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PRELIMINARY PHYTOCHEMICAL AND GCMS ANALYSIS OF *PSIDIUM GUAJAVA* L.LEAVES EXTRACT

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ABSTRACT

Psidium guajava L.commonly known as gauva has medicinal importance. The present study was carried out for the investigation of chemical compounds found in *Psidium guajava* L. by simple chemical tests and by GCMS analytical method. The extracts were prepared by using methanol and chloroform as solvents by Soxhlet method. The preliminary phytochemical analysis revealed the presence of Alkaloids, carbohydrates, tannins, proteins, saponins, fixed oils & fats, cardiac glycosides in both extracts however amino acids, anthraquinones & flavonoids were absent in the extracts of *Psidium guajava* (Linn) leaves. GCMS analysis of the methanol extract of leaf of *Psidium guajava* L. revealed the presence of sixteen compounds. The major compound was Caryophyllene with 71.07% peak area followed by 6,10-Dodecatrien-3ol,3,7,11trimethyl-[E]- and Napthalene1,2,3,4 tetrahydro- 1,6dimethyl-4-1-(methyl ethyl)-(1 S-cis)-. The Chloroform Extract extract of *Psidium guajava* L. showed the presence of eight compounds. Caryophyllene was found as the major [71.07%] compound followed by Caryophyllene oxide [37.02%].

KEYWORDS: *Psidium guajava* L, gauva, Soxhlet method, GCMS analysis, Caryophyllene.

INTRODUCTION

Plants show vast biodiversity and are rich in phytochemicals with potential medicinal value. Medicinal plant products are consumed daily by many people all over the world to maintain health. [Vaghasiya and Yogeshkumar, 2009]

The traditional system of medicine is practiced in the rural and the tribal areas where modern medicine is not accessible. Phytochemical and pharmacological investigation of the

traditionally used plants are still not available. Therefore, much of the information is not available to the scientific community. Many investigations are being currently on going to isolate the active component present in species showing high antibacterial activity during screening. When primary information is at hand then further studies can be carried out involving standardization of the extracts, identification and isolation of active components and pharmacological studies of isolated compound. Such scientific studies can be used to develop drugs for treatment of diseases. [Prabhuji *et al*., 2009].

Psidium guajava L. commonly known as guava belongs to family *Myrtaceae*. The guava tree is a low evergreen tree with wide spreading branches and downy twigs. The tree is native of tropical America. Today, the tree is cultivated in nearly all the countries of tropical world belt including India, China, Thailand, Malaysia, Inadonesia and Japan [Khare, 2007; Ahmed, 2010].

Psidium guajava L. has medicinal value. Every part of the plant like leaves, bark, fruit and roots is used to treat various diseases. Decoction of the root of Guava is used to treat ulcers, wounds, diarrhoea [Ismail Mohmmed, 2012]. A decoction of leaves is used as gargle to relieve toothache and gum boils. In India, young leaves of Guava are used as remedy against cough[Ahmed, 2010].

There is need of scientific documentation. Therefore, present study was carried out for the investigation of chemical compounds found in. *Psidium guajava* L. by GCMS analytical method.

MATERIAL AND METHOD

Plant material

Psidium guajava (Linn) leaves were collected from the Botany garden of Dayanand in the month of January. The leaves were identified in the department of Botany, D.B.F. Dayanand College of Science, Solapur [Maharashtra].

Preparation of plant extract

For preparation of plant extracts, plant leaves were washed under running tap water. They were then dried under shade and ground into coarse powder in the electronic grinder. Fifteen grams of powder was then extracted in methanol [300 ml] and chloroform [300 ml] by using

Soxhlet method. Ten cycles were run. The solvent from the extract was removed by evaporation at room temperature [28±2^{0}c]. The extracts were freezed till further use.

Phytochemical Analysis of Plant Extract [Raaman, 2006]

Physical characteristics of plant Extract

Physical characteristics of the plant extracts like colour, odour and consistency were studied.

Percentage Yield of Plant Extract

Percentage yield of plant extracts in methanol and chloroform was determined in terms of total quantity of powder in grams taken for preparation of extract.

