Biological Forum – An International Journal **14(1): 1199-1203(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Quantification of Bioactive Compounds in *Piper betle* Leaf extract by Gas Chromatography Mass Spectrometry (GC-MS) Technique

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(Received 18 November 2021, Accepted 22 January, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: *Piper betle* is a Piperaceae family scented perennial creeper. The leaves are high in bioactive compounds such as phenol, a compound with anti-tumor, anti-mutagenic, and immune modulatory activities. There are so many bioactive compounds present in betel leaves. To identify the major compound particularly the varieties grown Tamil Nadu is the major concern of the study. The aim of this study is to evaluate the bioactive compound in *Piper betle* leaves extract using Gas Chromatography Mass Spectrometry (GC-MS). Over 100 compounds were found in the GC-MS results, with 19 of them having a probability of greater than 30.The compound phentermine shows highest peak at retention time of 9.346 mins followed by Hexadecanoic acid, Tetradecanoic acid, Eugenol, Dodecanoic acid. Hexadecanoic acid shows the highest area% about 26.665% which indicate highest composition of hexadecanoic acid present in the betel leaf extract. The result revealed that the compound in betel leaf extract possess the medicinal properties.

Keywords: *Piper betle* leaves, Gas Chromatography Mass Spectroscopy, Retention Time and Area.

INTRODUCTION

Betel leaves (*Piper betle* L) belongs to the family Piperacea. *Piper betle* is an aromatic perennial creeper with heart shape leaf (Amonkar *et al*., 1986). The trace of usage of piper betle leaves was found in 5500 to 7000 BC in Thailand (Chaveerach *et al*., 2006). The vernacular names of betel vine are Nagarvallari in Sanskrit, Pan in Hindi, Vetrilai in Tamil, Nagballi in Telugu, Nagarbael in Gujarati, Nagbeal in Marathi, Tambol in Arabic (Balkrishna, 2008). The scientific classification of betel vine belongs to Kingdon: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, order: Piperales, family: Piperacea, Genus: *Piper*, species: *betle* (Pradhan *et al*., 2013). There are 1050 species divided into three genera in Asia (Suwanphakdee *et al*., 2016).

The essential oils are responsible for the flavour and aroma of betel leaves, as well as contributing to distinctive flavour. Essential oil in *Piper betle* ranges from 0.15% to 2%, based on the location and type of cultivation. The betel leaves contains Polysaccharides, Tannins, flavonoids and phenols. The major constituent of betel leaves is phenols and terpenes which also contain compound such as chavicol, allylprocatechol, chavibetol, phenyl alanine (Bajpai *et al*., 2010). In fresh betel leaves essential oil consists of 98.4% volatile compound whereas cured leaves essential oil is about 97.34%.

The leaves shows antibacterial activity against the microorganism such as *Mycobacterium smegmatis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Madhumitha *et al*., 2019). Eugenol, methyl eugenol, chavibetol, b-caryophyllene, estragole, hydroxycatechol, a- pipene, b-pipene and estragole 1,8 cineol are the phytochemical found in the betel leaves (Guha *et al*., 2019). An ethanol extract of Piper betel leaves was tested for antibacterial efficacy against human pathogenic microorganisms for both gram-positive and gram-negative bacteria (Datta *et al*., 2011). Gas chromatography- mass spectroscopy is an important analytical tool in area of herbal medicinal research, particularly for identifying and describing different mixture of organic compounds found in extracted material (Gu *et al*., 2004). The gas chromatography- mass spectroscopy is used to find the concentration of volatile compound present in plant material (Islam *et al*., 2020). There are different solvent used for extraction of essential oil from *Piper betle.* The solvents such as water, ethanol, methanol, hexane and chloroform (Guha *et al*., 2019).

The objective of the present study is to evaluate the bioactive compound of water extracted betel leaves by using gas chromatographic technique.

