"The antibacterial, antioxidant, and antiproliferative effects of guava fruit peel extracts against HT29 colon cancer cell lines"

Vino Udappusamy and RajanThinakaran*

*Department of Biochemistry, PSG College of Arts & Science, Coimbatore-641 014Tamilnadu, India.

* **Corresponding author: RajanThinagaran (E-mail: rajan@psgcas.ac.in)**

Abstract - The study used quantitative ecological approaches to quantify the number of species, variety, and how the environment affects plant evolution through time. *Psidium guajava* has many biological characteristics, including antimicrobial and anticancer properties. This project intends to uncover, define, and assess phytochemical substances as colon cancer antioxidants. The fruit peel extract contained alkaloids (35.5 mg/g), flavonoids (14 mg/g), phenols (39.5 mg/g), and tannins (35 mg/g). Fourier Transform Infrared Spectroscopy identified functional groups in ethanolic guava peel extract. Guava fruit peel extract's antimicrobial properties were examined. *Staphylococcus aureus* and *Enterobacter aerogenes* were particularly sensitive. *Psidium guajava* peel extract inhibited 76.29% of free radicals at 500 mg/ml. *Psidium guajava* extract was non-toxic to normal cells but harmful to HT29 colon cancer cells (IC50 = 75.51 µg/ml). According to the results, fruit peel extract could treat colon cancer.

Keywords: *Psidium guajava*, colon cancer, antimicrobial activity, Antioxidant activity

Abbreviations:

HCl- Hydrochloric acid
$FeCl_3$- Ferric chloride.
MHA- Mueller Hinton agar
H_2O_2-Hydrogen peroxide
KBr - Potassium bromide
BDI – Biodiversity index
BHT - Butylated hydroxytoluene
UV-Vis- Ultraviolet–visible spectroscopy
FTIR - Fourier-transform infrared spectroscopy
NO - Nitric oxide
DPPH - 2,2-diphenyl-1-picrylhydrazyl
µl - Microliter
DNA- Deoxyribonucleic acid
DMSO - Dimethyl sulfoxide
PBS- Phosphate-buffered saline
NCCS - National Centre for Cell Science
DMEM - Dulbecco's Modified Eagle Medium

PGPE- *Psidium guajava* Peel extract

INTRODUCTION

Herbal therapy has been the most common and safest technique for treating illness since prehistoric times. It has had a profound impact on the evolution of primary healthcare. Plant-based drugs can treat cancer because they are effective, affordable, and have few side effects [1]. This article describes the anticancer properties of *Psidium guajava*, Annona squamosa, Mangifera indica, Artocarpus heterophyllus, and Manilkara zapota fruit extracts. This study shows that a fruit extract from a common plant has anticancer properties and could be a promising treatment for breast cancer. The fruit of the *Myrtaceae* family tree *Psidium guajava L.* is high in vitamins, fibre, and minerals. Guava has anti-inflammatory, antitumor, antibacterial, anti-diabetic, and anticancer effects. Plants generate medicinal phytochemicals in their leaves, fruits, seeds, peels, pulp, bark, and oil [2]. According to new research, bioactive phytochemicals in guava have varying effects on various forms of human cancer. Numerous studies have shown that particular guava sections have anticancer benefits. However, there has never been a complete study of all available scientific data. This study aims to provide a comprehensive picture of how guava-derived products and chemicals can help prevent and treat cancer.

Cancer is a non-contagious disease that can drastically alter one's life. As a result of the rapid advancement of technology, there are an increasing number of fatal inherited conditions, such as cancer. In addition, long-term exposure to harmful substances can affect the way the body operates and cause genetic damage. Furthermore, smoking, excessive alcohol consumption, and a lack of consuming fruits and vegetables may enhance your chances of acquiring cancer [3]. Cancer cells spread to different tissues as a result of metastasis. Early detection and treatment cannot cure an illness but help people live longer and cope better. Cancer of the colon begins anywhere in the large intestine and spreads throughout the digestive tract. It is the world's third most frequent type of cancer. A change in a person's genes, surroundings or lifestyle, or bad diet may all contribute to colon cancer [4].

The major causes of colorectal cancer include chromosomal instability, CpG island methylator phenotype, and microsatellite instability (CRC). Colon cancer risk factors include the environment, how people eat when they are overweight, and how much energy they take. In recent studies, numerous chemicals from plant seeds, fruits, bark, roots, and leaves have been shown to suppress cancer development. Green-well and Rahman, 2015. However, cytotoxic medications, linked to many diseases, frequently affect malignant and healthy cells. As a result, there is an urgent unmet need to discover new cancer treatments, as current therapies are insufficient. The ultimate goal is to develop new cancer treatments that are more selective, effective, and without side effects. Phytochemicals, natural plant-based substances, have been used in traditional medicine to treat various ailments and issues. People understand that these compounds are better than chemical medicines since they are less expensive, safer, and can be taken orally. With recent advancements, research on medicinal plants has been undertaken to uncover bioactive compounds that combat cancer [5].

Based on what is known, plant leaves and bark have been used to cure many diseases for a very long time. On the other hand, fruits' antibacterial and antioxidant properties are less well understood. In addition, few studies have been conducted to determine whether or not

fruits and vegetables can combat germs. As a result, discovering fruits with therapeutic powers is critical [6]. The Magnoliophyta, Magnoliopsida, and *Myrtaceae* families all include *Psidium guajava*. The guava is another name for this fruit. The *Psidium guajava* plant's leaves, petals, bark, seeds, and other parts can cure many health problems. The guava fruit is rich in vitamin A, vitamin C, iron, phosphorus, and calcium. It also includes the active phytochemicals guaijavarin, quercetin, saponin, oleanolic acid, lyxopyranoside, and arabopyranoside, as well as the flavonoids saponin, oleanolic acid, lyxopyranoside, and arabopyranoside. The principal components of guava are ascorbic acid and citric acid, which play an essential role in the fruit's ability to prevent mutations. The skin of the guava fruit has more ascorbic acid than the flesh [7]. There have been few studies on utilizing *Psidium guajava* to cure cancer, but none on using fruit peel extract to treat colon cancer. Furthermore, previous research has not established that *Psidium guajava* ethanolic fruit peel extract has antioxidant properties. Therefore, this research aims to discover how the phytochemical components and antioxidant properties of *Psidium guajava* can help treat colon cancer.

MATERIALS AND METHODS

Field survey and Identification of specimens: Numerous plants were collected and identified using spot identification during the field trip. Botanical Survey of India, Southern Circle, Coimbatore, the presidency of Flora of Madras [8] and Excursion Flora of Central Tamil Nadu, India, confirm the plants. Taxonomists resolve other types of uncertainty.

Phytosociological Studies: The study used species area estimation and quadrant analysis [9]. The size of each quadrant was fixed using the species-area curve method, and seven quadrates were chosen randomly. Plant species and individual specimens from each quadrant were catalogued. The vegetation data were analyzed to calculate diversity indices, species richness, Shannon- Weiner diversity (H'), and the Simpson index [10].

Frequency, density and abundance were calculated using the following formulae:

$$\textbf{Frequency (F)} = \frac{\text{Number of quadrants in which the species present}}{\text{Total number of quadrates studied}} \text{ X100}$$

$$\textbf{Abundance (Ab)} = \frac{\text{Number of individuals of the species in all quadrants}}{\text{Number of quadrants of occurrence of the species}}$$

$$\textbf{Density (D)} = \frac{\text{Number of individuals of the species in all quadrants}}{\text{The total number of quadrants studied}}$$

$$\textbf{Relative density} = \frac{\text{Number of individuals of a species}}{\text{Total number of individuals}} \text{ X100}$$

$$\textbf{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Sum frequency of all species}} \text{ X100}$$

Diversity indices: A biodiversity Calculator for Simpson and Shannon Indexes was used. The BDI used in this study that was selected from young's manipulation is Alpha Biodiversity [α], known as the "Shannon Index" (short version) or "Shannon-Wiener Biodiversity Index" (full version) (BDI) [11] and https://www.alyoung.com/labs/biodiversity_calculator.html).

