Chemical Constituents of *Heliotropium hirsutissimum* Grauer (Heliotrope) (Sigil otu, Bambil) (Sonnenwende) and Ecotype

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The aim of this study is to examine the chemical constituents of *Heliotropium hirsutissimum* GRAUER naturally spreaded in Denizli and its ecotype spreaded a hotter region in Izmir (Bornova-campus) and to identify them by GC-MS. After the extraction of fresh leaves, one alkaloid and a mixture of phenols, essential oils: 2,3- butanediol, 2-3- dimethyl- (CAS) pinacol (other name: tetramethylethylene glycol), phenol, 4-(3-hydroxy-1-propenyl-2-methyl-(CAS) coniferyl alcohol (other name 4-hydroxy-3-methoxycinnamyl alcohol), 1H-pyrrolizine-7-methanol-2,3,5,7,a-tetrahydro-1-hydroxy-(IS-Cis)- (CAS)=heliotridine, Xycaine\$ Acetamide,2-(diethylamino)-N-(2,6-dimethylphenyl) and 2,6,10,14,18,22-tetracosahexaene (other name: squalene) were identified in the species spreaded naturally in Denizli. Izmir ecotype gave a mixture of essential oils and phenol 1-hexadecanol; tetracosane acid, butyl 2-methylpropyl ester, phenol: phenol, 4,4'-(1-methylethylidene)bis-(CAS) 2,2-bis(p-hydroxyphenyl)propane, ethanol, 2-2'-(1-2-ethanediol bis(oxy))bis-(CAS)triethylene glycol, octadecanoic acid (other name stearic acid), 2-hydroxy-1-(hydroxymethyl) ethyl ester (CAS) and 2-Monostearin. The main components of the two ecotypes was 1,2-benzene dicarboxylic acid and hexadecanol. The presence of triethylene glycol was found to be as the significant structural difference of Izmir ecotype. These results indicated that the composition of heliotrops highly dependent on the climate conditions.

Key Words: *Heliotropium hirsutissimum*, Ecotype, Chemical constituents.

INTRODUCTION

The genus *Heliotropium*, a known source of pyrrolizidine alkaloids, flavonoids and geranyl aromatic derivatives, is comprised of about 250 species distributed throughout both hemispheres¹, with 14 species and two of them endemic to Turkey². One of them *Heliotropium ferrugineogriseum* NAB is an Irano-Turanian the other is *H. haussknechtii* BUNGE. Mediterranean element and distributes throughout East Anatolia (mezopotamia) and South Anatolia region, respectively. This erect or decumbent annual or perennial semi shrubby herb and can grow up to 50 cm high and possesses purple flowers in scorpioid cymes. Leaves are alternate, calix parted, stamens are in the corolla tube and have short filaments, throat scales are absent².

This herbaceous plant is known to contain pyrrolizidine alkaloids (PAs)³⁻⁵ and has already been reported to be responsible for intoxications of horses, goats and sheep^{6,7}.

The Boraginaceae family is well known for containing pyrrolizidine alkaloids (PAs) that are responsible for several cases of human and cattle intoxication resulting in chronic hepatotoxicity. The pyrrolizidine alkaloids also induce important risks of carcinogenicity, mutagenicity and teratogenicity^{8,9}.

In this study, essential oil and alkaloid contents of *Heliotropium hirsutissimum* naturally spreaded on Denizli and also its ecotype spreaded a hotter region in Izmir (Bornova-campus) were identified by GC-MS. Genus *Heliotropium hirsutissimum* has a large distribution area in Turkey as being an South Mediterranean element spreaded also in Bulgaria, Greece, Aegean, N. Africa, Syria and Palestine¹⁰. Like many of the plants of this geographic area, they characteristically produce a resinous exudate that covers the leaves and stem¹. These exudates have been associated with a complex defense mechanism. There is experimental evidence indicating that the resins are part of a protective cover that prevents excessive water evaporation¹¹. These resins have been proposed to play an extensive role against phytophagous organisms and UV radiation in the range 180-320 nm^{12,13}. Researchers described the isolation and structural elucidation of rel-(8R,9R)-carrizaloic acid¹⁴ (**1**) 3-[rel-(8R,9R-9-hydroxy-9,13,13-trimethyl-12-oxo-10-cyclo-hexenyl)methyl]-4-methoxybenzoic acid, a new compound and three known flavonoids from the resin exudates of *Heliotropium huascoense*¹³. They have also studied the antifeedant, insecticidal and antifungal effects of **1** and its methyl derivative (**2**) against several divergent

