



GC-MS ANALYSIS OF PHYTOCHEMICAL FROM *PSIDIUM GUAJAVA* LINN LEAF EXTRACT AND THEIR *INVITRO* ANTIMICROBIAL ACTIVITIES

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ABSTRACT

In the present study, hot water, methanol, chloroform, hexane, ethyl acetate and acetone extracts of *Psidium guajava* leaves were extracted. The phytochemicals such as, saponins, tannins, flavonoids, alkaloids, carbohydrate, terpenoid and phenolic compound were determined in ethyl acetate extract. All six leaf extracts were used for antibacterial activity studies. Among the tested solvent, ethyl acetate extract showed more activity against most of the tested organisms. This extract showed high inhibition zone against *Entrobacter* sp. (15 mm), *Proteus vulgaris* (19 mm), *Pseudomonas aeruginosa* (15 mm) and *S. aureus* (18 mm). The methanol extract showed considerable activity (20 mm) against *E. coli* than other solvent. Acetone and ethyl acetate extract showed potential activity against *Candida albicans*, however, all tested extracts showed very little activity against *Aspergillus* sp. The GC-MS analysis confirmed the presence of compounds such as, 2-Isopropoxyethylamine, Bicyclo(7.2.0)undec-4-Ene, 4,11,11-Trimethyl-8-methylene-, Caryophyllene, Alpha-Farnesene, Trans-Z, alpha-Bisaboleneepoxid Alpha-bisabolol, β -carotene, Propanoic acid, 2-(Aminoxy)- and 2,4,6-Cycloheptatrien-1-one, 3,5-Bis-Trimethylsilyl.

KEY WORDS

Psidium guajava, antimicrobial, traditional medicine.

INTRODUCTION

Psidium guajava a low evergreen shrub or tree 6 to 25 feet high, with wide-spreading branches and square, downy twigs, is a native of tropical America. *P. guajava* L. belongs to the family Myrtaceae which is mainly distributed in warm and tropical temperature regions of the world [1]. This plant is rich in triterpens, tannins, essential oil, flavonoids, saponins, fiber, vitamins, fatty acids, alanine, palmaric acid, glutamic acid, quercetin and D-glucose [2]. *P. guajava* is mainly used as folk medicine many countries. The leaves and decoction prepared from this plant are used for the treatment of vomiting, dysentery, stomach upsets, bleeding gums, intestinal worms, prevention of hangovers, edema and

cough [3]. A decoction of the leaves or bark of *P. guajava* L. is used for the treatment of ulcer, wounds and eye infections [4]. *P. guajava* leaf extracts have improve myocardial function and antioxidant properties [5]. In various studies, *P. guajava* leaf and bark extracts showed potent antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi*, *Shigella* spp., *Salmonella paratyphi* A, B, C, *Bacillus* spp., *E. coli*, *Pseudomonas* spp. and *Clostridium* spp. This plant also showed potent antifungal, anti-amebic, anti-yeast and antimalarial properties [6, 7]. The present study was conducted to study the possible inhibitory effect of the leaf of *P. guajava* against Gram-negative and Gram-positive bacteria and selected fungi. Also, the

antimicrobial compounds were identified using Gas chromatography - mass spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

P. guajava leaves processing

Psidium guajava leaves were collected, washed and dried in shade at room temperature for two weeks. Upon drying, the plant material was blended and powdered using a mixer grinder. It was stored in an air tight container until use.

Preparation of plant extracts

The dried leaf powder was suspended in hot water, methanol, chloroform, hexane, ethyl acetate and acetone for overnight in sterile conditions. The extract was finally filtered using Whatman's filter paper no.1.

Preparation of hot water extract

Hot water extract was prepared by plain decoction method. For this 25 gm of leaf powder was taken into a beaker containing 200 ml of sterile double distilled water. This was heated in water bath till the volume of the extract reduced to less than 50 ml. The liquid was filtered using Whatmans's filter paper no.1. Then the water content was evaporated off completely to achieve dried form of extract.

Preparation of solvent extract

25 gm of powder was extracted with methanol, chloroform, hexane, ethyl acetate and acetone by mixing it for 24 h, resulting liquid was filtered using Whatman's filter paper no. 1.

