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A study on phytochemical profiling, antibacterial and anticancer activity of *Psidium guajava* L. fruit peel extracts against MCF-7 cell line

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Abstract
The aim of this study is to highlight some of the biological activities of methanol extract of *Psidium guajava* fruit peel. phytochemical screening of four extracts of *P. guajava* (Aqueous, Methanol, Ethyl acetate and Hexane) were analyzed, among the four extracts only in methanol more activities were noticed. The results of the methanolic extracts showed the presence of phenol, flavonoids, alkaloids and steroid. Where as in other extracts these compounds were not found. Antioxidant activity of *P. guajava* revealed the highest scavenging activity in methanol fruit peel extract when compare to other extracts using DPPH Assay. The results of antibacterial activity of the tested methanol fruit peel extract against four pathogenic bacterial strains such as *Salmonella typhi* (10.5±2.8), *Escherichia coli* (10.8±2.7), *Proteus mirabilis* (10.5±2.8) and *Shigella flexneri* (10.8±2.7) (Gram^{-ve}) followed by disc diffusion method. Anticancer activity of (breast cancer MCF-7) of *P. guajava* methanol fruit peel extract was carried out MCF-7 cell line against normal cell line. At end of 48 hrs of 125µg/ml, 49.20% cell viability was observed by following MTT Assay. Based on the anticancer activity the functional group of the active components present in the *P. guajava* fruit peel FTIR spectrum was obtained. From this study it is understood that the active compound present in the *P. guajava* fruit peel extract could be a potent source of natural antioxidants which are of great importance as therapeutic agent in preventing or slowing down the progress of ageing, age associated oxidative stress and related degenerative diseases. Further research is recommended for better characterization of important constituents responsible for antioxidant and anticancer activity.

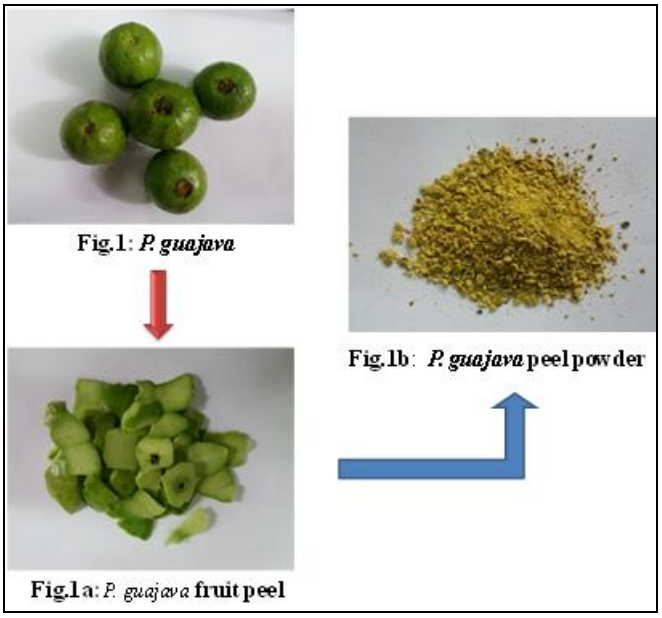
Keywords: *psidium guajava* l., phytochemical compounds, antibacterial activity, DPPH assay, anticancer activity (MCF-7)

Introduction
Guava **(***P. guajava Linn.***)** belonging to family Myrtaceae is a traditionally used plant because of its food and nutritional value. Guava is widely grown in tropical areas like India, Bangladesh, Florida, and West Indies. Different parts of the *P. guajava* are reported to be used in folk medicine [1, 2].

