Asian Journal of Applied Science and Technology (AJAST)
(Open Access Quarterly International Journal) Volume 1, Issue 9, Pages 414-420, 2017

# Qualitative Phytochemical Screening and Assessment of Antimicrobial Activity of *Psidium guajava* and its Cytotoxic Studies

S.R.Sukanya[1], K.Sinthu[2], J.R.Abima Shazhni[3] and F.Starlet Priya[4]

[1,2,3,4]*Department of Biochemistry, Lekshmipuram College of Arts and Science, Neyyoor – 629802, Tamilnadu, India.*

*Article Received: 21 August 2017* *Article Accepted: 30 September 2017* *Article Published: 14 December 2017*

ABSTRACT

This work is aimed at evaluating the phytochemicals and the antimicrobial activity of *Psidium guajava* leaf. Aqueous, chloroform and butanol extracts of dried and ground plant materials were prepared using Soxhlet apparatus. Qualitative phytochemical screening of the extracts showed positive for carbohydrates, chloride, tannins, alkaloids, flavanoids, saponins phenolic compounds and steroids. Antimicrobial activities of the extracts were evaluated by agar well diffusion method. The extracts exhibited significant antimicrobial effects against all the tested bacterial and fungal pathogens. The breast cancer (MCF-7) cell lines were used to screen the in vitro anticancer challenging with the *Psidium guajava* extracts. Concentration ranges from 6.25 to 100µg of *Psidium guajava* purified extracts were screened against (MCF-7) cells. The anticancer activity of *Psidium guajava* were performed by 3-[4, 5–Di methyl thiazol-2-yl]-2, 5-diphenyltetrazolium Bromide (MTT) assay. The cytotoxicity was highly significant in the 100 µg concentration of leaf extract in the breast cancer (MCF-7) cell lines. The results for this activity, confirmed the potential of guava leaves having anti-microbial and anti-cancer activity.

Keywords: Plant extracts, *Psidium guajava,* Phytochemicals, Antimicrobial activity and MTT assay.

## 1. INTRODUCTION

*Psidium guajava* has extensive use in folk medicine. Different parts of the plant can be used in the treatment of diseases such as wounds, lesions, ulcers, diarrhea, cholera, hypertension, obesity and the control of diabetes mellitus [1]. Guava leaves have been used to treat cough and pulmonary diseases [2]. They have also served as anti-inflammatory and haemostatic agent in china. Guavas are rich in dietary fiber and vitamin C with moderate levels of folic acid. Guava contains both carotenoids and polyphones like gallocatechine, leucocyanidin and amritoside. Phytochemical screening revealed the presence of alkaloids, saponins, cardenolides with steroided rings and cardenolides with deoxy sugar. The leaves has been used for treatment of diarrhea, malaria and dysentery. It has been used in the treatment of sore throats vomiting and menstrual complications. Tender leaves are chewed for bleeding gum and bad breath [3].

The leaves powder was successively extracted with petroleum ether, chloroform, ethanol, water, hydroalcoholic [4]. Phytochemical analysis of guava leaf contains following components alkaloids, anthocyanins, carotenoids, triterpenes and vitamin C [5]. The HSC-2 cells treatment with guava leaves ethanolic extract significantly reduced the proliferation and increased apoptosis of HSC-2 cells [6]. *P. guajava* leaf contains plenty of phenolic compounds which inhibit the peroxidation reaction in the body and so it can be expected to prevent various chronic diseases such as diabetes, cardiovascular disease and cancer.

Furthermore, decreasing of free radicals in the body by means that the polyphenols in the leaf of *P. guajava* can prevent atherosclerosis, cataract and also inhibits biological aging of the body and skin [7]. The therapeutic activity of extracts from guava Leaves against, microbial infections, and cancer have been studied.

## 2. MATERIALS AND METHODS

### *Plant sample*

The plant *Psidium guajava* was collected from Marthandam, Kanyakumari District, Tamil Nadu, India. The leaf of the plant used for this study were rinsed severally with clean tap water and shade dried in a dark place at room temperature for few days. The dried plant parts were cut into small pieces ground in electric chopper to get fine powder for further use.

### *Preparation of extracts*

The powdered plant materials were subjected to soxhlet extraction using aqueous, acetone, dimethyl sulfoxide, chloroform and ethanol. Each 5 g of powder of plant material was filled separately in the thimble and extracted successively with 60 ml of solvents using a soxhlet extractor for 3 h. After solvent evaporation, each of these solvent extract was weighed and preserved in room temperature until further use.

### *Qualitative analysis of phytochemical constituents*

All the extracts were subjected to systematic phytochemical screening for testing the presence of various phytochemical constituents by the method followed by standard protocols [8]. Phytochemical test includes carbohydrates, amino acids, proteins, vitamin C, chloride, tannin, alkaloids, flavonoids, phlobatannins, steroids, phenols and saponins.

