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Metabolites detection through GC MS in volatile and fixed oil of Betelvine leaf for potential application: Therapeutic and nutritional

PJ Rathod[1], S K Bhuva[2], HP Gajera[3]
[1] Associate professor, Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India
[2] Assistant Professor, Department of Horticulture, Junagadh Agricultural University, Junagadh, Gujarat, India
[3] Professor and Head, Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India

Abstract
The experiment was conducted in the Department of Biochemistry, JAU, Junagadh, with the aim of metabolite detection through Mass spectrometry by utilizing the extracted volatile oil of betelvine leaf (Nagarvel pan) for their nutritional, medicinal, or therapeutic properties and their potential application in the food industry. The extracted volatile oil showed 65 peak with various mass-to-charge ratios with the putative of 56 compounds with the class of Alcohol, Alkaloid derivative (tentative), Aromatic Acid, Aromatic Hydrocarbon, Ester, Fatty Acid, Ketone, Lignan, Fatty Acids (Monounsaturated Fatty Acid, Saturated Fatty Acid, Unsaturated Fatty Acid, Omega-3 Fatty Acid, Omega-6 Fatty Acid), Organic Acid, Phenolic Compound, Phenylpropanoid, Terpene and their Hydrocarbon, Alcohol, Ester, Sesquiterpene and their ester & alcohol, Monoterpene, Sesquiterpenoid, Terpenoid. >5% area detected of Nagarvel pan (Betelvine leaf) were majority of methyl eugenol groups with Damascenone (5.04%), phytol (27%), selin-11-en-4 alpha-ol (23.17%), and remaining were <5% area in chromatogram table with 62 compounds. Many volatile compounds and flavoring compounds were detected with o-cymene, caryophyllene, germacrene D-4-OL, chavicol, and khusinol. It has potential applications in the flavor and food industries.

Keywords: Fatty acids, piper betel, GC/MS

Introduction
The betelvine (*Piper betel* L.; Family: *Piperaceae*) is the leaf of a vine which includes pepper and kava. In India, it is known as 'pan' Betelvine is a perennial, dioecious, evergreen climber that is grown in tropics and subtropics for its leaves that are used as a chewing stimulant. Betel leaf grows on a perennial vine (*Piper betle L.*). It is consumed in fresh and raw state for digestive, refreshing, stimulating, aphrodisiac, etc. effects by about two billion people. These beneficial effects, taste, and aroma are mainly contributed by the essential oil present in the leaves. (Das *et al.*,2016) [1]
Many foods product already in market with flavour of tooth -pastes, cold drink skin emollients chocolates, Tooth Powder, as appetizers, digestive agents in Pan Masala, as Deodorants tonics and medicines, as Mouth freshness as Beauty and Cosmetics Facial Creams, Betel leaf essential oil used in anti-septic lotions and Ice-cream too.
The oil can be utilized as an excellent food preservative and organoleptic enhancer. According to claims made centuries ago, it might also have several health advantages. But in Gujarat, many people are addicted to eating pan masala with various juicy ingredients. In India, many hypothetical myths are also attached to social gathering and has the potential to be used as aphrosaidic. Whether these beliefs are true or any bioactive compounds are present in this or not. To know this experiment was formulated with the local betel leaf with self-grown crop species in our departmental field with truck of electric pole. Many food products have been developed by food processors with the addition of some volatile oils or raw extracts ins cupcakes, chocolate, biscuits, suji halwa, and lozenges; therefore, it is the most appropriate reason to know the metabolites or phytochemicals in oils of betel leaf, and of course it has a potential future in the world food sector. Therefore, it was imperative to study metabolite detection through high through put techniques like GC MS in volatile and fixed oil of betel leaf to know the potential Industrial application.

Materials and methods
GCMS conditions
Extracted oils were applied in autosampler tubes and used to create methyl ester and derivatives. GC-MS analyses were performed using a DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). The chromatographic runs were started as per the following details conditions [GC-2010]. The GCMS conditions as per table indicated: ionization mode, electron ionization (EI) mode; ionization current, 60 µA; ionization voltage, 70 eV. Each sample was analysed. The Wiley NBS, the NIST.
(http://www.nist.gov/srd/nist1a.cfm), the mass spectral data, PubMed, and other data sources were used to identify the eluted compoundsFatty acids and compounds were used for google search, NIST library for the potential application for food and industrial application.