Collection of samples and processing of fruit peel extract of *Psidium guajava*:

The Palani market in Dindigul district, Tamil Nadu, India, is where fresh *Psidium guajava* fruits are gathered. The fruits were washed with tap water before being rinsed with distilled water. The fruit's skin was then peeled and dried for 45 to 50 days before being ground with a mechanical blender. It is extracted in a soxhlet apparatus with benzene, acetone, ethyl acetate, ethanol, and water. After that, the extract was dehydrated in a desiccator. The extractive value of the various extracts was also calculated using the Harborne biochemistry method [12, 13].

Phytochemical analysis of an ethanolic extract of *Psidium guajava*: The dried fruit peels were evaluated for both qualitative and quantitative Identification of phytochemical constituents like alkaloids, flavonoids, glycosides, tannins, saponins, etc.

(i) Qualitative screening: The qualitative analysis of the ethanolic fruit peel extract of *Psidium guajava* was performed according to the procedure described by Harborne, [14] and Kaur and Arora [15]. Briefly, the alkaloid was detected with the addition of 5 ml of methanol, 5 ml of 2 N HCl and Meyer's and Wagner's reagent based on the formation of turbidity or precipitation. The tannins were identified by boiling 0.5 g of powdered sample in 20 ml of distilled water. Followed by this, three drops of 5% FeCl3 were added, and the development of brownish-green or blue-black colouration indicated a positive result. The flavonoids were determined by heating 1 g of powdered sample in 10 ml of ethyl acetate at 40 to 50^{o}C for 5 minutes. To which 1 ml of ammonia was added, the formation of yellow colour indicated the presence of flavonoids. To 2 g of sample, 10 ml of methanol and 2 ml of glacial acetic acid containing a drop of 5% $FeCl_3$ were added. The saponins are identified by boiling 1 g of the sample in 10 ml of distilled water for 15 minutes and shaking well to observe the formation of froths, indicating a positive result. The presence of glycosides was observed with the formation of a reddish-brown ring at the junction of two liquids.

(ii) Qualitative screening: The qualitative analysis of the ethanolic fruit peel extract of *Psidium guajava* was performed according to the procedure described by Harborne, 1973 and Khanal [16].

Test for Flavonoids: To 5 g of powdered sample, 100 ml of water and 2 ml HCL solution were added. Then the mixture was boiled for 30 minutes, cooled and filtered into Whatman No. 1 filter paper. The aqueous solution was discarded, and the residues in the filter paper were dried in an oven for 30 minutes at 60^{o}C. The total flavonoids present in the sample were calculated by using the following formula.

$$\text{Flavonoid (\%)} = ((W2-W1))/W1 \times 100$$

Where, W1 = weight of empty filter paper; W2 = weight of paper + flavonoid extract

Test for Alkaloids: To 5 g of powdered sample, 100 ml of 10% acetic acid was added and shaken well. The mixture was kept as such for 4 hours and filtered using Whatman No. 1 filter paper. The aqueous extract was evaporated to ¼th of its original volume using a magnetic stirrer. Followed by this, the concentrated ammonium hydroxide (NH_4OH), was

added dropwise to precipitate the alkaloid content. Then the solution was filtered again and washed with 1% NH_4OH. The filter paper containing residue was dried in an oven at 60^oC for 30 minutes, cooled and weighed.

$$\text{Alkaloid (\%)} = ((W2-W1))/W1 \times 100$$

Where, W1 = weight of empty filter paper; W2 = weight of paper + alkaloid extract

Total phenolic content (TPC): The crude extract of *Psidium guajava* peel's total phenol content (TPC) was determined using the Folin-Ciocalteu reagent technique. In a brief, 2.5 ml of 10% Folin-Ciocalteu reagent (1:1) and 2 ml of 2% sodium carbonate were mixed with 1 ml of plant extract (1 $mgml^{-1}$) as the solvent (Na_2CO_3). The mixture solution was let to stand for 15 minutes in the dark at room temperature for incubation. Using a calorimeter to measure the absorbance at 765 nm was the solution.

$$\text{phenolic content (\%)} = ((Wi-Wf))/Wi \times 100$$

Where, Wi= dried plant extracts;Wf= extracts after drying

Test for Tannins Content: The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract was added with 3.75 ml of distilled water and 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 - 0.5 mg/ml) were used as standard solutions. The results of tannins were expressed in terms of tannic acid in mg/ml of extract [17].

Characterization of phytochemical compound

FTIR analysis: FTIR analysis was performed to determine the functional groups involved in the antioxidant activity of fruit peel extract of *Psidium guajava*. Briefly, the dried samples were mixed with KBr (1:20; 0.02 g of sample with KBr at a final weight of 0.4 g). Then, infrared spectra were obtained using a Fourier-transform infrared spectrometer (Perkin Elmer FTIR spectra (system 2000) USA) within a scanning range of 400– 4000/cm [18].

Antimicrobial activity of fruit peel extract of *Psidium guajava*: The antimicrobial activity of ethanolic fruit peel extract of *Psidium guajava* was determined using the agar well-diffusion assay. Briefly, Mueller Hinton Agar (MHA) plates were prepared, and wells of about 6 mm diameter were made using a sterile cork borer. The microbial strains with 0.5 McFarland turbidity were swabbed onto the agar surface, respectively. Then, about 100 µg/ml concentration of ethanolic fruit peel extract was loaded in the well and subjected to incubation at 37^oC for 24 hours. After incubation, the zone of inhibition was measured and tabulated [19].

Antioxidant activity of ethanolic fruit peel extract of *Psidium guajava*

Hydrogen peroxide radical Scavenging Assay: It was decided to use the method described by Jayaprakash *et al.*, 2015. In a sodium phosphate buffer, a solution of hydrogen peroxide (20 mM) was produced (pH 7.4). Then, 1 ml extract or ascorbic acid (reference antioxidant) in ethanol was added to 2 ml (20 mM) hydrogen peroxide at various concentrations. After 10 minutes, the absorbance of extracts in sodium phosphate buffer without hydrogen peroxide was measured at 230 nm against a blank solution containing extracts in sodium phosphate buffer without hydrogen peroxide. The H_2O_2 scavenged by plant extract and the standard compounds ascorbic acid were calculated [20].

DPPH radical scavenging activity: The Quantitative measurements of the radical scavenging assay were performed according to the method described by standard procedure. Briefly, the reaction mixture contained 50 µl of fruit peel extract of *Psidium guajava* at concentration ranging from 0.031 to 1 mg/ml, and 5 ml of a 0.04% (w/v) solution of DPPH in 80% methanol was taken. The known antioxidant, butylated hydroxytoluene (BHT), was used as a positive control. The DPPH solution without fruit peel extract was used as a control, and 80% methanol was used as a blank solution. The discolouration of the solutions was measured at 517 nm using a spectrophotometer, followed by incubation for 30 minutes in the darkroom. The percentage of the DPPH free radical was determined by using the following equation,

$$\text{DPPH scavenging effect (\%)} = ((A0 - A1)/ A0) \times 100$$

where A0 was the absorbance of the control and A1 was the absorbance in the presence of the fruit peel extract of *Psidium guajava*. The decrease in the absorption induced by the test was compared with the positive controls. The IC_{50} (concentration providing 50% inhibition) values were calculated with the dose inhibition curve in a linear range by plotting the extract concentration versus the corresponding scavenging effect [21].

