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A Study on Guava Leaves Extract: A Folk Medicine

Ratul Deb*

Department of Biotechnology, JIS University, West Bengal, India

***Corresponding Author:** Dr. Ratul Deb, Department of Biotechnology, JIS University, West Bengal, India. Email: ratuldeb645@gmail.com

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Abstract

Antibiotics are generally used to kill micro-organisms. But nowadays, micro-organisms showed resistance against many antibiotics like benzyl penicillin, methicillin, vancomycin etc. To overcome this problem, we are in need of natural products with antimicrobial activity, as it shows no side effects like the antibiotics. The guava (Psidium guajava) is a phyto-therapeutic plant and it helps in various fields like treatment and manages of various diseases like malaria, gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, and a number of other conditions. As some of these diseases are due to bacterial infection, we in the present study try to evaluate the antibacterial activity of guava leaves extract. Myeloid leukemia's continue to be difficult to treat at this time too even various medicines is in the relevant field. Novel therapeutic strategies that exclusively target cells as well as various malignant cells. In search for natural product-based anticancer agent tried to evaluate the effect of guava leaf extract on Chronic Myeloid Leukemia (CML) cells. The preliminary studies showed potent anti-CML activity.

Keywords: Guava leaves, Anti-bacterial activity, Anti-leukemic activity

Introduction

Recently natural medicine takes place all in the attention field. Several fruits and fruit extract mainly tea extract from arrowroot [1] and caffeine [2], which helped in antimicrobial activity against the *E. coli* O157:H7 and it is proved in laboratory. Natural medicine manifest relatively high levels of antimicrobial action which can be used in inhibition of the growth of foodborne pathogens. Plant extracts can kill the bacterial cells by the rupture of cell walls and membranes and also extracts may be helpful in solve of the irregular disruption of the intracellular matrix.

The guava (*Psidium guajava*) is a Phyto-therapeutic plant and it helps in various fields like treatment and manages of various diseases like malaria, gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, and a number of other conditions [3-5]. This plant has a potential activity against diabetes, hypertension, and obesity. In this study, I aim to evaluate the total extracts of *P. guajava* leaves which are collected from JIS University Hostel. Also, I have tried to establish a connection of efficacy using various aqueous and organic solvents against killing or inhibiting the growth of foodborne bacterium *Staphylococcus aureus, Escherichia coli*, *Salmonella enteritidis*, and *Bacillus cereus* which are major cause of illness and spoilage [6-10].

Belongs to the family *Myrtaceae*, it is originated in South America tropical region. Guava plants are found and grown in tropical and subtropical areas of the world. The genus Psidium have at about more than 150 species of small trees and shrubs in which only 20 species can produce the edible fruits and the rest are moreover wild and non-edible in nature. The most local cultured species of Psidium is P. *guajava* L. which is the common guava as our local language. This species is utilized for regulation of vigor, fruit quality improvement management and growth of various pest and disease resistance control.

The guava leaves have a height of 2 to 6 inches with 1 to 2 inches in width. When crushed, it has an aromatic flavor and appears dull-green with stiff at it leaves which are clearly shown. Also, it has various bioactive components which can fight against various pathogens, weight loss process as well as regulation of blood glucose levels. The leaves of guava contain an essential oil rich in various types of substances like cineol, tannins, triterpenes, flavonoids, resin, eugenol, and a various number of other substances which are really helpful as a folk medicine. Maceration, infusion aqueous-alcoholic extraction by fermentation, percolation, digestion, decoction, Soxhlet extraction, counter-current extraction, supercritical fluid extraction, and microwave-assisted extraction, and phytanic extraction, ultrasound extraction is the basic extraction procedure. Maceration extraction is basically a crude extraction; in where solvents diffuse into solid plant and solubilize the various compounds which has similar polarity. Effect of the plant material is fully dependable on its origin as well as variations, the time, solvent concentration and polarity, temperature of extraction, various metabolite compositions present in following extract. Variations in extraction methods are found in areas of extraction period, the solvent used pH, particle size, enlisted temperature and the solvent-to-sample effective ratio.