MATERIALS AND METHODS

A. Plant material

Fresh betel leaves (Karpoora variety) were procured from the farmer's field, Namakkal. The fresh betel leaves were washed to clean the dirt and foreign matter. Then the washed betel leaves are air dried to remove the surface water. After the removal of surface water, betel leaves are dried in cabinet drier for 60°C until the moisture is removed (Pin *et al*., 2014). The temperature of cabinet drier is maintained at 60°C to reduce the loss phytochemicals in betel leaves whereas the high temperature cause the loss of phytochemicals. The dried leaves are powdered to uniform size particle by using the blender.

B. Extraction

Water solvent extraction method is used to extract the bioactive compound from the betel leaves. Here water is used as the solvent to extract materials. The dried betel leaves powder were mixed with the water in the ratio of 1:30 (1g gram of dried betel leaves powder with 30 ml water) (Pin *et al*., 2011). The dried sample and water are mixed thoroughly and kept in water bath at 60°C for 1 hour. Then the sample was filtered by using filter paper to collect the betel leaf extract.

C. Gas Chromatography-Mass Spectroscopy (GC-MS)

Perkin Elmer Clarus SQ8C Gas chromatography- Mass Spectroscopy (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India) was used for the analysis of bioactive compound in the piper betel leave extract. DB-5 MS capillary standard non polar column (Dimension: 30m × 0.25 mm ID, film thickness: 0.25 µm) was used to separate the bioactive compound from betel leaves extract and the sample injected about 1 microliter. Helium was used as the carrier gas with flow rate of 1.0mL min^{-1}. The mass spectrum was scanned from 0–480 m/z.

RESULT AND DISCUSSION

The GCMS resulted for betel leaf extract is given in Fig 1, which showed the peaks obtained respect to retention time. The peak indicates the concentration of the substance; where the retention time indicated the type of compound which responsible for the peak. There are more than 120 compounds were identified, in which 19 compounds showed the probability percentage above 30. Table 1 show the compound identified, Retention time, Area%, Molecular formula, Molecular mass, Molecular structure. The result from the GC-MS showed that betel leaf extract is predominantly constituted of essential oils and fatty acids such as dodecanoic acid, tridecanoic acid, tetradecanoic acid, oleic acid, pentadecanoic acid, palmitonic acid, n-hexadecanoic acid, octadecanoic acid, octadecadienoic acid. Phentermine, 2- myristynoyl pantethenine, 3-hydroxyl palmitate and octodecamide belongs to the amine group. Eugenol was the phenolic compound and phytol belongs to terpene group. The leaf extract also consists of ester group (Diisooctyl phththalate) and lignin (4 – allyl-1,2- diacetoxybenzene). Similar findings were observed in Junairiah *et al.* (2018) and the results showed that methanol extract of black betel leaves contained of alkaloids, terpenoids/steroid, flavonoids, polyphenols and tannins compounds.

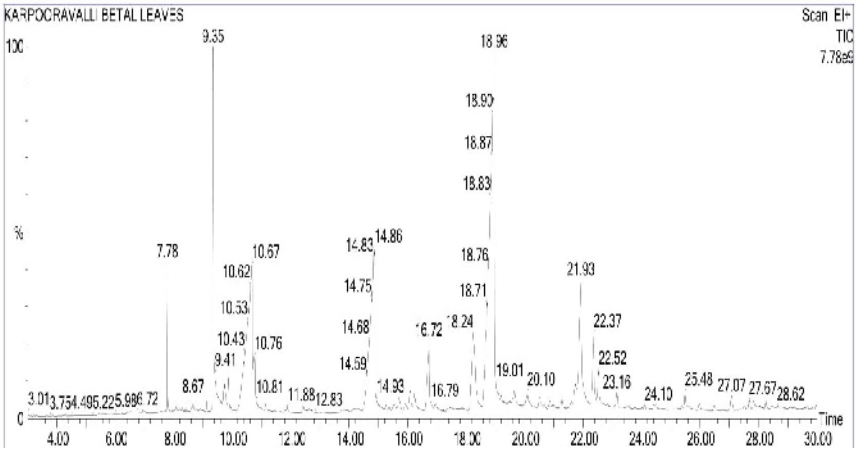


Fig. 1. Result obtained for *Piper betle* leaf extract from GC-MS.

