

## GC-MS Analysis and anti-microbial activity of *Psidium Guajava* (leaves) grown in Malva region of India

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### Abstract

The essential oil of the leaves of *Psidium guajava* grown at Ujjain M.P. (India) was isolated and analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The components of the essential oil were identified by comparing their retention indices and mass spectra fragmentation patterns with those stored on the MS-computer library and also from the published literatures. The present study describes the phytochemical profile and anti-microbial activity of essential oil of *P. guajava*. Furthermore, anti-microbial activity of oil was evaluated using agar well diffusion method. The anti-microbial test results showed that the oil had a potential anti-microbial activity against all twelve Gram+ve and Gram-ve bacterial strains such as: *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Lactobacillus spp.*, *Enterococcus aerogenes*, *Acinetobacter spp.* (Gram Positive) and *Escherichia coli*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Gram Negative). Essential oil showed maximum zone of inhibition and minimal inhibition concentration against *Bacillus subtilis* and *Escherichia coli* bacterial strains. These results permitted the conclusion to be made that, it is the first report of the GCMS analysis and anti-microbial activity on a *P. guajava*, a naturally growing species from Malva Region of India.

### Key words:

*P. guajava* (Leaves), Myrtaceae, Gas Chromatography Mass Spectrometry, Anti-Microbial Activity

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### INTRODUCTION

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active

substances that produce a definite physiological action on human body. The most important of these chemically active constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes [1-2]. Natural products, which come out from medicinal plants are important for pharmaceutical research and for drug development as a sources of therapeutic agents. At presents the demand for herbal or medicinal plant products has increasing significantly.

*P. guajava* (Guava) commonly called as "Amrude". In folk medicines, extracts of roots, bark, and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums and a number of other conditions [3]. *P. guajava* is one of the 150 species of *Psidium*. It is a small tree up to 33 ft in height with spreading branches. It is easy to recognize because of its smooth, thin copper colored bark that flakes off, showing the greenish layer beneath and also because of the attractive bony trunk which attains a diameter of 10 inches. Young twigs are quadrangular and downy. The leaves are when crushed, evergreen, opposite, short petiole, oval or oblong, elliptic, sometime irregular in outline, 2 and 1/2 to 6 inches wide, leathery with conspicuous parallel veins and more or less downy on the underside. Faintly fragrant, the white flowers, borne singly or in small clusters in the leaf axial. These flowers are 1 inches (2.5cm) wide, with 4 or 5 white petal, which shed quickly and a prominent tuft of perhaps 25 white stamens, tipped with pale yellow anthers.

The fruit sweet musky odor when ripe, may be round, ovoid or pear shaped, 2 or 4 inches long with 4 or 5 protruding floral remnants (sepals at the apex ) and the skin is light yellow and thin. Next to skin is a layer of granular flesh, 1/8 to 1/2 inch thick, white yellowish, light or dark pink or near red juicy, acid, sub acid or sweet and flavorful. The central pulp,

lightly darker in tone is juicy and normally filled with very hard yellowish seeds. Seed count ranges from 112 to 535 but some time some guava are seedless or nearly so [4-6].

The whole plant is very useful in different diseases [7] such as Anti Diarrheal[8-10], Anti Inflammatory Effect[11-12], Antispasmodic[10], CNS Activity[13-14], Conjunctivitis[10], Coughs[15], Diabetes[15], Kidney Problems[8], Malaria[16], Oral Care[17]etc. The different extracts of this plant have shown antifertility [18], antimicrobial [19-20], anti-hyperglycemic [21] and anticancer [22] activities.

Earlier the constituents of essential oil from the *P. guajava* Linn were analyzed by GC-MS qualitatively and quantitatively in which sixty compounds of the essential oils were identified [23]. In this work, we studied the chemical composition and antibacterial activity of essential oils of *n*-hexane fraction from *P. guajava* collected from University Campus, Ujjain M.P. (India) where people frequently use this plant in traditional medicine.

## MATERIALS AND METHODS

### *Plant Material*

The leaves of *P. guajava* were collected from University campus, Ujjain, altitude range of (3500 m) in the month of December, 2009 (Table 1) and identified by the authorities of Institute of Environment Management and Plant Sciences, Ujjain.

### *Extraction*

The leaves of *P. guajava* (2 kg) were properly shade dried, powdered and extracted serially in Soxhlet extractor with *n*-hexane, benzene, benzene- acetone, methanol, ethanol and water. Removal of solvent under reduced pressure afforded solid mass in case of benzene, ethyl acetate, acetone, and methanol while liquid (oil) in case of *n*-hexane.