Phytochemical screening from the leaf extract

Phyto-chemical analysis for the screening and identification of bioactive chemical constituents of extracts of the leaf was performed with the standard method with little modifications. The phytochemicals such as, saponins, phenolic compounds, tannins, terpenoids, flavonoids, alkaloids, carbohydrates and phenolic compounds were determined [8].

Evaluation of antimicrobial activity of *P. guajava*

Test organisms

The following pathogenic strains were used for the antimicrobial activity studies. The bacteria such as, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter*, *Proteus* sp., and *E. coli* were used. To analyze antifungal activity, *Candida albicans* and *Aspergillus* sp. were selected.

Preparation of inoculums

Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to

the nutrient broth (g/l) (peptic digest of animal tissue, 5.0; beef extract, 1.5; yeast extract 1.5 and sodium chloride, 5.0). The Erlenmeyer flasks were incubated at 37 °C for 18 h for bacterial culture and 72 h for fungal isolate and were used as the inoculum.

Antimicrobial assay of leaf extract by agar well diffusion method

The extracts (hot water, methanol, chloroform, hexane, ethyl acetate and acetone) were tested for antimicrobial activity using agar diffusion on Muller Hinton Agar. The media was sterilized by autoclaving at 121 °C at 15 lbs pressure for 15 min. The molten agar was allowed to cool at 45 °C and then 20 ml of Muller Hinton Agar was poured carefully into Petri dishes. The agar was allowed to set and harden. The test plates of each organism were prepared. Using sterile swabs lawn of the test organism was spread onto the Muller Hinton Agar plates. The wells were punctured in the center by using a sterile cork borer. Then the wells were filled with all six extracts. The plates were incubated at 37 °C for 24 h for bacterial isolates and 72 h for fungal isolates. After incubation the plates were observed for the zone of inhibition.

Gas chromatography - mass spectrometry (GC - MS) analysis

The GC-MS analysis of the ethyl acetate extract of *P. guajava* was carried out. Thermo GC-trace standard non-polar column (Dimension: 30 meters, film: 0.25 µM) was used for analysis. The injector temperature was set at 260 °C and 1 µl of sample injected into the instrument. The oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 50-650 (m/z) and the ionization voltage was 70eV. The spectrum of the components was compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

RESULTS AND DISCUSSION

Phytochemical screening of leaf extract

Phytochemical screening of ethyl acetate extracts revealed the presence of flavonoids, tannins, terpenoids, saponins, alkaloids, phenolic compounds and carbohydrates (Table 1). Guava leaves have several chemical constituents such as comarins, essential oils, flavonoids, triterpenes and ellagitannins which are known to have antimicrobial properties. Polyphenolic

compounds dominate Guava leaves are flavonoids (>1.4%) and tannins. Antibacterial compounds derived from plants could be phenolic substances such as flavonoids [9]. Tannins can be obtained from almost any kind of green plants; however, the quantity varies. Tannins are polyphenolic compounds that are in plants,

food and beverage [10]. Guava leaves contain tannin by 9%, which can be used as an antibacterial. Tannins can be used as an antibacterial substance because it has a phenol group, so that the tannins have properties like alcohol is an antiseptic that can be used as an antimicrobial component [9].

Table 1: Activity of *P. guajava* leaf extract against selected bacteria and fungi

Leaf extracts	Zone of inhibition (mm)						
	<i>E.coli</i>	<i>Enterobacter</i>	<i>Proteus</i> sp.	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Candia albicans</i>	<i>Aspergillus niger</i>
Acetone	20	15	17	14	16	19	6
Hexane	14	12	15	9	11	11	5
Methanol	15	11	13	12	12	9	7
Ethyl acetate	13	15	19	15	18	16	8
Chloroform	14	10	14	11	8	8	11
Water	13	9	16	14	11	9	12
Standard Amikacin/ Nystatin	22	25	25	29	31	26	23

Table 2: GC-MS analysis of active principles of *P. guajava* leaf extract with their retention time, scan, height, area (%) of compounds

