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Full Length Research Paper

Gas chromatography mass spectrometry (GC-MS) analysis of the hexane and benzene extracts of the *Piper betle* (leaf stalk) (Family: Piperaceae) from India

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The oily fraction of the leaf stalk of *Piper betle* of Indian origin, led to the identification of number of esters compounds. The hexane eluates of n-hexane extract of *P. betle* yielded a waxy fraction which was rechromatographed on silica gel column. Its hexane and 25% benzene eluates yielded waxy liquids, which were small in amount and was not separated by column chromatography. Hence, they were separated by gas chromatography mass spectrometry (GC-MS) analysis which revealed the presence of 19 compounds. The compounds were identified by comparing their retention time and covate indexes with that of literature and by interpretation of mass spectra. Many of them are used in industry for various applications like perfumes, flavors, deodorants, antiseptic and pharmaceuticals.

Key words: *Piper betle* (leaf stalk), piperaceae, esters, gas chromatography mass spectrometry.

INTRODUCTION

Piper betle Linn. (local name 'Pan') Piperaceae, a dioecious, perennial creeper, climbing by many short adventitious rootless, widely cultivated in hotter and damper parts of the country is widespread in damp forests and is cultivated in India and other countries in South-East Asia, such as Vietnam and China, and also in Central and South America and Africa. A concoction of indigenous Indian drugs containing *P. betel* dry extract was found to be an effective long-lasting oral contraceptive (Adams, 2004). The flowers of this area used as ingredient for the chewing food known as betel quid in South-East Asia (Ali and Mehta, 1970). Mouth washes and tablets containing pulverized betel nut were used for the treatment of dental and periodontal diseases (Bank, 1982). Betel leaves were reported to have high antioxidant effects (Bhattacharya et al., 2005; Chang et al., 1983) antidiabetic (Das, 1976) Radio protective (Dubey et al., 1987). Antibacterial Effect (Hwang et al., 1992), Pro-apoptotic effect (Massada, 1976).The leaves possess antibacterial properties and are beneficial in the treatment of purulent parodontosis in the form of a collutory made of the juice or extract. A poultice of the leaves and a wash with the decoction are used in treating wounds, burns, impetigo, furunculosis, eczema and lymphangitis. The leaves if topically applied to the chest cure cough and asthma and if applied to the breast arrest lactation. Friction of the spinal column with the leaves is recommended for treating colds. The roots (8 to 12 g) are used in treating rheumatism (Misra et al., 2009). The essential oil of *P. betel* showed hypertensive, cardiac and respiratory depressant effects (Nalina and Rahim, 2007). Eugenol was identified as antifungal principle in the oil (Nguen Van Dan, 1990).

The betel leaves, in vernacular pan, are used as leaf morsel by people in the Indian subcontinent. It is believed that more than 100 types are cultivated on a commercial scale in India. On the basis of flavor characteristics of the essential oil the existing types are grouped into five distinguishable cultivars, viz. 'Bangla', 'Desawari', Meetha' and 'Sanchi'. The distinction in flavor is marked by the varying concentration of mono- and sesquiterpenoids (Paresh and Normen, 1998). Till now no work done by any scientists that's why we have chosen for the study. In this paper, we report the compounds isolated from hexane and benzene fraction by gas chromatography mass spectrometry (GC-MS) and

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Table 1. Esters present in hexane ext. of *P. betel* (leaf stalk) from India.

Compounds		Retention time (min.)	Area%	Molecular mass	Molecular formula	Applications
Fraction 1 (Hexane)						
1	Methyl undecanote	5.6	6.10	200	$C_{12}H_{24}O_2$	Flavor and fragrance agents
2	Dodecanoic acid, methyl ester (methyl laurate)	6.73	9.23	214	$C_{13}H_{26}O_2$	In making soaps, insecticides,
3	Dodecanic acid, ethyl ester	7.52	11.62	228	$C_{14}H_{28}O_2$	Not known
4	Tridecanoic acid, methyl ester	7.84	6.03	228	$C_{14}H_{28}O_2$	Antibacterial, antifungal
5	Octanoic acid, 3-methyl butyl ester	8.08	8.61	214	$C_{13}H_{26}O_2$	Skin irritant
6	Tetradecanoic acid, methyl ester	8.93	8.70	242	$C_{15}H_{30}O_2$	Not known
7	Tetradecanoic acid, ethyl ester	9.49	1.48	256	$C_{16}H_{32}O_2$	Hypercholesterolemic
8	3-methyl butyl dodecanoate (isoamyl laurate)	10.17	5.97	270	$C_{17}H_{34}O_2$	Flavor and fragrance agents
9	9-Heptadecanone	10.44	2.39	254	$C_{17}H_{34}O$	Not known
10	Hexadecanoic acid, (Palmitic acid) methyl ester	11.00	16.25	270	$C_{17}H_{34}O_2$	Not known
11	Hexadecanoic acid, ethyl ester	11.62	4.20	284	$C_{18}H_{36}O_2$	Not known
12	10-Nonadecanone	12.62	0.86	282	$C_{19}H_{38}O$	Not known
13	Octadecanoic acid, methyl ester (Stearic acid methyl ester)	12.85	5.02	298	$C_{19}H_{38}O_2$	Co solvents, oil carrier
14	2-methyl undecanol	13.25	1.16	186	$C_{12}H_{26}O$	Not known
15	9-Heneicosanone	14.25	1.49	310	$C_{21}H_{42}O$	Not known
16	4-methyl Hexadecane	15.70	2.81	240	$C_{17}H_{36}$	Not known
17	Hexadecane (also called cetane)	16.81	1.71	226	$C_{16}H_{34}$	Measure of the detonation of diesel fuel
18	1-Dodecanol (lauryl alcohol)	17.36	1.84	186	$C_{12}H_{26}O$	Cosmetics, personal care products

their applications.

