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GC-MS profiling of methanolic extract of *Piper betle* (Karpoori Variety) leaf

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Abstract
Piper betle is an important species of the *Piperaceae* family with glossy heart shaped leaves. The leaves act as magnificent reservoirs of bioactive compounds. To detect the bioactive compounds in the *P. betle* (Karpoori variety) leaves, a study was conducted by GC-MS analysis. The preliminary quantitative phytochemical screening revealed the total quantity of alkaloids, flavonoids and phenol compounds in *P. betle* extract. The GC-MS analysis detected thirty two phytocompounds in methanolic extracts of *P. betle* (Karpoori variety) leaves. The present study forms the basis for biological characterization and phyto pharmaceutical significance of the identified compounds. The presence of various bioactive compounds justifies the usage of *P. betle* leaf as an herbal choice for treating various diseases in animals.

Keywords: *P. betle*, Karpoori variety, quantitative phytochemical analysis, GC-MS

Introduction
Piper betle commonly known as the betle vine is an important plant in Southeast Asia (Kumar *et al*., 2010) [8]. The leaves of *P. betle* have long been used in the Indian local system of medicine and it's generally found in hot and moist climatic condition. There are many varieties of *P. betle* based on the color, size, taste and aroma. In India, approximately 40 varieties are found and three varieties of *P. betle* leaves *viz*., Sirugamani, Karpoori and Vellaikodi are accessible mostly in Tamil Nadu (Bhattacharya *et al*., 2007) [2]. They are majorly cultivated during November to December and January to February seasons. The *P. betle* herb contains a wide variety of biologically active compounds whose concentration depends upon the variety of the herb, season and climate (Chauhan *et al*., 2016) [5]. Hence the identification of active constituents of locally available variety of *P. betle* herb will be useful to signify their use in the herbal medicine. Since Gas Chromatography and Mass Spectrometry (GC-MS) analysis could able to detect and quantify the compounds of herbs, the present study was conducted in locally available, Karpoori variety of *P. betle* leaves by GC-MS analysis.

Materials and methods
P. betle herb was collected from Paramathi Velur of Namakkal District, South India and authenticated by the Botanical Survey of India (No. BSI/SRI/5/23/2017/Tech/1921) Coimbatore, Tamil Nadu.

Preparation of the leaf extract
Freshly collected leaves of *Piper betle* were shade dried and the size reduced to powder with the use of mechanical grinder. 10 grams of the pulverized material were soaked in 100 mL of methanol and kept on a rotary shaker for 24 hrs. The extract was then filtered through Whatman No. 1 filter paper and the process was repeated till the extraction of all soluble compounds. The extract was concentrated in a rotary evaporator under reduced pressure. The dried material was collected and stored in refrigerator for further experimental procedures.

Quantitative estimation of Phytochemicals
The estimation of total alkaloids, total phenols and total flavonoids in *P. betle* (Karpoori variety) was carried out to find out the quantity of individual phytoconstituents present in the herb.

Estimation of Total Alkaloids
The total alkaloids content was measured as per the method of Harborne (1973) [7]. Briefly, 40 mL of 10% acetic acid in ethanol was added to 1 g of powdered sample of *Piper betle* allowed to stand for 4 hours.

The filtrate was then concentrated on water bath to one fourth of its original volume. Concentrated ammonium hydroxide was added drop wise to the extracts until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with diluted ammonium hydroxide and then filtered. The residue was dried and weighed to find out the total alkaloids content.

Estimation of Total Phenol

The total phenolic content was determined by the Folin-Ciocalteu assay described by Singleton *et al.* (1999) [11]. Fifty microliter of alcoholic extract of *Piper betle* was mixed separately with 250 µl of 10% Folin-Ciocalteu solution followed by addition of 750 µl of 7.5% (w/v) sodium carbonate and then the solution was incubated at room temperature for 2 hours in the dark. The absorbance was measured at 750 nm using a double beam UV-Visible spectrophotometer. A calibration curve was obtained using gallic acid as standard for the concentration ranging from 25 to 250 µg/ml as standard. The total phenolic content was expressed in terms of the milligrams of the gallic acid equivalent per gram of the dry mass (mg GAE/g).