The antimicrobial effect of essential oils, methanol, hexane, and ethyl acetate extracts was observed by Gonc, alves et al. Gottlieb and Magalhaes extracted essential oil from fresh leaves of guava using a Clevenger type doser. Plant produces non-nutritive phytochemicals for their own protection, but it has an extra- ordinary against various diseases of human. Isolation of two triterpenoids named as guavanoic acid and guava coumaric acid was isolated. Arima and Danno also identified four types of flavonoids which can inhibit the growth of *Salmonella enteritidis* and *Bacillus cereus* in partial way.

Materials and Methods

Preparation of plant extract: The leaf samples were collected from the guava trees. Random leaf samples were collected into plastic zip lock bags with appropriate labeling and stored in an ice cooler until being transported to the laboratory for extraction.

Extraction methods: 10gm of leaf samples were washed in tap water, dried, and crushed in the ice- chilled mortar-pestle, which was placed on an ice-bag, with 20 ml of 70% Ethanol. The mixtures were transferred to 50 ml falcon tubes and centrifuged at 6,000 rpm for 40 minutes at 4°C. The supernatant was collected by filtering with filter paper and stored at 4°C until use. Some amount of supernatant was

lyophilized at 45 psi. Then 5 mg of lyophilized material was mixed with 1 ml of DMSO.

Phytochemical analysis: Screening and identification of bioactive chemical constituents in the guava leaves were carried out with the extracts using the standard procedure and chemical test was done by regular method.

Test for Protein: The protein content of guava leaves extract was determined by Folin-Ciocalteu (Lowry) assay. Dilutions of BSA with concentrations of 200, 400, 600, 800 and 1000 µg/ml was done by transferring respective amount from the BSA solution (1mg/ml) and adjusting it to a total volume of 1 ml by distilled water. Reagent A (2% sodium carbonate in 0.1n sodium hydroxide) and Reagent B (0.5% copper sulphate in 1% potassium sodium tartrate) were added in the ratio of 49:1 to form Reagent C. Then 5 ml of Reagent C was added in all the tubes. The tubes were then incubated at dark for 10 minutes. After that, 0.5 ml of 2X Folin-Ciocalteu Reagent was added in all the tubes. The tubes were again incubated at dark for 30 minutes. Then the absorbance was recorded at 660 nm in the spectrophotometer. The same process was done in case of guava leaves extract with concentrations of 12.5, 25, 50 and 75 µg/ml.

Test for Phenols: The phenol content of guava leaves extract was determined by Folin-Ciocalteu (Gallic Acid) Assay. At first a stock solution of 6 ml of Gallic Acid was made of concentration 1 mg/ml. Serial dilution was taken with known volume (usually 3 ml) of stock and it was replaced into a known volume of distilled water (mainly 3 ml). This produced 6ml of the dilute solution and it was marked as T1. The concentration of T1 was 0.5mg/ml. Again, from T1, 3 ml of solution was taken and was placed into a 3ml of distilled water and it was marked as T2. The concentration of T2 was 0.25 mg/ml. In the same way, the process was continued for T3, T4 and T5, having concentration 0.125, 0.0625 and 0.03125 mg/ml respectively. Now, from the stock solution and all the dilutions (T1, T2, T3, T4 and T5), 0.1ml was taken out individually and was mixed with 1.9 ml of Folin-Ciocalteu Reagent. The tubes were incubated at dark for 10 minutes. Then the absorbance was recorded at 720 nm in the spectrophotometer. The same process was done in case of guava leaves extract with concentrations of 12.5, 25, 50, 75 and 100 µg/ml.

Test for scavenging activity: The scavenging activity of guava leaves extract was determined by Hydrogen Peroxide Scavenging Assay. At first, 100 ml of Potassium Buffer Saline (P.B.S) was prepared with 100ml double distilled water, 0.8 gm sodium chloride, 0.02 gm potassium chloride, 0.15 gm sodium hydrogen phosphate and 0.024 gm potassium hydrogen phosphate. The O.D of PBS is taken. From 100 ml of PBS, 45.3 µl of PBS was taken out and replaced with same volume of 4 mm Hydrogen Peroxide in the dark and O.D was taken. For, PBS, dilutions of samples with concentrations of 12.5, 25, 50, 75 and 100 µg/ml were done by transferring respective amount from the guava leaves extract and adjusting it to a total volume of 2.6 ml by P.B.S. For PBS+H_2O2, dilutions of samples with concentrations of 12.5, 25, 50, 75 and 100 µg/ml was done by transferring respective amount from the guava leaves extract and adjusting it to a total volume of 2.6 ml by PBS+H_2O2. In both cases, absorbance was taken at 230 nm in the spectrophotometer.

