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STUDIES ON ESSENTIAL OILS BEARING PLANTS OF BANGLADESH. PART VII. COMPOSITION OF THE RHIZOMES OIL OF ACORUS CALAMUS L. (SWEET FLAG)

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STUDIES ON ESSENTIAL OILS BEARING PLANTS OF BANGLADESH. PART VII. COMPOSITION OF THE RHIZOMES OIL *OF ACORUS CALAMUS* L. (SWEET FLAG)

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Abstract

Results on the composition of a Calamus oil obtained from plants growing in Bangladesh are reported. The oil has been isolated by hydrodistillation and has been fractionated by chromatography on a column packed with neutral alumina.

The whole oil and the fractions obtained have been analysed by GC and GC/MS. 59 components have been identified, that correspond to about 93% of the whole oil.

(Z)-Asarone is the main component, which represents about 81% of the oil.

KEY WORD INDEX: *Acorus calamus* L.; Essential oil composition; (Z)-Asarone.

GLI OLII ESSENZIALI DEL BANGLADESH. NOTA VII. LA COMPOSIZIONE DELL'OLIO ESSENZIALE ESTRATTO DAI RIZOMI DI *ACORUS CALAMUS* L. (SWEET FLAG)

Riassunto

Vengono riportati i risultati relativi alla composizione di un olio di Calamo estratto da piante cresciute in Bangladesh. L'olio, isolato per idrodistillazione, è stato frazionato mediante cromatografia su colonna di allumina neutra. L'olio come tale e le frazioni sono stati analizzati per GC e GC/MS.

Sono stati identificati 59 componenti pari a circa il 93% dell'intero olio. Il componente principale è lo (Z)-Asarone che rappresenta circa l'81% dell'olio.

PAROLE CHIAVE. *Acorus calamus* L.; Composizione dell'olio essenziale; (Z)-Asarone.

Introduzione

Acarus calamus L., a specie of the Araceae family, is a herbaceous, perennial plant, which comes from Northern Asia. It is widespread in Europe, in India and in Northern America. It usually grows near the shores of lakes or near a water course (1). The rhyzomes of Calamus and its estract have been used as medicine for their antispasmodic, relaxing, hipertensive, antiasthmatic, antibacterial and vermifuge properties. The essential oil extracted from rhyzomes has been used for flavoring alcoholic beverages, as insecticide and insect-repulsive (2-4).

Three varieties of the species *A. Calamus* can be distinguished (5,6): a diploid variety, fertile, morphologically rather omogeneous, with two chemotypes, widespread in Northern America; a triploid variety, sterile, morphologically and chemically omogeneous, wide-espread in Europe; a tetrapoid variety, partially fertile, of which it is

espread in Europe; a tetrapoid variety, partially fertile, of which it is possible to distinguish two principal ecotypes, the subtropical-tropical and the temperate one; and some eterotypes, widespread in East Asia, India and Japan. Moreover, Vashit and Handa (7) report information on an esaploid variety, that grows in the Kashmir region. Wulff and Stahl (8) and Cavazza (9) state that the different varieties of Calamus differ each other mainly for their content of (Z)-Asarone, that is present as trace in the oils of the diploid varieties, in moderate amount (2.5-9.5%) in the oils of the European triploid varieties, and it is the main component (77-82%) in the oils of the tetraploid plants. The reported content of (Z)-Asarone (4.6%) in the oils of the esaploid plants of Kashmir (7) is near to that of the oils of the triploid plants.

Until 1995, the wide literature on the composition of Calamus essential oil has been reviewed by Lawrence (3,10). More recent papers report the composition of oils extracted from plants coming from Hymalaya (11), India (12), Europe (12), China (13,14) and Mongolia (15).

This paper reports the results obtained with modern analytical techniques on the composition of Calamus oil extracted from rhyzomes of plants growing in Bangladesh. Only a paper, pubblished in 1984, of Manzoor-i- Khuda et al. (16), reports results obtained by TLC for Calamus oil from plants growing in Bangladesh.

