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The active potential of Guava *Psidium guajava* (L.) leaves extract and its anticancer activity

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Abstract
A literature search was conducted on Medline for research articles relating guava (*Psidium guajava Linn.*) to cancer, in order to determine any potential anticancer activity. Today, there is increasing interest in discovering new bioactive compounds derived from ethno medicine. Preparations of guava (*P. guajava L.*) leaves have traditionally been used to manage several diseases. The pharmacological research in vitro as well as in vivo has been widely used to demonstrate the potential of the extracts from the leaves for the co-treatment of different ailments with high prevalence worldwide, upholding the traditional medicine in cases such as diabetes mellitus, cardiovascular diseases, cancer, and parasitic infections. Moreover, the biological activity has been attributed to the bioactive composition of the leaves, to some specific phytochemical subclasses, or even to individual compounds. The purpose of this research was to study Guava (*P. guajava L.*) leaf extract as a potential activity for anticancer activity.

Keywords: *psidium guajava* (L.) extract, guava, anticancer activity

1. Introduction

Guava (*Psidium guajava Linn*.) family Myrtaceae is important plant used traditionally for medicinal purposes. Guava (*P. guajava L*.) used as an important food as well as a medicinal plant in tropical and subtropical countries, therefore its nickname as the poor man's apple. Guava contains dietary fiber, protein, calcium, phosphorus, potassium, copper, iron, vitamin A, vitamin B1, vitamin C, vitamin B2, vitamin B3 and folic acid. It is rich in antioxidant and protects cell damage. With this richness guava serves as both food supplement and also very useful medicine. Guava is rich in antioxidants compounds and contains a high level of ascorbic acid ranging from 174.2 to 396.7 mg/100 g fresh fruit [1].

Psidium guajava is a small tree which is 10m high with thin, smooth, patchy, peeling bark. Leaves are opposite, short-petiolate, the blade oval with prominent pinnate veins, 5–15 cm long. Flowers are somewhat showy, petals whitish up to 2 cm long, stamens numerous [2]. The ability of guava leaf extract on the treatment of various diseases has been proven scientifically, but the mechanism hasn't been fully explained. In general, biological properties of guava have been already associated with its polyphenolic compounds, such as protocatechuic, ferulic, ascorbic, gallic and caffeic acids and quercetin [3]. Polyphenols are secondary metabolites of plants. In the last decade, there has been much interest in the potential health benefits of dietary plant polyphenol as antioxidant [4].

The polyphenol compounds in the extract of guava fruits and leaves can act as an immunostimulant that may lead to an increase in the immune system. Increasing the body's immune system can keep the body from various infectious diseases. A well-functioning immune system is crucial for staying healthy. Therefore, the potential of natural substances to strengthen the immune system has long been the subject of investigation [5]. There were many synthetic and natural preparations claiming to be immunostimulants. They seemed to represent useful alternative to vaccination and chemotherapy in the control of disease. Immunostimulants from natural substances could enhance the specific immune respone [6].

A survey of the literature shows *Psidium guajava* is mainly known for its antispasmodic and antimicrobial properties in the treatment of diarrhoea and dysentery. It has also been used extensively as a hypo-glycaemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepato-protective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses [7]. The ability of this plant to exhibit antioxidant, hepatoprotective, antiallergic, antimicrobial, anticancer, cardioprotective, antidiabetic, ant cough, antidote properties.

1.1 In Vitro Anticancer Studies - Guava Leaf Extracts

Seven articles appeared between 2006 and 2010 relating guava leaf extracts to ant proliferation of cancer cell lines in vitro. One of the first studies was conducted by who tested the ant proliferative activity of essential oils from 17 Thai medicinal plants. Among all these plants, guava leaf oil showed the highest ant proliferative activity against the KB (human mouth epidermal) cancer cell line, and second highest in the P388 (murine leukemia) cancer cell line. Guava leaf also showed potent cytotoxic effects in the KB cancer cell line. The IC50 value was 0.0379 mg/ml which is 4.37 times more potent than the anticancer drug vincristine. The authors proposed that the main flavonoids in guava are myricetin and aligning, and that myricetin is a good antioxidant, ant mutagen, and potent ant carcinogen [8].

The effects of guava leaf extract on a human colon carcinoma cell line. Prostaglandin end peroxide H synthase (PGHS) is a

key enzyme for the synthesis of prostaglandins (PGs), which play important roles in inflammation and carcinogenesis[9]. Guava is known to have antiinflammatory, ant oxidative, and ant proliferative activities [8, 10, 11]. Guava leaf extract inhibited the PGE (2) synthesis and also suppressed the DNA synthesis rate in the PGHS-1- and PGHS-2-expressing cells. These results demonstrate the ant proliferative effect of guava leaf extract may be partially due to the inhibition of PGHS isoform catalytic activity [12, 13].

