

PHYTOCHEMICAL ANALYSIS OF SOME SELECTED VARIETIES OF PIPER BETLE L.

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ABSTRACT

Objective: To Analyse the major phytochemical components in selected varieties of *P. betle* leaves using GC-MS.

Methods: *P. betle* leaves were shade dried and pulverized to powder in a mechanical grinder. The powder was successively extracted with ethanol (40-60 °C). The extracts were concentrated under reduced pressure in a rotary evaporator. The ethanolic extracts of the plant leaves were used for GC-MS analysis.

Results: In the present study ten chemicals were identified and compared from the *P. betle* varieties, the major component being Eugenol, which is responsible for the flavour and aroma of the leaves.

Conclusion: Variety Karpoori possesses the highest content of Eugenol and could be used as a promising variety in the pharmaceutical industry. The components of essential oil can also serve as a determinant to distinguish different varieties of betle vine cultivars.

Keywords: GC-MS, Eugenol, Hybrid variety 1, Hybrid variety 2, Jaipur Bangla, Karpoori, Local variety, Sirugamani

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INTRODUCTION

P. betle is a tropical shade-loving perennial evergreen vine, with glossy heart-shaped leaves and white catkin. It may climb as high as 10-15ft. Sometimes the plant can also grow as a ground cover and it has a growth pattern similar to that of the pepper plant. Leaves are simple, alternate, ovate, cordate, acuminate or acute, entire and bright green. Flowers are dioecious. This plant has male spikes which are dense and cylindrical while female spikes are pendulous. Perianth 0. Stamens 2-4; filaments short; anthers 2 celled, the cells distinct. Ovary 1 celled; ovule solitary, style short or 0, stigma 5. Fruiting spikes up to 5 inches thick, male spikes 3-6 inches long very narrow. Roots arise from each node which aid in fixing the plant to the host tree [1, 2].

Phyto-constituents

The leaf contains Water (85-90%), Proteins (3-3.5%), Carbohydrates (0.5-6.1%), Minerals (2.3-3.3%), Fat (0.4-1%), Fibre (2.3%), Essential oil (0.08-0.2%), Tannin (0.1-1.3%), Alkaloid (arakene). It also contains different Vitamins like Vitamin-C (0.005-0.01%), Nicotinic acid (0.63-0.89 mg/100 gms), Vitamin-A (1.9-2.9 mg/100 gms), Thiamine (10-70µg/100 gms), Riboflavin (1.9-30µg/100 gms). It also contains minerals such as Calcium (0.2-0.5%), Iron (0.005-0.007), Iodine (3.4µg/100 gms), Phosphorus (0.05-0.6%), Potassium (1.1-4.6%). Leaves contain bitter compounds that constitute about 0.7-2.6%. The specific strong pungent aromatic flavour in leaves is due to phenol and terpene like bodies [3]. The total phenol content varies in the gender. The male plant contains three-fold higher total phenols content and two-fold higher thiocyanate content as compared to a female plant. The quality of the leaf depends upon the phenolic content, i.e., more the phenolic content better is the leaf quality [4]. Recently many research works show that *P. betle* leaves contain starch, diastases, sugars and an essential oil composing of safrole, allyl pyrocatechol monoacetate, eugenol, terpinen-4-ol and eugenyl acetate, as the major components [5]. Phytochemical investigation on leaves revealed the presence of Alkaloids, Carbohydrate, Amino acids, Tannins and Steroidal components [6]. The middle part of the main vein contains the largest quantity of Tannin. The terpenoids include 1, 8-cineole, cadinene, camphene, caryophyllene, limonene, pinene,

chavicol, allyl pyrocatechol, carvacrol, safrole, eugenol and chavibetol. Eugenol was identified as the antifungal principle in the oil. The fresh new leaves contain much more amount of essential oil diastase enzyme and sugar when compared to old leaves. Chavicol is four times potent as antiseptic agent as compare to carbolic acid [7].