### *Antimicrobial activity of plant extracts*

Antimicrobial activities of the plant extracts were determined by well diffusion method [9]. Four bacterial pathogens such as *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis*, and *Staphyloccocus aureus* and three fungal pathogens such as *Aspergilus niger, Aspergillus fumigatus* and *Penicillium* sp. were used for this investigation. The test bacterial strains were inoculated into Nutrient broth and incubated at 37°C for 24 h. After the incubation period, the culture tubes were compared with the turbidity standard. Fungal inoculums were prepared by suspending the spores of fungus (as previously cultured) in saline water mixed thoroughly, made turbidity standard and used. Fresh bacterial culture of 0.1 ml having 108 CFU was spread on Muller Hinton agar plates using sterile cotton swabs. The fungal strains were spread on Potato dextrose agar. Wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50µl of plant extracts using micro pipette in aseptic condition. The plates were kept in a refrigerator to allow pre-diffusion of extract for 30 min and then incubated at 37°C for 24 h and 28-30 37°C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

### *MTT assay*

MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilized formazan product

was measured at 540nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The cells was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at $37^0$C for 3 hours. MTT was removed by washing with 1x PBS and 200 µl of DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA).

## 3. RESULTS

### *Qualitative analysis of phytochemical constituents*

Phytochemical screening of the plant extracts were performed for the analysis of phytochemical constituents In the leaf material, aqueous extract showed positive results for carbohydrate, chloride, tannins, alkaloids, flavonoids, Phlobatannins, steroids, phenolic compounds and saponins; Chloroform extract showed positive results only for chloride, tannins, saponins and phenolic compounds; Butanol extract showed positive results for chloride, tannins and phenolic compounds (Table 1).

Table 1. Qualitative analysis of phytochemical constituents

| **Sl. No.** | **Chemical constituents** | **Leaf extracts** | | |
|---|---|---|---|---|
| | | Aqueous | Chloroform | Butanol |
| **1** | Carbohydrates | + | - | + |
| **2** | Protein | - | - | - |
| **3** | Amino acid | + | - | - |
| **4** | Vitamin C | + | - | - |
| **5** | Chloride | - | + | - |
| **6** | Tannins | + | + | + |
| **7** | Alkaloids | + | - | - |
| **8** | Flavonoids | + | | - |
| **9** | Phlobatannins | + | - | - |
| **10** | Steroids | + | - | + |
| **11** | Phenolic compunds | + | + | - |
| **12** | Saponins | + | - | - |

'+' presence of compound; '-'absence of compound

***Antimicrobial activity of plant extracts***

Antimicrobial activity of the plant extracts were determined by well diffusion method against various bacterial and fungal pathogens. The leaf of aqueous extract showed inhibition activity only on *K. pneumonia* (12 mm); chloroform extract showed activity on *E. coli* (12 mm), *S. aureus* (14 mm), *B. cereus* (12 mm), *A. fumigatus* (14 mm) and *A. niger* (15 mm); and butanol extract showed activity on *E. coli* (12 mm), *S. aureus* (16 mm), *B.cereus* (14 mm), *A. fumigates* (17 mm), *A.niger* (14 mm) and *Penicillium sp* (12 mm) (Table 2).

Table 2. Antimicrobial activity of Psidium guajava leaf extracts

| Sl. No. | Test organisms | Leaf | | |
|---|---|---|---|---|
| | | **Aqueous** | **Chloroform** | **Butanol** |
| 1. | *E.coli* | - | 12mm | 12mm |
| 2. | *K. pneumoniae* | 12mm | - | - |
| 3. | *S. aureus* | - | 14mm | 16mm |
| 4. | *B. cereus* | - | 12mm | 14mm |
| 5. | *A. fumigatus* | - | 14mm | 17mm |
| 6. | *A. niger* | - | 15mm | 14mm |
| 7. | *P. chrysogenum* | - | - | 12mm |

**Zone of inhibition in 'mm'**

***Anticancer activity***

The anticancer effect of *Psidium guajava* leaf extract was determined using MTT assay. The present finding indicated the percentage inhibition of MCF-7 cell lines (Fig.1). The (**Fig.2)** displayed the anticancer activity of leaf extracts of *Psidium guajava* in MCF-7cells. The aqueous extract illustrates higher cytotoxicity in 100 µg concentrations. Finally the present study suggests that the aqueous extract of *Psidium guajava* leaf comprised novel anticancer compounds which will be potent therapeutic agent for breast cancers.