Preliminary Phytochemical Analysis of Extract

Preliminary phytochemical analysis was done to find out the active chemical principle of the plant by simple chemical tests [Raaman, 2006] as follows.

Detection of Alkaloids: Solvent free extract [50 mg] was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various reagents a follows.

1. Mayer's test –To a few milliliter of filtrate, a drop of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates the test as positive.
2. Wagner's test –To a few milliliter of filtrate, few drops Wagner's reagent are added by the side of the test tube. A reddish brown precipitate confirms the test as positive.
3. Hager's test—To a few milliliter of filtrate 1 or 2 ml of Hager's reagent is added. A prominent yellow precipitate indicates the test as positive

Detection of Carbohydrates [Benedict's test] – To a 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 minutes. A characteristic colored filtrate indicates the presence of sugar.

Detection of Amino acids and proteins- The extract [100mg] was dissolved in 10 ml distilled water and filtered through Whatman no.1 filter paper and the filtrate was subjected to test for proteins and amino acids.

Biuret test-Two ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1ml. of ethanol was added followed by excess of potassium hydroxide pellets. Pink color in the ethanol layer indicates presence of proteins.

Ninhydrin test – Two drops of ninhydrin solution were added to 2 ml. of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

Detection of Saponins [Foam test] –The extract [50mg] was dissolved in 20 ml. of distilled water. The suspension was shaken in a graduated cylinder for 15 minutes. A two cm. layer of foam indicates the presence of Saponins.

Detection of Tannins [Ferric chloride test] –The extract [50mg] was dissolved in 5 ml of distilled water. To this few drops of 5% Ferric chloride were added. A dark green color indicates the presence of tannins.

Detection of flavonoids [Magnesium and hydrochloric acid reduction test] –The extract [50 mg] was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid [drop wise] were added. If any pink to Crimson color develops presence of flavonoids was inferred.

Detection of anthraquinones- The extract [50mg] was dissolved in distilled water. To 2 ml of extract, 1ml dilute ammonia solution was added and shaken vigorously. Pink color in ammonia layer indicates presence of anthraquinones.

Detection of Cardiac glycosides[Killer kiliani test] –The extract [50mg] was dissolved in distilled water and then filtered. To 2 ml of filtrate 1ml of glacial acetic acid and a drop of Ferric chloride and a drop of concentrated sulfuric acid was added. Green blue color to upper layer and reddish brown color at the junction of two layers indicates the presence of cardiac glycosides.

Detection of fixed oils and fats [Spot test]-A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

GCMS analysis of extracts

GCMS analysis of the extracts was carried out in Indian Institute of Technology, Mumbai. GCMS was performed by using Helwett Packard, GCD 1800 A model with electron ionization detector operated through a data system.1 µl of extract was used to inject in injection port of GC column.

Identification of compounds

The mass spectrum of unknown components was compared with spectrum of the known components stored in the NIST and Wiley library. Interpretation of mass spectrum of GCMS was done using data base of NIST library having more than 75,000 compounds [software-Turbomas 5.2]. The name, molecular weight and structure of components were then ascertained. The relative percentage was calculated by comparing its average peak area to the total area.

RESULT AND DISCUSSION

Physical characteristics of extracts

Physical characteristics of methanol & chloroform extracts of *Psidium guajava* (Linn) leaves are depicted in table 1.Methanolic extract was dark green in color while chloroform extract was faint green in color. Methanol extract was semi solid sticky in consistency while chloroform was dry powdery. The methanol extract had pungent, aromatic odor however chloroform had organic.

Table 1: Physical characteristic of extracts.

Sr. no.	Solvent used	physical characteristics		
		Colour	Odour	Consistency
1	Methanol	Dark green	Pungent, aromatic	semisolid sticky
2	Chloroform	Faint green	Organic	Dry Powdery

Table 2: Percentage yield of extracts of *Psidium guajava* (Linn) *leaves.*

Sr. no.	Solvent used	Weight of dry powder [gms]	Weight of dry extracts [gms]	percentage yield
1	Methanol	15	2.50	16.66
2	Chloroform	15	2.50	16.67

As shown in table 2 the percentage yield of methanol and chloroform extract was equal that is 16.67%.

