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GC-MS analysis of bioactive compounds in *Psidium guajava* leaves

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Abstract
The bioactive components of *Psidium guajava* leaves have been evaluated using GC/MS. The chemical compositions of *Psidium guajava* leaves were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched by the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanolic extract of *Psidium guajava* leaves revealed the existence of Alpha - bisabolol, 1, 2- Benzenedicarboxylic acid, buty, Hexadeca-2, 6, 10, 14-tetraen, Caryophyllene, Bis (2-ethylhexyl) phthalate, Nerolidol and Germacrene. The qualitative analysis of ethanolic and aqueous extract of *Psidium guajava* leaves showed that tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids, triterpenoids, carbohydrate, polyphenol and glycoside present in both extract. The quantitative determination of *Psidium guajava* leaves contain Phenol (9.33 mg/gm powder), Tannin (4.30 mg/gm powder), Flavonoids (6.42 mg/gm powder) and Saponin (3.67 mg/gm powder). The results of this study offer a platform of using *Psidium guajava* leaves an herbal alternative for various diseases.

Keywords: *Psidium guajava*, bioactive compounds, GC MS

1. Introduction
Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. Medical Plant constitutes an important therapeutic aid in alleviating ailments. Almost 80% of the world populations, particularly in the third world are fully dependent on medicinal plants for meeting their health care needs. The herbal medicines today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. In the primeval times, the Indian sagacious held the view that herbal medicines are the only resolution to treat numeral health related problems and diseases. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing the disease. Increasing interest in herbal products has today accelerated the growth of medicinal plant-based industries [1].

Traditional medicinal usage of herbs by humans, however imperfect and "unscientific" by modern standards, is the result of countless trial-and-error tests that people have conducted, and so traditional usage points the way to natural therapeutic usage. As we later stress, however, "natural" does not necessarily mean "safe." Some herbal products are extremely effective, but so dangerous that they should only be used in the hands of skilled medical professionals. Others, however, are sufficiently safe that they can be used by laypeople to help prevent or alleviate minor health problems. Sometimes the herbal drugs are preferable, but as we stress throughout this work, qualified medical personnel should always be consulted [2].

Psidium guajava Linn. (Family: Myrtaceae) has a rich ethnobotanical history. In many parts of Africa, the leaf, stem bark and roots are used traditionally for the management, control, and/or treatment of an array of human disorders. The leaf and bark extracts have been used for ages to fight diarrhoea and dysentery. The plant is indigenous to tropical America, widely distributed from Mexico to Brazil. It is also found all over Africa and Asia in semi cultivation. Other major ethnotherapeutic uses of the plant include the treatment of malaria with the leaves as an ingredient in the preparation of fever 'teas' or as part of the pot herbs used in steam treatment, and as mouth rinses and gargles in the treatment of stomatitis and phengingivitis. A weak decoction of the leaves and tender branches is also used as a tonic in psychiatric management [3].

Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals [4].

The aim of this study is to determine the organic compounds present in the *Psidium guajava* (Linn) leaves extract with the aid of GC-MS technique, which may provide an insight in its use in tradition medicine.

2. Materials and Methods

2.1 Plant material

Leaves of *Psidium guajava* were selected to screen its biopotentials based on its traditional usage. The fully mature *Psidium guajava* leaves were collected in April 2013 from Thanjavur, Tamil Nadu, India from a single herb. Care was taken to select healthy leaf.

2.2 Authentication of plant material

The collected leaves were identified and authenticated by a Botanist Dr. M. JEGADEESAN, Prof. and Head, Department of Environmental and Herbal Sciences, Tamil University, Thanjavur, Tamil Nadu. A Voucher specimen has been deposited at the Tamil University Herbarium. The leaves were cut into small pieces and shade dried at room temperature for 15 days.

2.3 Preparation of extract

The collected leaves of *Psidium guajava* were washed under running tap water and dust was removed from the leaves. The leaves were dried at room temperature for 15 days and coarsely powdered. The powder (2 gm) was extracted with 70% ethanol and 100% aqueous for 48 hours. A semi solid extract was obtained after complete elimination of alcohol and water under reduced pressure. The *Psidium guajava* leaves extract (PGLF) was stored in refrigerator until used. Chemical tests were carried out on the alcoholic and aqueous extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara [5], Trease and Evans [6] and Harborne [7].