MATERIALS AND METHODS

Plant material

The *P.r betle* plant material was collected from Kolkata (West Bengal). The leaf stalk studied was collected from plants grown in Kolkatta, West Bengal. A voucher specimen has been deposited at the Herbarium of Vikram University. The essential oil was obtained from the fresh leaf stalk by steam distillation for 4 h. The species produced yellowish oil with a pleasant odor in 0.20% yield (based on fresh weight).

Extraction

The air dried *P. betel* (leaf stalk) were Soxhlet extracted, successively with n-hexane, and benzene was fractionated on silica gel column. The column was eluted with different solvents in their increasing order of polarity.

Table 2. Compounds present in benzene ext. of *P. betel* (leaf stalk) from India.

Compounds		Retention time (min.)	Area%	Molecular mass	Molecular formula	Applications
Fraction 2 (Benzene)						
1	4-ethyl benzaldehyde	3.71	7.37	134	$C_9H_{10}O$	Not known
2	Iso Eugenol (2-methoxy-4(1-propenyl) phenol (4-Propenylguaiacol)	4.89	15.07	164	$C_{10}H_{12}O_2$	Perfumeries, flavorings, essential oils
3	Tetadecanoic acid(Myristic acid)	9.39	7.34	228	$C_{14}H_{28}O_2$	lubricant additive
4	1-methoxy-4 methyl benzene (p-Methylanisole)	9.88	5.07	122	$C_8H_{10}O$	food additive
5	Eugenol (2- methoxy-4- (2- propenyl) phenol (Allylguaiacol)	10.37	12.26	164	$C_{10}H_{12}O_2$	Local antiseptic and analgesic

Vacuum liquid chromatography

A portion (100 g) hexane extract was subjected to vacuum liquid chromatography (VLC) on silica gel 60 H using gradient of hexane: benzene: acetic acid (6/4/0.1, v/v) as solvent system to obtain two VLC fractions using a rotator evaporator at a maximum temperature of 40°C.

Preparation of trimethylsilyl (TMS) ether derivatives

The aliphatic compounds present in the VLC fractions of the hexane and benzene extract were converted to their trimethylsilyl derivatives. Each fraction was mixed with Tri-Sil reagent (0.1 ml) in glass sealed tubes using an ultrasonic bath for 2 min and then vortexing briefly. The tubes were then incubated at 60°C for 45 min.

Thereafter, the solvent was evaporated under a stream of nitrogen and the TMS ether derivatives were dissolved in 0.2 ml of n-hexane and benzene respectively, the tubes were sonicated in an ultrasonic bath for 2 min, vortexed and centrifuged for 3 min. The n-hexane and benzene layers were transferred to other tubes, avoiding any solid particles, and analyzed by the GC-MS. After derivatization, the tubes were stored at -20°C for subsequent analyses within 3 days.

Gas chromatography-mass spectrometry

A Hewlett-Packard 5890 Series II Chromatograph equipped with a FID detector and HP-2 fused silica columns (25 m × 0.32 mm, 0.25 µm film thicknesses) was used. The samples, dissolved in hexane, were injected in the split less mode into helium carrier gas. Injector and detector temperatures were maintained at 250°C. The column temperature was programmed from 60°C (after 2 min) to 220°C at 4°C/min, and the final temperature was held for 20 min. Peak areas and retention times were measured by electronic integration of by computer. The relative amounts of individual components are based on the peak areas obtained, without FID response factor correction.

GC-MS analyses were carried out on a Hewlett-Packard 5970A mass selective detector (MSD), directly coupled to HP 5790A gas chromatograph. A 26 m × 0.22 mm column, coated with 0.13 µm of CP-Sil 5CB was employed, using helium carrier gas. The oven temperature program was 60°C (3 min), then 5°C/min to 250°C (30 min). Other conditions were the same as described under GC. Electron ionization (EI) mass spectra were acquired over a mass range of 10-400 Da at a rate of 2/s.

Identification of the compounds

The identification of the compounds present in the VLC fractions of the hexane and benzene extracts were based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley 229, Nist 107, 21 Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Revenkar and Sen, 1978; Santhakumari et al., 2003; Sharma et al., 1989).

RESULTS AND DISCUSSION

The oily fraction of *P. betel* (leaf stalk) was isolated from the separation of hexane and benzene extracts. The n-hexane extract of *P. betel* yielded a waxy fraction which was rechromatographed on silica gel column. Hexane and benzene eluates yielded waxy liquids in very small amount and was not separated by column chromatography. Hence they were separated by GC-MS which revealed the presence of eighteen and five compounds respectively. The compounds were identified by comparing their retention time and covate indexes with that of literature and by interpretation of mass spectra.

The quantitative estimation of each peak was made by estimating area of the peak by computer, attached by GC-MS instrument. The results of GC-MS analysis are being given in (Tables 1 and 2).

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