RESEARCH ARTICLE

Quantification of Bioactive Compounds in *Piper Betle* Leaf Extract by Gas Chromatography-Mass Spectrometry (GC-MS)

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ABSTRACT

Piper betleis a Piperaceae family scented perennial creeper. The leaves are high in phenol, a compound with anti-tumor, anti-mutagenic, and immunomodulatory activities. This study aims 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 the highest peak at a retention time of 9.346 minutes, followed by Hexadecanoic acid, Tetradecanoic acid, Eugenol, Dodecanoic acid. Hexadecanoic acid shows the highest area% about 26.665%, indicating the highest composition of hexadecanoic acid in the betel leaf extract. The result revealed that the compound in betel leaf extract possesses medicinal properties.

Keyword: *Piper betle leaves; Gas Chromatography-Mass Spectroscopy; Retention Time; Area*

INTRODUCTION

Betel leaves (*Piper betle*.L) belong 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 from 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). Eugenol and hydroxychaxicol are the major constituents present in betel leaves.

The essential oils are responsible for the flavor and aroma of betel leaves and contribute to distinctive flavour. The essential oil in Piper betle ranges from 0.15% to 2%, based on the location and type of cultivation (Sharma *et al*., 1983). The betel leaves contain Polysaccharides, Tannins, flavonoids, and phenols. The major constituent of betel leaves is phenols and terpenes which also contain compounds such as chavicol, allylprocatechol, chavibetol, phenyl alanine (Bajpai *et al.,* 2010). The fresh betel leaves, essential oil consists of 98.4% volatile compound whereas cure leaves essential oil is about 97.34%.

The leaves show antibacterial activity against microorganisms 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 phytochemicals 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 the area of herbal medicinal research, particularly for identifying and describing a different mixture of organic compounds found in extracted material (Gu *et al.,* 2004). Gas chromatography- mass spectroscopy is used to find the concentration of volatile compounds present in plant material (Islam *et al*., 2020). There are different solvents used for the 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 the gas chromatographic technique.

MATERIALS AND METHODS

Plant material

Freshkarpuravalli betel leaves variety were purchased from the farmer in Paramathivellur, 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 surface water removal, betel leaves are dried in a cabinet drier for 60 ^{o}C until the moisture is removed (Pin et al., 2014). The temperature of cabinet drier is maintained at 60^{o}C to reduce the loss of phytochemicals in betel leaves whereas the high temperature causes the loss of phytochemicals. The dried leaves are powdered to uniform size particles by using the blender.

Extraction

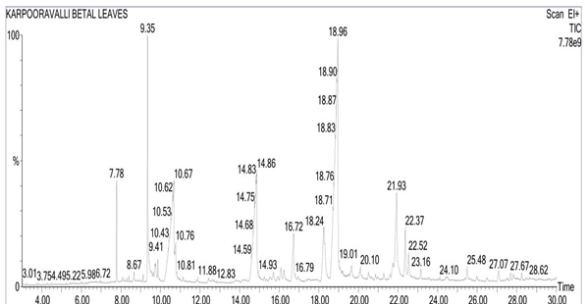
The water solvent extraction method is used to extract the bioactive compound from the betel leaves. Here water is used as the solvent to extract materials. The dried betel leaves powder was mixed with the water in the ratio of 1:30 (1g gram of dried betel leaves powder with 30 mL water) (Pin *et al*.,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

Gas Chromatography-Mass Spectroscopy (GC-MS)

Perkin Elmer Clarus SQ8C Gas chromatopgraphy-mass spectroscopy (Department of Agricultural Microbiology, 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 was injected about 1 microliter. Helium was used as the carrier gas with a flow rate of 1.0 mL min$^{-1.}$ The mass spectra were scanned from 0 – 480 m/z.

RESULTS AND DISCUSSION

The Gas Chromatography mass spectroscopy result for betel leaf extract is given in Figure 1, which shows the peaks obtained with respect to retention time. The peak indicates the concentration of the substance, whereas the retention time indicates the type of compound responsible for the peak. There are more than 120 compounds were identified, of which 19 compounds show a probability percentage above 30. Table 1 shows the compound identified, retention time, area%, molecular formula, molecular mass, and molecular structure. The result from the GC-MS shows 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-myristynoylpantethenine, 3-hydroxyl palmitate and octodecamide belong to the amine group. Eugenol was the phenolic compound and phytol belongs to terpene group. The leaf extract also consists of ester group (Diisooctylphththalate) and lignin (4 – allyl-1,2-diacetoxybenzene).