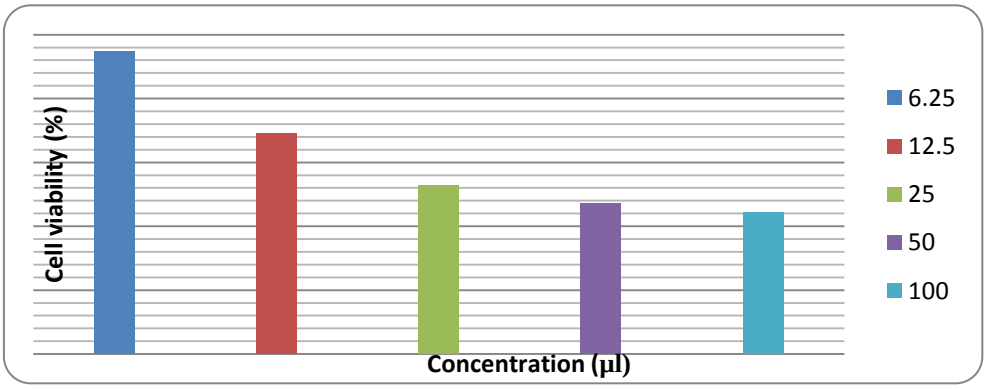


Fig. 1: Percentage viability of MCF 7 cell lines after the exposure with the leaf extract of *Psidium guajava*

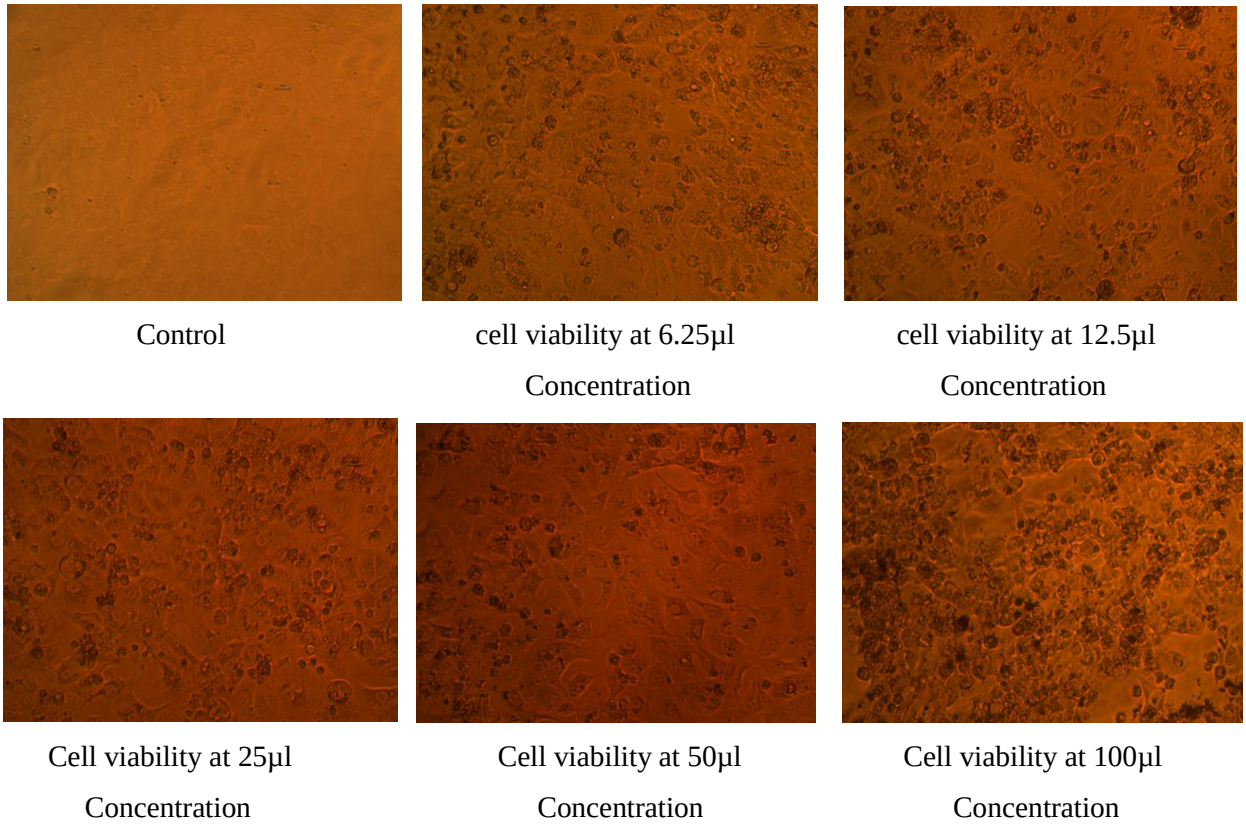


Fig. 2. Anticancer effect of *Psidium guajava* leaf extract on breast cancer cell lines

## 4. DISCUSSION

The present phytochemical study indicated the presence of carbohydrates, amino acids, chloride, tannins, alkaloids, flavanoids, phenolic compounds and steroids. Leaves extract of fruits plants are a good source of bioactive compounds which have some ethno medicinal applications were screened for their antibacterial activity against bacterial pathogen of human. Antimicrobial agents are essentially important in reducing the global burden of infectious disease. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties [10]. Guava and has been used traditionally as a medicinal plant throughout the world for a number of ailments [11]. Guava fruit is also rich in vitamin C, naturally occuring leaves of guava can be used as a potential supplement in the treatment of scurvy [12].