Instruments protocol for analysis of GCMS-FAME

Column Oven Temp.	40.0 °C	MS table:
Injection Temp.	:250.00 °C	Start Time :3.50min,
Injection Mode	: Split	End Time :30.00min,
Flow Control Mode	: Pressure	ACQ Mode: Scan,
Pressure	:49.5 kPa	Event Time :0.50sec,

Total Flow	:54.0 mL/min	Scan Speed 1428, Start m/z :50.00, End m/z :700.00, Sample Inlet Uni: GC "[GCMS-QP2010 Plus], Ion Source Temp :200.00 °C, Interface Temp.:290.00 °C, Solvent Cut Time :2.00 min, Detector Gain Mode: Relative Detector Gain: +0.00 kV, Threshold 100"
Column Flow	:1.00 mL/min	
Linear Velocity	:36.1 cm/sec	
Purge Flow	:3.0 mL/min	
Split Ratio	:50.0	
High Pressure Injection	: ON	
High Press. Inj. Pressure	:250.0 kPa	
High Press. Inj. Time	:1.00 min	
Carrier Gas Saver	: OFF	
Splitter Hold	: OFF	
Oven Temp. Program		
Rate	Temperature(°C)	Hold Time(min)
-	40.0	3.00
6.00	290.0	10.00

Results and discussion

Experimental results for volatile compounds in oils of betel are presented in Table.1 & Figure1. The data showed 65 peaks with varying amount of height and area %. And RT for compounds was ranges between 2.733 to 20.679. The more than fifty percentage area % covered by Isoeugenol while 5.04% detected in chromatogram was Damascenone. Their derivatives showed nearly sixty percentage mean only three compound covers this much composition in betel leaf. While remaining 62 compounds ranges between 0.001 to 3.26. The representation of all bioactive or key compounds are detected in the the range of 1.12 % (alpha. -Bourbonene) to 3.26% (Caryophyllene). The GC MS metabolites detected with same compound at different RT also because of their derivatives compounds. (Table.1 & Table 3). Similar findings also reported by Das *et al*., (2016) [] They showed betel oil is constituted by about 30–60 compounds which include eugenol, isoeugenol, methyl eugenol, safrole, chavicol, hydroxychavicol, (No. 57 of Table.1) and 19 No. of table 2 of this compounds also detected by Garg and Jain 1996 [2] chavibetol, anethole, estragole, germecrene-D, etc. These results are in agreement with the Das *et al*., (2016) [1] oil was extracted Betel and they found from range from 0.09% to 1.0% (wb) depending upon variety, extraction method, pretreatment, curing, agro-climatic conditions, etc. The Exact name and class of compounds identified with standard name in various pubchem, google search, chemical data, chemical abstract data base showed the exact mass. (Table.2). The data showed the viz., putative of 56 compounds with class of Alcohol, Alkaloid derivative (tentative), Aromatic Acid, Aromatic Hydrocarbon, Ester, Fatty Acid, Ketone, Lignan, Fatty acids (Monounsaturated Fatty Acid, Saturated fatty acid, Unsaturated Fatty Acid, Omega-3 Fatty Acid, Omega-6 Fatty Acid), Organic Acid, Phenolic Compound, Phenylpropanoid, Terpene and their Hydrocarbon, alcohol, ester, Sesquiterpene and their ester & alcohol, Monoterpene, Sesquiterpenoid, Terpenoid. This indicated that this leaf seed oils have potential application in food flavour and fragmented but need to be care full because presence of carcinogenic compounds too. (Table 3.) But It probably nullify with the this much potential compounds as antimicrobial, antifungal a, anti-inflammatory and anticancer property too. These results are in agreements of many researchers to who studied, Das *et al* (2016) [1] and showed that oil possesses good antioxidant and antimicrobial properties at a very low concentration (>0.20 µl/ml), and the susceptible microorganisms include gram-positive and gram-negative bacteria, and fungi, such as Aspergillus, Candida, Escherichia, Penicillium, Salmonella, Staphylococcus, Streptococcus, Vibrio, etc. species.

