.52 |
| 3 | Pulp | 19.00 |
| | | |

$IC_{50}$ = the least $IC_{50}$ value for 24 h i.e., 50%viability loss against HePG-2 cells was observed in methanol extract; $IC_{50}$ = Median lethal dose

### 3.4. Chemical analysis, Mineral and vitamin contents of graviola pulp

Table (6) displays the chemical analysis (protein, fat, crude fiber, ash, available carbohydrates, total soluble sugar, TSS) of the graviola pulp on dry matter. The protein content found for the graviola pulp was 8.02 ± 0.13%, higher than that established by **Souza *et al* [34]**, making this flour inadequate for incorporation into food formulations for protein enrichment. The ash content of a food product refers to the inorganic residue that remains from burning of organic matter. From the results, the ash content observed, around 3.51± 0.02%, associated with the presence of minerals in the pulp that was retained after the dehydration process, providing evidence that the production of flour from fruits and vegetables is rich in minerals necessary for human consumption **[1]**. The fat content of graviola pulp was found to be 3.97± 0.1%. The pulp contained 3.750.48% crude fiber, and fruits and vegetables are known to contain high fiber levels that can benefit the muscles of the large and small intestines in the human diet. Available carbohydrates, total soluble sugars, and TSS contents of the graviola pulp were 80.75±0.52, 33.7 ± 0.01, and 26.43±0.01%, respectively. Table 6. shows the mineral content of the dried graviola pulp. The obtained data showed that graviola pulp recorded the highest in elements such as Mg, K, Zn, and Ca were 564.57, 409.01, 250, and 227.7 mg/100g, respectively. The pulp of a sub-specie *Annona b.* contained calcium, magnesium, copper, manganese, phosphorus, iron, zinc, and sodium with values of 84.33, 56.78, 0.38, 0.33, 55.20, 20.73, 0.31, and 45.24%, respectively **[35]**. The results of this study assured that sufficient amounts of minerals,

such as calcium, in dried pulp, are crucial for strong bones and teeth. Essential minerals are also in charge of regulating blood pressure and preserving the body ideal pH balance. Table 6. presents the vitamin content of graviola pulp. The table revealed that fresh graviola pulp contains Vitamin A (2.72 mg), Vitamin B: Thiamin (B1) (27.5 mg), Riboflavin (B2) (28.34 mg), B12 (56.5 mg), Folic acid (B9) (2.64 mg), Pyridoxine (B6) (50 mg), and Vitamin C (473.33 mg) per 100 g of edible pulp. **Khan *et al* [36]** said that custard apple protects against illnesses such as heart attack and cancer due to different minerals and vitamins. The custard apple provides the body's water equilibrium and controls blood pressure fluctuations. A custard apple provides healthy skin due to the presence of vitamins and works to enhance eyesight by presenting vitamin custard apple fruit. The amount of magnesium in custard apples contributes to maintaining the body's water balance by reducing the risk of arthritis and taking away different acids due to high magnesium. Fruit consumption reduces the risk of vomiting and deficiency in vitamin B6. The custard apple is full of vitamin C antioxidants, which help to combat many diseases and enhance the immune system.

**Table (6):** Chemical analysis, Minerals, and vitamins contents of gravolia pulp (*Custard apple* L.)

| Gravolia analysis | |
|---|---|
| Chemical analysis g/100g (D.W.) | |
| Protein (×6.25) | 8.02 ± 0.13 |
| Ash | 3.51 ± 0.02 |
| Fat | 3.97±0.1 |
| crude fiber | 3.75±0.48 |
| TC | 80.75±0.52 |
| Total soluble sugar(mg/100g) | 33.7 ± 0.01 |
| TS S | 26.43±0.01 |
| Mineral mg/100g (D.W.) | |
| Ca | 227.7 |
| K | 409.01 |
| Mg | 564.57 |
| P | 80.3 |
| Zn | 250 |
| Mn | 1.72 |
| Cu | 215 |
| Vitamins (mg/100g) (F.W.) | |
| Vit. A | 2.72 |
| B1 | 27.5 |
| B2 | 28.34 |
| B12 | 56.5 |
| B9 | 2.64 |
| B6 | 50 |
| Vit. C | 473.33 |

D.W: on dry weight; F.W: on fresh weight; AC: Available Carbohydrates: calculated by difference; T.S.S: Total soluble solid; values expressed as mean± S.D.