Test for Flavonoids: The flavonoid content of guava leaves extract was determined by Colorimetric Assay. Dilutions of Catechin with concentrations of 12.5, 25, 50, 75 and 100µg/ml was done by transferring respective amount from the catechin solution (0.5mg/ml) and adjusting it to a total volume of 1.5 ml by Distilled water. Then 0.1ml of 10% aluminium carbonate was added in all the tubes. After that, 0.1 ml of IM Potassium Acetate was added in all the tubes. Then, 2.8 ml of Distilled water was added in all the tubes. The tubes were incubated at dark for 30 minutes. Then the absorbance was recorded at 415nm in the spectrophotometer. The same process was done in case of guava leaves extract with concentrations of 12.5, 25, 50 and 75µg/ml.

Test for Tannin: The flavonoid content of guava leaves extract was determined by Tannic Acid Assay. Dilutions of tannic acid with concentrations of 12.5, 25, 50, 75 and 100µg/ml was done by transferring respective amount from the tannic acid stock solution (1mg/ml) and adjusting it to a total volume of 1.5 ml by adding Ethanol. Then 0.5 ml of 25% sodium carbonate was added in all the tubes. After that, 0.25 ml of Folin- Ciocalteu reagent was added in all the tubes. Then, 2.75 ml of Distilled water was added in all the tubes. The tubes were incubated at dark for 30mins. Then the absorbance was recorded at 725nm in the spectrophotometer. The same process was done in case of guava leaves extract with concentrations of 12.5, 25, 50 and 75 µg/ml.

Antibacterial Activity: The antibacterial activity of guava leaves extract was determined by Agar Well Diffusion Method. At first nutrient agar solution was prepared by mixing nutrient agar powder and Agarose in Distilled water. The solution was then autoclaved at 121°C with 15 psi for 1 hour. The solution was then poured into 4 agar plates and let them sit still for solidification. After solidification, 10 µl of overnight inoculated Nutrient Broth solution of *Escherichia coli* K12 culture was poured respectively in 2 plates and 10 µl of overnight inoculated Nutrient Broth solution of *Pseudomonas geniculata* SMKP6 culture was poured respectively in 2 plates. The solutions were spread with a glass spreader and the plates were left in the laminar air flow for 15-30min for drying. After drying, 3 wells were made in each of the 4 plates. In case of E. coli plates, one plate consists of wells for Control, 25 and 50 µl of guava leaves extract, and the other plate consists of wells for Control, 100 and 200 µl of guava leaves extract. In case of Pseudomonas plates, one plate consists of wells for Control, 25 and 50 µl of guava leaves extract, and the other plate consists of wells for Control, 100 and 200 µl of guava leaves extract. Now the control was poured in the wells for control, and samples are poured in their wells according to their concentration in individual plates. The *Escherichia coli* K12 plates were kept in incubator at 37°C and *Pseudomonas geniculata* SMKP6 plates were kept in the dark at room temp for overnight to achieve the result.

Anti-leukemic Activity: The effects of guava leaves extract on leukemic cells were determined by conventional Trypan blue exclusion assay. For this assay, 5 mg of the lyophilized sample was mixed with 1 ml of DMSO to have a concentration of 5 mg/ml which was used for this assay. Chronic Myelogenous Leukemia (CML) cell line K-562 was used in this assay. Cells were seeded in triplicate at a density of 0.1 × 106 in 24 well plates. After that cell were treated with different concentrations ranging from 0, 2.5, 5, 10 and 20 µl/ml. After 48hours of incubation, cells were centrifuged at 2500 rpm for 7mins. The media was discarded & the pellet was dissolved in 1 ml RPMI (Roswell Park Memorial Institute) 1640 media. Now from this, 10 µl was taken in an Eppendorf and 40 µl of Trypan blue strain was added to make it 5X dilution. The total stained cells were taken and it was placed on haemocytometer. The cells were counted under inverted phase contrast microscope.