1.2 Possible Anticancer Guava Components

Guava, particularly its leaves, contains secondary plant metabolites with certain polyphenols with potential intrinsic antioxidant, anti-inflammatory, and antiviral properties [14, 11]. Several guava components have been postulated as having anticancer effects in vitro, and the most frequently reported are ascorbic acid (vitamin C), flavonoids (apigenin), and lycopene. Although only these three are now briefly discussed, many other possible substances in guava plant parts (leaves, fruits, bark, etc.) may have potential anticancer activity [12, 13].

2. Materials and methods

2.1 Collection of leaves of *Psidium guajava* Linn.

The leaves of *Psidium guajava L.* (Myrtaceae), were selected on the basis of ethnopharmacological and ethnobotanical literature survey. The plant materials were collected from the tropical region of Jawadhu Hills, Tiruvannamalai district (12°36′10″N, 78°53′07″E, and altitude 705 m), Tamil Nadu, India. The taxonomic identification was made through Mrs. S. Isabella Rosaline, Associate Professor, Department of Botany, Auxilium College, Katpadi, Vellore District and Tamil Nadu. The voucher specimen was numbered and kept in our research laboratory for further reference [15].

2.2 Preparation of plant extracts

The leaves of *Psidium guajava L.* were air-dried for 7-15 days in the shade at the environmental temperatures (27-37°C day time) and the dried leaves were powdered mechanically using commercial electrical stainless steel blender. Dry powder (250 g) was macerated in 1 litre of deionized water then kept for 24 h at room temperature. The resulting aqueous extract was filtered with Whatman filter paper no. 1. The filtrate was concentrated in a drying-room at 40°C for 24 h. The extract was stored at -20°C [16].

2.3 Anticancer activity

2.3.1 Media preparation (Sigma)

1. Co2 incubator- Thermo Fisher, USA
2. Multimode micro plate reader- Bio Tek, USA
3. Refrigerated centrifuge- Eppendorf Germany
4. Cell: He La - NCCS Pune
5. MTT,(3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide)
6. Fetal bovine serum
7. Trypsin
8. Penicillin
9. RPMI1640 medium
10. DMSO

The sachet (12.0g) was dissolved in 800 ML of sterile distilled water to which 2.5g of sodium bicarbonate was added. The beaker was covered with aluminum foil and stirred using magnetic stirrer for 10 minutes. The medium pH was adjusted to 7.2 using 0.1M Noah. The volume of the medium was made to 1000 ML and filtered through sterile 0.2µ membrane filter unit. The medium quality control was checked by incubating 5 ML of filtered medium in the CO2 incubator for 2 days. The antibiotics and serum was added before it was used for cell culture.

2.3.2 Cell culture and MTT assay Procedure

The He La human cancer cell line was purchased from NCCS Pune. The cells were grown in a RPMI1640 medium supplemented with 10% fetal bovine serum and antibiotics as mentioned earlier [17, 18]. Cell proliferation (MTT) assay was performed following the method described by and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated ones [19].

For the MTT assay, the cells were grown in 25 cm ×25 cm ×25 cm tissue culture flasks containing RPMI1640 medium as culture medium supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (GIBCO) and grown at 37°C under a humidified atmosphere of 95% air and 5% CO2. Cells were regularly passaged and maintained before including for the experiment. When a cell density in a culture flask reached 70-80% confluence, they were trypsinized and seeded in 96-well plates at varying cell number according to the size and shape of the cells were seeded in the density of 3000 cells per well in 100 µL and incubated for 24 hours at CO2 incubator. (Biorad, 680).

Test items were prepared as 20 mg/ml stocks by adding DMSO. The working stock of 2X (2000, 200. 20, 2.0 and 0.2 µg) concentration to the cell in 100 µL volume and the final concentration range were: 1000, 100, 10, 1.0 and 0.1 µg/ml. The plates were further incubated for 48 in the CO2 incubator. MTT solution was composed of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at 5 mg/ml in phosphate buffered saline (1.5 mM KH2PO4, 6.5 mM Na2HPO4, 137 mM NaCl, 2.7 mM KCl; pH 7.4), from this solution 50 µl was pipette out into each well to achive 1 mg/mL as final concentration. The plate was further incubated for 2.30 hours in incubator and the medium was carefully decanted. The Formosan crystals were air dried in dark place and dissolved in 100 µL DMSO and the plates were mildly shacked at room temperature and the OD was measured using Synergy HT micro plate reader at 570nm [20].

From the optical densities the percentage growths were calculated using the following formula:

Percentage growth= 100× [(T-T0)/(C-T0)]
If T is greater than or equal to T0, and if T is less than T0,
Percentage growth = 100× [(T-T0)/T0)],
Where T is optical density of test,
C is the optical density of control,
T0 is the optical density at time zero.

From the percentage growths a dose response curve was generated and GI50 values were interpolated from the growth curves.

2.3.3 Cell Imaging

The end of 48 hours' time point the images were captured before adding the MTT. Different concentration treated cells were observed under microscope for cell morphology analysis and images of each concentration was captured and recorded.