All parts of this tree, including fruits, leaves, bark, and roots, have been used for treating stomachache and diarrhea in many countries. Leaves, pulp and seeds are used to treat respiratory and gastrointestinal disorders, and as an antispasmodic, anti-inflammatory, as cough sedative, anti-diarrheic, in management of hypertension, obesity and in the control of *Diabetes mellitus*. It also possesses anticancer properties [3, 4]. The seeds are used as antimicrobial, gastrointestinal, anti-allergic and anticarcinogenic [5] to [9]. The aim of this study is to highlight some biological activities of four extracts of *P. guajava* fruit peels. Hence search for an effective chemo preventive agent has led to the identification of various naturally occurring compounds like flavonoid and alkaloids (*P. guajava* L. Myrtaceae) fruit peel which is known to possess number of pharmacologic properties such as antioxidant, antitumor, antiallergic, anti-inflammatory, antimicrobial and neuroprotective activities [11]. A thorough review of literature revealed that no work has been taken on the curative efficiency of *P. guajava* on human breast cancer (MCF-7). Hence, the present study was undertaken to fill in the lacunae and unravel the antioxidant and anticancer potential of various extracts of fruit peel of *P. guajava* against human breast cancer *in vitro* by using MCF-7 cell line.

Materials and Methods
Collection and Identification of Fruit
Fresh fruits *Psidium guajava* were purchased from Koyambedu fruit market, Chennai, Tamil Nadu, India, and were authentically identified by Prof. P. Jayaraman, Institute of Herbal Science (IHS), Plant Anatomy Research Centre (PARC), West Tambaram, Chennai, India.

Processing of medicinal Fruit Peel
The (*P. guajava*) diseased free fruit peel was used to prepare extracts for the study. The fruit were collected and washed thoroughly with running tap water and allowed it to remove the soil and unwanted dust particles. The peel was collected by using sharp knife. Then they were shade dried at room temperature for ten days and then the dried peel materials were powdered using kitchen blender. The fruit and fruit peel is exhibited in fig.1 to 1b.



Preparation of fruit peel Extract
The 50 gram of fruit peel powder was mixed with taken 500 ml of solvents like Methanol, Hexane, Ethyl acetate and Aqueous extracts respectively. The fruit peel was kept in orbiter shaker for 48 hrs. Then the extracts were filtered and dried in hot air oven at 37ºC. Then the extract was stored under refrigeration at 4ºC for further analyses.

Phytochemical Screening
All the four extracts were subjected to phytochemical screening for its phytoconstituents according to [10] to [11] methods.

Preparation of Sterile Disc
Each sterile disc (6mm) was incorporated individually with 5, 10, 15, and 20µg/ml of the extracts using micropipette. Ampicillin was used as standard (10µg/ml) and the precautions were taken to prevent the flow of the solvent extract from the disc to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After sometimes another doses of extracts were applied on discs. Then they were stored at 4ºC.

Standardization of Crude Fruit Peel Extracts against Human Pathogens by Disc Diffusion Method
The test microorganisms used for antibacterial analyses, were clinical isolates of *Salmonella typhi, Escherichia coli, Proteus mirabilis, S. felexneri* (Gram^{-ve}). The bacterial strains were maintained in the Nutrient Agar (NA) at the Department of Bacteriology, King Institute of Preventive Medicine, at Guindy, Chennai and were identified using Gram staining and biochemical analysis to confirm their nature. The 25 ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, 100µl of fresh culture of human pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at various concentrations (5, 10, 15 and 20µg/ml) and the plates were incubated for 24 hours at 37ºC. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) and recorded.

DPPH free Radical Scavenging
The ability of the extract to scavenge DPPH radical was determined by the method described by [12].

Cell line and Culture
MCF-7 cell lines were obtained from Veterinary College, Vepery, Chennai. The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50µg/ml CO_2 at 37°C.

Reagents
MEM was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT) and Dimethyl Sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All the other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.
In Vitro assay for Anti-Cancer activity (MTT assay) (Mosmann, 1983) Cells (1 × 105/well) were plated in 24-well plates and incubated in 37^0C with 5% CO_2. After the cell reaches the confluence, the sample was added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570 nm was measured with UV- Spectrophotometer using DMSO as a blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula:

% cell viability =
A570 of treated cells / A570 of control cells × 100

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

Statistical Analysis
The data were subjected to statistical analysis and the Mean ± SE for six individual observations was calculated. The percentage change between the Control/Standard and the experiment was calculated and presented in appropriate tables. The significance of the sample mean was tested by student's-'F' test and the differences were considered as significant at $p<0.01$ level. The analytical data along with Tables/ Figures/ Plates are presented in appropriate places in the text by [14].