### *Vacuum liquid chromatography*

A portion (100 g) of n-hexane extract was subjected to vacuum liquid chromatography (VLC) on silica gel 60 H using gradient of n-hexane: benzene: acetic acid (6/4/0.1, v/v) as solvent system to obtain VLC fraction using a rotator evaporator at a maximum temperature of 40°C.

#### *Preparation of trimethylsilyl (TMS) ether derivatives*

The aliphatic compounds present in the VLC fractions of the n-hexane extract was converted to their trimethylsilyl derivatives. Fraction was mixed with Tri-Sil reagent (0.1 ml) in glass sealed tubes using an ultrasonic bath for 2 min and then vortexing briefly. The tube was then incubated at 60°C for 45 min. Thereafter, the solvent was evaporated under a stream of nitrogen and the TMS ether derivatives was dissolved in 0.2 ml of n-hexane the tubes was sonicated in an ultrasonic bath for 2 min, vortexed and centrifuged for 3 min. The n-hexane layer was transferred to other tubes, avoiding any solid particles, and analyzed by the GC-MS. After derivatization, the tube was stored at -20°C for subsequent analyses within 3 days.

#### *Gas Chromatography-Mass Spectrometry*

A Hewlett-Packard 5890 Series II Chromatograph equipped with a FID detector and HP-2 fused silica columns (25 m × 0.32 mm, 0.25µm film thicknesses) was used. The samples, dissolved in n-hexane, were injected in the split less mode into helium carrier gas. Injector and detector temperatures were maintained at 250°C. The column temperature was programmed from 60°C (after 2 min) to 220°C at 4°C/min, and the final temperature was held for 20 min. Peak areas and retention times were measured by electronic integration of by computer. The relative amounts of individual components are based on the peak areas obtained, without FID response factor correction.

GC-MS analyses were carried out on a Hewlett-Packard 5970A mass selective detector (MSD),

directly coupled to HP 5790A gas chromatograph. A 26 m × 0.22 mm column, coated with 0.13 µm of CP-Sil 5CB was employed, using helium carrier gas. The oven temperature program was 60°C (3 min), then 5°C/min to 250°C (30 min). Other conditions were the same as described under GC. Electron ionization (EI) mass spectra were acquired over a mass range of 10-400 Da at a rate of 2/s.

#### *Identification of Compounds*

The identification of components present fractions of the of n-hexane (*P. guajava*) extract were based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley 229, Nist 21 Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [24-26].

#### **Microbial strains taken for anti-microbial activity**

##### **Gram Positive**

- *Staphylococcus aureus*
- *Streptococcus faecalis*
- *Bacillus subtilis*
- *Lactobacillus spp.*
- *Enterococcus aerogenes*
- *Acinetobacter spp.*

##### **Gram Negative**

- *Escherichia coli*
- *Proteus vulgaris*
- *Enterobacter aerogenes*
- *Salmonella typhimurium*
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*

### Anti-microbial screening

*In vitro* anti-bacterial activity of extract was studied against twelve bacterial strains by the agar well diffusion method as described by Perez and co-workers [27] with certain modifications. Nutrient agar (Hi Media, India) was used as the bacteriological medium. The antibacterial activity of essential oils was taken at different concentrations (5, 10, 20, 40 and 80 $\mu$ L/well). The nutrient agar was melted and cooled to 48-50°C and standardized inoculums of  $1 \times 10^6$  CFU/mL, (0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound was introduced in the well (8.5 mm). The plates were incubated overnight at 37°C. The anti-microbial spectrum of the oils was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, 20 $\mu$ L each of amoxicillin and streptomycin (5mg/mL of autoclaved distilled water). These are commonly used anti-biotic to treat infections caused by several Gram-positive and Gram-negative bacteria. For each bacterial strain positive controls were maintained. The experiment was performed three times to minimize the error and the mean values are presented.

### Minimal inhibition concentration

The essential oils that exhibited considerable activity were diluted with nutrient broth (1:1) in a series of twelve sets of three test tubes for different microorganisms [28]. An aliquot of 1mL of the bacterial suspension ( $1 \times 10^6$ ) was inoculated into each tube. The control tubes were inoculated with same quantity of sterile distilled water and 75% ethanol. All tubes were incubated at 37°C for 24hrs. The lowest concentration that did not permit any visible growth when compared with the control was considered as

the minimum inhibitory concentration. The contents of all tubes that showed no visible growth were cultured on nutrient agar, incubated at 37°C for 24hrs. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony.