Estimation of Total Flavonoid

The total flavonoid content in the extracts of *Piper betle* was estimated by the aluminium chloride colorimetric method (Chang *et al.,* 2002) [4]. Briefly, 0.25 ml extract of *P. betle* herb (10mg/ml) was separately mixed with 0.75 ml of ethanol, 0.05 ml of the 10% aluminium chloride, 0.05 ml of the 1M potassium acetate and 1.4 ml of the distilled water. The reaction mixture was incubated for 30 minutes at room temperature. The absorbance of the mixture was measured at 415 nm using double beam UV-Visible spectrophotometer. A calibration curve was obtained using rutin as standard with concentration ranging from 10 to 160 µg/ml. The total flavonoid content was expressed in terms of the milligram of rutin equivalent per gram of the dry mass (mg RU/gm).

Identification of bioactive components by Gas Chromatography – Mass Spectrometry (GC-MS) analysis

Gas Chromatography - Mass Spectrometry (GC-MS) was used in the present study to identify the bioactive components present in the alcoholic extracts of *P. betle* (Karpoori variety). The analysis was performed using GC-MS 5975 C Agilent System and Gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with elite – 1 fused silica capillary column.

For GC - MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51 ml/min and an injection volume of 1 ml was employed (Split Ratio: 10). The Injector and Ion source temperature were 240ºC and 200ºC, respectively. The oven temperature was programmed from 70ºC (isothermal for 2 min.), with an increase of 300ºC for 10 minutes. Mass spectra were taken at 70eV; a scan interval of 5 minutes with scan range of 40 – 1000 m/z. Total GC running time was 30 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Turbo Mass software was adopted to handle mass spectra and chromatograms (Adams, 2007) [1]. Interpretation of GC - MS results was carried out using Dr. Duke's phytochemical and ethno botanical database which is having more patterns on phytocomponents. The spectrum of the unknown components of the *P. betle* herb was compared with the spectrum of the known components stored in National Institute of Standards and Technology (NIST) library and identified.

Results and discussion

The quantitative analysis of *P. betle* showed the total alkaloid content of 62.00 ± 0.57 mg/g, total phenol content of 130.00 ± 1.15 mg of GAE (Gallic acid equivalent)/g and total flavonoid content of 7.68 ± 0.36 mg of RU (Rutin)/g of extract). The results of GC-MS analysis of *P. betle* extract is presented in figure 1 and table 1. Thirty two phytocompounds were identified in *P. betle by* GC-MS analysis of herbal extract. The phytocompounds present in the *P. betle* were proven for various biological activities as detailed in table 1.

Table 1: Components detected in GC-MS analysis of *P. betle* extract with their activities.