In different countries, the leaves are folded in different ways and generally, some calcium hydroxide is smeared inside. Slices of the dry *Areca nut* are on the upper left of the leaf and slices of the tender *Areca nut* on the upper right. The fold on the lower right contains tobacco—a relatively recent introduction. There is archaeological evidence that *P. betle* leaves have been chewed along with the *areca nut* since very ancient times. In most countries, the mixture of both has a ceremonial and highly symbolical value [8]. *P. betle* leaves are chewed together in the wrapped package along with the *Areca nut* and mineral slaked lime. Catechu called “Kattha” in Hindi and other flavouring substances and spices are also added. The lime acts to keep the active ingredient in its freebase or alkaline form, thus enabling it to enter the bloodstream via sublingual absorption. The *Areca nut* contains the alkaloid arecoline, which promotes salivation (the saliva is stained red), and is itself a stimulant. This combination, known as a *P. betle* quid has been used for over thousand years [9].

Properties of the ingredients and their traditional uses

P. betle leaf

P. betle leaves have a strong pungent aromatic flavour and is widely used as masticators. The presence of a fairly large quantity of diastase in the *P. betle* leaves is likely to play an important part in starch digestion. Large quantities of saliva produced by chewing *P. betle* leaf act as digestive and probably the presence of diastase enhance this activity. The leaves contain a good amount of B vitamins (particularly nicotinic acid) ascorbic acid and carotene.

P. betle leaf consists of more juice which cures pharyngitis, abdominal pain and abdominal distension. Ordinary *P. betle* leaf cures urticaria and the effects due to the derangement in the equilibrium of the three senses of humour namely Vatha, Pitha and Kabha. It gives a pleasant odour in the mouth. *P. betle* leaves possess an anti-oxidant action. The anti-oxidant effect is due to the presence of phenols particularly hydroxychavicol (4-allyl pyrocatechol). The

leaf produces an aromatic volatile oil containing a phenol called chavicol which has powerful antiseptic properties. The essential oil present gives rise to a sensation of warmth and well-being in the mouth and stomach. It is also known to produce a primary stimulation of the central nervous system, followed by a kind of inebriety in large doses. *P. betle* leaf is believed as a common household remedy for various ailments.

- A paste of *P. betle* leaves mixed with salt and hot water can be administered for filariasis.
- For treating obesity one *P. betle* leaf mixed with *Piper nigrum* is prescribed for two months.
- Juice with honey or a liquid extract is useful in coughs, dyspnoea, deranged phlegm and indigestion, common in children.
- The application of leaves smeared with oil is said to promote secretion of milk when applied on the breasts of lactating women.
- A local application is recommended for inflammatory swellings such as orchitis, arthritis and mastitis.
- In pulmonary affections of childhood and old age, leaves are soaked in mustard oil and warmed and applied to the chest in order to relieve a cough and dyspnoea.

Chewing *P. betle* leaf with *Areca nut* which has pungent and astringent taste removes derangement of humors in the body. Germs will die; it removes thirst and phlegm, prevents several diseases, reduces hunger, endears women; improves taste and cleans the abdomen. They sweeten the breath, improve voice, and remove fetor from the mouth. Also, they increase salivary secretion. *P. betle* chewing is considered as a good and cheap source of dietary calcium. The calcium ingested is reported to be well absorbed by the system [10].

MATERIALS AND METHODS

Source of plant material

The present investigation was carried out in six different varieties of *P. betle* viz., Hybrid 1 (HY1), Hybrid 2 (HY2), Jaipur Bangla (JB), Karpoori (KAR), Local variety (LV) and Sirugamani (SG). Plant cuttings were collected from Sugarcane Research station, Sirugamani, Tamil Nadu.

GC MS analysis

P. betle leaves were shade dried and pulverized to powder in a mechanical grinder. The powder was successively extracted with

ethanol (40-60 °C). The extracts were concentrated under reduced pressure in a rotary evaporator. The ethanolic extracts of the plant leaves were used for GC-MS analysis.

The GC-MS analysis was carried out on a Thermo GC-TRACE ultra ver: 5.0, Thermo MSDSQ II. This instrument was employed with following conditions: Column ZB 5-MS Capillary Standard non-polar Column (30 x0.25 mm), helium was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 1.0 ml was employed in an injector with a temperature of 260 °C. The oven temperature was programmed from 70 °C raised gradually to 260 °C at the rate of 6 °C/min [11].

