
Research Article

Major volatile hydrocarbons of rice paddy herb, *Limnophila aromatica* Lam. Merr as possible chemotaxonomic marker

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Abstract

Rice paddy herb, *Limnophila aromatica* Lam. Merr, is a tropical flowering plant in the plantain family, Plantaginaceae, and flourishes in a hot, watery environment, particularly rice fields. It is known to have a distinct flavour and aroma reminiscent of lemon/cumin, and used as salad in diet by Southeast Asian communities. The essential oil from *L. aromatica* from Tuaran (Sabah) was extracted and analysed, a total of 36 volatile organic constituents were identified. Detailed analysis revealed the presence of only seven major volatile compounds (amyl vinyl carbinol, terpinolene, β -linalool, sabinene, terpinen-4-ol, α -humulene, aromadendrene) in the range of 3.02 % to 30.06 %. These volatiles could be grouped into monoterpene (44.10 %), oxygenated monoterpene (32.15 %), sesquiterpene (20.87 %), oxygenated sesquiterpene (1.2 %) and oxygenated diterpene (2.27 %-1.5 %). Interestingly, the presence of sabinene, terpinen-4-ol and α -humulene were found in a high percentage and could be suggested as chemotaxonomical markers of this herb.

Keywords: *Limnophila aromatica*, Essential oil, Sabinene, Terpinen-4-ol, α -humulene

Introduction

The herbal plant of the genus *Limnophila* is an aromatic marsh herb comprising circa 37 species and occurring as wild weeds in paddy fields in Southeast Asian countries (Mabberley & Large, 1994). The aerial component of these plants is widely used as spice and as a medicinal herb (Do et al., 2013). One particular species, *Limnophila aromatica* Lam. Merr has been reported as the most extensively utilised and fragrant species. *Limnophila aromatica* belongs to Scrophulariaceae and is widely used as spice and medicinal herb among Southeast Asian communities. It is easily cultivated in the peripherals of a soggy rice field with negligible toxicity and it has been reported to possess diuretic, muscle relaxant and antispasmodic activities (Do 1999; Do et al., 2013).

In Thailand and Vietnam, *L. aromatica* is easily cultivated in flooded rice fields and strongly associated with Vietnamese cuisine, added in soup broth, sauces and consumed as a raw salad. Also known as “Vietnamese coriander,” this herb has two very distinct flavours of lemon oil and cumin. The lemony scent of this herb makes it a major ingredient in freshwater fish dish preparation, and is also commonly used in Vietnamese curries. In Sabah, *L. aromatica* is also found growing in lowland paddy fields and soggy land-use areas including in the urban landscape. It is widely collected by locals and often sold at weekend markets. The consumption of *L. aromatica* is documented among ethnic communities in Sabah and it is consumed raw or prepared as part of a dish.

However, almost no information is available pertaining to the chemical composition of fragrance or the volatile chemical constituents of *L. aromatica*. This paper reports on the chemical constituents of aromatic compounds from the oil of *L. aromatica* from Tuaran, Sabah. The objective of this investigation is to quantify the amount of essential oil and its chemical constituents via Gas Chromatography Mass Spectroscopic analysis and propose the major volatile chemicals as chemotaxonomic markers for this species.

Materials and Methods

Plant materials and extraction of essential oil

Specimens of *Limnophila aromatica* were collected from a Tuaran paddy field in January 2013. These specimens were identified based on their morphological appearances and were positively identified as *L. aromatica*. The plant materials were removed from mud, organic matter and washed in three changes of distilled water. Cleaned specimens (250 g) were then chopped and subjected to hydro distillation using a Clevenger-type apparatus for eight hours. Distilled oil was collected in pentane (Merck, Germany), dried over anhydrous sodium sulphate (Sigma, USA), concentrated *in vacuo* stored in airtight glass vial flushed with nitrogen (N₂) gas and kept at -81 °C prior to analysis.