### 3.5. Sensory evaluation

Table (7) announces the sample's sensory test and then its estimation to measure the acceptability of these products, revealing a final judgment on the preferences of the products relative to the control. The results indicated that pies with graviola pulp juice were substituted with dough water and the filling custard made from graviola juice had high sensory scores in comparison with the control. There was no difference ($P \leq 0.05$) between the control and the 75/50 pie sample. A significant difference ($P \leq 0.05$) could be detected between 75/75% of the pie samples. The most preferable of all the substituted pies samples are characterized by their rich flavor and

taste due to graviola pulp juice. The mean score for the overall acceptability of graviola pies varied from 8.59 to 8.98, where the maximum value was obtained for 50/50 % graviola pies. Also, samples substituted with 75/75% recorded a slightly significant difference for some attributes such as texture, odor, and OAA. **Souza *et al* [34]** indicated that custard apple bagasse flour (*Annona squamosa* L.) (CAB) was produced and incorporated into cookie formulations in different proportions (5 to 50%). The study also evaluated most of the measurements to evaluate the highest priority compared to the sample containing wheat flour, as it allows for increasing the nutritional value in the proposed formulations.

**Table (7):** Sensory evaluation of gravoila pies

| Samples | Appearance | color | Taste | Texture | Odor | OAA |
|---|---|---|---|---|---|---|
| Control | 8.8 ± 0.4 $^{a}$ | 9 ± 0.00$^{a}$ | 8.8 ± 0.4$^{a}$ | 9 ± 0.00 $^{a}$ | 8.8 ±0.4$^{ab}$ | 8.88±0.16$^{ab}$ |
| 25/25 | 8.8 ± 0.4 $^{a}$ | 9 ± 0.00$^{a}$ | 8.8 ± 0.4$^{a}$ | 9 ± 0.00$^{a}$ | 8.8 ±0.4$^{ab}$ | 8.88 ±0.16$^{ab}$ |
| 25/50 | 9 ± 0.00 $^{a}$ | 9 ± 0.00$^{a}$ | 8.67 ± 0.41$^{a}$ | 8.83±0.41$^{ab}$ | 8.83±0.41$^{ab}$ | 8.87 ±0.18$^{ab}$ |
| 25/75 | 8.83± 0.26$^{a}$ | 8.75 ±0.27$^{a}$ | 8.83 ±0.26$^{a}$ | 8.83±0.41$^{ab}$ | 8.92 ± 0.20$^{a}$ | 8.83 ±0.24$^{ab}$ |
| 50/25 | 8.83 ± 0.26$^{a}$ | 8.67 ±0.52$^{a}$ | 8.67 ± 0.52$^{a}$ | 8.67±0.52$^{ab}$ | 8.75±0.42$^{ab}$ | 8.72 ±0.44$^{ab}$ |
| 50/50 | 8.97 ± 0.08$^{a}$ | 8.97 ±0.08 $^{a}$ | 8.97 ±0.08$^{a}$ | 9 ± 0.00$^{a}$ | 9 ± 0.00$^{a}$ | 8.98 ± 0.05$^{a}$ |
| 50/75 | 8.83 ± 0.26$^{a}$ | 8.92 ± 0.20$^{a}$ | 8.92 ±0.20$^{a}$ | 9 ± 0.00$^{a}$ | 9 ± 0.00$^{a}$ | 8.93 ±0.16$^{ab}$ |
| 75/25 | 8.67 ± 0.52$^{a}$ | 8.67 ± 0.52 $^{a}$ | 8.67 ±0.52$^{a}$ | 8.67±0.52$^{ab}$ | 9 ± 0.00$^{a}$ | 8.73 ±0.41$^{ab}$ |
| 75/50 | 9 ± 0.00$^{a}$ | 9 ± 0.00 $^{a}$ | 8.97 ±0.20$^{a}$ | 9 ± 0.00$^{a}$ | 8.92 ± 0.20$^{a}$ | 8.97 ± 0.08$^{a}$ |
| 75/75 | 8.67 ± 0.52$^{a}$ | 8.83± 0.41$^{a}$ | 8.5 ± 0.55 $^{a}$ | 8.5 ± 0.45$^{b}$ | 8.47 ±0.52$^{b}$ | 8.59 ± 0.35$^{b}$ |