RESULTS AND DISCUSSION

Gas chromatography-mass spectroscopy analysis (GC-MS)

Gas chromatography Mass Spectroscopy, a hyphenated system which is very a compatible technique and the most commonly used technique for the identification and quantification of secondary compounds. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [12].

GC-MS analysis was carried out in the powdered leaf of *P. betle* varieties viz., HY1, HY2, JB, KP, LV and SG and the results are given in the table 1 and Figs (1 – 12). In general *P. betle* leaf contains the phytochemicals viz., Hydroxychavicol, Eugenol, Safrole, Caryophyllene, Caryophyllene oxide, Silicone oil, Campesterol, Stigmasterol, Vitamin E and Sitosterol [13]. HY 1 variety contains Hydroxychavicol, Eugenol, Safrole, Caryophyllene, Caryophyllene oxide, Silicone oil, Campesterol and Stigmasterol. Campesterol and Stigmasterol were absent in HY2. JB contains all the major compounds. Safrole, Silicone oil and Vitamin E were absent in Karpoori and Local Variety. Hydroxychavicol, Vitamin E and Sitosterol were absent in SG. Eugenol content is more in all the varieties and was highest in Karpoori (53%). Hydroxychavicol was highest in LV and absent in SG.

The leaves of *P. betle* possess a strong spicy aromatic flavour and widely consumed as a mouth freshener [14]. The leaves are credited with wound healing, digestive and pancreatic lipase-stimulant activities in traditional medicines. The most important factor determining the aromatic value of the leaf is the amount and particularly the nature of essential oil present. *P. betle* leaves from different regions vary in aroma and taste. The oil content of different Indian types also varies. Table 4 gives the profile of important chemical constituents.

Table 1: Phytochemicals present *Piper betle* varieties analysed by GC-MS P

S. No.	Compounds	Molecular formula	Molecular weight	Area %					
				HY1	HY2	JB	KP	LV	SG
1	Hydroxychavicol	C9H10O2	150	15.45	1.51	25.63	1.63	50.55	-
2	Eugenol	C10H12O2	164	15.36	17.01	29.37	35.04	6.95	21.24
3	Safrole	C10H10O2	162	0.91	1.22	0.79	-	1.52	1.22
4	Caryophyllene	C15H24	204	1.10	3.14	1.05	1.89	0.29	1.56
5	Caryophyllene oxide	C15H24O	220	0.42	0.51	0.31	0.52	0.19	0.27
6	Silicon oil	-	0	0.21	13.44	0.27	-	-	0.81
7	Campesterol	C28H48O	400	0.30	-	0.31	0.71	0.63	1.80
8	Stigmasterol	C29H48O	412	0.42	-	0.56	0.93	0.67	2.66
9	Vitamin E	C29H50O2	430	-	2.44	0.75	-	-	-
10	Sitosterol	C29H50O	414	-	0.84	0.33	0.25	0.26	-

In the present study, ten chemicals were identified and compared from the *P. betle* varieties, the major component being Eugenol, which is responsible for the flavour and aroma of the leaves [15-17]. Among the selected varieties Karpoori possesses the highest content of Eugenol and could be used as a promising variety and form a valuable source of eugenol, which is widely used in pharmaceutical industry. Eugenol is a Phenolic compound and its content also varies with gender. [18] Pradhan *et al.* (2013) reported that male plants contain three-fold higher content of this phenolic compound as compared to the female. In the present study also higher Eugenol content was present in HY2 (female) compared to HY1 (male). The components of essential oil can also

serve as a determinant to distinguish the gender and also different varieties of *P. betle* vine [19].