GC-MS analysis of the essential oil

Analysis of essential oil was performed using Shimadzu QP-2010 chromatography coupled with Shimadzu GCMSQP-2010plus detector (Shimadzu Corp., Japan) using SGE BPX-5 (30.0 m X 0.25 µm i.d., film thickness 0.25 µm) fused silica capillary column. High purity helium was used as the carrier gas at a constant flow rate of 0.8 ml/min. A 1 µl sample was injected (split ratio 100:1) into GCMS using an AOC5000 autoinjector to be analysed. The initial temperature was set at 50 °C, heated at a rate of 3 °C/min to 280 °C and held

isothermally for five minutes. Ion source temperature for analysis was set at 200 °C while interface temperature was set at 280 °C. The mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40 a.m.u to 450 a.m.u. at 0.25 scan/s.

Resolved chromatography peaks were identified by comparing their mass fragment patterns against two standard libraries; 1) National Institute for Standard and Technology (NIST) 1998, 2) Flavours and Fragrance of Natural and Synthetic Compounds (FFNSC) V 1.2. In addition, retention indices of the respective peaks were determined based on a homologous series of *n*-alkanes (C₈ - C₄₀) (Custom Retention Time Index Standard, Restek Corp, United States) external standard analysed under the same operating conditions and calibrated based on Automatic Adjustment of Compound Retention Time (AART) function of the GCMS. Relative concentrations of essential oil components were calculated based on GC peak area with the AART correction factors (Nagappan et al., 2012).

Results and Discussion

The essential oils obtained from hydro-distillation technique were fragrant, yellowish oils and yields were calculated as 2.17±0.15 % of the fresh *L. aromatica*. Gas Chromatography Mass Spectroscopic analysis gave a total of 36 well-resolved peaks positively identified to be highly volatile organic constituents based on their mass fragmentation pattern and retention indices (RI). Detailed identity of volatile organic constituents of *L. aromatica* studied is presented in Table 1 and Figure 1.

Analysis of the GCMS chromatogram revealed the presence of seven major volatile compounds (amyl vinyl carbinol, terpinolene, β-linalool, sabinene, terpinen-4-ol, α-humulene, aromadendrene) in the range of 3.02±0.21 % to 30.06±0.12 % apart from minor compounds of its essential oil. In addition, these volatile hydrocarbons could further be grouped into hydrocarbon monoterpene (44.1 0%), oxygenated monoterpene (32.15 %), hydrocarbon sesquiterpene (20.87 %), oxygenated sesquiterpene (1.27 %) and oxygenated diterpene (2.27 %-1.5 %). There were two hydrocarbon monoterpenes, 15 oxygenated monoterpenes, six hydrocarbon sesquiterpenes, five oxygenated sesquiterpenes and two hydrocarbon diterpene compounds present in the aromatic oil of *L. aromatica*. Interestingly, the presence of sabinene, terpinen-4-ol and α-humulene in a major metabolite in this plant could be used as the volatile chemotaxonomical markers of this herb species.

Table 1: Essential oil composition (%) of *Limnophila aromatica* Lam. Merr.