OAA: Overall acceptability
Means in the same column with different letters are significantly different ($p \leq 0.05$). Each mean value is followed by ± SD (standard deviation) for triplicate of samples.

### 3.6. The nutritive value

Table (8) shows the nutritional value of graviola pies at different substitution levels as a substitute for the kneading water and custard milk filling with graviola pies. The graviola pulp pies were composed of 11.01–16.79% protein, 1.78–2.22% ash, 21.48–28.19% fat, 2.00–2.65% crude fiber, and 52.98–59.86% available carbohydrates, depending on the substituted dough water and custard milk as filling with the product. The caloric value of the pies ranged from 489.81 to 523.47 Kcal/100g samples. The results in caloric value may be due to the effects of graviola pulp substituted in pies. The table included that 75/25% and 75/50% pie samples recorded the lowest values in caloric value compared with control pies due to a decrease in fat content. Graviola is naturally sweet and therefore makes an excellent nutritious snack and may even be added to desserts, particularly for children. The fruit is a comparatively high-calorie product and is thus included in the diet for weight gain and for athletes. Each 100g edible portion contains 104 calories.

**Table (8):** Nutritive value of pies made from gravoila pulp (on dry weight)

| Samples | Protein (g/ 100g) | Ash g/ 100g | Ether extract g/ 100g | Crude fiber g/ 100g | AC g/ 100g | Energy (Kcal/100g) |
|---|---|---|---|---|---|---|
| Control | 15.17 ± 0.34$^{e}$ | 2.06±0.01$^{bc}$ | 26.02 ±0.29$^{d}$ | 2.44±0.01$^{bcd}$ | 54.31 ± 0.61$^{e}$ | 512.1±1.53$^{bc}$ |
| 25/25 | 13.75 ± 0.07$^{f}$ | 2.06±0.00$^{bc}$ | 26.65 ±0.11$^{c}$ | 2.51±0.01$^{abc}$ | 55.04 ± 0.04$^{d}$ | 514.97±0.51$^{b}$ |
| 25/50 | 14.46 ±0.06$^{d}$ | 2.1 ± 0.12$^{b}$ | 28.19 ±0.59$^{a}$ | 2.27 ± 0.02$^{de}$ | 52.98 ± 0.16$^{f}$ | 523.47±4.43$^{a}$ |
| 25/75 | 14.15 ± 0.08$^{e}$ | 1.78 ±0.04$^{e}$ | 27.23±0.09$^{b}$ | 2.00 ± 0.03$^{f}$ | 54.85 ± 0.23$^{de}$ | 521.03±0.17$^{a}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50/25 | 16.79 ±0.01[a] | 2.22 ±0.0[a] | 22.47 ±0.58[b] | 2.59 ± 0.03[ab] | 55.94 ± 0.55[c] | 494.12±3.04[e] |
| 50/50 | 15.13 ±0.08[c] | 2.01±0.02[cd] | 24.54 ±0.17[f] | 2.23 ±0.19[e] | 56.09 ± 0.3[c] | 505.75±0.03[d] |
| 50/75 | 15.95 ±0.18[b] | 2.11 ± 0.02[b] | 26.24±0.11[cd] | 2.65 ± 0.04[a] | 53.05 ± 0.24[f] | 512.17±0.77[bc] |
| 75/25 | 14.26±0.16[de] | 2.07±0.05[bc] | 21.48 ±0.57[l] | 2.33±0.02[cde] | 59.86 ± 0.35[a] | 489.81 ±3.11[e] |
| 75/50 | 13.66 ±0.04[f] | 1.95± 0.01[d] | 23.76 ±0.06[g] | 2.04 ± 0.01[f] | 58.60 ± 0.01[b] | 502.82 ±0.36[d] |
| 75/75 | 11.01 ±0.11[g] | 1.95 ± 0.04[d] | 25.27 ±0.06[e] | 2.02 ± 0.02[f] | 59.76 ± 0.11[a] | 510.47 ± 0.50[c] |