SI No.	RT	Scan	Height	Area	Area %	Norm %
1	14.468	2333	8,520,143	3,012,499.2	38.623	88.64
2	16.374	2714	1,376,331	146,496.1	1.878	4.31
3	16.754	2790	4,146,486	607,429.2	7.788	17.87
4	16.874	2814	3,584,614	397,678.2	5.099	11.70
5	17.804	3000	6,923,308	3,398,719.0	43.574	100.00
6	25.948	4628	2,613,502	236,981.9	3.038	6.97

Table 3: Identified compounds from the ethyl acetate extract of *P. guajava* leaves and their activity

Retention Time	Compound	Biological activity
9.601	2-Isopropoxyethylamine	Antimicrobial activity
14.468	Bicyclo(7.2.0) undec-4-Ene,4,11,11-Trimethyl-8-methylene-, [IR-(IR*,4Z,9S*)]-	Antibacterial activity
16.374	Caryophyllene	Anaesthetic activity
16.754	Alpha-Farnesene	Antioxidant activity
16.874	Trans-Z, alpha-Bisaboleneepoxide	Antibacterial activity
17.804	Alpha-bisabolol	Anticancer activity, Immunosuppressant, Anti-inflammatory
19.850	B-carotene	Antioxidant activity, Anticancer activity
22.116	Propanoic acid, 2-(Aminooxy)-	Antioxidant activity
25.948	2,4,6-Cycloheptatrien-1-one,3,5-Bis-Trimethylsilyl-	Antioxidant activity

Antimicrobial activity of leaf extract

All six leaf extracts were used for antibacterial activity studies. Among the tested solvent, ethyl acetate extract showed more activity against most of the tested organisms. This extract showed high inhibition zone against *Enterobacter* sp. (15 mm), *Proteus vulgaris* (19 mm), *Pseudomonas aeruginosa* (15 mm), and *S. aureus* (18 mm). The methanol extract showed considerable activity (20 mm) against *E. coli* than other solvent (Fig. 1). In this case, ethyl acetate extract showed 15 mm zone of inhibition. Acetone and ethyl acetate extract showed potential activity against *Candida albicans*, however, all tested extracts showed very little activity against *Aspergillus* sp. (Fig. 2) (Table 1). The extracts of *P. guajava* leaves were tested for antibacterial potential and found to be effective against *Staphylococcus aureus*, *Streptococcus mutatis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Proteus* spp. *Shigella* spp. and *Escherichia coli*; the major causal agents of intestinal infections in humans [11, 12]. The methanolic root extract of *P. guajava* that consists of quercetin was also found to be fungicidal [12]. The bark tincture showed fungicidal activity at different concentrations but exhibited only fungistatic property in case of *Candida albicans* [13, 14]. The *in vitro* antibacterial activity of *P. guajava* leaf extract on *Staphylococcus aureus* was possibly due to protein degrading activity of the extracts [15]. Recently, antimicrobial and larvicidal properties of novel quinine compound was reported from *Aegle marmelos* (Linn) [16].

GC-MS analysis of ethyl acetate extract of *P. guajava* leaves

Nine compounds were identified in *P. guajava* leaves by GC-MS analysis (Fig. 3). The active principles with their retention time (RT), compound and their biological activity are presented in Table 2. The biological activity of the identified compound was tabulated (Table 3). The GC-MS chromatogram shows the elution profile of ethyl acetate extract of *P. guajava* leaves. The GC-MS method confirms that *P. guajava* contains the compounds such as, 2-Isopropoxyethylamine, Bicyclo (7.2.0) undec-4-Ene,4,11,11-Trimethyl-8-methylene-, [IR-(IR*,4Z,9S*)]-, Caryophyllene, Alpha-Farnesene, Trans-Z, alpha-Bisaboleneepoxide, Alpha-bisabolol, B-carotene, Propanoic acid, 2-(Aminooxy)- and 2,4,6-Cycloheptatrien-1-one,3,5-Bis-Trimethylsilyl. GC-MS analysis was widely used to determine the phytochemical from the medicinal plants [17 – 19]. Fu et al. [20] identified nine compounds such as, ursolic acid, 2 alpha-hydroxyoleanolic acid, 2 alpha-hydroxyursolic acid, quercetin, hyperin, 1- O- galloyl-beta- D- glucose morin- 3- O- alpha- L- arabopyranoside, myricetin- 3- O- beta- D- glucoside and quercetin- 3- O- beta- D-glucuronopyranoside were identified from the leaf extract of *P. guajava*. Jebashree et al. [21] carried out GC-MS analysis using ethyl acetate extract to find the anticancer molecules. Thirteen compounds were identified from the extract of *P. guajava* and caryophyllene is a known compound for its curative efficiency as an antibacterial activity and anti-inflammatory and remarkable [22].