Esperimental

The rhyzomes were harvested in the experimental field of BCSIR Laboratoires, Chittagong, Bangladesh. The rhyzomes were washed, set free from roots, dryed, cleaned, cut into small pieces, crushed and homogenized. Then the essential oil was extracted by hydrodistillation in a Clevenger system. The oil was collected, dryed on anidrous sodium sulphate and then analysed by HRGC/FID and HRGC/MS.

The essential oil was separated by open column chromatography

using neutral allumina as stationary phase. The fractions obtained were then analysed by HRGC/FID and by HRGC/MS under the same experimental conditions used for the whole oil.

Column chromatography:

The essential oil was fractioned by chromatography on a column filled with neutral alumina, using petroleum ether, mixtures of petroleum ether and diethyl ether, diethyl ether and ethyl alcohol as eluents, under the following experimental conditions: glass column, 1.6 cm i.d.; adsorbent, neutral alumina, activity grade II, 20 grams; eluents: fraction 1, petroleum ether, 50 ml; fraction 2, petroleum ether: diethyl ether, 95:5, 50 ml; fraction 3, petroleum ether:diethyl ether, 90:10, 50 ml; fraction 4, petroleum ether:diethyl ether, 80:20, 50 ml; fraction 5, petroleum ether:diethyl ether, 50:50, 50 ml; fraction 6, diethyl ether, 50 ml; fraction 7, ethyl alcohol, 50 ml. Amount of oil used for fractionation, 100µl.

GC/FID analysis:

A 5160 Mega Series Gas Chomatograph (Fisons Instruments, Milan, Italy) equipped with a data processor Shimadzu C-R3A, was used with a SE-52 fused silica column, 25 m x 0.32 mm, 0.40-0.45 µm film thickness (Mega, Legnano, Italy); column temperature, 45°C (6 min) to 250°C at 3°C/min; injector temperature, 250°C; detector temperature, 250°C; injection mode, split; split ratio 1:50; volume injected, 1µl of the oil; carrier gas, He, 100 Kpa.

GC/MS analysis

A gas chromatograph mass spectrometer (quadrupole) system MD 800 (Fisons Instruments, Milan, Italy) equipped with Adams' library (17) and a home made bank FFC (Flavor and Fragrance Components) (18) was used with a DB-5 fused silica column, 30 m x 0.25 µm film thickess (J & W, Folson, California, U.S.A.); column temperature,

