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Research Article

Trachyspermum ammi Hydrosol, An Almost Pure Source of Thymol; Analysis of the Recovered Essential Oil from Different Samples

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ABSTRACT

Essential oil and hydrosol of *Trachyspermum ammi* (L.) Sprague (Apiaceae) are traditionally applied for chronic pain and neural ailments such as tremor, paralysis, and palsy. Unlike numerous studies on the chemical composition of *T. ammi* essential oil, no certain report has been performed on the hydrosol chemical compositions. Current study aimed to chemically evaluate different *T. ammi* hydrosols. Ten hydrosol samples collected from Fars (Iran) markets and as well as a standard hydrosol were subjected to GC and GC/MS following recovering by a liquid extractor. Hierarchical Cluster Analysis and Principal Component Analysis methods were performed to determine the similarity of those samples. Out of 14 constituents identified in studied sample, thymol (84.33-95.62%) was found as the main component (97.77% for the standard hydrosol). Cluster Analysis divided the samples into two main groups. As a by-product in the extraction of plants' essential oil, *T. ammi* hydrosol can be considered as an almost pure, but cheap and easy producible resource of natural thymol. In accordance with the pharmacological properties of thymol, this preparation can be applied in numerous relevant clinical and phytopharmaceutical approaches.

Keywords: Aromatic water, Hydrosol, *Trachyspermum ammi*, Thymol, GC, GC/MS

INTRODUCTION

Herbal aromatic waters, also spoken as hydrosols or distillates, are aqueous products usually obtained via hydro or steam distillation of different plants parts. In regard of the aromatic plants, respective hydrosols are yielded during the distillation procedure and essential oil extraction[1]. Generally, via water distillation, some water soluble parts of the yielded essential oil may transfer to the distillated aqueous phase and produce plant's aromatic water[2,3]. Physicochemically, herbal hydrosols are high diluted and acidic liquids with pleasant to unpleasant and dissimilar to similar odor to the respective essential oil extracted simultaneously[4].

Hydrosols are generally recognized for the commercial and medico-pharmaceutical applications[5]. Medical, pharmaceutical and cosmetic applications of plants essential oils and respective hydrosols date back to very long time ago[6]. Currently, numerous herbal hydrosols are commercial, pharmaceutically or medically by people throughout the world.

Trachyspermum ammi (L.) Sprague (TA) –from the family Apiaceae- is one of the most well-known medicinal herbs with both medical and nutraceutical properties. Clinical aspects of TA essential oil and hydrosol in medieval manuscripts of Persian medicine involve the application for chronic pain and neural ailments such as tremor, paralysis, and palsy. Management of ophthalmic and otic infections, gastrointestinal and respiratory complications is other traditionally reported applications of TA[7,8]. With reference to the current findings on TA essential oil, antimicrobial and antiviral, analgesic, antitussive, anti-inflammatory, antioxidant, antitumor and bronchodilatory activities have been demonstrated[9-14].

Other than the clinical and pharmaceutical applications, numerous studies have been released on the chemical composition of TA essential oil[14-15]. On the contrary, there are no certain reports on the chemical compositions of TA hydrosol which is generally extracted concurrent with the essential oil. As the mentioned hydrosol is extensively marketed and applied ethnomedically in Iran and nearby countries, performing a practical analysis on the composition of this pharmaceutical hydrosol could be of interest. Accordingly, the current study aimed to evaluate the chemical constituents of different TA hydrosols.