### Results and Discussion

The essential oil obtained by distillation from the leaves of wild growing *P. guajava*, with a yield of 0.45%, was pale yellowish and possessed a strong odour. By gas chromatography mass spectroscopy, 7 compounds were identified. The major constituents recorded from essential oil of *P. guajava* were: Methyl 2, 6, 10 –trimethyltridecanoate (28.86%) and Methyl octadecanoate (22.18%). The different constituents of essential oil of *P. guajava* are well represented with their relative area percentage (Table 2).

Anti-microbial activity showed that, the inhibition zones were found increased considerably when the concentration rate increased. Therefore it can be said that quantity of the oil was important for inhibition effect. Among all Gram-positive bacterial growths, the maximum zone of inhibition was recorded against *B. subtilis* i.e. 34mm, followed by *Lacobacillus spp.* i.e. 28mm and 27mm zone of inhibition against *S. faecalis*. On the other hand four different Gram negative bacterial strains were tested and among these microorganisms *E. coli* showed maximum zone of inhibition i.e. 36mm, followed by *Enterobacter aerogenes* i.e. 33mm.

The minimal inhibition concentration (MIC) was 3 $\mu$ L recorded in Gram-negative strain *E. coli* followed by a Gram-positive strains *B. subtilis* showed 2.5 $\mu$ L of minimal inhibition concentration (MIC) (Table 4).

From these it is concluded that the essential oil showed maximum zone of inhibition and minimal inhibition concentration against *B. subtilis* and *E. coli* bacterial strains, which indicate that *P. guajava* essential oil has capacity to inhibit the growth of both

Gram-positive and Gram-negative bacterial strains when used in a higher amount.

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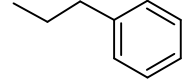
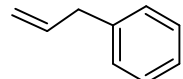
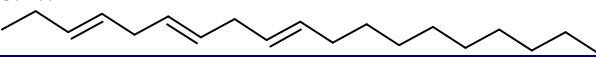
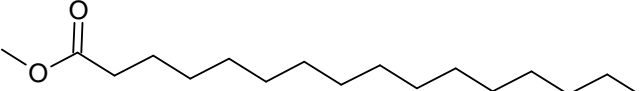
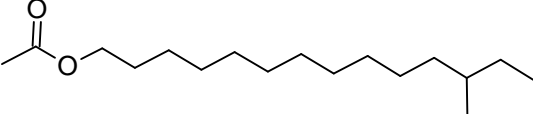
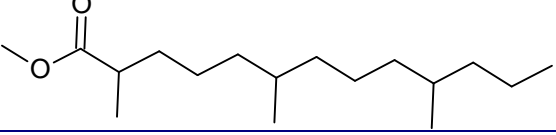
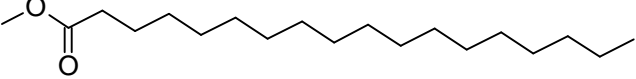
### Acknowledgements

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**Table 1:** Collection details and essential oil yield of *P. guajava* from study area of School of Studies in Chemistry, Ujjain Malva Region of India.

Species name	Place of collection	Altitude of study area (m)	Month & year of collection	Oil yield (%)
<i>Psidium guajava</i>	University Campus , Ujjain M.P. India	3500	December (2009)	0.45

**Table 2** Compounds present in *n*-hexane ext. of *P. guajava* (Leaves).

No.	Name of Compounds	Retention Time (sec.)	Area (%)	Molecular Formula	Molecular Mass
1.	Propyl benzene 	7.62	9.52	C <sub>9</sub> H <sub>12</sub>	120
2.	Allyl benzene 	7.71	1.89	C <sub>9</sub> H <sub>10</sub>	118
3.	3,6,9 Nonadecatriene 	14.38	15.37	C <sub>19</sub> H <sub>34</sub>	264
4.	Methyl hexadecanoate 	14.44	9.32	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
5.	Methyl tetradecyl acetate 	14.48	12.86	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
6.	Methyl 2, 6, 10-trimethyltridecanoate 	14.53	28.86	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
7.	Methyl octadecanoate 	15.63	22.18	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298

**Table 3:** Anti-microbial activity of essential oil of *P. guajava* against Gram-positive and Gram-negative bacteria strains.