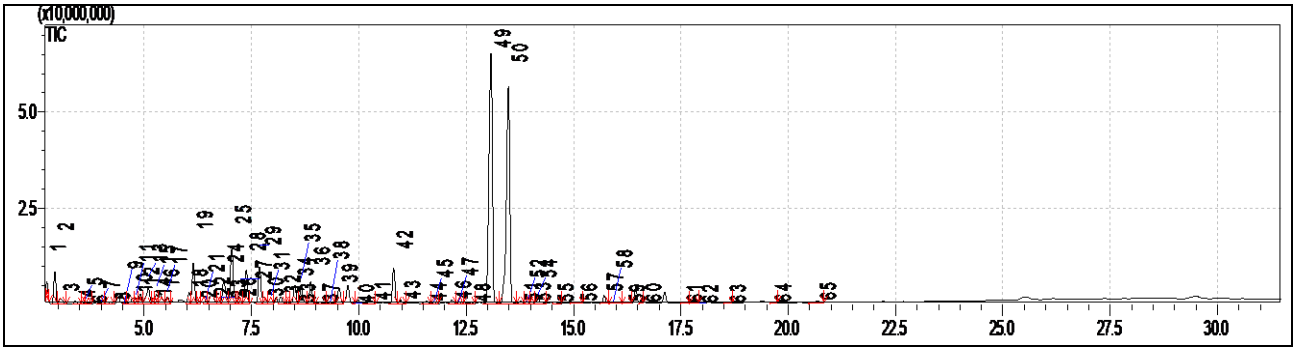

Fig 1: Chromatograph of fatty acids methy ester of oil extracted from belevine leaf

Table 1: No of peak identified from the volatile and fixed oils of betel vine green leaves

Peak No	RT (min)	Area (%)	Compound Identified
1	2.733	0.51	gamma-Terpinene
2	2.919	2.24	o-cymene
3	3.037	0.66	(+)-4-Carene
4	3.398	0.25	Linalyl isovalerate
5	3.6	0.06	3-(2-Bromocyclobutylidene)-2-methylpropionic acid
6	3.697	0.12	Thujopsene-(I2)
7	3.986	0.15	13,16-Octadecadiynoic acid
8	4.221	0.19	Tetrahydro-22-desoxy-tomatillidine

9	4.541	0.01	LINALOOL OXIDE
10	4.734	0.65	4-[1,3]Dioxolan-2-yl-3,4-dimethyl-cyclohex-2-enone
11	4.813	0.47	Caryophyllene
12	4.91	0.8	β.-Humulene
13	5.095	1.86	Copaene
14	5.198	0.51	Valencene
15	5.289	0.75	EUDESMOL ACETATE
16	5.371	1.12	α.-Bourbonene
17	5.538	0.67	linalool
18	6.05	0.98	β-Elemene
19	6.146	3.26	Caryophyllene
20	6.248	0.28	DRIMA-7,9(11)-DIENE
21	6.426	0.27	β-cadinene
22	6.567	1.44	gamma-gurjunene
23	6.747	0.39	delta-cadinene
24	6.856	2.39	.alpha.-Caryophyllene
25	7.04	5.04	MUUROLENE
26	7.157	0.34	Nerolidyl acetate
27	7.251	1.23	EPIZONARENE
28	7.375	3.14	α.- **Copaene**
29	7.687	3.06	CADINENE
30	7.795	0.68	CURCUMENE
31	7.952	0.59	alpha.-Cubebene
32	8.179	0.64	Dodecanoic acid
33	8.351	0.13	Damascenone
34	8.507	1.56	Calamenene
35	8.657	1.57	2,4-Octadienoic acid
36	8.88	**1.74**	Isosafrole
37	9.088	0.25	Gurjunene
38	9.335	0.22	Himachalene
39	9.54	1.45	Germacrene d-4-ol
40	9.879	0.06	Muurolene <14-oxy-alpha->
41	10.281	0.49	Guaiol acetate
42	10.81	2.86	Methyl eugenol
43	10.972	0.35	Tetradecanoic acid
44	11.578	0.34	Caryophyllene alcohol
45	11.749	0.14	Cubenol
46	12.163	0.36	Cerulignol
47	12.329	0.16	Cerulignol
48	12.623	0.14	Spathulenol
49	13.077	27	Isoeugenol
50	13.482	23.17	Isoeugenol
51	13.768	0.26	delta-Cadinol
52	13.924	0.15	CADALENE
53	14.032	0.17	Homovaretric acid
54	14.171	0.68	Hexadecanoic acid
55	14.566	0.15	Selin-11-en-4 αol
56	15.11	0.3	Caryophyllene oxide
57	15.713	0.64	Chavicol
58	15.926	0.18	Isoaromadendrene epoxide
59	16.231	0.09	Khusinol
60	16.563	0.16	Nerolidol
61	17.559	0.09	Octadecanoic acid
62	17.838	0.05	9-Octadecenoic acid
63	18.578	0.03	9,12-Octadecadienoic acid
64	19.615	0.07	9,12,15-Octadecatrienoic acid
65	20.679	0.24	Phytol

Table 2: Name and class of compounds identified with standardize name in chemical abstract and data base with exact mass