Nitric oxide radical scavenging activity: Nitric oxide radical scavenging activity of fruit peel extract of *Psidium guajava* was determined by Griess Ilosvay reaction using sodium nitroprusside. Briefly, the reaction mixture containing 2 ml of sodium nitroprusside (10 mM) and 0.5 ml of phosphate buffer (pH-7.4) was mixed with 0.5 ml of fruit peel extract and incubated at 25°C for 150 minutes. After the incubation, 0.5 ml of nitrite was pipetted out, and 1ml of sulphanilic acid reagent (0.33% of sulphanilic acid in 2% glacial acetic acid) was added and left undisturbed for 5 minutes. Then, 1 ml of 1% naphthyl ethylene diamine dihydrochloride (NEDD) was added and kept at 25°C for 30 minutes. The pink colour solution was measured spectrophotometrically at 540 nm. The percentage of nitric oxide inhibition was calculated by using the formula,

$$\text{Nitric oxide radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100.$$

where A_0 was the absorbance of the control, and A_1 was the absorbance of the treated sample [22].

DPPH dot blot assay: Nasseri *et al.* described a dot-blot DPPH staining technique for determining the radical scavenging activity of *Psidium guajava* fruit peel extract. A five-by-five-centimetre UV-fluorescence indicator-impregnated silica gel plate was obtained. Ascorbic acid and DPPH were used as controls and standards, respectively. First, DPPH was applied to the plate, followed by the fruit peel extract. The scavenging was then determined qualitatively based on the colour change of the spot from purple (blank, no antioxidant activity) to yellow or white, indicating a positive result for antioxidant activity [23& 27].

Total antioxidant capacity by phosphomolybdenum method: The total antioxidant capacity of *Psidium guajava* fruit peel extract was determined using the phosphomolybdenum method described by El-Sayed Saleh Abdel-Hameed [24]. First, 3 ml of the reagent containing 0.6 M sulphuric acid, 29 mM sodium phosphate, and 4 mM ammonium molybdate was mixed with 0.3 ml of extract and 100 mg/ml of ascorbic acid. A control of three millilitres of reagent solution was used. Next, the mixture was sealed in tubes and incubated for 90 minutes at 95°C in a boiling water bath. After cooling to room

temperature, the absorbance at 695 nm was measured with a UV-Vis spectrophotometer. Next, the antioxidant activity was measured using ascorbic acid equivalents.

In-vitro cytotoxic activity by MTT assay: The current study used human colon cancer cells (HT-29). Cell lines were purchased at the National Centre for Cell Science (NCCS) in Pune, India. They were kept in a 37°C incubator with 5% CO_2 and cultured in DMEM (Dulbecco's Modified Eagle's Medium) medium containing 10% foetal bovine serum, 1% antibiotic, and antimitotic solution. HT-29 cells ($1x10^4$ cells/well) were seeded into 96-well plates and treated for 24 hours with serum-free medium, and various concentrations of *Psidium guajava* Peel extract (5, 10, 25, 50, 75, 100, 150, and 200 g/ml). Following the treatment period, cells were extracted from the used medium. The culture medium was removed and 100 mL MTT was added to each well and incubated at 37 1C for 4 h. Then, the supernatant was removed and 50 mL DMSO was added to each well and kept for 10 min in order to solubilize the formazan crystals. Using an ELISA multi-well plate reader (ROBONIK, India), the optical density was measured at 620 nm. The results were used to estimate the percentage viability of cells by the following formula

% viability = (OD value of experimental sample/OD value of experimental control) x 100

RESULTS AND DISCUSSION

Floristic composition of edible fruits at Ayakudi village

The research was carried out in the Tamil Nadu village of Ayakudi, near Palani in the Dindugal District. The first survey of edible fruits was documented as part of this effort. This study documented the presence of five edible fruit species representing five genera and five distinct families (**Table 1**). The floristic study revealed five species: *Psidium guajava,Annona squamosa, Mangifera indica, Artocarpus heterophyllus, and Manilkara zapota.* The *Psidium guajava* species was discovered to be more prevalent and denser at Ayakudi. As a result, we chose *Psidium guajava* for its anti-colon cancer properties.

Phytosociological parameters and Diversity indices of edible fruits in Ayakudi

Ayakudi is well-known for its guava, this is because nature abounds on Kodaikanal's slopes. However, the lack of scientific data on the species composition and vegetation structure of edible fruits in Ayakudi has made it more difficult to implement conservation strategies to protect this for increased productivity, and the quality of the fruits has pushed farmers to seek a higher market price. As a result, the primary goal of this research was to fill this void. Five locations were chosen, and the quadrat method was used to collect data on fruit species composition and regeneration potential at each site. The floristic study revealed the presence of five true edible species; their frequency, density, abundance, relative frequency, and relative density are listed in **Table 2.**

Diversity indices of True Mangroves in Ayakudi

The species diversity in Ayakudi followed an exponential distribution pattern, a similarity test was carried out between species diversity using the various diversity index formulas for edible fruits in Ayakudi. The calculated results were analyzed as follows: the higher the BDI value, the more divergent the community, i.e., the richness of a specimen is more significant a species is closer to a balanced density status. If all specimen densities are comparable, the Index is maximal and entirely dependent on species richness. These indices

are computed using the entire sample of data provided to the calculator. These diversity indices compare sample regions for "similarity" and other biodiversity correlations between different quadrants (**Figure 3a&3b**).

Processing of fruit peel extract of *Psidium guajava*

The extraction value of the bioactive material using various solvents and water, according to Arawande[26]. aids in creating a potential pharmaceutical, including the extract. **Table 3** shows the extraction value of *Psidium guajava* fruit peel extract using different solvents and distilled water. It appears that ethanol at 3.5% concentration had the highest extraction value and was chosen as a suitable solvent for future investigation.

Phytochemical analysis of an ethanolic extract of *Psidium guajava*

(i) Qualitative screening

Table 4 shows the phytochemical evaluation of an ethanolic extract of *Psidium guajava.* The extract contained alkaloids, flavonoids, steroids, triterpenes, tannins, thiols, anthraquinones, volatile oils, and phenols but no glycosides or saponins. Because the extract's biochemicals are polar, the ethanol extract is more likely to dissolve them. Furthermore, ethanol extract has been shown to penetrate cell membranes and promote faster physiological absorption of plant components [26].

(ii) Quantitative screening

The quantitative analysis of phytochemicals discovered in the ethanolic extract of *Psidium guajava* is shown in the table (**Table 5**). It was revealed that phenols, alkaloids, and tannins are the most abundant phytochemicals in the ethanolic extract, while flavonoids are comparably less abundant. The significant pharmacological effects of phenols could be attributed to the prevention of a number of illnesses, including cancer, atherosclerosis, and age-related degenerative brain disease [28]. Furthermore, higher amounts of phenolic compounds in fruit peel extract were revealed to play an important function in metabolic activity and treating chronic disorders.

Characterization of phytochemical compound

The FTIR analysis was performed to evaluate the functional group in the active phytochemical components of *Psidium guajava* fruit peel extract. Table 6 and Figure 4 show the results. The ethanolic extract of *Psidium guajava* contains a variety of functional groups, including aromatic compounds, aliphatic alkanes, aliphatic amines, alcohols, phenols, alkyl groups, alkanes, aromatics, alkynes, and ethers.