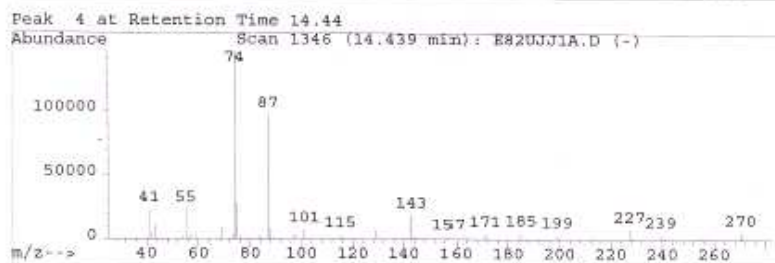
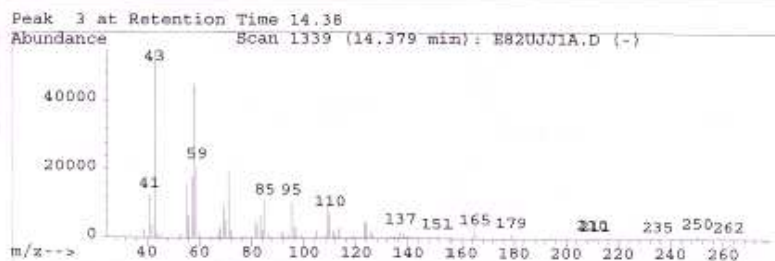
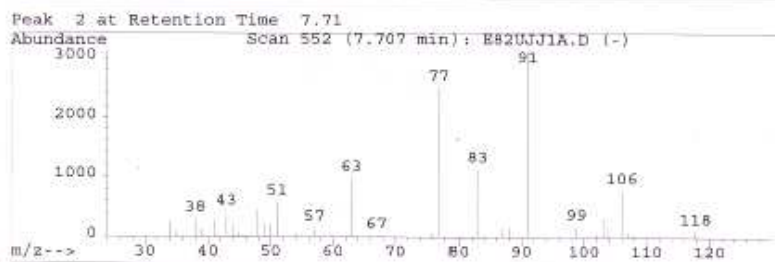
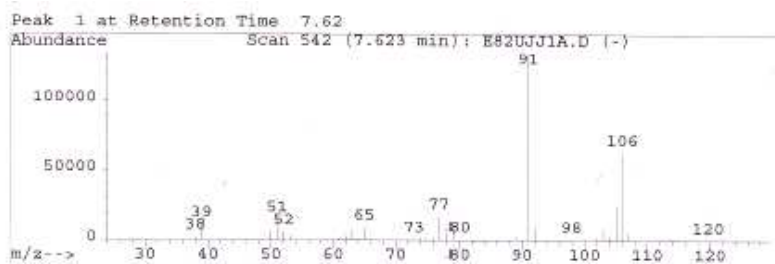
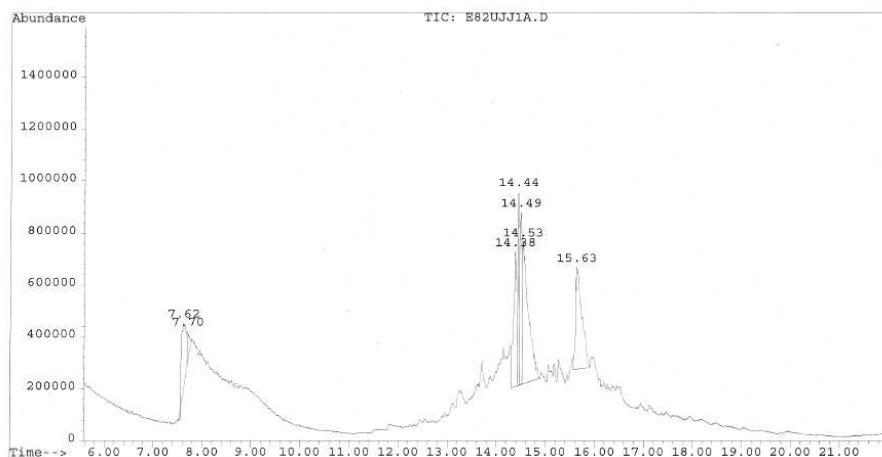
S. No.	Nature of Bacterial Strain	Micro-Organism	Diameter of inhibition zone (mm) of essential oil concentration used for anti-microbial analysis ( $\mu\text{L}/\text{well}$ ) ( $n=3$ )					Control +ve ( $n=3$ )	
			5 $\mu\text{L}$	10 $\mu\text{L}$	20 $\mu\text{L}$	40 $\mu\text{L}$	80 $\mu\text{L}$	Amoxicillin 5mg/mL	Streptomycin 5mg/ mL
1	Gram +ve	<i>Staphylococcus aureus</i>	N.A	2.2	6.4	12.0	24.0	31	29
2		<i>Streptococcus faecalis</i>	0.5	2.9	4.0	11.0	25.0	27	24
3		<i>Bacillus subtilis</i>	0.8	3.2	5.0	13.0	27.0	34	32
4		<i>Lactobacillus spp.</i>	N.A	N.A	0.5	4.0	8.3	28	26
5		<i>Enterococcus aerogenes</i>	N.A	1.5	4.0	10.0	21.0	22	20
6		<i>Acinetobacter spp.</i>	1.3	2.6	6.1	11.0	23.0	26	30
7	Gram -ve	<i>Escherichia coli</i>	2.0	5.0	7.0	11.0	27.0	36	34
8		<i>Proteus vulgaris</i>	N.A	2.8	6.1	13.0	27.0	21	20
9		<i>Enterobacter aerogenes</i>	0.6	1.7	5.7	12.0	25.0	33	32
10		<i>Salmonella typhimurium</i>	N.A	N.A	0.7	1.6	3.2	27	24
11		<i>Pseudomonas aeruginosa</i>	0.6	3.9	4.0	7.8	11.8	28	26
12		<i>Klebsiella pneumoniae</i>	N.A	2.3	3.4	5.3	8.9	22	23