Means in the same column with different letters are significantly different ($p \leq 0.05$). Each mean value is followed by ± SD (standard deviation) for a triplicate of samples

### 3.7. TPC, AOA, and mineral content

Table (9) shows the TPC, AOA, and mineral content of grvoila pies. According to Table 9., the TPC for extracts of pie, which substituted dough water and custard milk with graviola pulp juice at different percent from 0% to 75%, varying from 235.20 to 577.92 mg GAE/g. The pie samples of graviola pulp with 50/75% juice presented higher concentrations of phenolic compounds than the other pie samples. In this way, graviola pulp had a significant number of phenols able to act as natural antioxidants capable of reducing degenerative diseases such as arteriosclerosis, cardiovascular disease, and cancer **Bhat and Yahya [37]**. The results observed for the sequestering AOA of the ethanol extracts showed a variation of 8.97–29.70%, the lowest values recorded for control pies. The phenolic compounds are associated with vitality functions in plants, including pigmentation and defense, as well as in fruit peels and seeds, and to a lesser extent in pulp, respectively. **Souza *et al* [34]** studied that custard apple bagasse flour (*Annona squamosa* L.) (CAB) was produced and incorporated into cookie formulations in varying proportions (5-50%) phenolic compound evaluation. The results showed that this phenolic composition detected in CAB flour and cookie preparations showed values ranging from 200 to 658 mg GAE/100 g. The flour and formulations made from tropical fruit residues are similar in results. DPPH inhibition showed a contrast of 9.68-10.75%. Table 9. shows the mineral content in graviola pies. The mineral contents of samples that replaced dough water and custard milk with graviola pulp juice at various percentages ranging from 0% to 75%, as shown in the table, varied across all minerals and had the highest values when compared to the control. Graviola pulp pie samples with 75/50% juice had higher mineral content values than the other pie samples. Mineral values discovered include the incorporation of graviola pulp as a supplement and nutrient supplementation in promising food products such as wheat cereal flours used in bread products, which are generally deficient in minerals essential to human nutrition **Bhat and Yahya [37]**.

**Table (9):** TPC, AOA, and Mineral content of gravoila pies

| Samples | TPC (mg/g Gallic acid) | AOA* | Mineral (mg/100g) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ca | k | Mg | Zn | Mn | Fe |
| Control | 235.20 ±0.72[i] | 8.97 ± 0.18[f] | 97.73 | 156.56 | 80.93 | 1.13 | 0.84 | 2.01 |
| 25/25 | 395.83±0.87[b] | 19.19 ± 0.45[e] | 83.03 | 164.17 | 65.69 | 1.13 | 0.82 | 2.62 |
| 25/50 | 440.43f±4.87[f] | 21.26 ± 0.54[d] | 85.49 | 203.74 | 71.02 | 1.11 | 0.82 | 2.86 |
| 25/75 | 569.00±3.63[b] | 24.31 ± 0.44[b] | 88.2 | 207.5 | 79.04 | 1.2 | 0.88 | 2.88 |
| 50/25 | 459.32±2.96[e] | 21.26 ± 0.07[d] | 85.52 | 204. 85 | 82.74 | 1.54 | 0.87 | 2.88 |
| 50/50 | 492.5± 5.13[d] | 23.31 ± 0.41[c] | 85.67 | 206.76 | 84.05 | 1.58 | 0.88 | 2.73 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 50/75 | 577.92±2.56[a] | 29.70 ± 0.42[a] | 93.59 | 227.7 | 85.42 | 1.76 | 0.89 | 2.91 |
| 75/25 | 422.49±3.21[g] | 21.27 ± 0.51[d] | 89.18 | 214.2 | 85.4 | 1.8 | 0.84 | 2.93 |
| 75/50 | 541.20±1.51[c] | 23.61 ± 0.18[c] | 98.14 | 300.97 | 102.6 | 1.54 | 0.89 | 2.97 |
| 75/75 | 571.28±0.53[b] | 24.63 ± 0.09[b] | 92.52 | 224.3 | 75.3 | 2.23 | 0.87 | 2.47 |