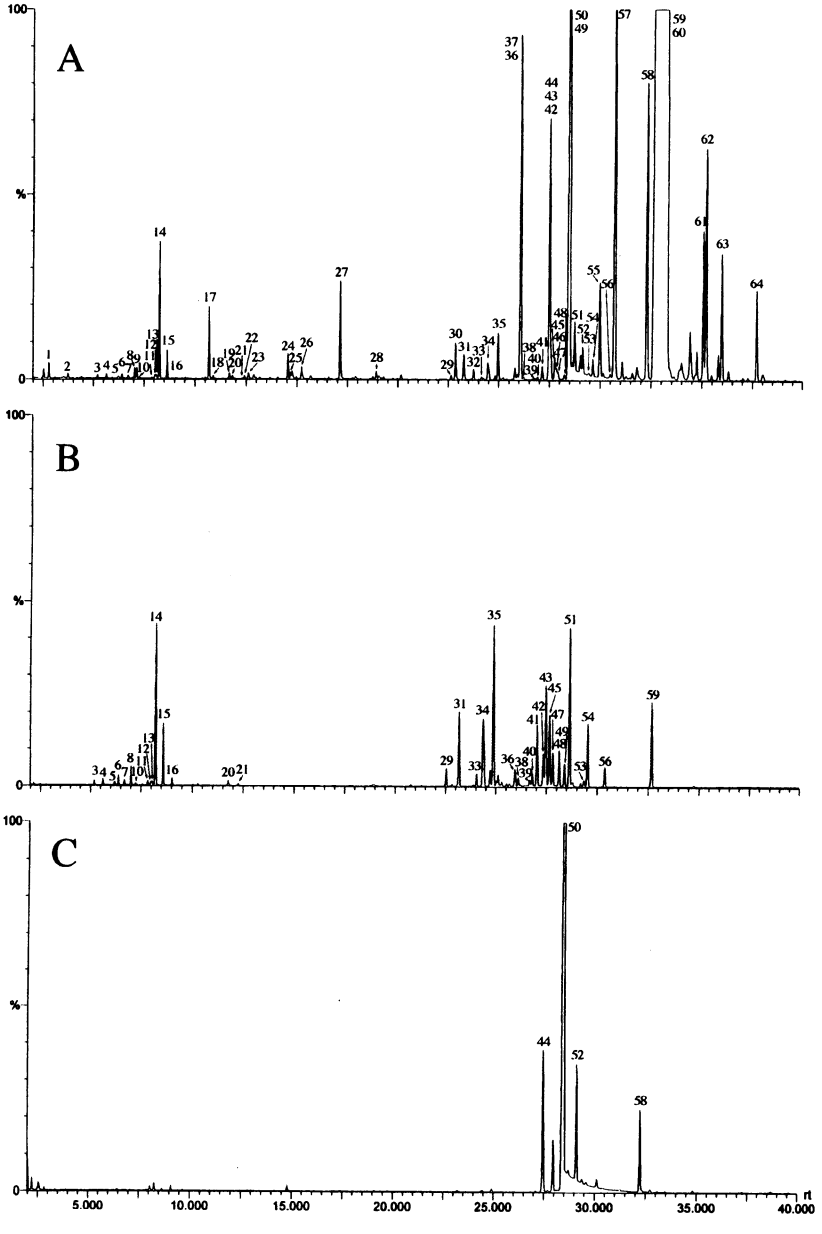
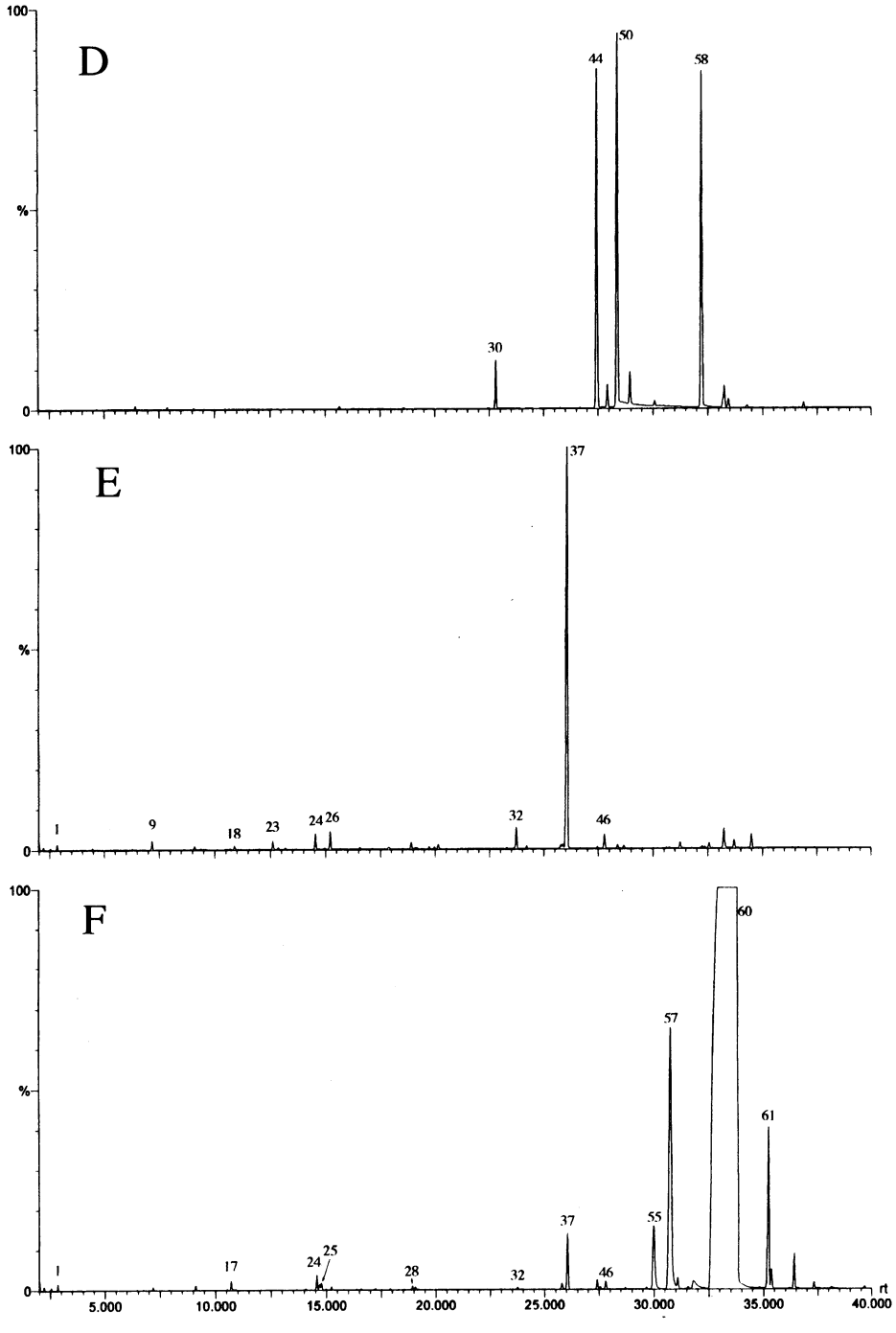