2.4 Quantitative determination of the chemical constituency

2.4.1 Preparation of fat free sample

2 g of the sample was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h.

2.4.2 Determination of total phenols by spectrophotometric method [8]

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

2.4.3 Flavonoid determined by the method of Bohum and Kocipai-Abyazan [9]

Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.4.4 Tannin determination by Van-Burden and Robinson [10] method

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

2.4.5 Saponin determination

The method used was that of Obdoni and Ochuko [11]. The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55 ^{0}C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90 ^{0}C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant.

2.5 GC-MS method

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 µ Mdf, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9min isothermal at 280 ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0 [12].

2.5.1 Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Results and Discussion

Table 1 and 2 represent the phytochemical constituents present in guava leaves. The qualitative analysis of ethanolic and aqueous extract of *Psidium guajava* leaves contain tannin, phlobatannins, sapanoin, flavonoids, steroids, terpenoids, triterpenoids, carbohydrate, polyphenol and glycoside present

in both extract. The quantitative determination of an ethanolic extract of *Psidium guajava* leaves contain high concentrations of phenol (9.33 mg/gm), tannin (4.30 mg/gm), flavonoids (6.42 mg/gm) and saponin (3.67 mg/gm) than the aqueous extract. The results of the study demonstrated that the ethanolic extraction had a higher content of the phytochemicals than the aqueous extract.

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom. Some isoflavones widely used in insecticides. They might also play a role in disease resistance. Some flavonoids such as quercetin and rutin, are known to support human health by serving anti-inflammatory, antihistaminic and antiviral agents [13]. Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids, such as glycyrrhizin and chrysin [14] against HIV. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity [15]. Flavonoids have been referred to as nature's biological response modifiers, because of inherent ability to modify the body's reaction to allergies. It possesses various pharmacological roles including anti-allergic, anti-inflammatory, cardio-protective, anti-microbial and anti-cancer activities [14].

Phenols are the secondary metabolites that are ubiquitously present in plants. They have been suggested to play a role in the antioxidant function. Phenolic compounds have antioxidant properties because of their ability to scavenge free radicals. The phenolic compounds of plant origin showed their antioxidative effect by various mechanisms, including their ability to scavenge free radicals or activate various antioxidant enzymes and inhibit oxidizes [16].

Thirty three compounds were identified in *Psidium guajava* leaves by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 3 and Fig 1). The GC-MS method confirms that *Psidium guajava* contain Alpha.-bisabolol, 1, 2-Benzenedicarboxylic acid, buty, Hexadeca-2, 6, 10, 14-tetraen, Caryophyllene, Bis (2-ethylhexyl) phthalate, Nerolidol and Germacrene are present. The biological activities listed (Table 4) are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA [17]. The results confirm the presence of constituents which are known to exhibit medicinal value as well as physiological activities.

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Therefore, the date generated from these experimental has provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. The results of this study offer a platform of using *Psidium guajava* leaves as herbal alternative for various diseases including diabetic, cancer, cardiovascular etc.

4. Acknowledgement

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Table 1: Phytochemical screening of *Psidium guajava* leaves

S. No	Secondary metabolites	Ethanol	Aqueous
1.	Tannin	++	++
2.	Phlobatannins	++	++
3.	Sapanoin	++	++
4.	Flavonoids	+++	++
5.	Steroids	++	+
6.	Terpenoids	+++	++
7.	Tri terpenoids	…….	…….
8.	Alkaloids	…….	…….
9.	Carbohydrate	++	++
10.	Protein	…….	…….
11.	Anthroquinone	……..	……..
12.	Polyphenol	+++	++
13.	Glycoside	++	++

(-) Absence; (+) Presence; (+++) High concentrations

Table 2: Quantitative analysis phytochemicals in *Psidium guajava* leaves

S. No	Secondary metabolites	Ethanol (mg/gm)	Aqueous (mg/gm)
1.	Phenol	9.33 ±0.65	8.0 ±0.56
2.	Tannin	4.30 ±0.30	4.80 ±0.30
3.	Flavonoids	6.42 ±0.44	5.34±0.36
4.	Saponin	3.67 ±0..25	4.24 ±0.29