Chandra vikash *et al.* (2012) [13] reported the main constituents as Hydroxychavicol (HC)/Hydroxychavicol acetate (HCA), Allylpyrocatechol (APC), Chavibetol (CHV) and Piperbetol. Other constituents are arecoline, carvacrol, caryophyllene, piperitol, piperbetol, eugenol, isoeugenol, allylpyrocatechol, chavicol, safrole, anethole, chavibetol, cadinene-hydroxychavicol, β -sitosterol, β -sitosteryl palmitate, dotriacontanoic acid, tritriacontane, stearic acid, cepharadione, piperine, piperlonguminine, chavibetol acetate, allylpyrocatechol monoacetate, allyldiacetoxy benzene, benzene,

estragole, methyl eugenol and hydroxycatechol, methylpiperbetol, piperol A, piperol B, cavacrol, eugenol acetate and allyl pyrocatechol diacetate. Leaves posses pharmacological properties like antibiotic,

antiulcer and platelets aggregation antifertility, cardiotoxic, antitumor, antimutagenic, respiratory depressant anthelminthetics which may be attributed to the presence of these chemicals.

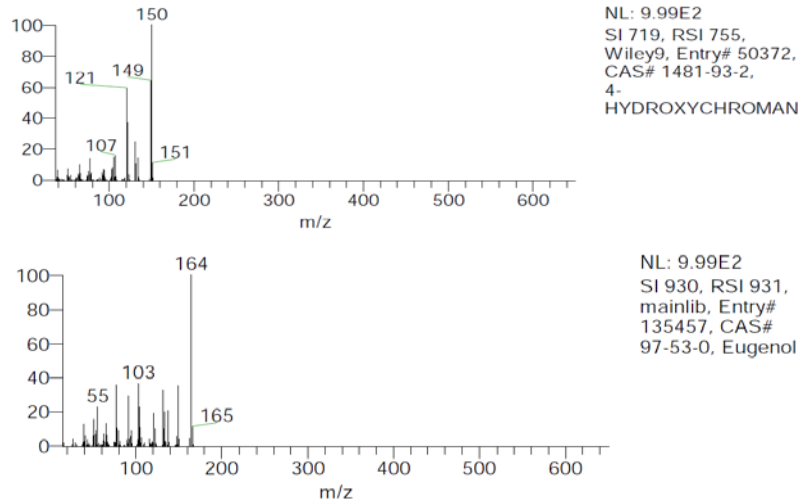


Fig. 1-2: GC-MS chromatogram profile of hybrid 1 (HY1)

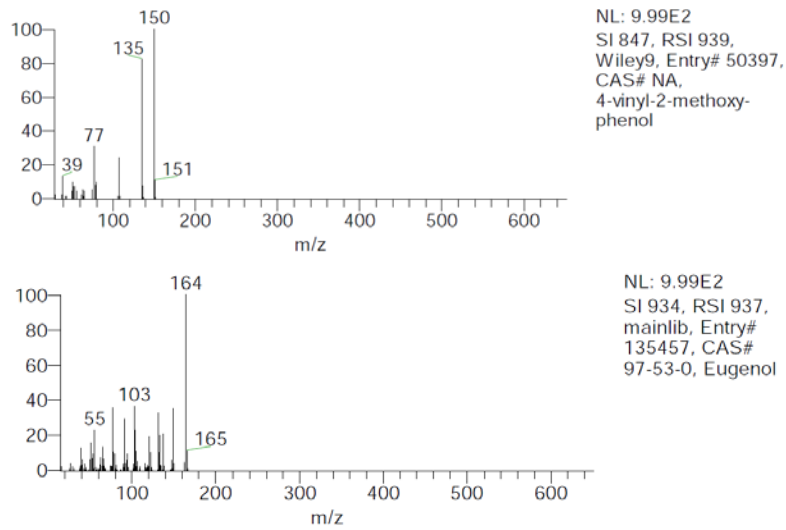


Fig. 3-4: GC-MS chromatogram profile of hybrid 2 (HY2)

