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Essential oil composition of fruits and leaves of *Zanthoxylum nitidum* grown in upper Assam region of India

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ABSTRACT

Essential oils isolated by hydro-distillation from the fruits and leaves of *Zanthoxylum nitidum* (Roxb.) DC (Rutaceae), growing in upper Assam region of North-East India were analyzed by GC and GC-MS. The fruit volatile oil contained 17 components amongst which 75% were monoterpenes, 12.5% were sesquiterpenes and 12.5% were straight chain hydrocarbons; whereas the leaf volatile oil contained 16 components out of which 60% were monoterpenes, 13.3% were sesquiterpenes, and 26.7% were straight chain hydrocarbons. Linalool (23.3%), limonene (12.9%), α -terpineol (8.3%), α -pinene (7.9%) were the predominant monoterpenes of the fruit oil and the main monoterpenes in the leaf oil were limonene (33.1%), geraniol (10.6%) and carvone (9.6%).

Key words: *Zanthoxylum nitidum*, essential oil, monoterpenes, linalool, limonene.

INTRODUCTION

Zanthoxylum nitidum (Roxb.) DC (Rutaceae), is a morphologically variable plant species occurring in south-east Asia and in Australia (1, 2). In India, it is found as a large prickly shrub mainly in North-East Indian states (Sikkim, Assam, and Nagaland). In India, different parts of the plant are traditionally used for different medicinal purposes. The root is used in toothache, stomachache, fever, rheumatism, paresis, boils and as an insecticide and piscicide. The fruit is used in the treatment of stomachache, cough, colic, vomiting, diarrhoea, and paresis and as an aromatic, stimulant and piscicide. The small branches, seeds and stem bark are prescribed in fever, diarrhoea and cholera (2, 3). The stem bark and root are reported to possess antibacterial activity (3). The fruits are used as a condiment in India and Nepal. Almost all parts of the plant are aromatic and hence, supposed to possess essential oil. However, in India, at present the plant has got no commercial importance (4, 5).

The components of essential oil isolated from the fruits of the plant grown in some other regions of the world have already been reported. Linalool has been reported as the major constituent of the fruit essential oil. Previous workers obtained linalool, β -phellandrene, phenyl ethyl acetate, methyl cinnamate

from the essential oil from the fruits of *Z. nitidum* in Jammu, India. Although the occurrence of essential oil in leaves was already reported, the studies on composition of essential oil from leaves of the plant however, have not been reported yet (4-6). The essential oil composition can provide much more knowledge regarding the medicinal properties and active constituents of this plant. The present paper aims at the studies on the composition of the essential oil from fruits and leaves of *Z. nitidum* grown in the upper Assam region of North-East India and also for comparative studies of this oil collected from plants grown in other parts of the world.

MATERIALS AND METHODS

Plant material: Fresh fruits and leaves from the mature plant of *Zanthoxylum nitidum* were collected during March-April 2008 from upper Assam region (Dibrugarh district) of India. The species was identified by Dr. S. J. Phukan, Taxonomist, from Botanical Survey of India, Eastern Circle, Shillong, India and a voucher specimen (DUPS-06-003) was deposited at Department of Pharmaceutical Sciences of Dibrugarh University for future reference. After collection the fruits and leaves were cut into small pieces and immediately subjected to isolation of essential oil.

Isolation of the essential oil: Freshly collected leaves (250 g) and fruits (300 g) were hydro-distilled for 5 h using an all-glass Clavenger type apparatus and the essential oils were isolated and collected. The percentage yield of the fruit oil and the leaf oil were found to be 5.12 % v/w (refractive index: 1.451) and 0.76 % v/w (refractive index: 1.472) respectively. After collection, the oils were dried over anhydrous sodium sulphate and stored at 4-8°C in the dark until tested and analyzed.

GC-MS Analysis: The GC-MS analysis of the oils were performed by employing a GCD 1800 A, Hewlett Packard gas chromatogram using fused capillary column (HP-1, column length: 30 m, internal diameter: 0.25 mm, film thickness 0.25 µm). The instrument was programmed at 50-200°C at a rate of 4°C/min; and then increased at 20°C/min up to 280°C. The carrier gas used was helium at a flow rate of 0.8 ml/min; split ratio, 1: 60; ionization energy 70 eV; scan time, 1 s; mass range 40-300 amu.

Identification of the components: The components were identified by comparing the retention time of the peaks on HP-1 column matching against the standard library spectra of pure substances and components of known essential oils, and finally confirmed by comparison of mass spectral fragmentation patterns with the published data (7-11).

RESULTS

The essential oil components of the fruit and leaf oil of *Z. nitidum*, the percentage of each constituent and their retention index (RI) values are summarized in Table 1 and 2 respectively. The components are arranged in order of their elution pattern on the HP-1 column.