phytophagous insects (the beetle *Leptinotarsa decemlineata*, the lepidopteran *Spodoptera littoralis* and the aphid *Myzus persicae*) and plant pathogens (*Fusarium moniliforme* and *Aspergillus niger*). The related compound filifolinol¹⁵, an aromatic geranyl derivative isolated from *H. filifolium* has been included in the biological tests for comparison purposes. The aim of this study is to describe the chemical constituents of *H. hirsutissimum* Grauer, spreads in Denizli, which is hexaploid ($2n = 48$) and never occurs in thermal habitats and affinities clearly lie with the annual *Heliotropium thermophilum* and Izmir ecotype of *H. hirsutissimum* Grauer spreads in a hotter region than Denizli ecotype. Tan et al.¹⁶, was described *H. thermophilum* as a species new to science and illustrated with its diploid chromosome number of $2n = 16$. It was restricted to the province of Aydin bordering on Denizli in SW Anatolia and was of interest on account of its unusual habitat, which was a geothermal area with ground temperatures of 55-65 °C. Micromorphological, ecological and ecophysiological data for the new taxon have been provided. Ecological isolation has allowed the evolution and differentiation of a new and distinct species¹⁶.

EXPERIMENTAL

Plant material: *Heliotropium hirsutissimum* was collected during the flowering season in October 2008, in Denizli West Anatolia and also was collected in campus of University of Ege in Izmir and identified by Prof. Dr. Yusuf Gemici from Herbarium Centre of University of Ege.

Extraction and isolation: The resinous exudates of *H. hirsutissimum* were obtained by dipping 100 g of each fresh plant in cold MeOH, EtOH, CHCl_3 , petroleum ether and distilled water for 15-20 times. The extracts were concentrated to afford 172.41 mg, (EtOH, MeOH), 200 mg (petroleum ether), 166 mg (CHCl_3) of residues and concentrates of campus ecotype gave 172.41 mg (MeOH), 161.3 mg (EtOH), 147 mg (CHCl_3) residues.

100 g of each fresh plant extracted in a blender with 300 mL cold distilled water and waited for 10 days by shaking sometimes. Then, this samples in water were taken in Clevenger apparatus to being the distillation process obtained distillate were partitionated to with dichloromethane and extracts were evaporated to dryness to afford 0.06 g for Denizli ecotype and 0.02 g residues for Izmir (campus) ecotype of plant.

Each residue were placed on 60 °C water both to add % HCl and filtrated partition were extracted with petroleum ether. After separation this mixture alkali watered partition were obtained and 25 % NH_3 were added by shaking until pH were regulated to 13. This solution was extracted with CHCl_3 and total alkaloids were obtained after with anhydrous Na_2SO_4 filtration as 3.14 g from Denizli ecotype and 2.54 g from Izmir ecotype.

A portion of this resinous residues (3.14 g in MeOH soluble Denizli) was fractioned by flash column chromatography on Silica gel [RP-C18 (25-40 μm) Merck], using eluent system of CH_2Cl_2 together with increasing amounts of CH_3OH to obtain 134 fractions, each of 100 mL. The fractions were monitored by TLC on Si gel (Silica Gel 60 F254) using the same solvent systems $\text{C}_6\text{H}_5\text{CH}_3\text{-CH}_3\text{OH-0.1 mL NH}_3$ (95:5),

$\text{C}_6\text{H}_5\text{CH}_3\text{-CH}_3\text{OH-CH}_2\text{Cl}_2$ (70:10:20), $\text{C}_6\text{H}_5\text{CH}_3\text{-CH}_3\text{OH-CH}_2\text{Cl}_2$ (50:20:30) and 100 % MeOH for each of them. Visualization was effected with H_2SO_4 (25 % v/v) by heating the samples of TLC plates. Same fractions were combined and examined with GC-MS.