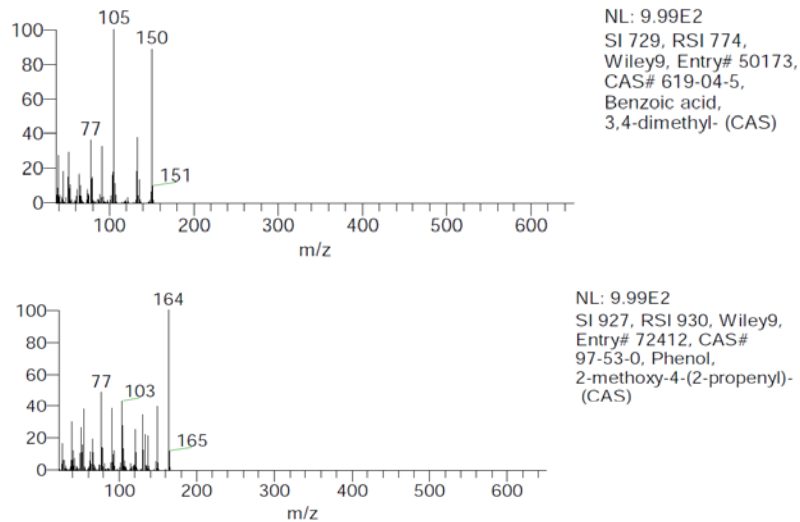


Fig. 5-6: GC-MS chromatogram profile of Jaipur Bangla (JB)

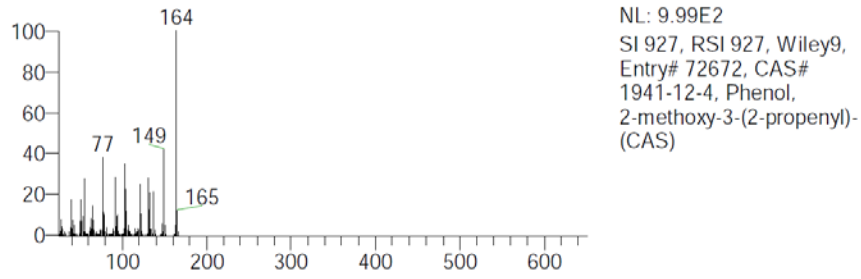
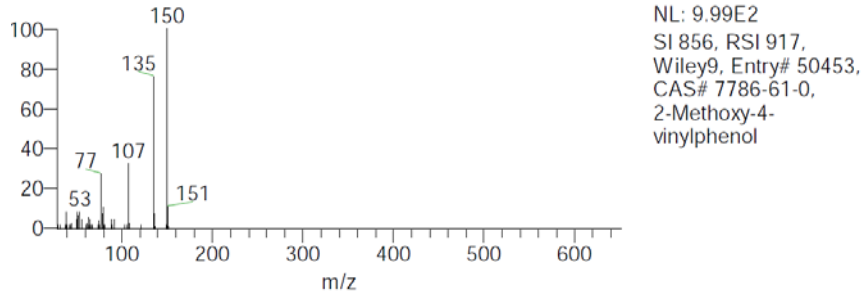


Fig. 7-8: GC-MS chromatogram profile of karpoori (KP)