RT (min)	RRI	Compounds	Concentration (%)	Identification mode
8.10	927	α -thujene	1.43 \pm 0.04	MS, FFNSC
9.83	1042	8-nonen-2-one	0.20 \pm 0.19	MS, NIST
10.30	969	Amyl vinyl carbinol	5.04 \pm 0.26	MS, NIST
10.57	958	β -myrcene	0.45 \pm 0.03	MS, NIST
11.85	1018	α -terpinene	0.44 \pm 0.17	MS, FFNSC
12.40	1030	Limonene	0.36 \pm 0.20	MS, FFNSC
13.09	1046	β -E-Ocimene	0.58 \pm 0.74	MS, FFNSC
13.70	998	γ -terpinene	1.04 \pm 0.05	MS, NIST
14.35	1041	3-carene	2.29 \pm 0.94	MS, NIST
14.93	1086	Terpinolene	6.01 \pm 0.44	MS, FFNSC
15.73	1082	β -Linalool	7.64 \pm 0.89	MS, NIST
15.80	1014	Terpinene-4-acetate	1.99 \pm 0.66	MS, NIST
16.55	1113	Phenethyl alcohol	0.43 \pm 0.22	MS, FFNSC
16.90	972	Sabinene	30.06 \pm 1.03	MS, FFNSC
17.62	1082	cis-sabinol	0.41 \pm 0.07	MS, NIST
18.15	1121	Camphor	0.49 \pm 0.16	MS, NIST
19.60	1137	Terpinen-4-ol	14.06 \pm 0.08	MS, NIST
19.97	1189	ρ -cymen-8-ol	0.59 \pm 0.62	MS, FFNSC
20.31	1308	α -terpinyl formate	1.23 \pm 0.12	MS, FFNSC
21.61	1136	Trans-3-carene-2-ol	0.23 \pm 0.03	MS, NIST
22.65	1212	Linalyl formate	0.18 \pm 0.05	MS, FFNSC
24.37	1314	2-pinen-10-ol	0.19 \pm 0.77	MS, NIST
26.43	1072	Isomyrcenol	0.21 \pm 0.94	MS, FFNSC
28.74	1356	ρ -acetanisole	0.79 \pm 0.26	MS, FFNSC
30.11	1494	Caryophyllene	3.82 \pm 0.11	MS, FFNSC
31.33	1452	β -farnesene	1.49 \pm 0.82	MS, FFNSC
31.67	1454	α -humulene	12.05 \pm 1.01	MS, FFNSC
35.83	1508	β -bisabolene	0.42 \pm 0.12	MS, FFNSC
36.92	1438	Aromadendrane	3.42 \pm 0.04	MS, FFNSC
37.33	1673	α -trans-bergamol	0.21 \pm 0.67	MS, NIST
38.06	1507	Caryophyllene oxide	0.17 \pm 0.08	MS, NIST
40.43	1576	Spathulenol	0.28 \pm 0.93	MS, FFNSC
40.56	2009	Eicosane	1.32 \pm 0.41	MS, NIST
45.32	1834	Napthalen-2-one	0.26 \pm 0.39	MS, FFNSC
48.94	1925	Methyl palmitate	0.14 \pm 0.15	MS, FFNSC
54.66	18.36	Neophytadiene	0.19 \pm 0.31	MS, FFNSC

Composition of grouped volatile compounds (%)

<i>Monoterpenes (hydrocarbon)</i>	44.10
<i>Monoterpenes (oxygenated)</i>	32.15
<i>Sesquiterpenes (hydrocarbon)</i>	20.87
<i>Sesquiterpenes (oxygenated)</i>	1.01
<i>Diterpenes (hydrocarbon)</i>	1.51
Total (%)	99.64

RRI, retention indices on BPX 5; Compounds are listed in order of elution based on BPX 5; Volatile compounds were identified based on their MS fragment pattern and retention index as compared with NIST and FFNSC data bases.

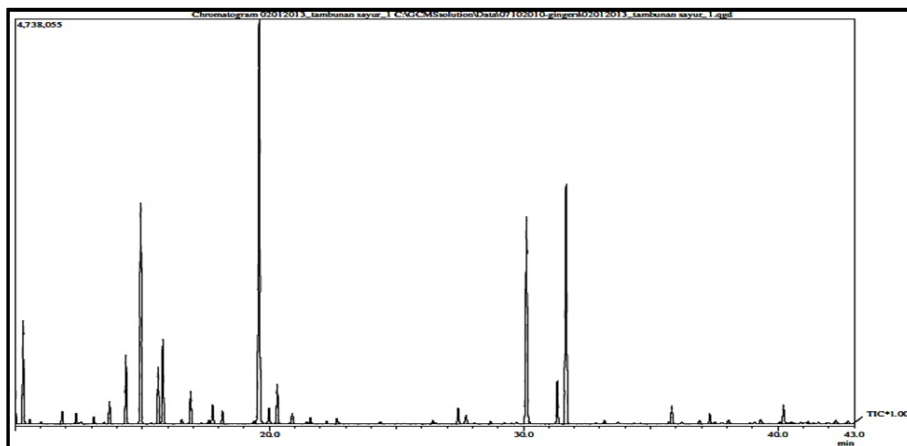


Figure 1. Gas-chromatography profile of *Limnophila aromatica* Lam. Merr.