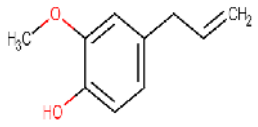
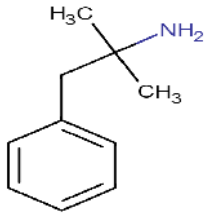
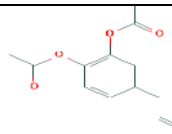
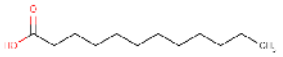
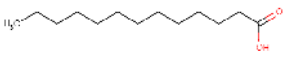
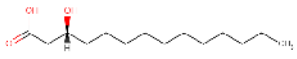
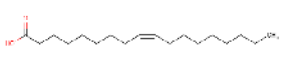
At a retention time of 9.346 minutes, the component phentermine produced the highest peak, indicating that betel leaf extract has a significant concentration of phentermine. The compound n-hexadecanoic acid had a high percentage area of 26.665%, indicating that the extract's current composition. Hexadecanoic acid inhibits phospholipase and has anti-inflammatory properties (Aparna *et al*., 2012). Dodacanoic acid, which is used as an antimicrobial agent, has a reported area of 13.842 percent. Lauric acid is another name for dodecanoic acid. Eugenol, a phenolic compound, produced a peak with a retention time of 7.78 minutes and an area of 1.764 %. The presence of eugenol in betel leaf extract has been discovered in several studies (Madhumitha *et al*., 2019).

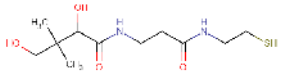
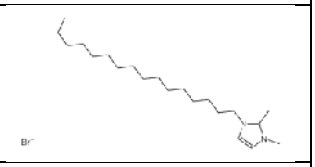
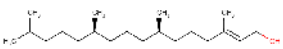
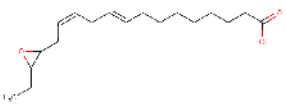
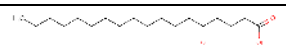
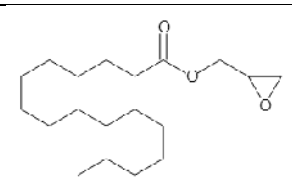
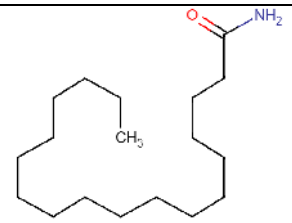
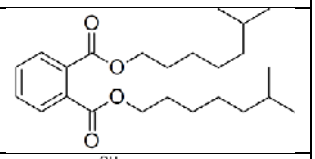
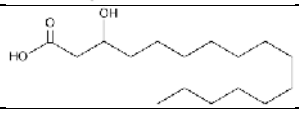
The phenolic compound 4-Allyl-1,2-diacteoxybenzene has a retention time and area of 9.746 minutes and 0.739 %, respectively. Anti-inflammatory, antioxidant and antibacterial activities are found in this compound (Madhumitha *et al*., 2020). Tridecanoic acid, Tetradecanoic acid, Pentadecanoic acid and Oleic acid are saturated fatty acids with different numbers of

carbon atoms- 13, 14, 15, and 18. The retention time & area for Tridecanoic acid, Tetradecanoic acid, Pentadecanoic acid and Oleic acid were 12.427 mins & 0.329%, 13.763 mins & 0.248%, 15.918 mins & 0.192% and 15.553 mins & 0.299% respectively. The antimicrobial compound tridecanoic acid has antibacterial and antifungal properties against pathogenic microorganisms (Chowdhury *et al*., 2021). Tetradecanoic acid is also known as mystric acid. Palmitoleic acid is a monounsaturated fatty acid with a retention time of 18.254 minutes and an area of 5.127 %. Bacteria and yeast are used in emerging technologies to produce palmitoleic acid (Bae *et al*., 2007). Phytol is a diterpene alcohol with 20 carbons which has retention time of 21.276 minutes and area of 0.242%. The phytol has antinociceptive and antioxidant properties (Santos *et al*., 2013).