TPC: Total phenolic content expressed as milligrams of Gallic acid equivalents (GAE); AOA*: antioxidant activity-calculated as percent discoloration of 2,2-diphenyl-1-picrylhydrazyl (DPPH),
Means in the same column with different letters are significantly different ($p \leq 0.05$). Each mean value is followed by ± SD (standard deviation) for triplicate of samples.

## 4. Conclusion

This study aimed to assess the functional properties of different parts of the graviola in terms of their phytochemical composition, minerals, vitamins, and health benefits. The phenolic compounds analyzed by chromatography were oleuropein, chlorogenic, and gallic. These were the significant components. Moreover, luteolin 7-glucose and quercetin were the most dominant identified flavonoids. In addition, isorhamnetin was a major identified isoflavone. A biological experiment showed that 10.52, 15.52, and 19.00 µg/ml were the lethal and the lowest doses for liver cancer. Functional pies were rich in nutritional composition and antioxidant activity and minerals was obtained. In conclusion, we draw the authorities concerned with these benefits to consider them useful as a nutritional and health pie helping in complementary treatment for liver tumor HePG-2.

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## 6. References


1. Clerici, M. T. P. S., Kallmann, C., Gaspi, F. O. G., Morgano, M. A., Martinez-Bustos, F. and Chang, Y. K. Physical, chemical, and technological characteristics of *Solanum lycocarpum* A. St.-HILL (Solanaceae) fruit flour and starch. *Food Research International*, 44(7), 2143-2150(2011). doi:10.1016/j.foodres.2011.01.060
2. Kumar, M., Changan, S., Tomar, M., Prajapati, U., Saurabh, V., Hasan, M., Sasi, M., Maheshwari, C., Singh, S., Dhumal, S., Radha; Thakur, M.; Punia, S.; Satankar, V.; Amarowicz, R. and Mekhemar, M. Custard Apple (*Annona squamosa* L.) Leaves Nutritional Composition, Phytochemical Profile, and Health-Promoting Biological Activities. *Biomolecules*, 11(5), 614-636 (2021).
3. Virgen-Ceceña, L. J., Anaya-Esparza, L. M., Coria-Téllez, A. V., García-Magaña, M. D. L., García-Galindo, H. S., Yahia, E. and Montalvo-González, E. Evaluation of nutritional characteristics and bioactive compounds of soursop-yogurt and soursop-frozen dessert. *Food Science and Biotechnology*, 28 (5),1337-1347(2019).
4. Al-Nemari, R., Al-Senaidy, A., Semlali, A., Ismael, M., Badjah-Hadj-Ahmed, A. Y. and Ben Bacha, A. GC-MS profiling and assessment of antioxidant, antibacterial, and anticancer properties of extracts of Annona squamosa L. leaves. BMC Complementary Medicine and Therapies, 20(1), 1-14 (2020). [CrossRef] doi: 10.1186/s12906-020-03029-9
5. Anaya-Esparza, L. M., de Lourdes Garcia-Magana, M., Domínguez-Ávila, J. A., Yahia, E. M., Salazar-López, N. J., González-Aguilar, G. A. and Montalvo-González, E. Annona's: Underutilized species as a potential source of bioactive compounds. *Food Research International*, 138, 109775 (2020). [CrossRef]
6. Mariod, A. A., Abdelwahab, S. I., Elkheir, S., Ahmed, Y. M., Fauzi, P. N., and Chuen, C. S. Antioxidant activity of different parts from *Annona squamosa*, and *Catunaregam nilotica* methanolic extract. *Acta Scientiarum Polonorum. Technologia Alimentaria*, 11(3), 249- 258 (2012). PMid:22744945.
7. Rady, I., Bloch, M. B., Chamcheu, R. C. N., Banang Mbeumi, S., Anwar, M. R., Mohamed, H., Babatunde, A. S., Kuiate, J., Noubissi, F. K., El Sayed, K. A., Whitfield, G. K. and Chamcheu, J. C. Anticancer Properties of Graviola (*Annona muricata*): A Comprehensive Mechanistic Review. *Oxidative medicine and cellular longevity*, 11(2), 1-39 (2018). https://doi.org/10.1155/2018/1826170.
8. Frankish, H. 15 million new cancer cases per year by 2020, says WHO. *The Lancet*, 361(9365), 1278 (2003).
9. Nussbaumer, S., Bonnabry, P., Veuthey, J. L. and Fleury-Souverain, S. Analysis of anticancer drugs: a review. *Talanta*, 85(5), 2265–2289 (2011).
10. Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M. and Kadir, H. A. *Annona muricata* (Annonaceae): a review of its traditional uses, isolated acetogenins, and biological activities. *International Journal of Molecular Sciences*, 16 (7), 15625–15658. (2015).
11. Peter, B., Bosze, S. and Horvath, R. Biophysical characteristics of proteins and living cells exposed to the green tea polyphenol epigallocatechin-3-gallate (EGCg): a review of recent advances from molecular mechanisms to nanomedicine and clinical trials. *European Biophysics Journal*, 46(1), 1–24 (2017).
12. Gullett, N. P., Ruhul Amin, A. R. M., Bayraktar, S., Pezzuto, J. M., Shin, D. M., Khuri, F. R., Aggarwal, B. B., Surh, Y.- J. and Kucuk, O. Cancer prevention with natural compounds. *In Seminars in Oncology*, 37(3), 258–281(2010). WB Saunders. doi: 10.1053/j.seminoncol.2010.06.014
13. Torres, M. P., Rachagani, S., Purohit, V., Pandey, P., Joshi, S., Moore, E. D., Johansson, S. L., Singh, P. K., Ganti, A.K. and Batra, S. K. Graviola: a novel promising natural-derived drug that inhibits tumorigenicity and metastasis of