Majority of volatile chemotypes in this herbal plant were found to be in classes of oxygenated monoterpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes. Presence of oxygenated monoterpenes and oxygenated sesquiterpenes in the oil of *L. aromatica* have been associated to its bioactive potential in inhibiting the growth of pathogens and microbes. Volatile constituents such as sabinene, α -terpineol, linalool and terpinen-4-ol are an important discovery to justify their importance in this plant. Each of these four major compounds is a reflection of the fragrance and spiciness exhibited by this plant. Sabinene is a natural bicyclic monoterpene with a strained ring system with cyclopentane fused rings to a cyclopropane ring. It has also been identified as the chemical component that contributes to spiciness of black pepper and is a major constituent of carrot seed oils, tea tree oil and in nutmeg. In addition, terpineol is a natural monoterpene alcohol that is found in cajuput, pine and petitgrain oils (Shan-Shan et al., 2005). There are four isomers, *alpha*-, *beta*-, *gamma*-terpineol and terpinen-4-ol. *Beta*- and *gamma*-terpineol differ only by the location of the double bond. Terpineol is usually a mixture of these isomers with *alpha*-terpineol as the major constituent. Terpineol has a pleasant odour similar to lilac and is a common ingredient in perfumes, cosmetics and flavours. α -Terpineol is one of the two most abundant aroma constituents of lapsang souchong tea; α -terpineol originates in the pine smoke used to dry the tea (Yuasa & Yuasa, 2005).

In addition, linalool is a naturally occurring terpene alcohol chemical found in many flowers and spice plants with many commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of

spiciness). It has other names such as β -linalool, linalyl alcohol, linaloyl oxide, *p*-linalool, allo-ocimanol and 2,6-dimethyl-2,7-octadien-6-ol. Meanwhile, terpinen-4-ol is a terpene and is considered the primary active ingredient of tea tree oil (Hammer et al. 2012). It is also the compound of highest concentration in the essential oil of nutmeg.

A majority of volatile chemotypes in this herbal plant were found to be in classes of oxygenated monoterpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes. Presence of oxygenated monoterpenes and oxygenated sesquiterpenes in the oil of *L. aromatica* have been associated to its bioactive potential in inhibiting the growth of pathogens and microbes. Volatile constituents such as α -terpineol, linalool and terpinen-4-ol were reported to show efficient activity against bacteria and fungi. Garcia & Langenheim (1991) reported sabinene and terpinene works in synergistically against endophytic fungi. Findings also revealed mixtures of terpinen-4-ol, α -terpineol and 1,8-cineole are responsible in combating gram-negative bacterium *Pseudomonas aeruginosa* (Thomsen et al., 2013).

As terpinen-4-ol is valued as a natural botanical medicine, it has been formulated for the treatment of vaginal thrush among women. When tested, this monoterpene constituent was found to inhibit strains of *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermis* and *Staphylococcus aureus* (Williams, 1998). In general, oxygenated monoterpenes are significantly more active than hydrocarbon monoterpene. The cytotoxicity of essential oil is demonstrated through bacterial membrane damage that occurs when essential oil passes the wall and cytoplasmic membrane resulting in disruption of different layers of polysaccharides, fatty acids and phospholipids (Costa et al., 2013). Rota & co-workers (2008) suggested synergistic effects of terpinolene, β -linalool, sabinene, terpinen-4-ol, α -humulene and aromadendrene together with phenolic compounds would give rise to antibacterial properties as well. As for the presence of variation in percentage of monoterpene and sesquiterpene chemotypes, it is reportedly due to propagation, geographical isolation and effects of microclimate on physiology of the plant (Southwell & Russell, 2003). In conclusion, the major volatile aromatics in *L. aromatica* were identified to consist almost 70 % of the essential oil composition. Their composition was proven to be aromatic, spicy and bioactive. In addition, these compounds could also be used as this specie's chemotaxonomic markers.

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