S. No	Name of the Component	Retention Time Value (Minutes)	Area %	Chemical group & Activity
1	a. 3 allyl 6 methoxy phenol (Chavibetol) b. Eugenol c. Eugenol	10.497	2.70	a. Phenol Antioxidant, Antimicrobial, Antimutagenic, Antiseptic and Antiviral (Rathee *et al*., 2006 and Tripathi *et al*., 2011) [10, 14] b. Oil compound Anti-oxidant, Antimicrobial, Analgesic, Antiviral, Anticancer & Antidepressant (Lee *et al*., 2007) [9]
2	a. Benzoic acid, 3, 5 dimethyl. b. 4, allyl-1, 2-diacetoxy benzene c. 4 carbamoyl -2-5 dimethyl pyridine	12.322	70.97	a. Acid Antimicrobial and Preservative* b. Phenol Cancer chemopreventive, Antimicrobial and Xanthine oxidase enzyme inhibitor (Tanaka *et al*., 1997) [12] c. Amide - No report on biological activity
3	a. Benzene propanoic acid, 2-Octyl ester b. 4 Chromanol c. Benzoic acid, 2, 5 dimethyl	12.578	6.31	a. Acid - No report on biological activity b. Alcohol – Antioxidant (Foo *et al.*, 2015) [6] c. Benzoic acid compound - Antimicrobial and Preservative (Foo *et al.*, 2015) [6]
4	a. 9 octadecyne b. 6-octen-1-ol,3,7-dimethyl-propanaote c. Bicyclo (3.1.1) heptane, 2, 6, 6-trimethyl	16.151	0.93	a. Fatty acid - No report on biological activity. b. Fattyacid - No report on biological activity. c. Hydrocarbon - No report on biological activity.
5	a. Phytol b. Phytol c. Phytol	18.821	2.18	A & b & c – Diterpene Antioxidant, Anticancer, Anti-Inflammatory, Anti-microbial, Antinociceptive and Diuretic (Camila *et al*., 2013) [3]
6	a. Linoleic acid ethyl ester b. (R)-(-)-14 methyl-8-hexa decyn-1-ol c. 9, 12 – Octadeca dienoic acid	19.261	0.57	a. Linoleic acid ethyl ester Antihistaminic, Antiarthritic, Hepatoprotective and Hypocholesterolemic* b. Alcohol - No report on biological activity. c. Fatty acids Anti-inflammatory, Hypo cholesterolemic, Cancer preventive and Hepato protective*

7	a. 9,17 octa decadienal b. 2-methyl-Z, Z-3, 13-octadecadienol c. 9, 12-Octadecadienoic acid (z, z)	19.314	0.795	a. Aldehyde – Antimicrobial*. b. Alcohol- Anticancer* c. Fatty acid Anti-inflammatory, Hypo cholesterolemic, Cancer preventive and Hepato protective*
8	a. Phytol, acetate b. Cyclopropane octanal, 2-octyl-cis-13-octadecanoic acid c.Z-B-Methyl-9-tetradecenoic acid.	19.725	1.18	a. Diterpene Antioxidant, Anticancer, Anti-Inflammatory, Anti- microbial, Antinociceptive and Diuretic (Camila *et al*., 2013) [3] b. Ester No report on biological activity c. Acid No report on biological Activity
9	a. Cyclopropane octanal a. b.2-octyl-cis-13-octadecenoic acid b. Z-B-Methyl-9-tetradecenoic acid	20.444	0.60	a. Ester - No report on activity b. Acid Anti-inflammatory * c. Acid No report on activity
10	a. Cyclodo decyne a. b.2-methyl-Z, Z-3, 13-octadecadienol b. 1, 2-15, 16-Diepoxy hexadecane	21.907	3.62	a. Fatty acid No report on biological activity b. Alcohol - Anticancer * c. Epoxide Anti-inflammatory and antitumor *
11	a. Vitamin E, b. Gamma tocopherols c. O-methyl dl alpha tocopherol	26.557	1.17	a & b & c. Vitamin E Antioxidant and Hepato protective (Traber and Alkinson, 2007) [13]
12	a. Silicic acid, diethyl bis(trimethylsilyl) ester b. Methyl-2- Phenyllindolizine c. Methyltris (Trimethyl siloxy) silane	27.359	1.71	a. Ester No report on activity b. Indolizine No report on activity c. Trisiloxane No report on activity
13	a. Methyltris silane b. N-methyl-1-adamantine acatamide	27.573	1.88	a. Trisiloxane No report on activity b. Amide No report on activity
14	a. Gamma sitosterol b &c. Beta sitosterol	28.126	6.20	a & b & c - Steroid. Antimicrobial, Anti-inflammatory, Anticancer, Diuretic and Hepato protective *