Results

Protein estimation in the absorbance and graph for Standard Protein (B.S.A) concentrations are given below in (Table 1) (Figure 1).

B.S.A Conc. (µg/ml)	O.D at 660 nm
200	0.194
400	0.399
600	0.584
800	0.781
1000	0.991

Table 1: Absorbance for (B.S.A) concentrations of 660 nm.

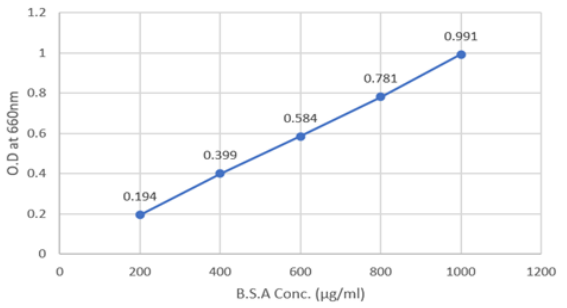

Figure 1: Absorbance for (B.S.A) concentrations of 660 nm.

In (Table 2) (Figure 2) it shows the absorbance for guava leaf samples of different concentrations at 660 nm.

Guava Leaves Extract (µl/ml)	O.D at 660 nm
12.5	0.17
25	0.33
50	0.67
75	0.98

Table 2: Absorbance for guava leaf samples. Therefore, the protein concentration for extract of concentrations: 12.5 µl/ml: 0.17/(0.001 × 12.5)=13.6 µg/µl; 25 µl/ml: 0.33/(0.001 × 25)=13.2 µg/µl; 50 µl/ml: 0.67/(0.001 × 50) = 13.4 µg/µl; 75 µl/ml: 0.98/(0.001 × 75)=13.1 µg/µl.

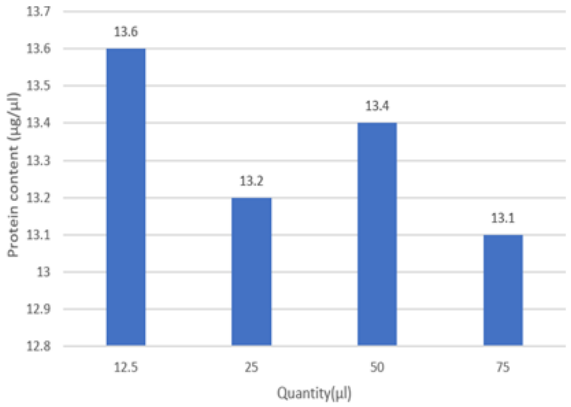

Figure 2: Total protein content of different concentrations of guava leaves extract.

Phenol estimation

The absorbance and graph for Gallic acid concentrations are given below in (Table 3) (Figure 3):

Gallic Acid Conc. (µg/ml)	O.D at 720 nm
1000	0.999
500	0.497
250	0.248
125	0.123
62.5	0.065
31.25	0.032

Table 3: Absorbance of Gallic acid concentration at 720 nm.

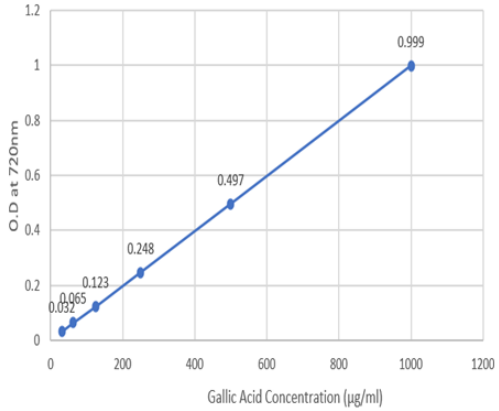

Figure 3: Absorbance of Gallic acid concentration at 720 nm.

Now, the absorbance for guava leaf samples of different concentrations is given below in (Table 4) (Figure4).

Guava Leaves Extract (µl/ml)	O.D at 720 nm
12.5	0.124
25	0.242
50	0.49
75	0.73
100	0.985

Table 4: Phenol concentration for extract of concentrations. Therefore, the phenol concentration for extract of concentrations: 12.5 µl/ml:0.124/(0.001 × 12.5)=9.9 µg/µl; 25 µl/ml:0.242/(0.001 × 25)=9.7 µg/µl; 50 µl/ml:0.49/(0.001 × 50)=9.8µg/µl; 75 µl/ml: 0.73/(0.001 × 75)=9.7 µg/µl; 100 µl/ml:0.985/(0.001 × 100)=9.9 µg/µl.