GC-MS identification: Shimadzu GCMS QP 5050A System with HP-1 fused silica capillary column (25 m, 0.32 mm i.d., 0.17 μm film thickness % dimethylpolysiloxane) was used. Injection heat was 250, inter space heat was 280, ionization mode was EI, energy of electrons were 70 eV and mass space was 40-500 m/z. Heat was programmed as 80, 10, 220 and 5 °C/min, 250 °C/10 min in helium gase with an fluent rate of 1.2 mL/min.

RESULTS AND DISCUSSION

Elution of 2.54 g residue (Izmir ecotype) with solvent system of Toluene-MeOH-0.1 mL NH_3 (95:5) gave a mixture of essential oils and phenol named: 1,2-benzenedicarboxylic acid diethyl ester; 1,2-benzenedi-carboxylic acid, butyl 2-methylpropyl ester; 4,4-(1-methylethylidene)*bis* phenol; 1, 2-benzenedicarboxylic acid, *bis*(2-ethylhexyl) ester; (0.0050 g) (Fig. 1).

$\text{C}_6\text{H}_5\text{CH}_3\text{-CH}_3\text{OH-CH}_2\text{Cl}_2$ (70:10:20) gave a mixture of phenols, assential oils and alkaloid: 2-3-butanediol, 2-3-dimethyl-(CAS) pinacol; phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-(CAS) coniferyl alcohol; 1*H*-pyrrolizine-7-methanol, 2,3,5,7-a-tetrahydro-1-hydroxy-(1*S-cis*)-(CAS) heliotridine; xycaine (acetamide, 2-(diethylamino)-N-(2,6-dimethylphenyl)(CAS)2-(diethylamino)-N-(2,6-dimethyl) (0.0043 g) (Fig. 2).

$\text{C}_6\text{H}_5\text{CH}_3\text{-CH}_3\text{OH-CH}_2\text{Cl}_2$ (50:20:30) gave a mixture of essential oils 1-2-benzene dicarboxylic acid, diethyl ester (CAS) ethyl phthalate; hexadecanoic acid, methyl ester (CAS) methyl palmitate; 1,2-benzene dicarboxylic acid, *bis*(2-methyl propyl)ester (CAS) isobutylphthalate 1-hexadecanol (CAS) cetol hexacosane (CAS) *n*-hexacosane; 1,2-benzenedicarboxylic acid, *bis*(2-ethylhexyl) ester (CAS) *bis*(2-ethylhexyl)-phthalate; 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-(CAS) squalene from Denizli ecotype (0.0311 g) (Fig. 3).

The elute obtained with $\text{C}_6\text{H}_5\text{CH}_3\text{-CH}_3\text{OH-0.1 mL NH}_3$ (95:5) gave a mixture of essential oils named ethanol, 2,2-(1,2-ethanediolbis(oxy))*bis*-(CAS) triethylylene glycol; 2,5,8,11,14-pentaoxa hexadecane-16-ol (CAS) pentaethylene glycol monomethyl ether; 9-octadecenamide, (Z)-(CAS) oleoamide (Fig. 4).

$\text{C}_6\text{H}_5\text{CH}_3\text{-CH}_3\text{OH-CH}_2\text{Cl}_2$ (70:10:20) gave a mixture of assential oils and phenol 1-hexadecanol (CAS) cetol; tetracosane acid, butyl 2-methylpropyl ester (CAS) N-butyl isobutyl phthalate, phenol, 4,41-(1-methylethylidene)*bis*-(CAS) bisphenol A(*p,p*-isomer) (Fig. 5).

100 % Methanol gave a mixture of essential oils named 1,2-benzenedicarboxylic acid, diethyl ester (CAS) ethyl phthalate; ethanol, 2,2-(1,2-ethane diylbis (oxy)) *bis*-(CAS) triethylene glycol; 2,5,8,11,14-pentaoxahexadecan-16-ol (CAS) pentaethylene glycol monomethyl ether; 9-octadecenamide, (Z)-(CAS) oleoamide; octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (CAS) 2-monosterain from Izmir ecotype (Fig. 6).