Fig. 1 Total ion gas chromatograms of Calamus essential oil (A) and of the fractions obtaiuned by column chromatography on neutral allumina (B-H).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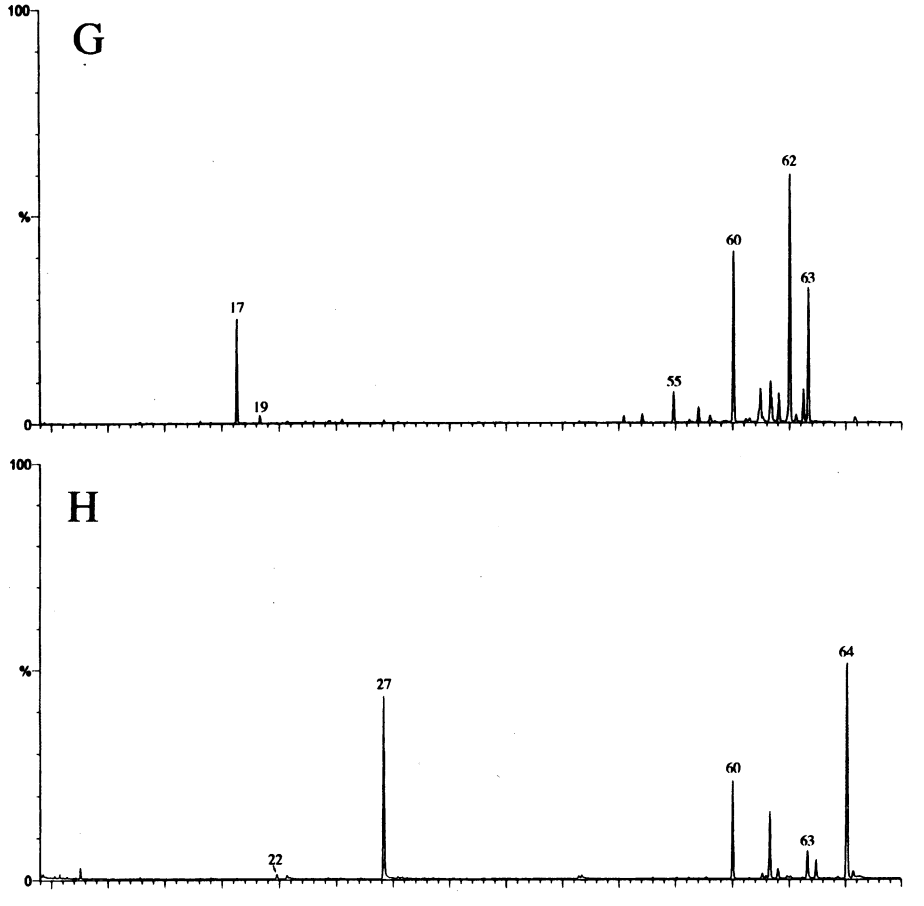
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Tab. 1 - Composition of *Acarus Calamus* Oil.