Table 3: Preliminary Phytochemical Analysis of Extracts of *Psidium guajava* (Linn) *leaves.*

Sr no.	Phytochemicals	Name of test	methanol	Chloroform
1	Alkaloids	Mayer test	+	+
		Wagner test	+	+
		Hager test	+	+
2	Carbohydrates	Benedict test	+	+
3	Saponins	Foam test	+	+
4	Proteins	Biuret test	+	+
5	Amino acids	Ninhydrin test	-	-
6	Anthraquinones	-	-	-
7	Tannins	Ferric chloride test	+	+
8	Flavonoids	Magnesium & HCL reduction test	-	-
9	Fixed oils & fats	Spot test	+	+
10	Cardiac glycosides	Killer Kiliani test	+	+

Table 3 shows the preliminary phytochemical analysis of extracts of *Psidium guajava* leaves. Alkaloids, carbohydrates, tannins, proteins, saponins, fixed oils & fats, cardiac glycosides were present in both extracts however amino acids, anthraquinones & flavonoids were absent in the extracts of *Psidium guajava* (Linn) leaves.

Singh *et al.,* (2012) noted the presence of Flavinoids, alkaloids, terpenoids, tannins, saponins and glycosides in the methanolic leaf extract of *Psidium guajava* L. The present study also revealed the presence of alkaloids, carbohydrates, saponins, proteins, tannins, fatty acids and oils and carbohydrates except Flavinoids. This is in accordance with reports of Singh *et al*., (2012).

GCMS Analysis

GCMS is one of the best techniques to identify the constituents of volatile matter, Long chain & branched chain hydrocarbons, alcohols, acids, esters. The more precise information in qualitative analysis can be obtained by gas chromatography coupled with mass spectroscopy [GCMS] [Cong *et al.,* 2007].

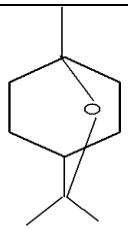
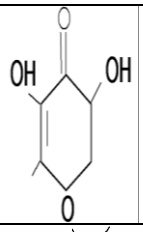
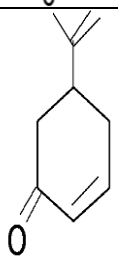
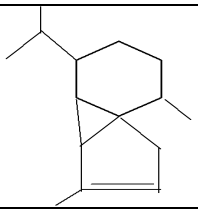
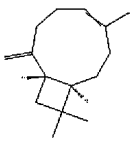
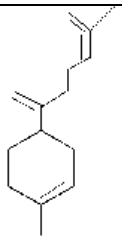
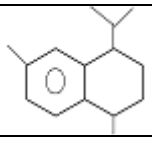
Table 4: Gas chromatographic and Spectral data for methanol extract of *Psidium guajava* L. Leaf.