All values are mean of triplicates ( $n=3$ ); Gram +ve: Gram-positive; Gram - ve: Gram negative; N. A: No Activity.

**Table 4:** Minimal inhibition concentration (MIC) ( $\mu\text{L}$ ) of essential oil of *P. guajava*

S. No.	Nature of Bacterial Strain	Micro-Organism	MIC ( $\mu\text{L}$ )
1	Gram +ve	<i>Staphylococcus aureus</i>	7.0
2		<i>Streptococcus faecalis</i>	8.5
3		<i>Bacillus subtilis</i>	2.5
4		<i>Lactobacillus spp.</i>	4.0
5		<i>Enterococcus aerogenes</i>	9.6
6		<i>Acinetobacter spp.</i>	12.0
7	Gram -ve	<i>Escherichia coli</i>	7.0
8		<i>Proteus vulgaris</i>	9.0
9		<i>Enterobacter aerogenes</i>	4.5
10		<i>Salmonella typhimurium</i>	10.0
11		<i>Pseudomonas aeruginosa</i>	6.0
12		<i>Klebsiella pneumoniae</i>	12.0

Gram +ve: Gram-positive; Gram .ve: Gram-negative; MIC: Minimal Inhibition Concentration.



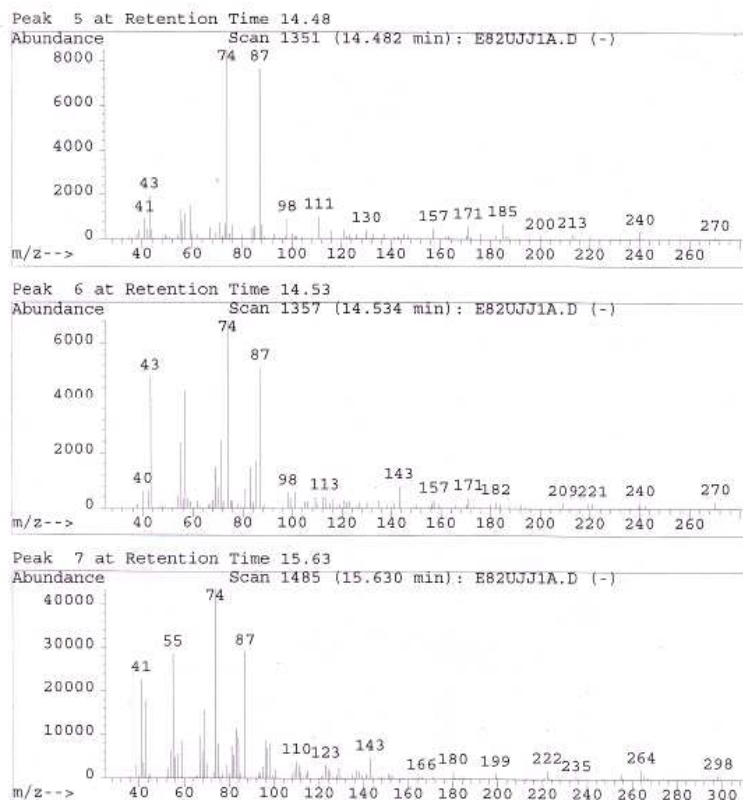


Figure 1: GC-MS chromatogram of *P. guajava.*, a naturally growing species from Malva Region of India.

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