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PHYTOCHEMICAL INVESTIGATION OF *THYMUS DECASSATUS* L. 1. FLAVONOIDS AND VOLATILE OIL

By

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دراسة كيميائية لنبات الثيمس ديكساتس (الزعتر)
١ - الفلافونيدات والزيوت الطيارة

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أسفرت دراسة الفلافونيدات لنبات الزعتر عن فصل وتعريف كل من الثيمونين ٥ ، ٦ ، ٤َ - تراى هيدروكسي -٧ - ٣َ ، داى ميثوكسي فلافون وكذلك الابيجينين .

وأظهرت دراسة الزيت الطيار بواسطة كروماتوجرافيا الغاز عن التعرف على ٤١ مركب متضمنة مادة الثيمول الذي تم فصلها من جزء الزيت الطيار .

Key Words: Thymus decassatus, L., flavonoids: thymonin, 5,6,4'-trihydroxy-7-3'-dimethoxy flavone, apigenin, volatile oil, thymol.

ABSTRACT

Investigation of the flavonoidal constituents of *Thymus decassatus,* L. resulted in the isolation and identification of thymonin, 5,6,4'-trihydroxy-7,3'-dimethoxyl flavone, and apigenin. Investigation of the volatile oil by GC revealed the identification of 41 components. Moreover, thymol as the major component was isolated from the volatile oil fraction.

INTRODUCTION

The genus *Thymus;* family: *Lamiaceae (Labiatae);* is represented in Egypt by four species *viz, Thymus bovei,* Benth., *T capitatus* L., *T. decassatus* L., and *T. vulgaris* Benth. (Tackholm, 1974) which are characterized by the occurrence of flavonoids, isoflavonoids, coumarins, triterpenes, saponins, tannins, resins and volatile oil.

Thymus species are commonly used in folk medicine since a long time as a remedy for common cold, whooping cough and asthma (Morton, 1977). *Thymus* plants (Thyme) showed antimicrobial, antifungal, antiparasitic and antitussive activities (Perez-Arblaez, 1956; Van Den Broucke *et al.,* 1980). *Thymus* species are used in many galinical preparations of antitussive action against infections of respiratory organs (Van Den Broucke *et al.,* 1980). They are also used as flavouring agents for many kinds of food products.

Thymus decassatus L. is one of the indigenous species growing in Sinai.

EXPERIMENTAL

Plant Material

Thymus decassatus L. was collected from St. Catherine, South of Sinai in June 1990. the plant was kindly identified by Prof. Dr: K.H. Batanouny, Faculty of Science, Cairo University. The aerial parts of the plant were air dried and grounded into fine powder.

Apparatus and Techniques:

Melting points were measured on Kopfler microscope. GLC was carried out on Perkin-Elmer Gas Chromatograph (6° C DAN/3900) with a gradient programmer using the following conditions: Glass column; fused silica gel (25 x

0.25 mm i.d) packed with 30% 0V-101, carrier gas; Helium (0.6 ml/min), detector MS 30 FID, chart speed, 0.5 cm/min., temperature programme basically at 65° C then to 220° C at 3° C/min. MS were obtained by A.E.I., using MS-902 mass spectrometer.

Extraction and Fractionation of the Flavonoids

About 1.5 Kg of the defatted powdered plant was extracted with methanol. The solvent free extract was taken with hot water (1500 ml), filtered and extracted with chloroform (3 x 500 ml) followed by ethyl acetate (3 x 500 ml). Treatment of the chloroform extract (7 gm) with methanol afforded a yellow precipitate which was shown to be a mixture of two flavonoidal substances (thymonin and 5,6,4'-trihydroxy-7,3'-dimethoxy flavone). Separation of both compounds was achieved by column chromatography using sephadex LH-20 and eluting with 70% methanol in water and preparative TLC (polyamide, benzene: ethyl methyl ketone: methanol (8:2:2)).

About 10 gm of the ethyl acetate fraction were chromatographed on silica gel column. Elution was affected with benzene, chloroform, ethyl acetate, and acetone mixtures. Apigenin was isolated from chloform/ethyl acetate fraction. (1:1).

TLC was carried out using polyamide developed with benzene: ethyl methyl ketone: methanol (4:3:3:) PC was carried out using 3 MM paper chromatography developed with 30% acetic acid. Detection was carried out by UV light before and after exposure to ammonia (Mabry, T.J. *et al.*, 1970).

Flavonoidal Substance I (Thymonin)

Fractions (15-35) eluted with 70% methanol (chloroform fraction, sephadex LH-20 column), gave after crystallization from methanol a yellow crystalline substance; m.p. 221-222° C, which has been identified by TLC, UV and MS (M^+ at *m/e* 360 and fragment ion peaks at *m/e* 346, 345, 302, 197) as 5,6,4'-trihydroxy-7,8,3'-trihydroxy flavone (Thymonin), (Van-Den Broucke, *et al.*, 1982).