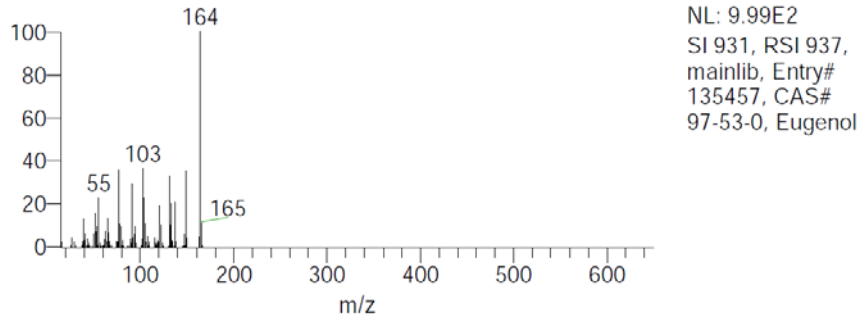
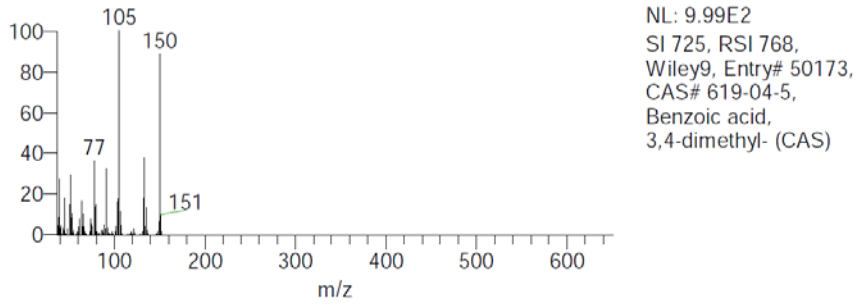
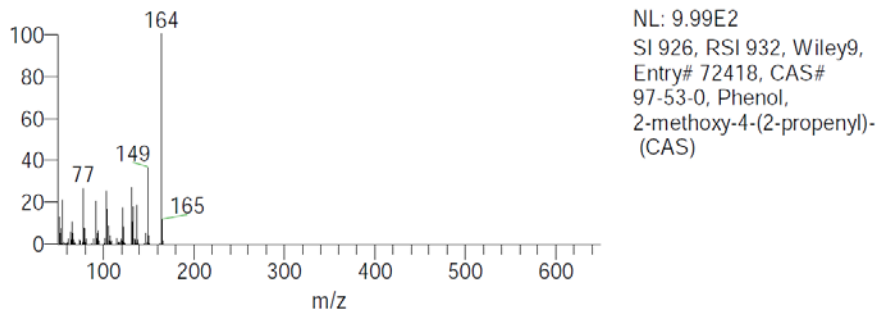


Fig. 9-10: GC-MS chromatogram profile of local variety (LV) 9



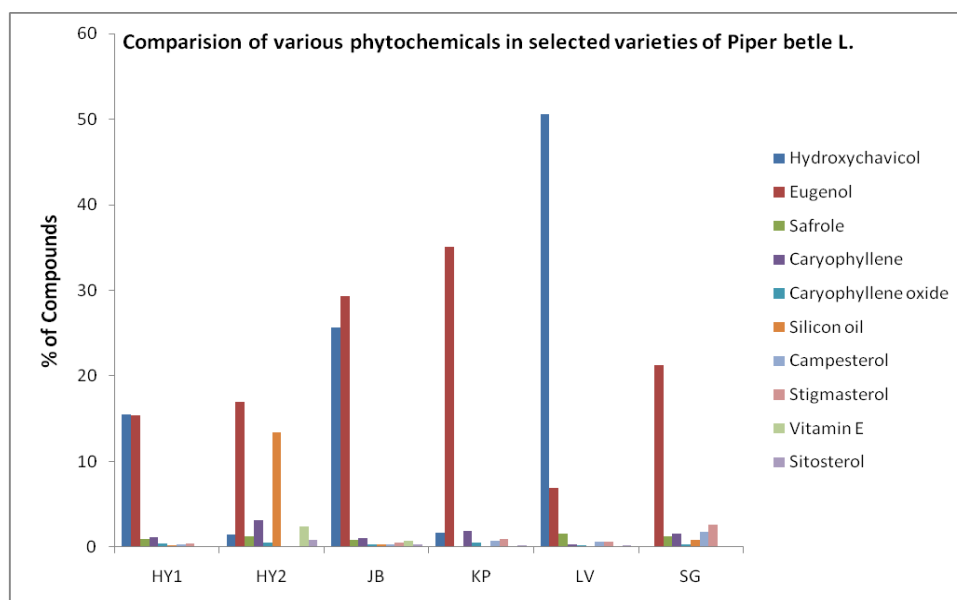


Fig. 11-12: GC-MS Chromatogram profile of sirugamani (SG)

CONCLUSION

In the present study, ten major chemicals were identified and compared. Among the chemical compounds, the major component is Eugenol, which is responsible for flavour and aroma of the leaves. Variety Karpoori possesses the highest content of Eugenol and could be used as a promising variety in the pharmaceutical industry. The components of essential oil can also serve as a determinant to distinguish different varieties of betle vine cultivars.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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