Fig. 1. Chromatograms spectra of Denizli 1 (toluene + methanol + 0.1 mL NH₃)

Fig. 2. Chromatograms spectra of Denizli 2 (toluene + methanol + dichloromethane 70:10:20)

Fig. 3. Chromatograms and spectra of Denizli 3 (toluene + methanol + dichloromethane 50:20:30)

Fig. 4. Chromatograms and spectra of Kampus 1 (toluene + methanol + 0.1 mL NH₃)

Fig. 5. Chromatograms and spectra of Kampus 2 (toluene + methanol + dichloromethane 70:10:20)

Fig. 6. Chromatograms and spectra of Kampus 3 (100 % methanol)

Denizli ecotype: 1-2-Benzenedicarboxylic acid di-ethyl ether (other names: phthalic acid). 1-2-Benzenedicarboxylic acid (other names: phthalic acid). Buthyl 2-methyl propyl ester (other names: phthalic acid). Phenol 4,4'-(1-methylethylidene) (other names: phthalic acid). 2,3-Butanediol, 2-3-dimethyl-(CAS) pinacol (other name: tetramethylethylene glycol). 4-(3-Hydroxy-1-propenyl-2-methyl-(CAS) coniferyl alcohol (other name 4-hydroxy-3-methoxycinnamyl alcohol). 1*H*-Pyrrolizine-7-methanol-2,3,5,7, α -tetrahydro-1-hydroxy-(IS-*cis*)-(CAS)=heliotridine. Xycaine [acetamide, 2-(diethylamino)-N-(2-6-dimethyl)]. Hexadecanoic acid (other name: palmitic acid). Methyl ester (CAS) methyl palmitate. 1-Hexadecanol (CAS) cetol. Hexacosane (CAS) *n*-hexacosane. 2,6,10,14,18,22-Tetracosahexaene (Other names: squalene). 2,6,10,15,19,23-Hexamethyl-(CAS) squalene.

Izmir (campus) ecotype: Ethanol, 2-21-(1-2-ethanediol bis(oxy)) bis(CAS) triethylene glycol. 2,5,8,11,14-Pentaoxahexadecan-16-ol (CAS) pentaethylene glycol monomethyl ether. 9-Octadecenamide, (Z)-(CAS) oleoamid. 1-Hexadecanol (CAS) cetol. Tetracosane (CAS) *n*-tetracosane, 1,2-benzenedicarboxylic acid diethyl ester. 2,2'-(1-2-Ethanediyl bis(oxy)) bis(CAS) triethylene glycol. 2,5,8,11,14-Pentaoxohexadecan-16-ol (CAS) pentaethylene glycol monomethylether. 9-Octadecenamide, (Z)- (CAS) oleoamid. Octadecanoic acid (other name stearic acid). 2-Hydroxy-1-(hydroxymethyl) ethyl ester (CAS) 2-monostearin, phenol, 4,4'-(1-methylethylidene) bis-(CAS) 2,2-Bis(*p*-hydroxyphenyl)-propane, tetracosane (CAS) *n*-tetracosane

TABLE-1
RETENTION TIMES AND RELATIVE PER CENT OF
CHROMATOGRAMS AND SPECTRUMS

Retention time	Relative (%)	Retention time	Relative (%)
8.148	1.58	18.745	15.36
10.292	2.56	20.437	8.07
12.112	1.14	22.097	11.62
13.072	8.77	24.057	7.30
14.497	1.22	26.583	7.32
15.653	15.39	29.971	2.55
16.021	7.54	34.418	2.05
17.226	4.13	—	—

It should be noted that the experimental RI values for volatile oil compounds were different from the literature values¹⁷ e.g., 1,2-benzene dicarboxylic acid was the main component of the two ecotypes and hexadecanol was the major constituent instead of 1-dodecanol by Machan *et al.*¹⁷. But the presence of triethylene glycol was found to be as the significant structural difference of Izmir ecotype.