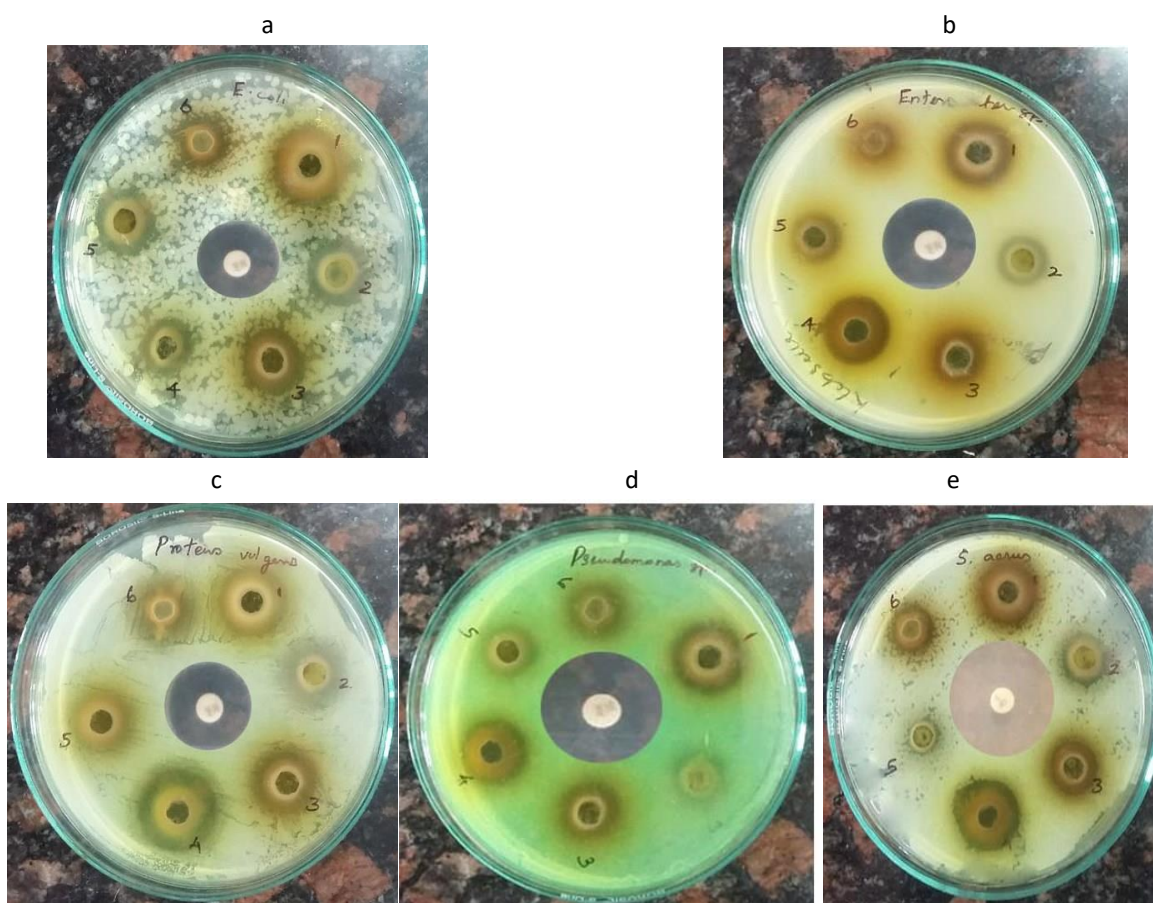


Figure 1: Antibacterial activity of *P. guajava* leaf extracted with various solvent against *E. coli* (a), *Enterobacter* (b), *Proteus* (c), *Pseudomonas* (d), *Staphylococcus* (e) (1-Acetone, 2-Hexane, 3 – Methanol, 4 – Ethyl acetate, 5 – Chloroform, 6- Water).