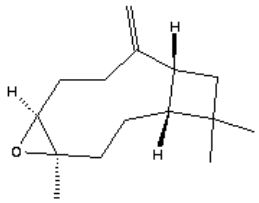
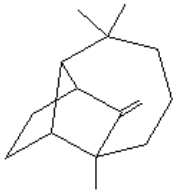
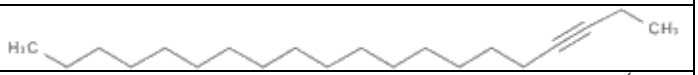
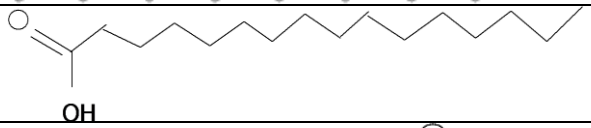
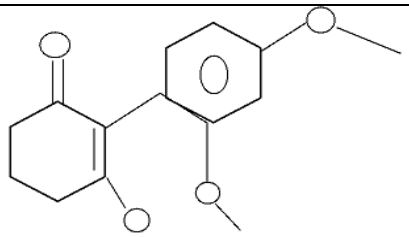
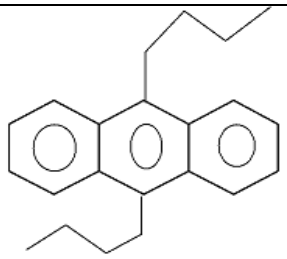
Peak No.	RT(min)	Name of Compound	Molecular formula	Molecular weight	%Peak area
1.	4.2	Eucalyptol	$C_{10}H_{18}O$	154	9.5%
2.	6.0	4H-pyran-4-one2, 3dihydro-3,5 -dihydroxy 6 methyl	$C_6H_8O_4$	144	3.3%
3.	7.9	2-Cyclohexene-1-one, 2methyl 5- (1-methyl ethenyl)-	$C_{10}H_{14}O$	150	8.9%
4.	11.7	A Cubebene	$C_{15}H_{24}$	180	2.6%
5.	12.1	Caryophyllene	$C_{15}H_{24}$	180	71.07%
6	13.5	Longifolene-(V_4)	$C_{15}H_{24}$	180	13.33%
7.	13.9	Napthalene1,2,3,4 tetrahydro- 1,6 dimethyl-4-1-(methyl ethyl) -(1 S-cis)-	$C_{15}H_{22}$	178	15.10%
8.	14.2	Napthalene 1,2,3,4 tetrahydro-1,6dimethyl 1,4-(1-methyl ethyl)-(1s-cis)-	$C_{15}H_{22}$	178	14.08%
9.	14.5	1,6,10-Dodecatrien-3 ol ,3,7,11trimethyl-[E]-	$C_{15}H_{26}O$	208	24.13%
10.	14.8	Caryophyllene oxide	$C_{15}H_{24}O$	196	8.1%
11.	14.9	1,6,10 Dodecatrien-3-ol ,3,7,11trimethyl-(E)	$C_{15}H_{26}O$	198	9.4%
12.	15.6	Caryophyllene oxide	$C_{15}H_{24}O$	196	4.5%
13.	17.9	3-Eicosyne	$C_{20}H_{38}$	278	3.9%
14.	19.3	nHexadecanoic acid	$C_{16}H_{32}O_2$	256	0.59%
15.	26.4	2cyclohexen-1-one,3methoxy-2-[2,4,5-tromethoxy phenyl]-	$C_{16}H_{20}O_2$	292	0.75%
16.	26.7	Anthracene9,10 dibutyl-	$C_{22}H_{26}$	290	0.75%

The GCMS profile of methanol extract of *Psidium guajava* L.leaf is depicted in Table 4. It revealed the presence of variety of phytochemicals. The major compound was Caryophyllene with 71.07% peak area followed by 1,6,10-Dodecatrien-3ol,3,7,11trimethyl-[E]- and Napthalene1,2,3,4 tetrahydro- 1,6dimethyl-4-1-(methyl ethyl)-(1 S-cis)-. Other compounds present were Napthalene 1,2,3,4 tetrahydro-1,6dimethyl 1,4-(1-methyl ethyl)-(1s-cis)-, Longifolene-(V_4), Eucalyptol, 1,6,10 Dodecatrien-3-ol, 3,7,11trimethyl-(E), 2-Cyclohexene-1-one, 2methyl 5-(1-methyl ethenyl)-, Caryophyllene oxide, 3-Eicosyne, 4H-pyran-4-one2,3dihydro-3,5-dihydroxy 6 methyl, α Cubebene, 2cyclohexen-1-one, 3methoxy-2-[2,4,5-tromethoxy phenyl]-, Anthracene9,10 di butyl, n Hexadecanoic acid.

Structures of compounds found and identified in methanol extracts of *Psidium guajava* L. are shown in Table 5. Figure1 shows GCMS chromatogram of methanol extract of *Psidium guajava* L. Leaf.

Table 5: Structures of compounds found in methanol extracts of *Psidium guajava* L.