The acetone extracts of guava branch (GBA) had cytotoxic effects on HT-29 cells. GBA inhibits the growth of HT-29 cells [13]. The result from this work has revealed the medicinal potential of this plant in the treatment of various diseases.

## 5. CONCLUSION

Based on the results obtained, it can be concluded that the *Psidium guajava* possesses significant antimicrobial and anticancer activity. Due to this observed pharmacological effects *Psidium guajava* remains a species with tremendous potential and countless possibilities for further investigation.

**REFERENCES**

[1] Tatiane Vieira Braga, Rosana Goncalves Rodrigues Das Dores, Camila Soncin Ramos, Fernanda Cristina Gontijo Evangelista, Leticia Marciada Silva Tinoco, Fernando de Pilla Varotti, Maria das Gracas Carvalho, Adriano de Paula Sabino, **"**Antioxidant, Antibacterial and Antitumour activity of Ethanolic extract of the *Psidium guajava* leaves," *American journal of plant sciences*, vol. 5, pp. 3492 – 3500, 2014.

[2] O. O Udemezue, Ukoha Ukoha, D. N Ezejindu, J. I Okafor, and A. D Obilor, " The effects of leaf extract of Guava on the Liver enzymes of adult wistar rats," *International journal of scientific and Reasearch puplications*, vol. 4(8), pp. 2250 – 3153, 2014.

[3] I. Elekwa, S. C Okereke and B. O Ekpo. **"**Preliminary phytochemical and Antimicrobial investigations of the stem, bark and leaves of *Psidium guajava L*," Journal of medicinal plants research, vol. 3(1), pp. 45 – 48, 2009.

[4] Vikrant Arya, Narender Thakur, C. P Kashyap, **"**Preliminary phytochemical analysis of the extracts of *Psidium* leaves," *Journal of pharmacognosy and phytochemistry*, vol. 1, pp. 2278 – 4136, 2012.

[5] O. O Udemezue, Ukoha Ukoha, D. N Ezejindu, J. I Okafor, and A.D Obilor. "The effects of leaf extract of Guava on the Liver enzymes of adult wistar rats," *International journal of scientific and Reasearch puplications*, vol. 4(8), pp. 2250 – 3153, 2014.

[6] Evie Lamtiur Pakpahan, Alveolie Gabriella, Melisa, Mettasari Wardoyo, Rakhmawati Caesaria, **"**In vitro anticancer activity of guava against HSC - 2 Human Oral Cancer cells," *Dental student's Research*, 2011.

[7] Vijaya Anand, Manikandan, Vijaya Kumar, Sampath Kumar, Pushpa, Agaath Hedina, **"**Phytopharmacological overview of psidium guajava," *Pharmacognosy Journal*, vol. 8(4), 2016.

[8] J.B Harborne, "Phytochemicals Methods. Chapman and Hall Ltd., London," pp. 49-188, 1973.

[9] C. Anushia , P. Sampathkumar, L. Ramkumar, "Antibacterial and antioxidant activities in *Cassia auriculata*," *Global Journal of Pharmacology*, vol. 3(3), pp. 127-130, 2009.

[10] Rinky Bisht, Sheetal Chanyal and Pavan Kumar Agrawal, **"**Antimicrobial and phytochemical analysis of leaf extract of medicinal fruit plants," *Asian J Pharm Research*, vol. 9(4), pp. 131 – 136, 2016.

[11] Sandra M. Barbalho1, M. V Flávia, Farinazzi-Machado, Ricardo de Alvares Goulart, Anna Cláudia Saad Brunnati, Alda Maria Machado Bueno Ottoboni and Cláudia Cristina Teixeira Nicolau "*Psidium Guajava* (Guava) A Plant of Multipurpose Medicinal Applications," *Med Aromat Plants*, vol. 1:4 , pp. 1-6, 2012.

[12] Anurag Mondal, **"**Psidium guajava (Guava) leaves as a potential treatment of scurvey," *Research Journal of recent sciences*, vol. 5(8), pp. 51 – 55, 2016.

[13] Sang-Bong Lee and Hae-Ryong Park, "Anticancer activity of guava (*Psidium guajava L*.) branch extracts against HT-29 human colon cancer cells," *Journal of Medicinal Plants Research,* vol. 4(10), pp. 891-896, 2010.
ez-Alco, C., Torres, N.V., *Biotechnol. Bioeng.* 2000**,** *70*, 82-108.