No	Standardize name	Mol. Formula	Exact Mass(g/mol)	Class
1	13,16-Octadecadiynoic acid	$C_{18}H_{30}O_2$	282.227	Fatty Acid
2	2,4-Octadienoic acid (Sorbic acid)	$C_8H_8O_2$	136.052	Unsaturated Fatty Acid
3	3-(2-Bromocyclobutylidene)-2-methylpropionic acid	$C_{10}H_{13}BrO_2$	245.020	Organic Acid
4	4-[1,3]Dioxolan-2-yl-3,4-dimethyl-cyclohex-2-enone	$C_{12}H_{16}O_3$	208.111	Ketone
5	Alpha-Linolenic acid (ALA)/ 9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278.227	Omega-3 Fatty Acid
6	Linoleic acid (9,12-Octadecadienoic acid)	$C_{18}H_{32}O_2$	280.252	Omega-6 Fatty Acid

7	Oleic acid (9-Octadecenoic acid)	$C_{18}H_{34}O_2$	282.463	Monounsaturated Fatty Acid
8	Cadinene	$C_{15}H_{24}$	204.186	Terpene
9	Elemene	$C_{15}H_{24}$	~204	Terpene
10	Bourbonene	$C_{15}H_{24}$	~204	Terpene
11	Cadalene	$C_{15}H_{24}$	~204	Terpene
12	Cadinol	$C_{15}H_{26}O$	~222	Alcohol
13	Calamenene	$C_{15}H_{24}$	~204	Terpene
14	Carene	$C_{10}H_{16}$	~136	Terpene
15	Caryophyllene	$C_{15}H_{24}$	204.368	Sesquiterpene
16	Caryophyllene Alcohol	$C_{15}H_{26}O$	222.396	Sesquiterpenoid
17	Caryophyllene Oxide	$C_{15}H_{24}O$	220.368	Sesquiterpenoid
18	Cerulignol	$C_{16}H_{18}O3$	258.128	Lignan
19	Chavicol	$C_{10}H_{12}O_2$	164.08	Phenylpropanoid
20	Copaene	$C_{15}H_{24}$	204.368	Sesquiterpene
21	Cubebene	$C_{15}H_{24}$	204.368	Sesquiterpene
22	Cubenol	$C_{15}H_{26}O$	222.396	Sesquiterpenoid
23	Curcumene	$C_{15}H_{22}$	202.35	Sesquiterpene
24	Cymene	$C_{10}H_{12}$	132.20	Monoterpene
25	Damascenone	$C_{13}H_{14}O$	198.10	Ketone
26	Dodecanoic Acid (Lauric Acid)	$C_{12}H_{24}O_2$	200.32	Fatty Acid
27	Drima–7,9(11)-Diene	$C_{15}H_{24}$	204.368	Terpene Hydrocarbon
28	Epizonarene	$C_{14}H_{18}$	186.30	Aromatic Hydrocarbon
29	Eudesmol Acetate	$C_{14}H_{24}O_2$	224.36	Terpenoid Ester
30	Germacrene D-4-ol	$C_{15}H_{24}O$	224.186	Terpene
31	Guaiol acetate	$C_{12}H_{16}O_3$	208.11	Terpenoid
32	Gurjunene	$C_{15}H_{24}$	204.19	Sesquiterpene
33	Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256.424	Fatty Acid
34	Himachalene	$C_{15}H_{24}$	204.19	Sesquiterpene
35	Homovaretric acid	$C_{14}H_{22}O_3$	250.17	Aromatic Acid
36	Humulene	$C_{15}H_{24}$	204.19	Sesquiterpene
37	Isoaromadendrene epoxide	$C_{15}H_{24}O$	224.186	Terpene
38	Isoeugenol	$C_{10}H_{12}O_3$	180.20	Phenolic Compound
39	Isosafrole	$C_{10}H_{10}O_2$	162.18	Phenylpropanoid
40	Khusinol	$C_{15}H_{24}O$	224.186	Terpene
41	Linalool	$C_{10}H_{18}O$	154.23	Alcohol
42	Linalool oxide	$C_{10}H_{18}O_2$	170.25	Terpenoid
43	Linalyl isovalerate	$C_{13}H_{24}O_2$	212.36	Ester
44	Methyl eugenol	$C_{11}H_{14}O_3$	194.23	Phenolic Compound
45	Muurolene	$C_{15}H_{24}$	204.189	Sesquiterpene
46	Nerolidol	$C_{15}H_{26}O$	222.201	Sesquiterpene alcohol
47	Nerolidyl acetate	$C_{17}H_{30}O_2$	270.227	Sesquiterpene ester
48	Stearic acid (Octadecanoic acid)	$C_{18}H_{36}O_2$	284.463	Saturated fatty acid
49	Phytol	$C_{20}H_{40}O$	296.315	Terpenoid alcohol
50	Selin-11-en-4-alpha-ol	$C_{15}H_{24}O$	220.189	Sesquiterpene alcohol
51	Spathulenol	$C_{15}H_{24}O$	220.189	Sesquiterpene alcohol
52	Myristic acid(Tetradecanoic acid)	$C_{14}H_{28}O_2$	228.424	Saturated fatty acid
53	Tetrahydro-22-desoxy-tomatillidine	$C_{19}H_{31}NO_3$ (tentative)		Alkaloid derivative (tentative)
54	Thujopsene	$C_{15}H_{24}$	204.189	Sesquiterpene
55	Terpinene	$C_{10}H_{16}$	136.241	Monoterpene
56	Valencene	$C_{15}H_{24}$	204.189	Sesquiterpene