Heliotridine, a novel pyrrolizidinediols¹⁸ that have in common the azacyclo (3:3:0) octane ring system have been reported to act as antitumor, hypotensive, local anesthetic, antispasmodic, antiinflammatory, carcinogenic or hepatotoxic agents¹⁹ has been isolated as a major product of *H. hirsutissimum* Denizli ecotype from the aerial parts of plant. Heliotridine is the most common necine base in American and Indian *Heliotropium* spp.²⁰, probably found free together with the esters, e.g., lasiocarpine and heliosupine²¹.

Between the 20 *Heliotropium* species collected in Mexico and USA and one species from Spain, heliotridine was found only in one species^{22,23}. *H. bovei*, *H. floridum*, *H. megalantum*, *H. sinuatum*, *H. strygosum* was reported to contain heliotridine²⁴, phenol, 4,4'-(1-methylethylidene)bis-(CAS) 2,2-Bis(*p*-hydroxyphenyl)propane was present in a higher ratio (57.06%) in Denizli ecotype rather than Izmir ecotype (15.73%). Denizli ecotype was rich in phenol contents with the presence of additive phenol, 4-(3-hydroxy-1-propenyl)- 2-methyl-(CAS) coniferyl alcohol content

Isoprenoid compounds like as isoprene, monoterpenes and sesquiterpenes are synthesized and emitted by some plant species (e.g., *Quercus ilex*), but not all plant species have this ability²⁵. Recent work indicated that isoprene acted on photosynthetic membranes to protect against thermal damage²⁶. Monoterpenes and sesquiterpenes which have diverse ecological functions, such as antiherbivory and antimicrobial defence or pollinator attraction²⁷ are non essential isoprenoids. Besides these abscisic acid (C15), gibberellic acid (C20) as being hormones and sterols and carotenoids are essential isoprenoid compounds which have protective functions in photosynthetic systems. Plant triterpenes, such as oleanolic acid^{28,29}, ursolic acid²⁹, a-hederin^{28,29} and glycyrrhizin³⁰ are aliphatic polycyclic compounds based on a skeleton with 30 carbon atoms and they are synthesized from squalene as the precursor³¹. Squalen, a triterpene sterol, was found in Denizli ecotype of *H. hirsutissimum*. This compound first isolated from fish liver oils but is also found in plant oils and mammalian fats³². Because of its unsaturated bonds, the squalene structure features reactivity and thermolability³³. So, we can explain the reason of *H. hirsutissimum* Grauer affinities why clearly lie with the annual *H. thermophilum*, which spreads in a geothermal area with ground temperatures of 55-65 °C

n-Tetracosane found in Izmir ecotype of *H. hirsutissimum* has been identified in the unsaponifiable matter of a plant wax and a fatty oil. An isotope labeling study in humans³⁴ concluded that the fraction of dietary stearic acid oxidatively desaturated to oleic acid was 2.4 times higher than the fraction of palmitic acid analogously converted to palmitoleic acid. Also, stearic acid was less likely to be incorporated into cholesterol esters. In epidemiologic and clinical studies stearic acid was associated with lowered LDL cholesterol in comparison with other saturated fatty acids³⁵. These findings may indicate that stearic acid is healthier than other saturated fatty acids. Hydrogenation of palmitic acid yields cetyl alcohol, which is used to produce detergents and cosmetics. The underlying method of drug delivery is similar to that used with decanoic acid found in Izmir ecotype also to deliver long-acting depot medication, in particular, neuroleptics such as haloperidol decanoate.

Application of earlier drought stress induction by polyethylene glycol solutions of elevated strength (8 and 16 %) severely reduced growth and yield attributes of soybean when compared to its later application. Furthermore, increased in the endogenous contents of jasmonic acid, salicylic acid and abscisic acid in response to drought stress demonstrated the involvement of these hormones in drought stress resistance^{36,37}. The most common dehydration method used for natural gas is

the absorption of water vapor in the liquid desiccant, triethylene glycol. For this reason, the presence of triethylene glycol, pentaethylene glycol monomethylether in Izmir ecotype of *H. hirsutissimum* revealed that the plant was under drought stress.

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