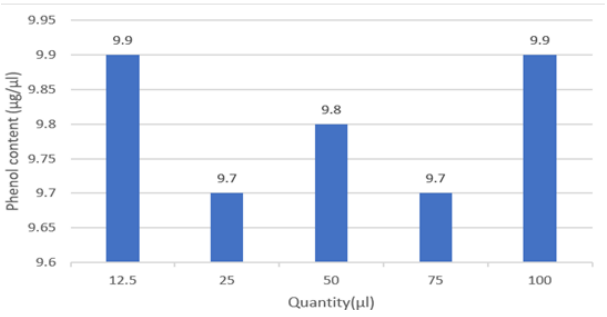

Figure 4: Total phenol content of different concentration of guava leaf extract.

It was observed that the guava leaf has high phenol content. High phenol generally contributes to the leaf's antioxidant potential.

Scavenging Activity

Now, the absorbance for guava leaf samples of different concentrations with blank (P.B.S) are given below in Table 6.

Guava leaves extract (ml)	P.B.S	O.D at 230 nm
0.0125	2.5875	0.03
0.025	2.575	0.19
0.05	2.55	0.34
0.075	2.525	0.52
0.1	2.5	0.69

Table 6: Absorbance for guava leaf samples of different concentrations.

Now, the absorbance for guava leaf samples of different concentrations with control (P.B.S+H_2O_2) is given below in Table 7.

Guava leaves extract (ml)	P.B.S+H2O2	O.D at 230 nm
0.0125	2.5875	0.48
0.025	2.575	0.55
0.05	2.55	0.634
0.075	2.525	0.732
0.1	2.5	0.816

Table 7: Samples of different concentrations with control (P.B.S+H_2O_2).

Conclusion

Psidium guajava is a common tree in tropical and subtropical region. Its leaves are not commonly taken as D food but yet it has a high nutritional profile. The macronutrients and micronutrients were analysed by various types of standard methods. The biochemical studies revels that the concentration of Carbohydrate, Protein, Starch and Amino Acid present in the sample is very high than their RDA value. It is also determined that guava leaves are the good source of Vitamin C and Vitamin B, Calcium, Magnesium, Phosphorus and Iron and various substances. Comparison between the guava leaf and guava fruit based on concentration of micronutrients showed result that, the leaves have more concentration in Vitamin B, Iron, Calcium, Magnesium, Phosphorus and various substances. So, I can conclude the leaves are rich in nutrients than fruit. Raw guava can help to decrease blood pressure and blood lipid in body as it has high Vitamin C and soluble fibre content. Guava leaves play an important role in improving body immunity and helps in keeping small blood vessels healthy as vitamin C is present in high range. Guava leaves contain Vitamin B complex that is helpful in improving blood circulation areas leads to the brain, also effect the stimulating cognitive function and relaxing the nerve cells. Guava leaves can help against diseases such as osteoporosis, hypo-calcemia, hypophosphatemia etc because of the enrichment of high concentration od Calcium and phosphorus. It is also used in hair regrowth as it is rich in vitamin B and C which really nourish the follicles and help in hair growth. The vitamin C helps to boost the collagen activity which helps hair grow out faster and healthier. Guava leaf helps in relieving cough and cold as it helps get rid of mucus due to high presence of iron compound as well as it also disinfects the respiratory tract, throat and lungs. Though it is cheaply and easily available right in our surroundings, we do not make any use out of it. Guava leaves. Beside all of these, the research shows that guava leaves could be used to prepare organic tea that lower cholesterol levels and diabetics and it is ongoing in different shadow. We can bring back this folk medicine and reuse them in broader and specific manner which is much better than those chemical drugs. The present study showed that guava leaf is a reservoir of potent secondary metabolites. Those metabolites make the extract a potential antibacterial and probable anti-cancerous agent. I need to go further specific studies on this ground to evaluate more accurate and specific molecular pathway involved with all of the processes.

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