1 Hexanal	0.05	33 α-Gurjunene	tr
2 Hexanol	0.01	34 β-Caryophyllene	0.08
3 Tricyclene	0.01	35 β-Gurjunene	0.16
4 Camphene	0.01	36 α-Humulene	0.02
5 Sabinene	tr	37 (Z)-Methyl-isoeugenol	1.11
6 β-Pinene	0.01	38 allo-Aromadendrene	tr
7 Myrcene	0.01	39 β-Cadinene	tr
8 δ-2-Carene	0.03	40 γ-Muurolene	tr
9 Octanal	0.02	41 Germacrene D	0.04
10 α-Phellandrene	tr	42 β-Selinene	0.02
11 p-Cymene	tr	43 Valencene	0.06
12 Limonene	tr	44 Shyobunone	0.96
13 β-Phellandrene	tr	45 Bicyclogermacrene	0.04
14 (Z)- β-Ocymene	0.36	46 (E)-Methyl-isoeugenol	0.01
15 (E)- β-Ocymene	0.08	47 α-Muurolene	0.02
16 γ-Terpinene	tr	48 Germacrene A	0.02
17 Linalol	0.19	49 γ-Cadinene	0.01
18 Nonanal	tr	50 epi-Shyobunone	3.31
19 cis-p-Menth-2-en-ol	0.01	51 δ-Cadinene	0.16
20 allo-Ocimene	0.01	52 Kessane	0.06
21 neo-allo-Ocimene	tr	53 α-Cadinene	tr
22 trans-p-Menth-2-en-1-ol	0.01	54 α-Calacorene	0.08
23 Camphor	0.02	55 Elemicin	0.29
24 (Z)-4-Decanal	0.09	56 β-Calacorene	tr
25 (E)-4-Decanal	0.03	57 (Z)-iso-Elemicin	1.72
26 Decanal	0.04	58 Unknown	0.92
27 Geraniol	0.34	59 Unknown sesquiterpene	0.06
28 Undec-10-en-1-al	0.03	60 (Z)-Asarone	81.27
29 α-Copaene	0.01	61 (E)-Asarone	0.52
30 Geranyl acetate	0.11	62 Unknown	0.73
31 β-Elemene	0.07	63 Unknown	0.34
32 Methyl-eugenol	0.03	64 Unknown	0.25

60°C to 240° at 3.0°C/min; injector temperature, 250°C; injection mode, split; split ratio, 1:20; volume injected, 1μl of a solution 1/20 in pentane of the oil; carrier gas, He, 83 Kpa; linear velocity, 35 cm/sec at 60°C; interface temperature, 250°C; source temperature, 200°C; EI^+ acquisition; mass range, 41-300 amu.

Results

The separation on neutral alumina allowed the isolation of 7 fractions that contained components of increasing polarity.
Figure 1 reports the total ion chromatograms of Calamus oil (A) and of the fraction separated by column chromatografy (B-H). Table 1 reports the composition of the oil. The GC and GC/MS analysis of the fraction separated on alumina allowed the identification and the quantitative analysis even of those components coeluted in the cromatogram of the whole oil, such as α-humulene coeluted with (Z)-methyl isoeugenol, β-selinene and valencene coeluted with shyobunone, γ-cadinene coeluded with epishyobunone.

As can be seen from the chromatograms reported in Figure 1, 59 components have been identified, that represent about 93% of the oil. (Z)-Asarone is the main component of the oil, with a percentage of more than 81% of the whole oil. In addition to (Z)-asarone, the oil contains about 3% of alchenyl polymetoxybenzenes ((Z)-methyl isoeugenol, (E)-methyl isoeugenol, elemicin, (Z)-isoelemicin and (E)-asarone). Epishyobunone (3.31%), (Z)-isoelemicin (1.72%) and (Z)-methyl isoeugenol (1.11%) are the components other than (Z)-asarone, present in amounts greater than 1%. The sesquiterpene hydrocarbon fraction is qualitatively very rich, and contains 21 components. β-Gurjunene and δ-cadinene are the main components of this fraction.

On the whole, the quantitative composition of the oil, at least for the main components, is similar to that of Calamus oils extracted from tetraploid plants grown in India (6,11,12).

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