Antimicrobial activity of fruit peel extract of *Psidium guajava*

The antibacterial activity of various plant extracts is documented in **Table 7** and **Figure 5**. The fruit peel extract of *Psidium guajava* was tested for antibacterial activity against Gram-positive and gram-negative bacteria using the excellent diffusion method. The results showed that ethanolic was potentially beneficial in inhibiting the microbiological growth of clinical bacterial pathogens, albeit with varying potency. At a 100 µg/ml concentration, the ethanolic extract of *Psidium guajava* suppresses bacterial growth, with Staphylococcus aureus being the most susceptible pathogen. The inclusion of many phytochemicals, including alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids, accounts for the extract's significant antibacterial activity [29]. *Psidium guajava*

exhibited the highest antibacterial activity against Gram-negative and Gram-positive infections, according to Redfern *et al.*2014 .'s study [30].

Antioxidant activity of ethanolic fruit peel extract of *Psidium guajava*

Hydrogen peroxide radical Scavenging Assay

Hydrogen peroxide enters the body via vapour or mist inhalation and contact with the eyes or skin. It decomposes quickly into oxygen and water, producing hydroxyl radicals (OH-), which can cause lipid peroxidation and DNA damage [29]. Medications that neutralize the hydrogen peroxide radical are more effective for treating stress-related disorders.

As previously stated, the methanolic extract of *Psidium guajava* has strong anti-hydrogen peroxide agents. Our findings have a high connection with the previous investigations. Venkatachalam and colleagues reported that a methanolic extract of *Psidium guajava* has increased hydrogen peroxide radical scavenging activity (100-500ul). This finding suggests that the extract has a high antioxidant capacity. The IC50 of the ethanol fraction of *Psidium guajava* peel was 72.06µg/ml. Ascorbic acid had a standard value of 74.59µg/ml.

DPPH radical scavenging activity

The ethanolic extract of *Psidium guajava* fruit peel displayed dose-dependent DPPH radical scavenging activity. The extract demonstrated a scavenging activity of 44.46% at 100 g/ml, while the standard activity was 40.55%. The ethanolic fruit peel extract of *Psidium guajava* had scavenging activity comparable to a reference chemical (Ascorbic acid). The IC50 value for *Psidium guajava* ethanolic fruit peel extract was 181.06g/ml, while standard ascorbic acid had an IC50 value of 170.91µg/ml (**Figure 6**). According to our data, guava peel extract has the highest level of antioxidant activity. According to Bouchoukh et al., the most antioxidant activity was found in ethyl acetate extract.

Nitric oxide radical scavenging activity

Vasodilation, antibacterial activity, and anticancer activity are physiological processes that depend on the nitric oxide radical. The minimum nitric oxide scavenging activity of the ethanolic fruit peel extract of *Psidium guajava* at 100 micrograms per millilitre was 32.07 per cent. The maximum activity was 76.29 per cent at 500 micrograms per millilitre. The proportion of inhibition increased as the ethanolic fruit peel extract concentration grew. Normal ascorbic acid was far more active than our ethanolic fruit peel extract (**Figure 6**). The IC50 value for traditional ascorbic acid was 179.6 g/ml, while for the ethanolic fruit peel extract, it was 169.11 g/ml. According to Ebrahimzade [31]. The production of peroxynitrite anion is responsible for the high specificity of NO radicals in superoxide radical reactions.

DPPH dot blot assay

The DPPH dot blot assay is a qualitative TLC-based evaluation used to detect a sample's antioxidant capacity based on the mechanism of free radical suppression by antioxidants present in the sample. The colour change from purple to yellow substantiated the scavenging ability of *Psidium guajava* fruit peel extract (**Figure 6**). The ethanolic fruit peel extract of *Psidium guajava* was found to be particularly effective at inhibiting the generation of free radicals as concentration was raised. Because ethanol extract contains the most biological components, free radicals are considerably reduced, resulting in the best antioxidant action. An ethanolic extract of *Psidium guajava* demonstrated the best antioxidant activity against DPPH in this investigation. Cedric et al. discovered that the ethanolic extract

of *Psidium guajava* leaves has the highest antioxidant activity as determined by DPPH, which was confirmed by our research [27].

Total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of plant extracts was evaluated using the phosphomolybdate procedure. The plant extracts converted Mo (VI) to Mo (V), resulting in a green complex with the highest absorbance at 695 nm. In various doses, *Psidium guajava* peel ethanolic fruit peel extract revealed its overall antioxidant ability (**Figure 6**). At a 500 g/ml concentration, 79 percent inhibition for the fruit peels extract and 77.8 per cent for ascorbic acid was recorded. The IC50 for the standard was 189.17µg/ml and 195.19µg/ml for the fruit peel extract.

In-vitro cytotoxic activity by MTT assay

PGPE derived from *Psidium guajava* peel was used to grow HT-29 colon cancer cells in vitro. Their cytotoxic activities were assessed using the MTT test. Cells were given varying dosages of PGPE, with untreated cells as the control. Cell viability was determined after treatment with different concentrations of PGPE (5,10, 25, 50, 100, 150, and 200 µg/ml). Cell viability reduced when PGPE concentration increased, according to the MTT experiment. The IC50 value of 75.51µg/ml is shown in **Figure 7**. The colon cancer cells treated with PGPE had a different morphology than the control cells, indicating that PGPE can kill cancer cells and be used as an anticancer agent for colon cancer. A study was conducted to assess the effect of PGPE on the cytotoxicity of HT-29 cells. Colon cancer is the third highest cause of death in the globe. This study aims to determine the anti-angiogenic capability of *Psidium guajava* (guava) leaf extracts and their potential anticancer effects against colorectal cancer. The guava plant's fruit is highly appreciated as food and nutrition, while its leaves and bark have a long history of medical use [32]. In addition to various useful biological substances such as phenolics, flavonoids, carotenoids, terpenoids, and triterpenes, the guava plant's broad availability in the middle- and low-income nations makes it ideal for the creation of low-cost cancer medicines. SW480, HT-29, and Caco-2 human colon cancer cell lines were resistant to the anti-proliferative effects of the flavonoid apigenin from guava leaf extract [33].

CONCLUSION

Guava has been used to treat a number of chronic ailments since ancient times. They can assist in reducing the side effects of pharmaceutical drugs because they contain clinically beneficial components, including vitamin C and flavonoids. Our studies established the presence and function of these biological molecules. In addition, our research found that guava is helpful against free radicals. The antioxidant activity of the sample should be assessed in a variety of ways at the same time, as different processes may produce different results. According to the findings of this study, phytochemicals and antioxidants were more numerous in the *Psidium guajava* peel extract. The FTIR spectrum contains unique peaks for aliphatic amines, alkenes, alcohols, alkanes, and aromatic chemicals. Guava ethanolic extract can also limit cancer cell proliferation by preventing their intermediate products from being used in biochemical processes. Our subsequent study will examine how *Psidium guajava* ethanolic extract inhibits cancer cell proliferation via several expression mechanisms. Based

on our findings, we are confident that these guava-derived biochemical components are crucial for maintaining homeostasis and can be used as antioxidants.