Results and Discussion
Phytochemical Analysis
Table.1 represents the phytochemical analysis of all the four extracts of *P. guajava* fruit peel. The qualitative phytochemical analyses carried out in methanol revealed the presence of flavonoids, Saponins, phenol, alkaloids, tannins,

Carbohydrates and Steroids. In the hexane extract of *P. guajava* the results were positive for three compounds (flavonoids, phenol, anthraquinones and steroids). The *P. guajava* aqueous extract shows the positive results for the three compounds (alkaloids, terpenoids and glycosides). In the ethyl acetate extract of *P. guajava* the results were positive for two compounds (alkaloids and glycosides (Table. 1) and totally absent for all other compounant such as saponins, alkaloids, terpenoids, tannins, carbohydrates, glycosides and protein. All species of *P. guajava* contains abundant bioactive substances such as tannins, xanthones, xanthenes, anthocyannins, flavones, phenolic compounds, phytosterols and so on [13, 19, 18 17].

Table 1: Phytochemical analyses of different extracts of *P. guajava* fruit peel

Phytochemical Compounds	Methanol	Hexane	Aqueous	Ethyl acetate
Flavonoids	+	+	-	-
Saponins	+	-	-	-
Phenol	+	+	-	-
Tannins	+	-	-	-
Alkaloids	+	-	+	+
Terpenoids	-	-	+	-
Carbohydrates	+	-	-	-
Anthraquinones	-	+	-	-
Glycosides	-	-	+	+
Steroids	+	+	-	-
Protein	-	-	-	-

'+' – Present '-' – Absent

Antibacterial Activity

The methanol fruit peel extract of *P. guajava* was evaluated for antibacterial activity against medically important bacterial strains such as *Salmonella typhi, Escherichia coli, Proteus mirabilis* and *S. felexneri* respectively. These five different pathogenic bacterial species have also been tested with commercially available antibiotics Amoxicillin ($C_{16}H_{19}N_3O_5S$) and the results were indicated in the Fig.2 and Table. 2. Maximum zone of Inhibition (MIC) was observed in the methanol fruit peel extract of *P. guajava* was tested by F- test and the differences were showed as significant at $p<0.01$ level. When compared to the other organism in methanol fruit peel extracts of *P. guajava* were showed as significant at $p<0.05$ level. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. Steroids have been reported to have antibacterial properties [20] and [21].

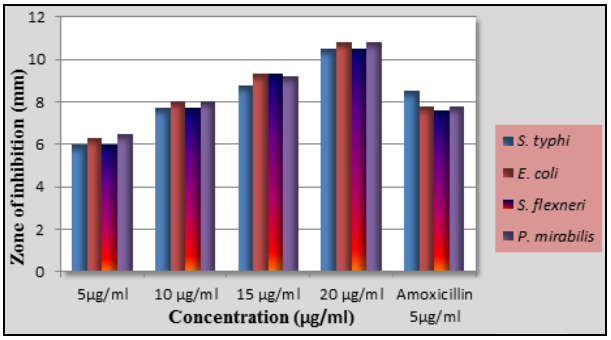


Fig 2: Antibacterial activity of methanol extract of *P. guajava* fruit peel

Table 2: Antibacterial activity of methanol extract of *P. guajava* fruit peel

Organism	5µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	Amoxicillin 5µg/ml
S. typhi	6±0.3*	7.7±0.6*	8.8±1.8*	10.5±2.8*	8.5±0.2
E. coli	6.3±0.4*	8±0.6*	9.3±1.6*	10.8±2.7*	7.8±0.1
S. flexneri	6±0.3*	7.7±0.6*	9.3±1.6*	10.5±2.8*	7.8±0.1
P. mirabilis	6.5±0.4*	8±0.6*	9.2±1.7*	10.8±2.7*	7.8±0.1