* - Dr. Duke's phytochemical and ethno botanical database

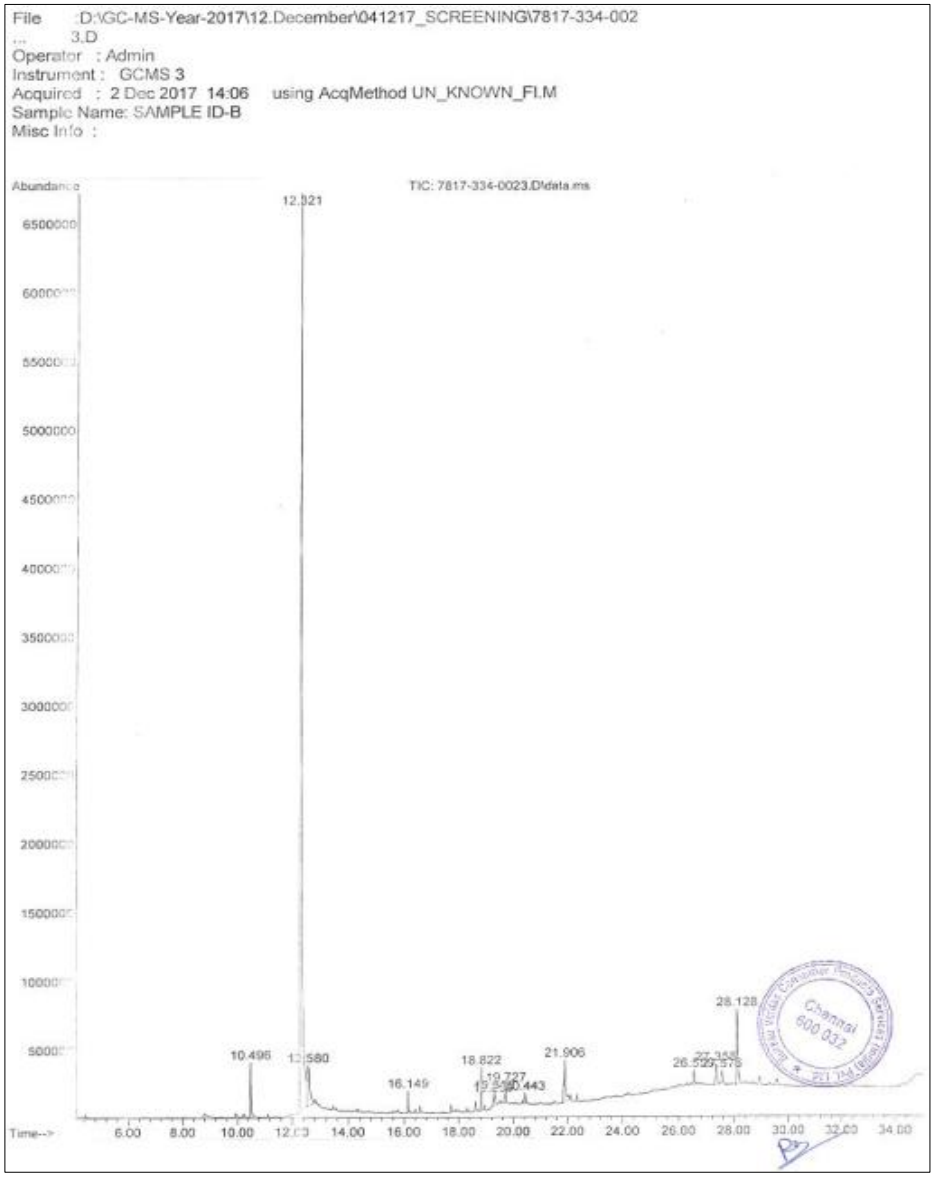

Fig 1: GC-MS chromatogram of methanolic extract of *P. betle* (Karpoori variety)

The results revealed that the methanolic leaf extract of *P. betle* (Karpoori variety) has number of bioactive phytoconstituents, which are responsible for numerous therapeutic activities. The compounds identified by GC-MS are medicinally valuable and possess wide variety of pharmacological applications. Further testing of individual phytoconstituents by *in vitro* and *in vivo* experiments will validate the biological activities of *P. betle* herb.

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