ACKNOWLEDGEMENTS

RT and VU would like to thank the Department of Biochemistry, PSG College of Arts & Science, Coimbatore-641 014Tamilnadu, India for providing the facilities required to write this paper.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or non-profit sectors.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

DECLARATIONS ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. O. S. Oladeji, K. A. Odelade, and J. K. Oloke, "Phytochemical screening and antimicrobial investigation of *Moringa oleifera* leaf extracts," *African Journal of Science, Technology, Innovation and Development*, vol. 12, no. 1, pp. 79–84, 2019.https://journals.co.za/doi/abs/10.1080/20421338.2019.1589082
2. Kumar, M., Tomar, M., Amarowicz, R., Saurabh, V., Nair, M. S., Maheshwari, C., &Satankar, V. (2021). Guava (*Psidium guajava* L.) leaves: nutritional composition, phytochemical profile, and health-promoting bioactivities. *Foods*, *10*(4), 752 https://doi.org/10.3390/foods10040752
3. Hartati, R., Nadifan, H. I., &Fidrianny, I. (2020). Crystal Guava (Psidium guajava L."Crystal"): Evaluation of In Vitro Antioxidant Capacities and Phytochemical Content. The Scientific World Journal, 2020.https://doi.org/10.1155/2020/9413727
4. Malki, A., ElRuz, R. A., Gupta, I., Allouch, A., Vranic, S., & Al Moustafa, A. E. (2020). Molecular mechanisms of colon cancer progression and metastasis: recent insights and advancements. *International journal of molecular sciences*, *22*(1), 130.https://doi.org/10.3390/ijms22010130
5. Naik, A. V., &Sellappan, K. (2021). Assessment of Genotoxic potential of Annonacin and Annona muricata L. extracts on human breast cancer (MCF-7) cells. *Advances in Traditional Medicine*, *21*(4), 779-789.https://doi.org/10.1007/s13596-020-00517-8
6. Abdelmalek, S., Mohsen, E., Awad, A., & Issa, R. (2016). Peels of Psidium guajava fruit possess antimicrobial properties. *The International Arabic Journal of Antimicrobial Agents*, *6*(3:1), 1-9.https://doi.org/10.3823/791

7. Naseer, S., Hussain, S., Naeem, N. *et al.* The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clin Phytosci* **4,** 32 (2018).https://doi.org/10.1186/s40816-018-0093-8
8. Gamble, J. S., & Fischer, C. E. C. 1954. Flora of the Presidency of Madras. Part 11. Addenda, corrigenda, indexes, etc. *Flora of the Presidency of Madras. Part 11. Addenda, corrigenda, indexes, etc.https://www.cabdirect.org/cabdirect/abstract/19360700979*
9. Michael, A.J. 1998. Determination of stress from slip data: Faults and folds. Journal of Geophysical Research,89: 11,517-11,526.https://doi.org/10.1029/JB089iB13p11517
10. Legendre, P. and Legendre, L. 1998. Numerical ecology, 2nd English edition. Elsevier Science, 853 pp. https://www.tandfonline.com/loi/uaar20
11. Young, T.M. (2017) Biodiversity Calculator for the Simpson and Shannon Indexes. Copyright 2017 by Tanner, M., Young All Rights Reserved, Page Loaded 14 Days Ago, Formula and Manual for the Calculation of Shannon—Wiener Index Alpha Biodiversity [α], 4 p.
12. Arawande, J. O., Akinnusotu, A., &Alademeyin, J. O. (2018). Extractive Value and Phytochemical Screening of Ginger (zingiber officinale) and Turmeric (curcuma longa) Using Different Solvents. *International Journal of Traditional and Natural Medicines Int. J. Trad. Nat. Med, 8*(1), 13–22.
13. Sowmya, B. H., & Usha Anandhi, D. (2021). Screening of in vitro free radical scavenging activities of the fruit extracts of Psidium guajava L. *Indian Journal of Traditional Knowledge*, *20*(3), 716-722.
14. Harborne JB (1973) Phenolic compounds. In: Phytochemical methods (pp. 33-88). Springer, Dordrecht. DOI: 10.1007/978-94-009-5921-7_2
15. Kaur, G.J., Arora, D.S. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermumammi* . *BMC Complement Altern Med* **9,** 30 (2009). https://doi.org/10.1186/1472-6882-9-30
16. Khanal, S. (2021). Qualitative and Quantitative Phytochemical Screening of Azadirachta indica Juss. Plant Parts. *International Journal of Applied Sciences and Biotechnology*, *9*(2), 122-127 https://doi.org/10.3126/ijasbt.v9i2.38050
17. Ogidi, O. I., George, D. G., &Esie, N. G. (2019). Ethnopharmacological properties of Vernonia amygdalina (Bitter Leave) medicinal plant. *Journal of Medicinal Plants*, *7*(2), 175-181.
18. Gaber, N. B., El-Dahy, S. I., &Shalaby, E. A. (2021). *Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues. Biomass Conversion and Biorefinery.* doi:10.1007/s13399-021-01386-0
19. N.S. Al-Zoreky (2009). *Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. , 134(3), 244–248.* doi:10.1016/j.ijfoodmicro.2009.07.002
20. Al-Amiery, A. A., Al-Majedy, Y. K., Kadhum, A. A. H., & Mohamad, A. B. (2015). Hydrogen peroxide scavenging activity of novel coumarins synthesized using different approaches. *PloS one*, *10*(7), e0132175.

21. Sihag, S., Pal, A., & Saharan, V. (2022). Antioxidant properties and free radicals scavenging activities of pomegranate (Punica granatum L.) peels: An in-vitro study. *Biocatalysis and Agricultural Biotechnology*, *42*, 102368https://doi.org/10.1016/j.bcab.2022.102368
22. Ali, B. M., Boothapandi, M., & Nasar, A. S. (2020). Nitric oxide, DPPH and hydrogen peroxide radical scavenging activity of TEMPO terminated polyurethane dendrimers: Data supporting antioxidant activity of radical dendrimers. *Data in brief*, *28*, 104972 https://doi.org/10.1016/j.dib.2019.104972
23. Nasseri, M. A., Keshtkar, H., Kazemnejadi, M., &Allahresani, A. (2020). Phytochemical properties and antioxidant activity of Echinopspersicus plant extract: Green synthesis of carbon quantum dots from the plant extract. *SN Applied Sciences*, *2*(4), 1-12.
24. El-Sayed Saleh Abdel-Hameed (2009). *Total phenolic contents and free radical scavenging activity of certain Egyptian Ficus species leaf samples. , 114(4), 1271–1277*. doi:10.1016/j.foodchem.2008.11.005
25. Khorrami, S., Zarepour, A., &Zarrabi, A. (2019). Green synthesis of silver nanoparticles at low temperature in a fast pace with unique DPPH radical scavenging and selective cytotoxicity against MCF-7 and BT-20 tumor cell lines. *Biotechnology reports*, *24*, e00393.https://doi.org/10.1016/j.btre.2019.e00393
26. Arawande, J. O., Akinnusotu, A., &Alademeyin, J. O. (2018). Extractive Value and Phytochemical Screening of Ginger (zingiber officinale) and Turmeric (curcuma longa) Using Different Solvents. *International Journal of Traditional and Natural Medicines Int. J. Trad. Nat. Med*, *8*(1), 13–22.
27. Mapfumari, S., Nogbou, N. D., Musyoki, A., Gololo, S., Mothibe, M., & Bassey, K. (2022). Phytochemical Screening, Antioxidant and Antibacterial Properties of Extracts of Viscum continuum E. Mey. Ex Sprague, a South African Mistletoe. *Plants*, *11*(16), 2094 https://doi.org/10.3390/plants11162094
28. Fawehinmi, A. B., Lawal, H., Chimezie, E. U., & Ola-Adedoyin, A. T. (2022). Quantitative and Qualitative Phytochemical Screening and Anti-Microbial Activities of Argemone mexicana Linn. *Journal of Pharmaceutical Research International*, *34*(54B), 33-45 https://doi.org/10.9734/jpri/2022/v34i54B7241
29. Atere, T. G., Akinloye, O. A., Ugbaja, R. N., Ojo, D. A., &Dealtry, G. (2018). In vitro antioxidant capacity and free radical scavenging evaluation of standardized extract of Costusafer leaf. Food Science and Human Wellness, 7(4), 266-272.https://doi.org/10.1016/j.fshw.2018.09.004.
30. Ahmad, I., Mir, M. A., Srivastava, S., Shati, A. A., Elbehairi, S. E. I., Irfan, S.,& Srivastava, P. (2021). Phytochemical screening and in-vitro antibacterial and anticancer activity of crude extract of Matricariaaurea. *Current Pharmaceutical Design*, *27*(1), 69-79.https://doi.org/10.2174/1381612826666201207105620
31. Ebrahimzadeh, M. A., Pourmorad, F., &Hafezi, S. (2008). Antioxidant activities of Iranian corn silk. *Turkish Journal of biology*, *32*(1), 43-49.https://journals.tubitak.gov.tr/biology/vol32/iss1/7/

