NOTE

Chemical Composition of the Essential Oil of Clausena heptaphylla W&A from Assam, India¶

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The essential oil obtained by hydrodistillation from the leaves of *Clausena heptaphylla* (W&A) from Assam, India was analyzed by GC and GC-MS. 16 Compounds representing 97.8 % of the total oil were identified of which (E)-anethole (92.6 %), (Z)-anethole (1.2 %) and methyl chavicol (1.6 %) were the major components. Eight minor components were unreported so far.

Key Words: Clausena heptaphylla, Rutaceae, Volatile oil composition.

Clausena heptaphylla W&A (Rutaceae) commonly exist as bushy shrubs. It grows well in some of the areas of North Eastern India upto an altitude 1330 m¹ where the leaves are known traditionally for chewing with betel leaves and flavouring tobacco². The leaves of the plant possess a strong and pleasant aromatic odour. Recently, a report has been made from U.K. on this species indicating that the predominant component of the essential oil, obtained from its leaves was found to be anethole³. Variations of major component in the leaf essential oil of another Clausena species viz., C. anisata Hook have been often reported. According to Guenther's classical reference, anethole was listed as a major component in the essential oil of C. anisata⁴. The same phenyl propanoid was also reported to be the main component from the leaves of *C. anisata* grown in Java⁵. However, methyl chavicol was recorded as dominant constituent from the similar plant sources in Philippine⁶ and Nigeria⁷. The other species of *Clausena* (C. dunniana Levl.) leaves being grown in China contained isoanethole (93.1 %), as major constituent⁸. Methyl chavicol followed by (E)-anethole as major components in the leaf and fruit oil of C. heptaphylla growing wild in northeast, India have been reported^{9,10}. There has also been a report on an anethole rich leaf oil of this same species¹¹ of northeastern region of India. Based on these reports, a study was undertaken on indigenous C. heptaphylla growing wild in north Eastern part of India. Its major and minor components of the leaf oil have been studied in the light of earlier reports, but to

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the best of our knowledge no detailed GC-MS analysis of *C. heptaphylla* leaf oil from north eastern India has been carried out with respect to the minor components.

GC analysis was performed on Perkin-Elmer AUTOXL gas chromatograph using PE-Wax, fused silica capillary (30 m \times 0.32 mm, film thickness 0.25 μ) and FID detector. Oven temperature was programmed from 50 to 230 ° at 5 °C/min; injector temperature 220 °C; detector temperature 250 °C; carrier gas hydrogen with inlet pressure of 6.5 psi.

GC-MS investigation of the oil was performed on Perkin Elmer Turbomass/auto XL system. A PE-wax fused silica capillary column (50 m \times 0.3 mm, 0.25 μ film thickness) was employed. GC condition: injection 220 °C, split ratio 1:20, helium carrier flow 1 mL/min and oven temperature 50 to 230 °C @ 5 °/m. MS condition: ion source 250 °C and ionizing voltage 70 eV.

Identification of compounds: Compound identification was made on the basis of their mass spectra, followed by Wiley/NIST Library search and retention time of reference compounds available.

Fresh leaves of *C. heptaphylla* were collected from Assam, India after the proper identification of the plant material. A voucher specimen has been deposited in R & D centre for Medicinal & Aromatic Plants, Assam India.

Isolation of the essential oils: The leaves of *C. heptaphylla* were subjected to hydrodistillation in a conventional Clevenger type apparatus for 4 h and yielded 1.2 % light yellow coloured oil. The oil was dried over anhydrous sodium sulphate and stored at 4 °C until analyzed.

Chromatography of the oil: The essential oil (1 mL) was column chromatographed over 20 g silica gel (60-120 mesh) in order to get fractions enriched in minor components; the following fractions were collected (each fraction 5 mL). Fr. No. 1-6 eluted in hexane and frs. 7-10 eluted in hexane:EtOAc and 10-12 eluted in hexane:EtOAc (9.8:0.2). Minimum anethole was found in the last fraction which was enriched with eight minor constituents unreported so far.

The volatile oil was obtained by conventional hydrodistillation of *C. heptaphylla*, which gave an oil in 1.2 % yield on fresh weight basis. Isolated fractions from column chromatography after GC and GC-MS analysis resulted in the identification of sixteen constituents representing 97.8 % of the oil. The relative concentrations of the volatile components are presented in Table-1 according to their elution order on the PE-Wax column. The major constituents in the oil from the Assam are (E)-anethole (92.6 %), (Z)-anethole (1.2 %), methyl chavicol (1.6 %). On comparing our results with earlier reports on *C. heptaphylla* oils from the northeastern region of India^{3,9-11} it was evident than out of 16 constitutents identified by

GC-MS, eight minor constitutents were unreported so for *e.g.*, *p*-cymene (0.1%), β -ocimene (0.2%), farnesol (0.2%), sesquiterpene alcohol (0.1%), dehydro-aromadendrene (0.1%), eugenol (0.2%), anisyl ketone (0.3%), guaiol (trace).

TABLE-1 CHEMICAL COMPOSITION (%) OF THE LEAF OIL OF Clausena heptaphylla FROM ASSAM, INDIA

| Components | Retention time | Percentage (%) |
|--|----------------|----------------|
| α-Pinene | 2.01 | 0.1 |
| β-Pinene | 2.66 | 0.1 |
| Myrcene | 4.86 | 0.1 |
| β-Ocimene | 5.22 | 0.2 |
| p-Cymene | 6.77 | 0.1 |
| Linalool | 13.59 | 0.2 |
| Methyl chavicol | 16.34 | 1.6 |
| (Z)-Anethole | 18.38 | 1.2 |
| (E)-Anethole | 20.17 | 92.6 |
| Farnesol | 23.42 | 0.2 |
| Anisaldehyde | 24.26 | 0.7 |
| Sesquiterpene alcohol [M ⁺ 222] | 26.29 | 0.1 |
| Dehydro-aromadendrene | 26.75 | 0.1 |
| Eugenol | 27.27 | 0.2 |
| Anisyl ketone | 28.18 | 0.3 |
| Guaiol | 28.62 | Trace |

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