Flavonoidal Substance II (5,6,4'-trihydroxy-7,3'-dimethoxy Flavone)

Fractions (50-110) eluted with 70% methanol (chloroform fraction, sephadex LH-20 column) gave after crystallization from methanol, a yellow crystalline substance; m.p. 245-250° C. It has been identified by UV spectra and MS (M^+ at *m/e* 330 and fragment ion peaks at 312, 285, 284, 151, 133) as 5,6,4'-trihydroxy-7,3'-dimethoxy flavone (Thymonin derivative).

Flavonoidal Substance III (Apigenin):

Fractions (101-108) eluted with chloroform: ethyl acetate (1:1) (ethyl acetate fraction, silica gel column) gave, after crystallization from methanol a substance; m.p. 345-346° C both alone and when admixed with authentic apigenin. UV and MS (M^+ at *m/e* 270, and fragment ions peaks at 242, 213, 153, 152, 124) confirmed its identity as apigenin.

Preparation of the Volatile Oil:

About 250 gm of the fresh plant material (leaves and flowers) were subjected to water distillation for about three hours (Guenther, 1949) (0.24% V/W).

Isolation of Thymol:

About 2 ml of the volatile oil fraction were dissolved in chloroform (10ml) and then shaked with 5% KOH (3 x 5 ml). The total phenates were acidifed with dilute HCl and extracted with chloroform (3 x 10 ml). Crystallization of the chloroform extract from methanol afforded a substance; m.p. 52-53° C which showed a single spot on TLC as authentic thymol. GLC of the isolated substance revealed the presence of main peak (97.5%) corresponding to that of thymol in addition to a minor one (2.5%) corresponding to that of carvacrol.

RESULTS AND DISCUSSION

The isolation of the flavonoids was carried out by extracting the defatted powdered plant material with methanol followed by solvent fractionation using chloroform and ethyl acetate.

Investigation of the chloroform fraction revealed the separation of a crystalline substance which was proved by TLC to be a mixture of two flavonoids. Successive column chromatographic fractionation using sephadex LH-20 followed by preparative TLC on polyamide succeeded in the separation of thymonin and 5,6,4'-trihydroxy-7,3'-dimethoxy flavone.

Column chromatographic fractionation followed by paper chromatography of the ethyl acetate fractions succeeded in the isolation of apigenin, in addition to thymonin and 5,6,4'-trihydroxy-7,3'-dimethoxy flavone.

Investigation of the volatile oil fraction by GLC analysis revealed the presence of 41 components (65.67%), their identification was compared by comparing retention times with authentic substances (Table 1). Thymol represents the main constituents amounting to 69.76% of the oil.

Table 1
Volatile oil isolated from *Thymus decassatus* L.

Compound	Rel. %	RT (min.)
Cyclene	0.181	1.50
α- Pinene	0.295	1.65
Camphene	0.542	2.13
β-Pinene	0.180	2.42
Sabinene	1.424	3.09
Myrcene	1.731	4.38
Carene	0.597	4.84
α- Terpinene	1.932	5.39
P-Cymene	0.645	6.07
Limonene	1.731	6.79
β-Phellandrene	2.062	8.87
Cis-β-Ocimene	0.418	9.58
Trans-β-Ocimene	0.629	10.01
λ-Terpinene	1.747	11.22
Linalool	1.235	13.01
Camphor	0.270	14.03
Linalyl acetate	0.366	14.04
Myrtenol	0.416	14.73
Borneol	0.798	16.21
D.H. Carvone	0.596	17.24
Pulegone	0.087	19.03
Carvone	0.543	19.80
P-Allyl anisaldehyde	0.036	25.81
Cuminyl alc.	0.098	26.87
Bornayl acetate	0.092	30.2

Table 1 Contd.

Compound	Rel. %	RT (min.)
Carvacryl acetate	0.017	31.30
α- Elemene	0.177	32.47
β- Elemene	0.061	33.27
Caryophyllene	0.095	33.81
Elemone	0.140	36.28
Humulene	0.057	37.68
Neral	0.138	42.58
Carvacrol	1.780	43.75
Thymol	69.67	44.84
Lauric acid	0.749	47.22
Cadinol	0.722	48.57
Bisabolol	0.631	49.74
Ilicic acid	0.554	51.34
Maaliene	0.241	52.94
Eudesmol	0.294	54.52
Elemenon	0.117	57.90
Germacrone	0.143	71.04

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