Sr.no.	Name of compound	Structure of compound
1	Eucalyptol	
2	4,4-pyran-4-one2,3dihydro-3,5-dihydroxy6methyl	
3	2cyclohexane-1,2methyl-5-(1-methyl ethyne)	
4	A-cubebene	
5	Caryophyllene	
6	Cyclohexene,1 methyl -4-(5-methyl-1-methylene-4-hexenyl)(s)-	
7	Napthelene 1,2,3,4-tetrahydrol,6-dimethyl-4(1methyl ethyl)-(1scis)	
8	1,6,10 dodecatrien-3oi 3,7,11-trimethyl	

9	Caryophyllene oxide	
10	Longi Folene	
11	3 Eicosyne	
12	nHexadecanoic acid	
13	2cyclohexene-1-one,3methoxy-2-(2,4,5trimethoxy phenyl)	
14	Antracene,9,10dibutyl	

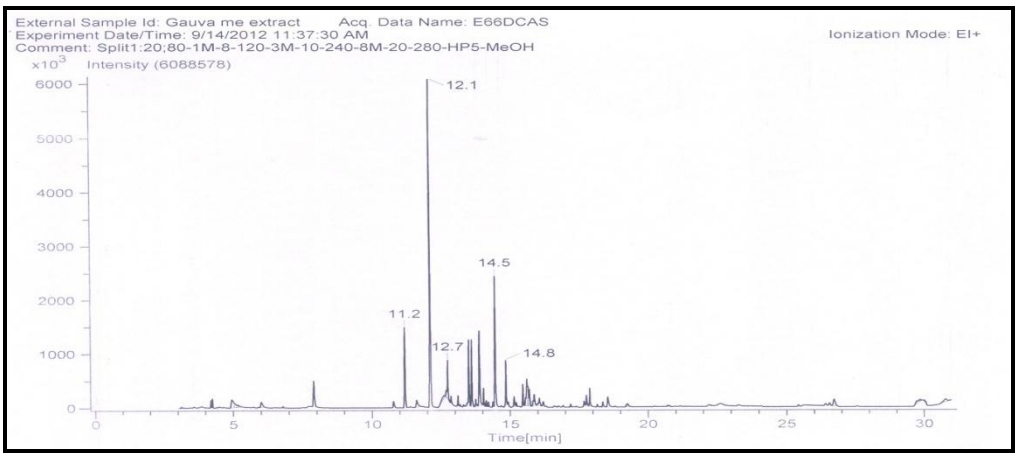


Figure 1: GCMS chromatogram of methanol extract of *Psidium guajava* L. Leaf.

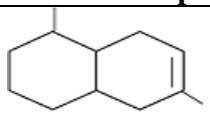
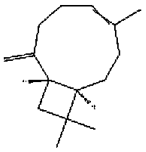
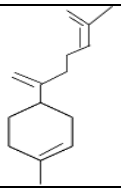
Table 6: Gas chromatographic and Spectral data for Chloroform extract of *Psidium guajava L.* Leaf.

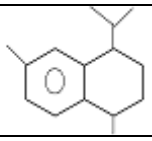
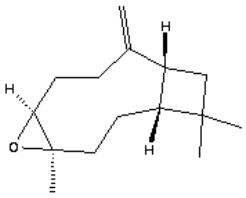
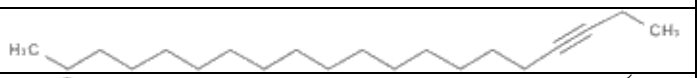
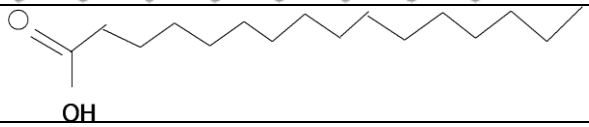
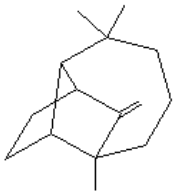
Peak No	R.T (min)	Name of compound	Molecular Formula	Molecular Weight	%Peak area
1.	7.9	2-Cyclohexene-1-one, 2methyl 5- (1-methyl ethenyl)-	$C_{10}H_{14}O$	150	8.9%
2.	11.2	Copene	$C_{15}H_{24}$	184	16.07%
3.	12.1	Caryophyllene	$C_{15}H_{25}$	180	71.07%
4.	13.5	Longifolene-(v_4)	$C_{15}H_{29}$	180	18.5%
5.	13.9	Napthalene 1,2,3,4 tetrahydro1,6 dimethyl-4-1-(methyl ethyl-(1scis)-	$C_{15}H_{22}$	178	2.1%
6.	14.8	Caryophyllene oxide	$C_{15}H_{25}O$	196	37.02%
7.	17.9	3Eicosyne	$C_{20}H_{38}$	278	6.14%
8.	19.3	nHexadecanoic acid	$C_{16}H_{32}O_2$	256	3%