Note: 1. Triplicate of mean value ± Standard error.
2. * denotes significant at 5% level

DPPH free Radical Scavenging

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and [16] peroxidation, involves a series of free radical mediated chain reaction processes, it is also associated with several types of biological damage. Therefore much attention has been focused on the use of natural antioxidants to protect from damage due to free radicals. In the present study, the methanolic Fruit peel extract of *P. guajava* alone showed 50% inhibition when compared to other fruit peel extracts of *P. guajava*. The data obtained reveal that the activity of *P. guajava* in DPPH radical activities were found to possess higher antioxidant activity when compared to fruit peel extracts as shown in the (fig- 3). *P. guajava* species is one of the most important fruit that have a potential source of bioactive chemical compounds. *P. guajava* species are widely used by many people food and as traditional medicine [16].

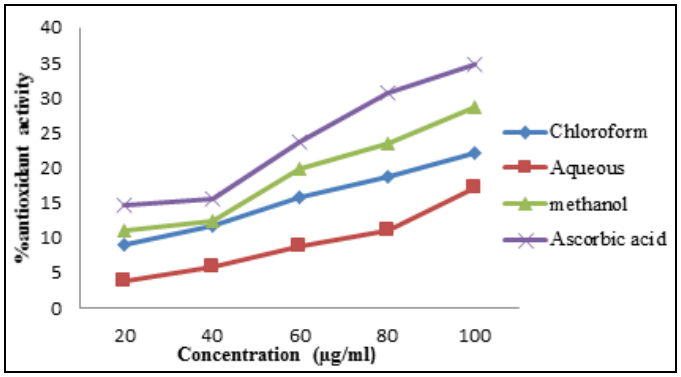


Fig 3: Antioxidant activity of *P. guajava* fruit peel extracts

MTT Assay

The MTT assay results showed methanol extract of fruit peel extract at 125µg/ml had 49.20% viability loss against MCF-7 cells

Anti-proliferation of the cells was assessed by MTT assay for 48 hrs in the *P. guajava* methanol fruit peel extract and the values are presented in Fig.4. The values revealed that anti-proliferative activity was seen in the MCF-7 cells when treated with different concentrations of fruit peel extract; the cell anti-proliferation being directly proportional to concentration. Per cent cell viability of MCF 7 cells were assessed for 48 hrs in the fruit peel extract at varying concentrations. The control cells were 100% viable and the viability decreased significantly with increase in concentration

of the fruit peel extract. The per cent decrease in cell viability was indirectly proportional to the concentration of fruit peel extract. At 100 µg/ml concentration of the *P. guajava* methanol fruit peel extract at the end of 48 hrs, 125µg/ml, 49.20% viability of the cells were observed. From the results it is pragmatic that fruit peel has profound effect in controlling MCF-7 cell proliferation. The values altogether depict that the fruit peel significantly controls cell proliferation of MCF-7 cells even at low concentrations. Hsieh and Peng (2010) [22] studied the action mechanism and signal pathways of *Psidium guajava* peel extract in killing prostate cancer (MCF-7) cells. Lee and Park (2010) [23] reported anticancer activity of *P. guajava* branch extracts against HT-29 human colon cancer cells.

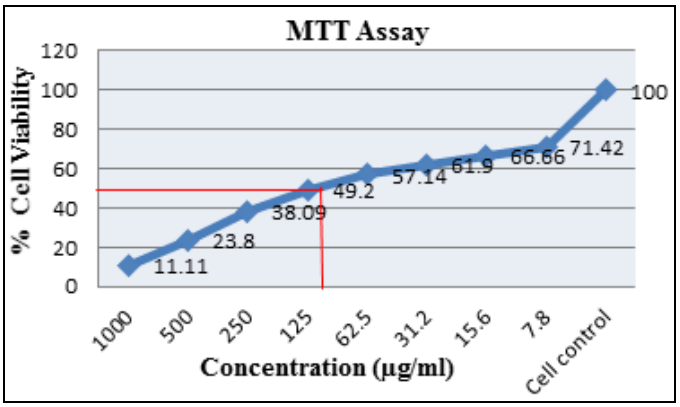


Fig 4: Anticancer effect of *P. guajava* fruit peel on MCF-7 Cell line

Cell Morphological Study (MTT Assay)