32. Cedric, Y., Payne, V. K., Nadia, N. A. C., Kodjio, N., Kollins, E., Megwi, L., Kuiate, J.-R., &Mbida, M. (2018). In vitro Anticoccidial, Antioxidant Activities and Cytotoxicity of Psidium guajava Extracts. *Research Journal of Parasitology*, *13*(1), 1–13. https://doi.org/10.3923/jp.2018.1.13
33. Esmeeta, A., Adhikary, S., Dharshnaa, V., Swarnamughi, P., Maqsummiya, Z. U., Banerjee, A., &Duttaroy, A. K. (2022). Plant-derived bioactive compounds in colon cancer treatment: An updated review. Biomedicine & Pharmacotherapy, 153, 113384 https://doi.org/10.1016/j.biopha.2022.113384.
34. Jayaprakash, R., Ramesh, V., Sridhar, M. P., & Sasikala, C. (2015). Antioxidant activity of ethanolic extract of Tinospora cordifolia on N-nitrosodiethylamine (diethylnitrosamine) induced liver cancer in male Wister albino rats. Journal of Pharmacy & Bioallied Sciences, 7(Suppl 1), S40. 10.4103/0975-7406.155791

Table 1. Floristic Composition of edible fruits at Ayakudi village

S.No	Name of the Species	Family	Habit	Common Name	Local Name
1.	*Psidium guajava* L.	Myrtaceae	Tree	Guava	Koyyaa
2.	*Annona squamosa* L.	Annonaceae	Tree	Sugar Apple	Sitapalam
3.	*Mangifera indica* L.	Anacardiaceae	Tree	Mango	Ma
4.	*Artocarpus heterophyllus* Lam	Moraceae	Tree	Jackfruit	Palaa
5.	*Manilkara zapota* (L.) P. Royen	Sapotaceae	Tree	Chikoo	Chappotta

Table 2. Phytosociological parameters of edible fruits in Ayakudi village

Name of the Species	Frequency (%)	Density (m^2)	Abundance	Relative frequency (%)	Relative density (%)
Psidium guajava L.	100	19.4	19.4	21.74	31.6
Annona squamosa L.	100	13.8	0.22	21.74	22.47
Mangifera indica L.	80	7.6	12.37	17.39	12.38
Artocarpus heterophyllus	80	5	6.25	17.39	8.14
Manilkara zapota (L.) P.Royen	100	15.6	15.6	21.74	25.41

Table 3. Extractive value for fruit peel extracts of *Psidium guajava*

Solvents used	Extractive value (%) w/v
Petroleum benzene	2.70
Acetone	1.03
Ethyl acetate	1.27
Ethanol	3.50
Distilled water	3.10

Table 4. Qualitative screening of phytochemicals for fruit peel extracts of *Psidium guajava*

Phytochemicals	Petroleum Benzene	Acetone	Ethyl-acetate	Ethanol	Water
Alkaloids	+	-	+	+	+
Flavonoids	+	+	-	+	+
Cardiac Glycosides	+	+	-	-	+
Steroids	+	+	+	+	+
Triterpenes	+	+	-	+	-
Saponins	-	-	+	-	_
Tannins	+	++	+	+	+
Thiols	-	-	+	+	+
Anthraquinones	+	-	-	+	+
Volatile oils	-	-	-	+	-
Phenols	+	-	-	+	+

Table 5. Quantitative screening of phytochemicals for fruit peel extracts of *Psidium guajava*

Phytochemicals	Quantity (mg/g)
Phenols	39.50
Flavonoids	14.00
Alkaloids	35.50
Tannins	35.00

Table 6. FTIR characterization of fruit peel extract of *Psidium guajava*

Peak Band (cm^{-1})	Functional groups	Intensity
3302	N-H stretch	Strong
2978	C-H stretch (alkane)	Medium
2885	C-H stretch (-CHO)	Weak
2260	C-N triple bond stretch,(Aliphatic groups)	Weak
1635	C=C stretch, (Alkenes)	Weak
1381	C-N stretch (Aromatic amines)	Medium
1273	C-N stretch (Aliphatic amines)	Weak
1049	C-O stretch (Alcohol)	Strong
678	C_H stretch (Aromatic compounds)	Medium

Table 7. Antimicrobial activity for fruit peel extracts of *Psidium guajava*

Bacterial pathogens	**Zone of inhibition (mm)**
Staphylococcus epidermis	19±1
Staphylococcus aureus	22±1
Streptococcus pyogenes	17±1
Bacillus subtilis	21±1
Escherichia coli	14±1
Proteus vulgaris	18±1
Pseudomonas aeruginosa	19±1
Enterobacter aerogenes	20±1