pancreatic cancer cells in vitro and in vivo through altering cell metabolism. *Cancer Letters*, 323(1), 29-40 (2012).
14. Clarkson, J. Pie: A Global History, Reaktion Books (2009).
15. Rajesh, S., Sivakumari, K., Ashok, K. and Abitha, A. R. Anti-cancer activity of *Cardiospermum halicacabum* Linn. leaf extracts against hepatocellular carcinoma cell line (Hep-G2). *World J Pharm Sci*, 5(3), 1133-1154 (2016).
16. Onoja, S. O., Omeh, Y. N., Ezeja, M. I. and Chukwu, M. N. Evaluation of the in vitro and in vivo antioxidant potentials of *Aframomum melegueta* methanolic seed extract. *J. Tropical Med.*, Article ID 159343, 1-6 (2014). **https://doi.org/10.1155/2014/159343.**
17. Pongjanta, J. Cereal Product Technology Laboratory Manual. *Lampang Agricultural Research and Training Center, Raja Mangala Institute of Technology. Lampang. Thailand.* (2002).
18. Watts, B. M., Ylimaki, G. L., Jeffery, L. E. and Elias, L. G. Basic sensory methods for food evaluation. IDRC, Ottawa, ON, CA. (1989).
19. Association of Official Agriculture Chemists (AOAC) *AOAC Official Methods of Analysis*,17th ed., AOAC, Washington, DC. (2000).
20. Latimer, G.W.J. International A., and Cunniff P., *Official Methods of Analysis of AOAC International*. (2016).
21. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T. and Smith, F. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356 (1956).
22. Singleton, V.L. and Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic- phosphor tungstic acid reagents. *Am. J. Enol. Vitic.*, 16(3), 144-158 (1965).
23. Zhishen, J., Mengcheng, T. and Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals", *Food Chemistry*, 64 (4), 555-559 (1999).
24. Blois, M. S. Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199-1200 (1958). ..**https://doi.org/10.1038/1811199a0**
25. Khalil, M. Y., Moustafa, A. A. and Naguib, N. Y. Growth, phenolic compounds, and antioxidant activity of some medicinal plants grown under organic farming conditions. *World Journal of Agricultural Sciences*, 3(4), 451-457 (2007).
26. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *JNCI: Journal of the National Cancer Institute*, *82*(13), 1107-1112 (1990).
27. Ott, L. An introduction to statistical methods and data analysis. 3rd ed. PWS-Kent, Boston, M.A. (1988).
28. Nandhakumar, E. and Indumathi, P. In vitro Antioxidant Activities of Methanol and Aqueous Extract of Annona squamosa (L.) Fruit Pulp. *J. Acupunct Meridian Stud*, 6 (3), 142- 148 (2013). **http://dx.doi.org/10.1016/j.jams.2012.09.002**