The analysis of the oil by GC and GC-MS resulted in the identification of 17 and 16 components from the fruits and leaves oil, comprising 94.1% and 93.8% of the total volatiles respectively. Interestingly, the fruit oil and leaf oil compositions were found to be varied considerably. Quantitatively, the fruit oil and leaf oil were characterized by a high proportion of monoterpenes 75% and 60%; sesquiterpenes 12.5% and 13.3%; and straight chain hydrocarbons 12.5% and 26.7% respectively. The main monoterpenes in the fruit oil were linalool (23.3%), limonene (12.9%), α -terpineol (8.3%), α -pinene (7.9%), γ -terpinene (6.6%), cis- β -ocimene (6.2%), terpinen-4-ol (4.7%), and isomenthone (2.0%). Among the twelve monoterpenes, there were four monoterpene hydrocarbons, six alcohols and two ketones. Only two sesquiterpenes were identified in the fruit oil, of which one is

sesquiterpene hydrocarbon (caryophyllene) and other one is caryophyllene epoxide.

On the other hand, among the monoterpenes of leaf oil, the predominant was limonene (33.1%), followed by geraniol (10.6%), carvone (9.6%), 7-hydroxy-3,7-dimethyloctanal (4.3%), geranial (3.9%) and nerol (2.2%). Out of nine monoterpenes, there was one monoterpene hydrocarbon, two alcohols, two aldehydes, two acids, one ketone and one ester. Cis- β -farnesene and n-tetracosane-3-ol are the two sesquiterpenes found in the leaf oil. Except limonene, the fruit oil and leaf oil compositions were found to be different from each other. One component each from fruit oil and leaf oil remained partially identified.

DISCUSSION

Zanthoxylum (Rutaceae) is a large genus of aromatic prickly trees or shrubs distributed pan-tropically and 13 species of it are found in India. Several Indian species of *Zanthoxylum* are medicinally active. The fruits of a number of species yield essential oil. The oils from *Z. acanthopodium*, *Z. armatum* and *Z. nitidum* are potential sources of linalool (2, 5). The essential oils collected from plants growing in different parts of the world show a great variation in the composition as well as content of particular constituent(s) due to geographical or environmental factors. The chemical variability of essential oil with compositions was characterized by the presence of high percentage of linalool (41.1%) and β -phellandrene (25%) along with phenyl ethyl acetate, methyl cinnamate in the fruit essential oil of *Z. nitidum* grown in Jammu, India (5, 6). While in contrast, β -phellandrene, phenyl ethyl acetate, methyl cinnamate were not detected in the fruit oil of the plants grown in upper Assam region of India and the linalool content was found to be in less amounts (23.3%) in this oil along with other constituents like limonene (12.9%) which were reported to be absent in that Jammu oil. The presence of some amounts of linalool, an important perfumery material, could make this oil some commercial interest. The fruit and leaf oil compositions were found to be considerably different (see results). The leaf essential oil can be used as a source of limonene but the poor oil content may limit the feasibility.

Indian workers reported linalool and limonene as major components of essential oil from *Zanthoxylum armatum* seeds in India (12). Chinese workers reported the essential oil composition of fruits from *Zanthoxylum nitidum* var. *Fastuosum* grown in China and found that sesquiterpenes were the major

Table 1: Chemical composition of *Z. nitidum* fruit oil

Pk	Retention Index	Percentage	Components
1	-	3.41	Isobutane
2	-	6.59	n-butane
3	924	7.82	α -pinene
4	1014	6.18	cis- β -ocimene
5	1022	12.90	Limonene
6	1043	6.57	γ -terpinene
7	1086	23.23	Linalool
8	1125	1.72	3-thujanol
9	1143	4.70	Terpinen-4-ol
10	1145	2.36	Menthone
11	1151	2.00	Isomenthone
12	1161	1.19	Lavandulol
13	1161	4.99	Carvomenthone isomer*
14	1162	8.29	α -terpineol
15	1163	1.01	Menthol
16	1403	5.95	Caryophyllene
17	1550	0.95	Caryophyllene epoxide

Pk: Peaks, *Partially identified component

Table 2: Chemical composition of *Z. nitidum* leaf oil

Pk	Retention Index	Percentage	Components
1	-	10.34	Isobutane
2	-	4.57	n-butane
3	600	6.44	Cyclohexane
4	-	3.07	E-n-non-3-ene
5	1014	33.12	Limonene
6	1206	9.60	Carvone
7	1218	2.22	Nerol
8	1237	10.64	Geraniol
9	1260	3.86	Geranial
10	1285	5.27	C ₁₀ H ₂₀ , MW156*
11	1300	0.82	Citronellic acid
12	1316	1.72	Nerolic acid
13	1341	4.32	7-hydroxy-3,7-dimethyloctanal
14	1365	1.98	Geranyl acetate
15	1446	1.75	cis- β -farnesene
16	1510	0.29	n-tetracosane-3-ol

Pk: Peaks, *Partially identified component

chemical constituents, whereas linalool and limonene were not detected (13). However, this variety of *Z. nitidum* is not reported to occur in India (4, 5). The knowledge in essential oil components of *Z. nitidum* may be helpful in studying its biological activities as per ethnomedicinal applications. The enantiomer detection (D or L or mixture) of limonene and menthol present and the biological evaluation of the essential

oils obtained are presently underway. To our knowledge, the present study is the first report of the composition of the fruit and leaf essential oil of *Zanthoxylum nitidum* (Roxb.) DC grown in North-East India.

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