Klebsiella pneumonia	14±1

FIGURES

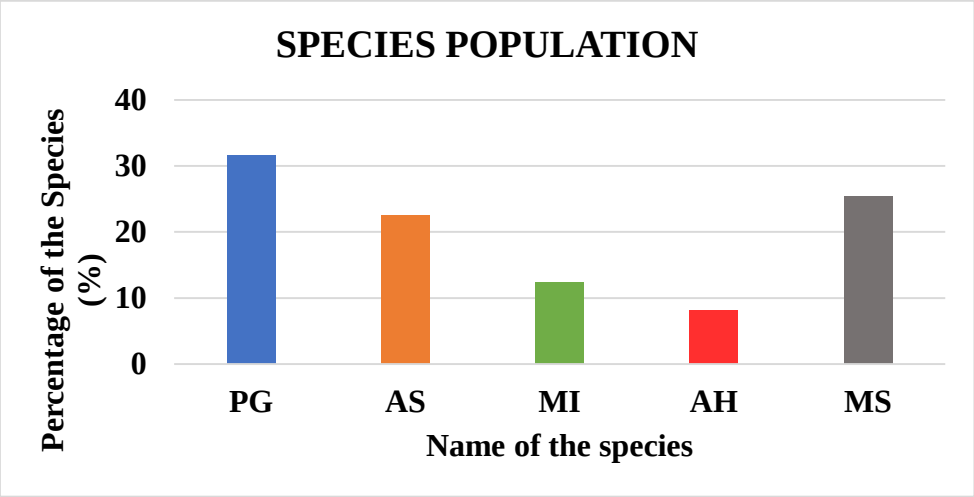


Figure 1. Species Population of edible fruits in Ayakudi

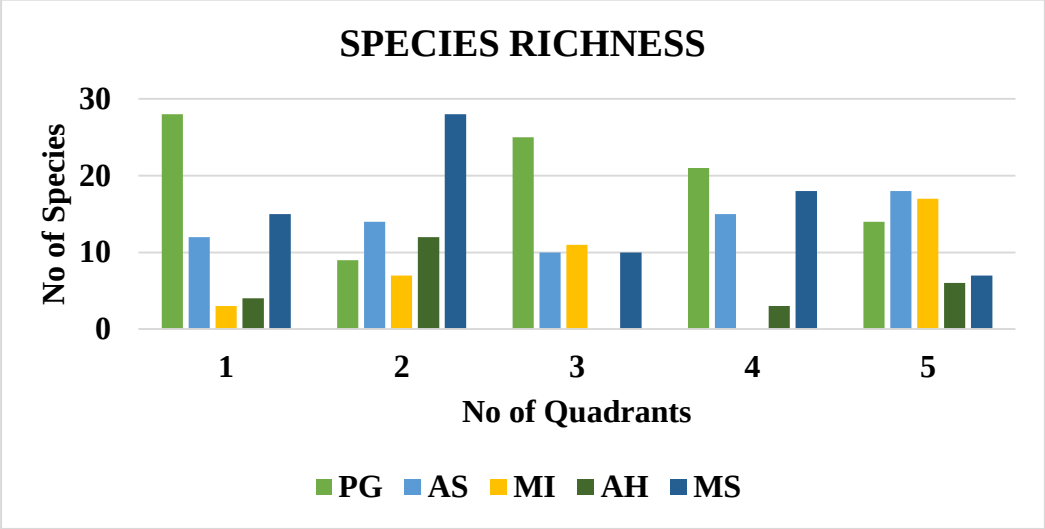


Figure 2. Species richness of edible fruits in Ayakudi

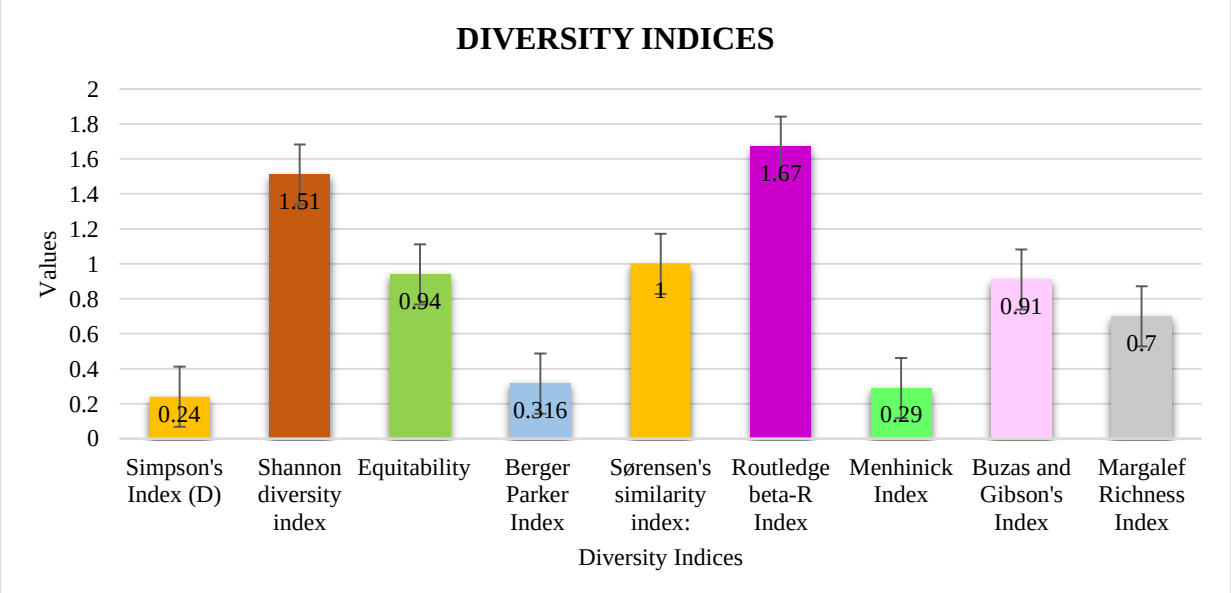
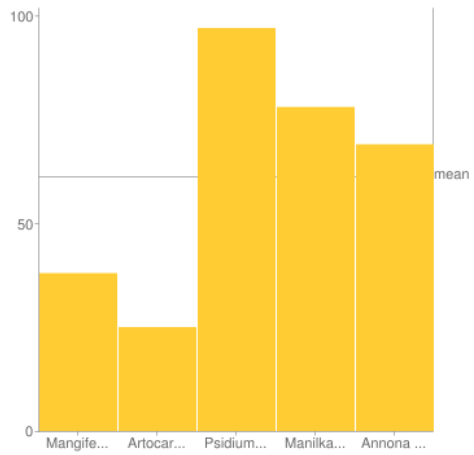
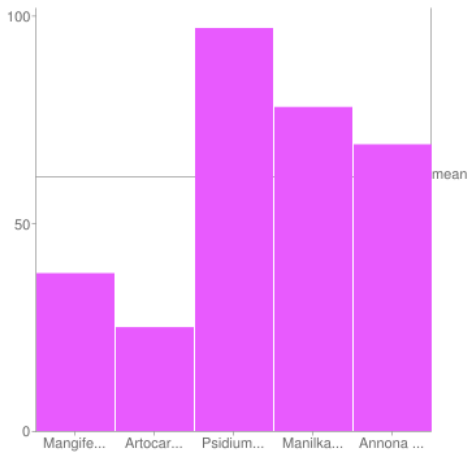


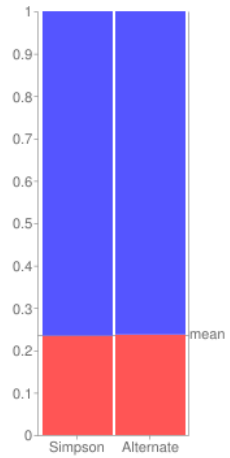
Figure 3a. Diversity indices of edible fruits in Ayakudi