29. Tiencheu, B., Egbe, C. A., Achidi, U. A., Tenyang, N., Tiepma, N. E. F., Djikeng, F. T. and Fossi, T. B. Nutritional, Organoleptic and Phytochemical Properties of Soursop (*Annona muricata*) Pulp and Juice after Postharvest Ripening. *Eur. J. Nutr. Food Saf*, 13(1), 15-28 (2021).
30. Jimenez, V. M., Gruschwitz, M., Schweiggert, R. M., Carle, R. and Esquivel, P. Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization mass spectrometric detection. *Food Research International*, 65, 42-46 (2014). **http://dx.doi.org/10.1016/j.foodres.2014.05.05**
31. Yang, C., Gundala, S. R., Mukkavilli, R., Vangala, S., Reid, M. D. and Aneja, R. Synergistic interactions among flavonoids and acetogenins in Graviola (*Annona muricata*) leaves confer protection against prostate cancer. *Carcinogenesis*, 36 (6), 656–665 (2015). **doi:10.1093/carcin/bgv046**
32. Hemalatha, G., Sivakumari, K., Rajesh, S. and Shyamala Devi, K. Phytochemical profiling, anticancer and apoptotic activity of graviola (*Annona muricata*) fruit extract against human hepatocellular carcinoma (HepG-2) cells. *Int. J. Zool. Appl. Biosci*, 5(1), 32-47(2020). **https://doi.org/10.5281/zenodo**
33. Valencia, L., Muñoz, D. L., Robledo, S. M., Echeverri, F., Arango, G. J., Vélez, I. D. and Triana, O. Actividad tripanocida y citotóxica de extractos de plantas colombianas. *Biomédica*, 31(4), 552–559(2011).
34. Souza, F. T. C., Santos, E. R., Silva, J. D.- C., Valentim, I. B., Rabelo, T. C. B., de Andrade, N. R. F. and Silva, L. K. D.- S. Production of Nutritious Flour from Residue Custard Apple (*Annona squamosa* L.) For the Development of New Products. Journal *of Food Quality*, Volume 2018 |Article ID 5281035, pages 10 (2018). **https://doi.org/10.1155/2018/5281035**
35. Shehata, M. G., Abu-Serie, M. M., Abd El-Aziz, M. N. and El-Sohaimy, S. A. Nutritional, phytochemical, and in vitro anticancer potential of sugar apple (*Annona squamosa*) fruits. *Scientific Reports*, 11(1), 1-13 (2021). **https://doi.org/10.1038/s41598-021-85772-8**
36. Khan, N. M, Noreen, S., Hassan, F., Ismail, M. A., Ambreen, A., Iftikhar, R. and Riaz, K. Nutritional and pharmacological potential and Health benefits of Annona cherimoya (custard apple*)*: A Review. *Bioscience Research*, 18m (3), 2348-2354 (2021).
37. Bhat, R. and binti Yahya, N. Evaluating belinjau (*Gnetum gnemon* L.) seed flour quality as a base for development of novel food products and food formulations. *Food Chemistry*, 156, 42-49 (2014).