Table 3: Potential Application of detected betel leaf compounds

Peak No.	Compound name	Biological activity	Ref. compound detected by
24	Cymene	prevent coughs and eliminate phlegm Antimicrobial, Anti-inflammatory, Antioxidant, Anticancer	Islam *et al* (2020) [4]
13	Copaene	Insect Repellent, Antimicrobial, Antifungal,	Das *et al* (2016) [1]
16	Bourbonene	primarily used in fragrance applications	Das *et al* (2016) [1]
11, 19, 24, 44, 56	Caryophyllene & its derivatives	Anti-inflammatory (Significantly reduces inflammation), Analgesic (Provides pain relief), Anticancer (Shows potential in cancer therapy, particularly in reducing tumor growth) Antimicrobial (Exhibits activity against bacteria and fungi), Cannabinoid Receptor	Das *et al* (2016) [1]
22,37	Gurjunene	Antimicrobial, Antifungal, Potential Insect Repellent (May act as an insect repellent in certain formulations.	Das *et al* (2016) [1]
25,28,40	Muurolene	used in fragrance formulations	Islam *et al* (2020) [3,4]
27	Epizonarene	used in fragrance applications	Das *et al* (2016)

21,23,29	Cadinene	Antimicrobial& Anti-inflammatory, Potential Anticancer	Das *et al* (2016) [1]
34	Calamenene	primarily in fragrances	Das *et al* (2016) [1]
35	2,4 Octadienoic Acid	Antimicrobial & Antioxidant also used in flavors and fragrances	Das *et al* (2016) [1]
36	Isosafrole	potential carcinogenic effects and is regulated in some contexts, Antimicrobial and Utilized in the chemical synthesis of other compounds.	Das *et al* (2016) [1]
39	Germacrene D-4-OL	Antimicrobial, Antioxidant, Anti-inflammatory and also Found in essential oils and used in perfumery.	Das *et al* (2016) [1]
42,49,50	Methyl Eugenol	Antimicrobial, Antioxidant, Insect Repellent (Used in pest control and as an insect attractant in traps), Potential Carcinogen	Das *et al* (2016) [1]

Conclusion

The experiments with leaf of betel in GC MS showed 65 peaks with putative of More than 56 compounds with maximum health beneficial compounds in oils of betel leaf. It may have potential application as Antimicrobial& Anti-inflammatory, Insect repellents and also has potential anticancer properties

References

1. Das S, Parida R, Sandeep I Sriram Sandeep, Basudev Kar, Sanghamitra Nayak & Sujata Mohanty.. Chemical composition and antioxidant activity of some important betel vine landraces. *Biologia,*2016:71(2):128–132.
2. Garg S, Jain R. Chavicol rich essential oil of *Piper betle* L., cultivar Sagar *Bangla. Euro Cosmet,*1996:5:27–28
3. Islam Md Atikul, In Min Hwang, Naeem Khan, Ok Yeon Song, Ji Young Jeong, Ji Hyeon Son, Nargis Jamila and Kyong Su Kim Authentication of Leaves and Petioles of Piper betle L. Varieties via Elemental Composition and Multivariate Chemometric Analysis. *Analytical Letters*,2021:54(11):1794.
4. Islam Md Atikul, Keun Young Ryu, Naeem Khan, Ok Yeon Song, Ji Young Jeong, *et al* Determination of the Volatile Compounds in Five Varieties of *Piper betle L*. from Bangladesh Using Simultaneous Distillation Extraction and Gas Chromatography/Mass Spectrometry (SDE-GC/MS) *Analytical Letters*,2020:53(15):2413.
5. Muhamad Faris Osman, Soo Yee Lee, Shahrul Razid Sarbini, Siti Munirah Mohd Faudzi, Shamsul Khamis, Badrul Hisyam Zainudin and Khozirah Shaari Metabolomics-Driven Discovery of an Introduced Species and Two Malaysian *Piper betle L*. Variants. Plants,2021:10(11):2510