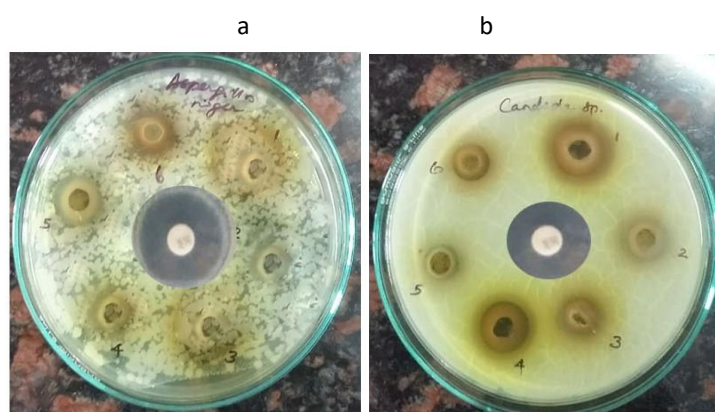


Figure 2: Antifungal activity of *P. guajava* leaf extracted with various solvent against *Aspergillus* (a) and *Candida* (b) (1-Acetone, 2-Hexane, 3 – Methanol, 4 – Ethyl acetate, 5 – Chloroform, 6- Water).

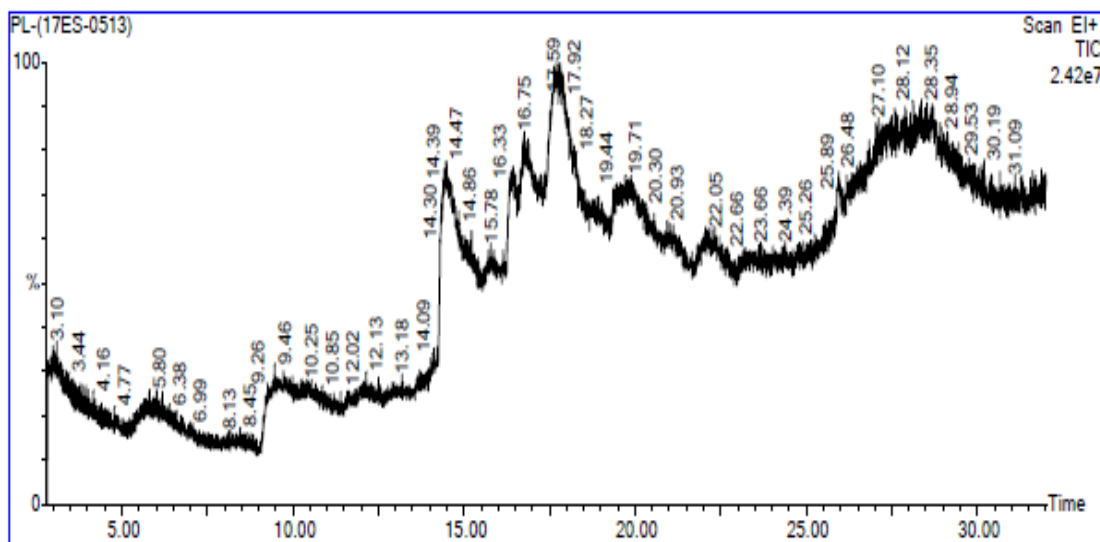


Figure 3: Gas Chromatography – Mass Spectrophotometry profile of compounds from the ethyl acetate extract of *P. guajava* leaves.

CONCLUSION

As the continuous emergence of multi drug-resistant bacteria necessitates the search of novel antimicrobials, naturally available secondary metabolites may act as alternative for chemotherapeutic agents and antibiotics in certain circumstances. To conclude that the *P. guajava* leaf extracts have a potent antibacterial and antifungal activity against both Gram +ve and Gram –ve bacteria. Also showed potent activity against *C. albicans* and found to be less effective against *A. niger*. The antimicrobial property of *P. guajava* may help to explore the novel chemical classes of antibiotics that could serve potent agents for infectious diseases. The information obtained in this study may provide validation for its reported medicinal applications.

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