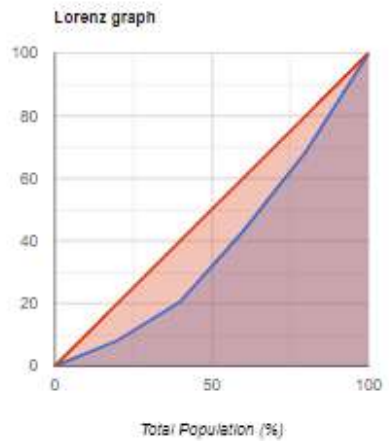
All Populations



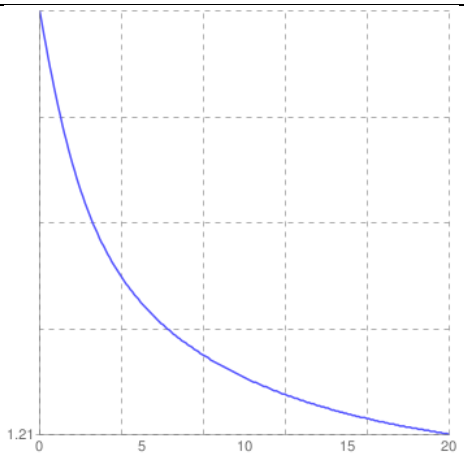
Preston diagram



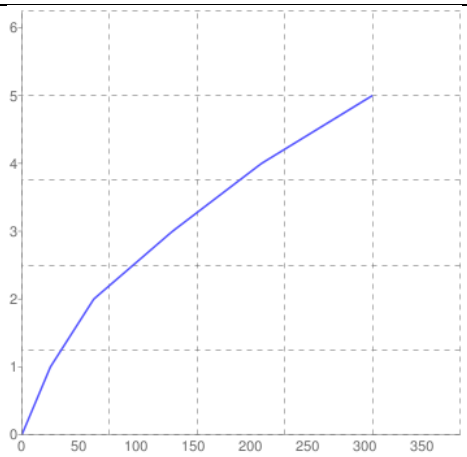
Simpson Index



Lorenz graph



Renyi/Hill graph



Rarefaction Curve

Figure 3b. Diversity indices of edible fruits in Ayakudi

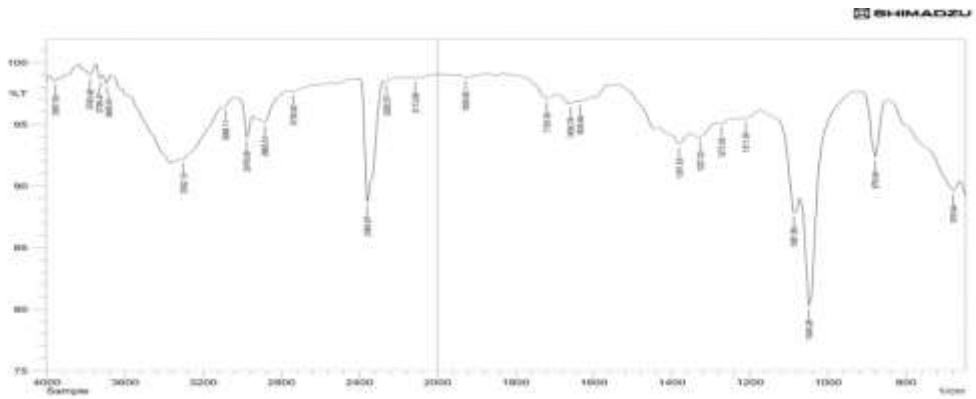


Figure 4. FTIR spectrum of fruit peel extracts of *Psidium guajava*

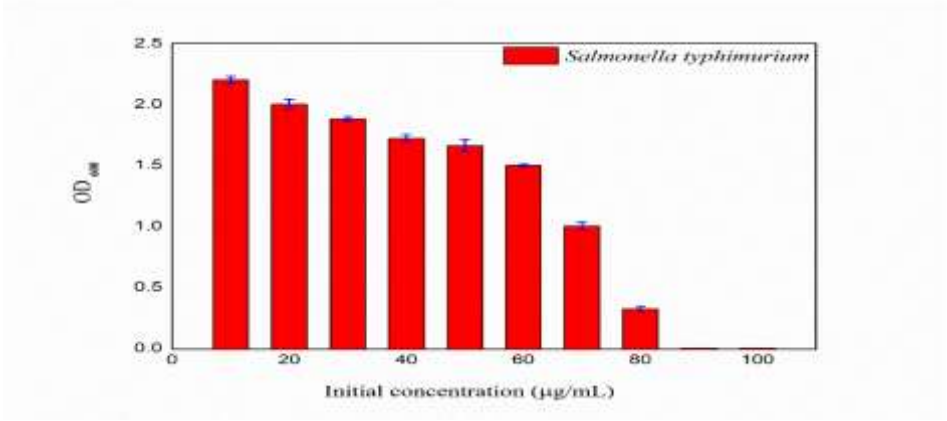


Figure 5. Antimicrobial activity for fruit peel extracts of *Psidium guajava*ODvaluein different concentration

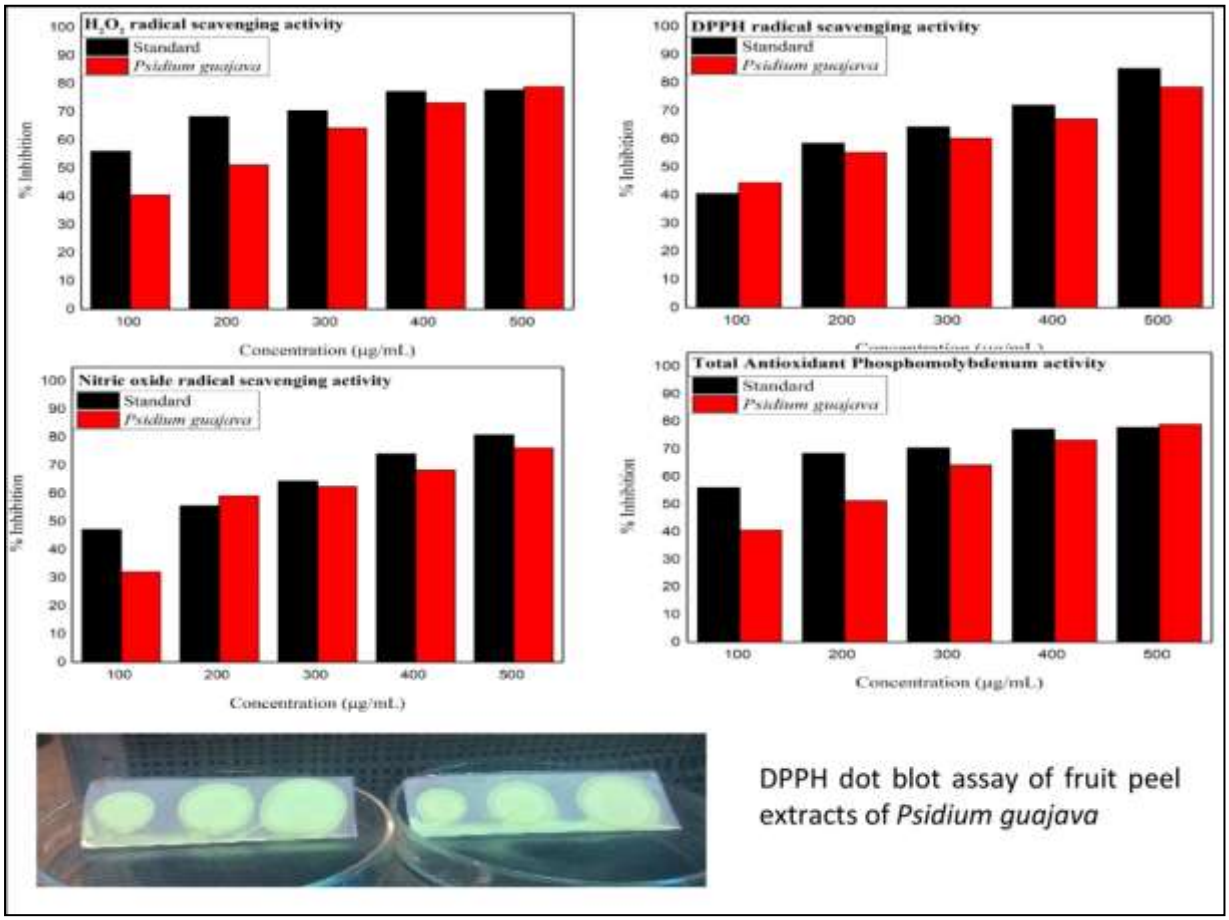


Figure 6Total antioxidant and free radical scavenging activity of fruit peel extracts of *Psidium guajava*

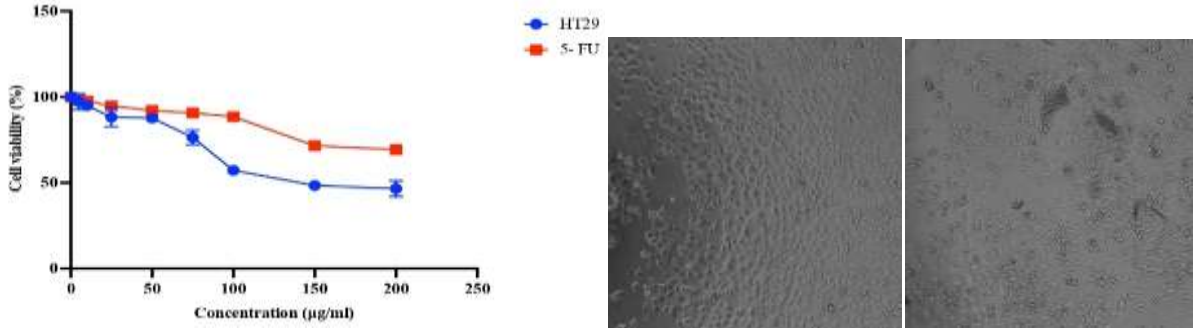


Figure7. Histogram represents the inhibitory effect against HT-29 cancer cells.