Values are expressed as Mean ± SD for triplicates

Table 3: GC MS analysis of *Psidium guajava* leaves extract

Peak#	R. Time	Area %	Name	Molecular formula	Molecular weight
1	3.582	0.37	Butanoic acid, 2-methyl-, methyl este	$C_6 H_{12} O_2$	116
2	8.436	0.57	dl-Limonene $$ Cyclohexene, 1-me	$C_{10} H_{16}$	136
3	8.534	1.37	1,8-Cineole $$ 2-Oxabicyclo[2.2.2]	$C_{10} H_{18} O$	154
4	11.783	0.20	(E)-2,6-Dimethyl-5,7-octadien-2-ol	$C_{10} H_{18} O$	154
5	14.013	0.34	Cyclohexasiloxane, dodecamethyl-	$C_{12} H_{36} O_6 S_{I6}$	444
6	15.555	0.22	Alpha.-Copaene	$C_{15} H_{24}$	204
7	16.776	19.76	Trans-Caryophyllene	$C_{15} H_{24}$	204
8	17.793	2.44	Alpha.-Humulene	$C_{15} H_{24}$	204
9	18.438	1.12	Germacrene D	$C_{15} H_{24}$	204
10	19.115	7.48	Trans-alpha.-bisabolene	$C_{15} H_{24}$	204
11	19.233	0.44	Aromadendrene 2 $$	$C_{15} H_{24}$	204
12	19.351	9.75	Beta.-Bisabolene	$C_{15} H_{24}$	204
13	19.949	1.19	Delta.-Cadinene	$C_{15} H_{24}$	204

14	20.124	0.36	(-)-Endo-2,6-dimethyl-6-(4-methyl-	$C_{15}H_{24}$	204
15	20.381	1.09	CIS-.alpha.-bisabolene $$	$C_{15}H_{24}$	204
16	20.923	21.87	Nerolidol B (CIS OR TRANS	$C_{15}H_{26}O$	222
17	21.803	10.55	(-)-Caryophyllene oxide	$C_{15}H_{24}O$	220
18	22.174	0.35	Trans-Caryophyllene	$C_{15}H_{24}$	204
19	22.444	0.59	Humulene oxide $$	$C_{15}H_{24}O$	220
20	22.807	0.69	Germacrene D	$C_{15}H_{24}$	204
21	22.917	0.68	Tricyclo[3.3.1.13,7]decane, 2-brom	$C_{10}H_{15}$	214
22	23.099	3.29	(+)-Aromadendrene	$C_{15}H_{24}$	204
23	23.202	4.93	Torreyol $$ 1-Naphthalenol	$C_{15}H_{26}O$	222
24	23.441	1.91	Globulol $$ (-)-Globulol	$C_{15}H_{26}O$	222
25	23.631	1.46	Beta.-Bisabolol	$C_{15}H_{26}O$	222
26	23.963	1.36	Alpha.-bisabolol	$C_{15}H_{26}O$	222
27	25.711	0.20	2-Methyl-6-(trimethylsilyl)benzophe	$C_{17}H_{20}O$	268
28	26.512	0.92	8-Acetyl-3,3-epoxymethano-6,6,7-t	$C_{14}H_{20}O_3$	236
29	27.223	0.35	1,2-Benzenedicarboxylic acid, dibut	$C_{16}H_{22}O_4$	278
30	28.671	1.89	1,2-Benzenedicarboxylic acid, buty	$C_{20}H_{30}O_4$	334
31	32.600	0.71	Propionic acid, 2-isopropo	$C_7H_{14}O_3$	146
32	38.268	0.82	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390
33	48.521	0.72	Hexadeca-2,6,10,14-tetraen	$C_{20}H_{34}O$	290

Table 4: Biological Activity of *Psidium guajava* leaves extract components identified by GC MS

S. No	Name of the Compound	Biological Activity**
1.	1,2-Benzenedicarboxylic acid, dibut	Antimicrobial, Antifouling
2.	Alpha.-bisabolol	Antiulcer, Antiphlogistic effects, Antimicrobial, Antioxidant, Anti-inflammatory, Promotes wound healing
3.	1,2-Benzenedicarboxylic acid, buty	Antimicrobial, Antifouling
4.	Hexadeca-2,6,10,14-tetraen	Antimicrobial, Anti-inflammatory
5.	Caryophyllene	Anti-tumor, Analgesic, Antibacterial, Antiinflammatory, Fungicide
6.	Bis(2-ethylhexyl) phthalate	Cytotoxic
7.	Nerolidol	Flavouring agent, antileishmaniasis, arginase inhibition
8.	Germacrene	Antibacterial activity