3. Results

3.1. Cell growth inhibition property

The leaves of *Psidium guajava L.* exhibited good growth inhibition in tested cell line with 78.7 µg as GI50. The *Psidium guajava* leaves extract of each concentration was performed in quadruplicate and cumulative variation were maintained less than 20% between the data points. Three set of cell lines were tested in a 96 well plate as described in the below 96 well format.

Tables and Figures

Table 1: Percentage growth of HeLa cells against the *Psidium guajava* leaves extract

Compound	Percentage Growth					Growth Inhibition in µg		
	1000 µg	100 µg	10 µg	1.0 µg	0.1 µg	GI50	TGI	LC50
Psidium guajava leaves extract (SPGT)	45	94	100	79	79	78.7	1000.0	1000.0

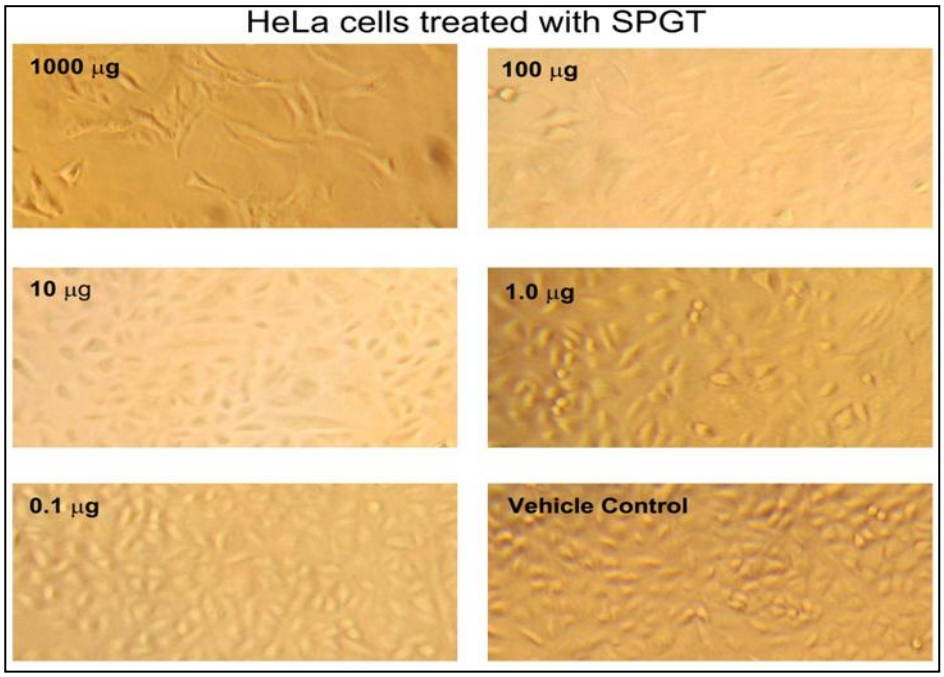

Fig 1: He La cells treated with *Psidium guajava* leaves extract (SPGT) for 48 hours

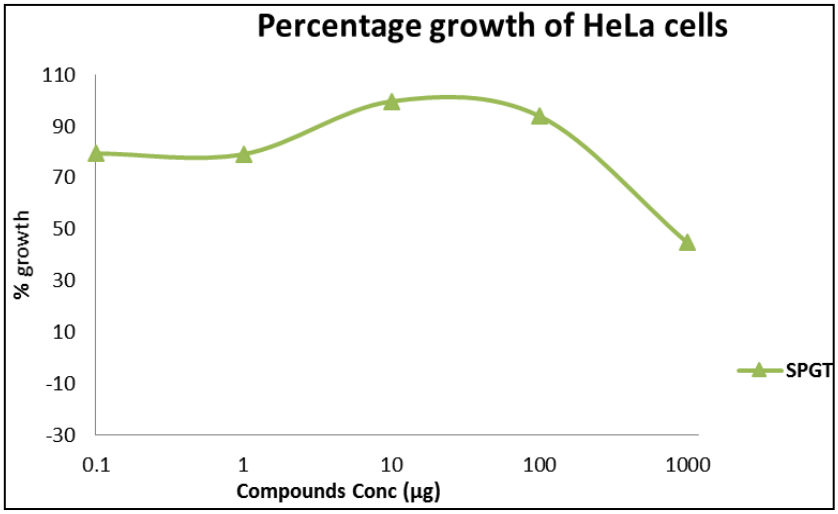

Fig 2: Percentage growth curve of HeLa cells against the *Psidium guajava*(SPGT) leaves extract

4. Conclusions

Seven in vitro studies indexed on Medline have been conducted on guava leaf extracts, and these researchers all suggest the presence of potential anticancer activity. The high antioxidant values of guava were noted by almost half the researchers as a possible contributing factor, but so were other constituents and mechanisms that continue to be researched. We studied and testing guava leaves extract against the proliferation of cancer cell lines. In these and other future studies, the amount of antioxidant tested should be compared

to what is actually biologically available in the blood after the guava fruit is consumed.

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