Treatment of MCF-7 cells with these *P. guajava* fruit peel methanol extracts even at low doses induced morphological changes in the MCF -7 cells, which had similar effect on cell morphology. It was observed that most of the cells became round in shape and were not attached to substratum after treatment with the extracts (1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8 µM), which was in dose-dependent manner (Fig 5). From the above results we can assume that extracts of *P. guajava* might have affected cell-cycle and induced apoptosis pathways.

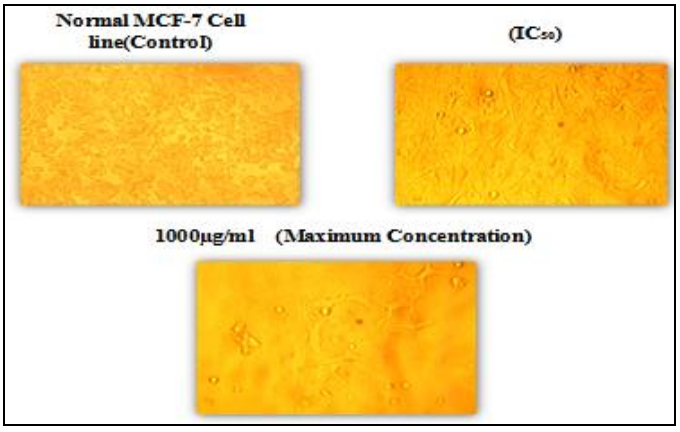


Fig 5: cell morphology of MCF-7cells when treated with IC50 concentrations of fruits peel methanol extract of p.guajava

FTIR analysis

The FTIR spectrum was used to identify the functional group of the active components present in the fruit peel extract. The active compounds and functional groups were identified based on the peak value obtain in the spectrograph and they were illustrated in the fig.6. The graphs of fruit peel extract were revealed the presence of phenols and alcohols, Nitro Compounds, Amines and Amides.

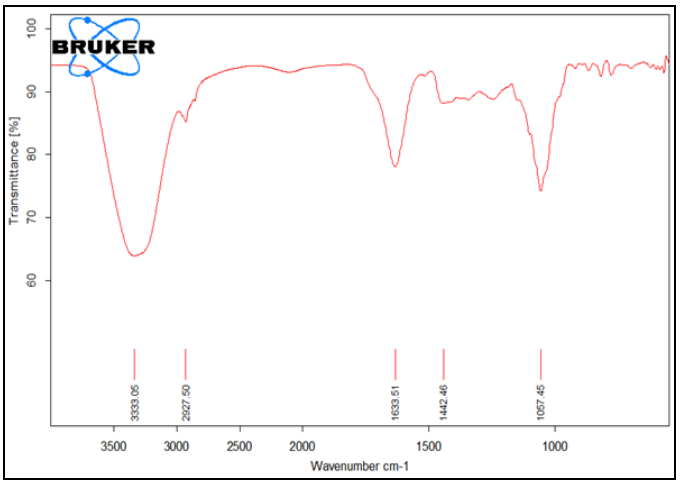


Fig 6: FTIR spectra of various functional groups (3500 to 1000 cm^{-1}) obtained for fruit peel (Methanol extract) of *P. guajava*

Conclusion

In conclusion, the methanol extract of *P. guajava* fruit peel has demonstrated promising antioxidant and anticancer properties against human breast cancer cells by *in vitro* method. Increasing awareness, promotion and utilization of this fruit for public benefits are highly encouraged and identification of active phytoconstiuents in the extracts will serve as a natural cytotoxic agent against various cancers.

Acknowledgement

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