****Source:** Dr. Duke's phytochemical and ethnobotanical databases [Online database] [17].

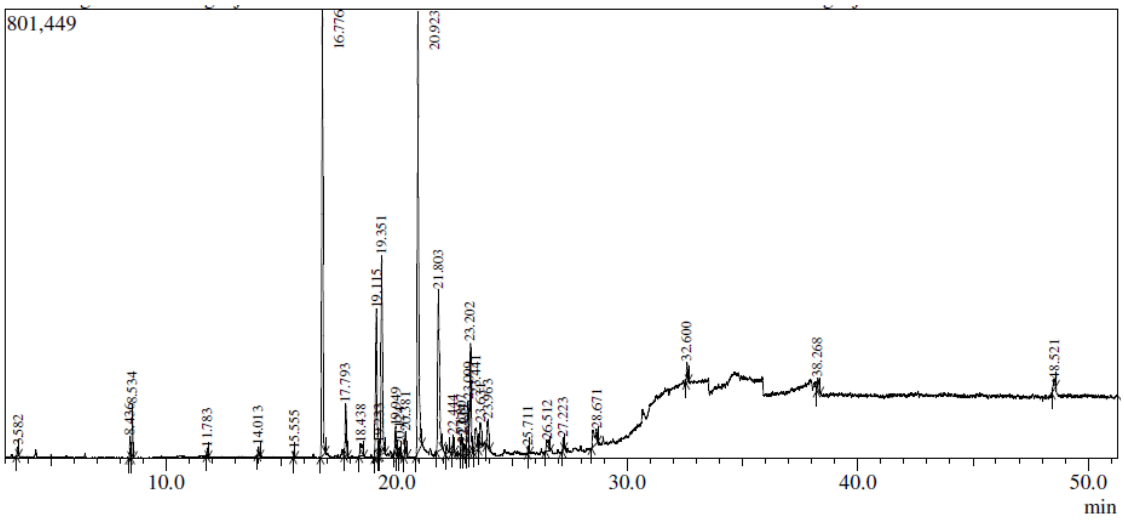

Fig 1: Chromatogram obtained from the GC/MS with the extract of ***Psidium guajava*** leaves

5. References

1. Barnes J, Anderson LA, Phillipson JD. Herbal Medicines. A guide for Healthcare Professionals, third ed. Pharmaceutical Press, London, 2007.
2. Downum KR, Romeo TR, Stafford HA. (Eds.). Phytochemical potential of tropical plants. Recent Advances in Phytochemistry, Plenum Press, New York, 1993, 27.
3. Iwu MM. Handbook of African Medicinal Plants. CRC Press, 1993, 223–224.
4. Roberts JKM, Xia JH. High-resolution NMR methods for study of higher plants. Methods Cell Biol 1995; 49:245–258.
5. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria, 1993, 289.
6. Trease GE, Evans WC. Pharmacognsy. Edn 11, Brailliar Tiridel Can. Macmillian publishers, 1989.
7. Harborne JB. Phytochemical methods, London. Chapman and Hall Ltd, 1973, 49-188.
8. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4(7):685-688.
9. Bohum BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian vaccinium

vaticulatum and V. calycinium. Pacific Sci 1974; 48:458-463.
10. Van-Burden TP, Robinson WC. Formation of complexes between protein and Tannin acid. J Agric Food Chem 1981; 1:77.
11. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J Pure Appl Sci 2001; 8:203-208.
12. Srinivasan K, Sivasubramanian S, Kumaravel S. Phytochemical profiling and GC-MS study of Adhatoda vasica leaves. Int J Pharm Bio Sci 2013; 5(1):714-720.
13. Okwu DE. Phytochemical and vitamin content of indigenous spices of south eastern Nigeria. J Sustain Adric Environ 2004; 6(1):30-37.
14. Duraipandiyan VM, Ayyanar L, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants. Asian Journal of Microbiologr 2006; 5:334-337.
15. Del-Rio A, Obdululio BG, Casfillo J, Masin FG, Ortuno A. Uses and properties of citrus flavonoids. J Agric Food Chem 1997; 45:4505-4515.
16. Leslie W, Boxin k. Antioxidant activity and phenolic Content of Oregon Caneberris. J Agric Food Chem 2002; 50:3495-3500.
17. Dukes. Phytochemical and Ethnobotanical Databases. Phytochemical and Ethnobotanical Databases. www.ars-gov/cgi-bin/duke/. 2013.