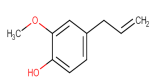
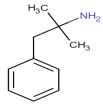
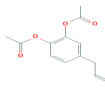
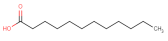
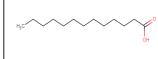
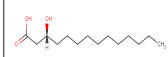
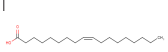
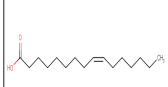


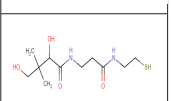
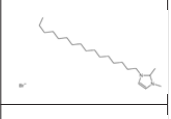
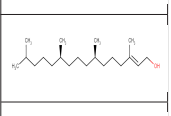
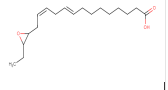
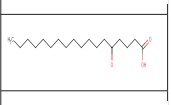
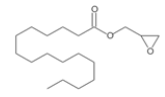
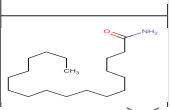
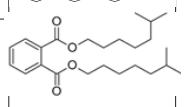
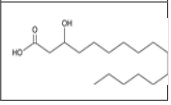
At a retention time of 9.346 minutes, the component phentermine produced the highest peak, indicating that betel leaf extract has a significant concentration of phentermine. Obesity patients are prescribed phentermine to help them lose weight (Ryder *et al*., 2016). The compound n-hexadecanoic acid had 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 is used as an antimicrobial agent and has a reported area of 13.842 percent. In humans, it lowers the risk of heart disease and cancer (Niknamain 2016). 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). Eugenol is used to help with dental problems(Sarrami *et al*., 2002).

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 minutes& 0.248%, 15.918 minutes& 0.192 % and 15.553 minutes& 0.299% respectively. The antimicrobial compound tridecanoic acid has antibacterial and antifungal properties against pathogenic microorganisms (Chowdhury *et al.,* 2021Tetradecanoic acid is also known as mystric acid. As a cardiac imaging agent, pentadecanoic acid is employed (Antarm *et al.*, 1986). 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). Diabetic risk is reduced by palmitoleic acid (Mozaffarin *et al*., 2013). Phytol is a diterpene alcohol with 20 carbons which has a retention time of 21.276 minutes and an area of 0.242%. The phytol has antinociceptive and antioxidant properties(Santos *et al.*, 2013).

Octadecanoic acid belongs to the amide of steric acid which is used as a metabolite. The octadecanoic acid was reported at a retention time of 25.972 minutes and an area of about 0.235%. Glycidyl palmitate is an ester reported at retention time and area of about 24.492 minutes and 0.231%. Glycidyl palmitate is also konoen as lysophosphatide acid. The retention time and area for octadecanoic acid were 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).

Table 1. Compound in betel leaf extract Retention time, Area, Molecular mass, Molecular formula and Molecular structure.

Sl.No	Compounds	Retention time (mins)	Area%	Molecular mass	Molecular formula	Molecular structure
1.	Eugenol	7.780	1.764	164.2011	C10H1202	
2.	Phentermine	9.346	5.602	149.2328	C10H15N	
3.	4-Allyl-1,2- diacetoxybenzene	9.746	0.739	234.25	C13H1404	
4.	Dodecanoic acid	10.676	13.842	200.3178	C12H2402	
5.	Tridecanoic acid	12.427	0.329	214.3443	C13H2602	
6.	Tetradecanoic acid	13.763	0.248	244.3703	C14H2803	
7.	Oleic acid	15.553	0.299	282.4614	C18H3402	
8.	Pentadecanoic acid	15.918	0.192	242.3975	C15H3002	
9.	Palmitoleic acid	18.254	5.127	54.4082	C16H3002	

10.	n-Hexadecanoic acid	18.960	26.665	256.4241	C16H32O2	
11.	2-Myristynoyl pantetheine	19.480	0.141	278.368	C11H22N2O4S	
12.	1H-Indene-1-hexade-cyl-2,3-dihydro	20.970	0.157	342.6010	C25H42	
13.	Phytol	21.276	0.242	296.539	C20H40O	
14.	9,12-octadecadienoic acid	21.741	0.869	294.429	C18H30O3	
15.	Octadecanoic acid	22.366	1.895	298.4608	C18H34O3	
16.	Glycidyl palmitate	247.492	0.231	312.5	C19H36O3	
17.	Octodecanamide	25.972	0.235	283.4925	C18H37NO	
18.	Diisooctyl phthalate	27.03	0.547	390.6	C24H38O4	
19.	3-hydroxyl palmitate, TMS derivative	27.518	0.154	256.42	C16H32O2	

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 acids. The GC-MS results revealed phentermine and hexadecanoic acid were found in higher concentrations. These compounds are used in the weight reduction of obese patients and have anti-inflammatory properties. Phentermine shows 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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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

The manuscript submitted was entirely original work not used others work.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the

MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail sathiav0055@gmail.com

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