Octadecanoic acid belongs to amide of steric acid which is used as metabolite. The octadecanoic acid reported at retention time of 25.972 minutes and area about 0.235%. Glycidyly palmitate is an ester reported at retention time and area about 24.492 minutes and 0.231%. Glycidyl palmitate is also known as lysophosphatide acid. The retention time and area for octadecanoic acid was found to be 22.366 mins and 1.895% respectively. Some studies of octadecanoic acid observed the antibacterial activity against the bacterial strains (Pu *et al*., 2013). A study conducted by Sushma *et al.*, (2020) confirmed the presence of alkaloids, polyphenols, flavonoids and tannins. On quantification IPB was found to contain 4.2±1.661 mg/ml and 1.523±0.156 mg/ml of flavonoids and tannins respectively.

Table 1: Compound in betel leaf extract by GC- MS (Retention time, Area, Molecular mass, Molecular formula and Molecular structure).

Sr.No.	Compounds	Retention time (mins)	Area%	Molecular mass	Molecular formula	Molecular structure
1.	Eugenol	7.780	1.764	164.2011	$C_{10}H_{12}O_2$	
2.	Phentermine	9.346	5.602	149.2328	$C_{10}H_{15}N$	
3.	4-Allyl-1,2-diacetoxybenzene	9.746	0.739	234.25	$C_{13}H_{14}O_4$	
4.	Dodecanoic acid	10.676	13.842	200.3178	$C_{12}H_{24}O_2$	
5.	Tridecanoic acid	12.427	0.329	214.3443	$C_{13}H_{26}O_2$	
6.	Tetradecanoic acid	13.763	0.248	244.3703	$C_{14}H_{28}O_3$	
7.	Oleic acid	15.553	0.299	282.4614	$C_{18}H_{34}O_2$	
8.	Pentadecanoic acid	15.918	0.192	242.3975	$C_{15}H_{30}O_2$	

9.	Palmitoleic acid	18.254	5.127	54.4082	$C_{16}H_{30}O_2$	
10.	n-Hexadecanoic acid	18.960	26.665	256.4241	$C_{16}H_{32}O_2$	
11.	2-Myristynoyl pantetheine	19.480	0.141	278.368	$C_{11}H_{22}N_2O_4S$	
12.	1H-Indene-1-hexadecyl-2,3-dihydro	20.970	0.157	342.6010	$C_{25}H_{42}$	
13.	Phytol	21.276	0.242	296.539	$C_{20}H_{40}O$	
14.	9,12-octadecadienoic acid	21.741	0.869	294.429	$C_{18}H_{30}O_3$	
15.	Octadecanoic acid	22.366	1.895	298.4608	$C_{18}H_{34}O_3$	
16.	Glycidyl palmitate	247.492	0.231	312.5	$C_{19}H_{36}O_3$	
17.	Octodecanamide	25.972	0.235	283.4925	$C_{18}H_{37}NO$	
18.	Diisooctyl phthalate	27.03	0.547	390.6	$C_{24}H_{38}O_4$	
19.	3-hydroxyl palmitate, TMS derivative	27.518	0.154	256.42	$C_{16}H_{32}O_2$	

CONCLUSION

The compounds identified in the *Piper betle* leaf extract most have medicinal properties and some have antibacterial activities. Mostly, betel leaf extract consists of fatty acid compounds and also contains phenols, terpenes, amide groups and esters. The extract contains both saturated and unsaturated fatty acid.

The GC-MS results revealed phentermine and hexadecanoic acid were found in higher concentrations. Phentermine showed the highest peak at a retention time of 9.346 minutes, whereas hexadecanoic acid recorded the highest percentage area of 26.665%. The water solvent extraction mostly extracts fatty acids from the *Piper betle* leaf extract than the other

compounds. Hence, it can be used for further processing like encapsulation.

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How to cite this article: P. Geetha, V. Sathiamoorthy and M. Balakrishnan (2022). Quantification of Bioactive Compounds in *Piper betle* Leaf Extract by Gas Chromatography Mass Spectrometry (GC-MS) Technique. *Biological Forum – An International Journal*, *14*(1): 1199-1203.