Table 6 shows the gas chromatographic and Spectral data for Chloroform extract of *Psidium guajava* L. Eight peaks were found in the Chloroform Extract extract of *Psidium guajava* L. which shows the presence of eight compounds in the extract. Caryophyllene was found as the major [71.07%] compound followed by Caryophyllene oxide [37.02%]. Other compounds present in Chloroform extract of *Psidium guajava* L. were Copene, Longifolene-(v_4), 2-Cyclohexene-1-one, 2methyl 5-(1-methyl ethenyl)-, 3Eicosyne, nHexadecanoic acid and Napthalene 1,2,3,4 tetrahydro1,6 dimethyl-4-1-(methyl ethyl-(1s cis)-.

Structure of compounds found and identified in chloroform extracts of *Psidium guajava* L. are shown in Table7. Figure 2 shows GCMS chromatogram of Chloroform extract of *Psidium guajava* L.

Table 7: Chemical structures of compounds found and identified in chloroform extracts of *Psidium guajava* L.

Sr.no.	Name of compound	Structure of compound
1	Copene	
2	Caryophyllene	
3	Cyclohexene,1 methyl-4-(5-methyl-1-methylene-4-hexenyl)-	

4	Napthelene 1,2,3,4-tetrahydrol,6-dimethyl-4(1methyl ethyl)-(1scis)	
5	Caryophyllene oxide	
6	3 Eicosyne	
7	nHexadecanoic acid	
8	Longi Folene	

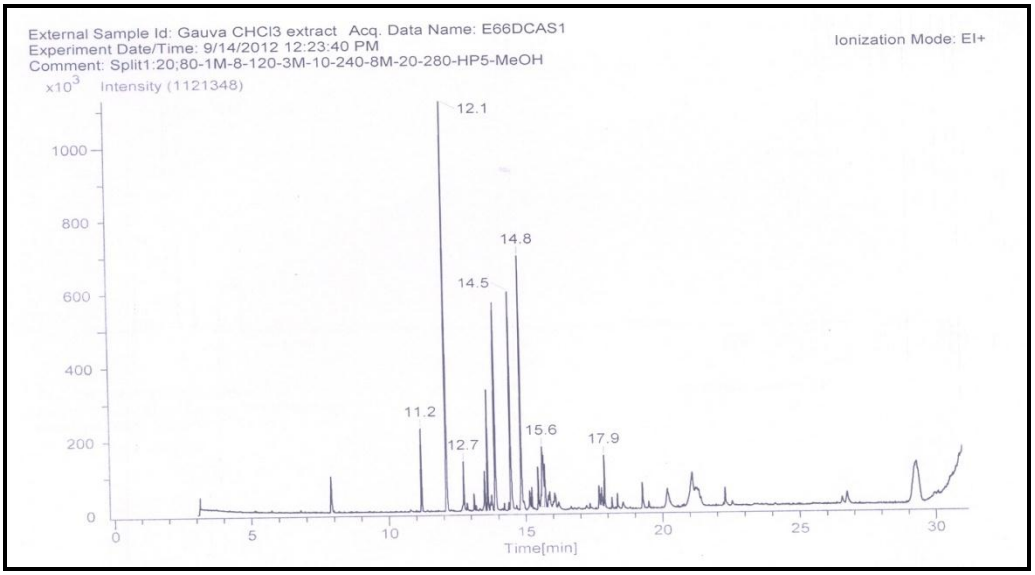


Figure 2: GCMS chromatogram of Chloroform extract of *Psidium guajava* L.

S. Thenmozhi and S. Rajan (2015) evaluated the chemical compositions of Psidium guajava leaves by using using Perkin-Elmer Gas Chromatography– Mass Spectrometry. They have reported that 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. In the present investigation methanol and chloroform were used as solvent for eextraction. In both Caryophyllene was the major compound found which correlates with the studies by S. Thenmozhi and S. Rajan(2015).

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and *Psidium guajava* L plant proves to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Therefore, this plant has therapeutic value 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, cardiovascularand microbial infections.

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