MATERIALS AND METHODS

Sample preparation

Ten different samples of TA hydrosols were purchased from traditional medical markets around the Fars province (Iran). These samples were numbered from 1 to 10. On the

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Table 1: Chemical composition of different TA hydrosol samples

Compounds	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	St	KI^C	KI^R
p-Cymene	-	0.73	-	-	-	-	-	-	-	-	-	1025	1024
Alpha-Terpinolene	-	-	-	-	-	-	-	-	0.45	-	-	1100	1096
Linalool	-	-	2.72	-	-	-	-	-	-	-	-	1101	1098
Terpinene-4-ol	0.49	1.86	0.87	-	-	0.81	-	0.56	0.5	-	-	1180	1177
Dill ether	-	-	-	-	-	0.69	-				-	1193	1186
Dihydro carveol-neo	-	-	1.61	-	-	-	-	-	-	-	-	1197	1194
Cuminaldehyde	-	-	-	-	-	0.68	-				-	1245	1241
Carvotanacetone	-	-	3.34	-	-	0.83	0.62	-	-	1.38	-	1250	1247
Anethole-E	-	-	1.05	-	-	-	-	-	-	1.52	-	1288	1284
Thymol	88.32	93.23	85.56	88.66	94.64	92.20	90.61	95.62	84.71	84.33	97.77	1296	1290
Carvacrol	8.78	2.34	4.85	9.84	1.82	3.47	6.52	1.68	9.38	1.67	0.91	1304	1299
Piperitenone	-	-	-	-	-	-	-	0.57	-	-	-	1345	1343
Dill apiole	-	-	-	-	-	-	0.68	-	0.74	7.76	-	1629	1620
Unknown	2.40	1.16	-	1.50	3.54	1.33	1.57	1.57	4.21	3.73	1.32	1965	-
Identification	97.60	98.84	100	98.50	96.46	98.67	98.43	98.43	95.79	96.27	98.68		

Compounds were identified by combination of both mass spectra and retention indices. KI^C represents the retention indices which were calculated against C_8-C_{22} *n*-alkanes on mentioned column (KI^R). Compounds have been sorted regarding retention indices on DB-5 MS capillary column. S_1-S_{10}: different collected samples; St: standard hydrosol

other hand, a sample of TA seed was subjected to hydrodistillation and the yielded hydrosol was collected and considered as a standard sample. All samples were kept in the fridge (4ºC) for further steps.

Essential oil recovering and concentration

Using a liquid extractor, the essential oil fractions of all collected samples were recovered in two stages. Firstly, 500 ml of each sample was mixed with 500 ml of petroleum ether as a solvent. The solvent was then heated to 45ºC for about 150 min to recover the essential oil from an aqueous phase to the organic phase. The remains of organic phase from the first step were removed and subsequently additional amount (500 ml) of fresh petroleum ether was added to the system. As for the first stage, the sample was heated for 150 min. The stage carrying out of this step was to increase the essential oil yield in the organic phase (1). The recovered essential oil respective to each sample was then individually concentrated in a basic rotary evaporator equipped with a vacuum pump. Each concentrated sample was kept in amber glass vials for further investigation.

Gas chromatography and analysis

GC/FID analysis of the recovered volatile oil samples was carried out on a gas chromatograph Agilent technologies (7890A) apparatus attached to HP-5 column (25 m length × 0.32 mm i.d.; film thickness 0.52 µm) connected to flame ionization detector (FID). Nitrogen was used as carrier gas (flow rate: 1 ml/min, split ratio: 1:30). The injector and detector temperatures were adjusted at 250 and 280°C, respectively. Column temperature was linearly programmed from 60 to 250°C (at rate of 5°/min) and subsequently was held at 250°C for 10 min. Essential oil samples in dichloromethane (~1%) were consecutively injected to the system.

The adjusted GC/FID method and condition were employed for GC/MS analysis. The process was performed via an Agilent technologies (7890A) gas chromatograph equipped with a HP-5MS capillary column (coated with phenyl methyl siloxane, 30 m × 0.25 mm i.d.) and connected to a mass detector (Agilent technologies model 5975C). The carrier gas (Helium) flow rate was selected as for the GC/FID. The mass spectrometer was acquired in EI mode (70 eV) in a mass range of 30-600 m/z. The interface temperature was 280°C. The data from GC/MS were used to identify the samples' constituents. This process compared the resulting Kovats indices (KI) calculated by using a homologous series of *n*-alkanes C_8-C_{22} as well as

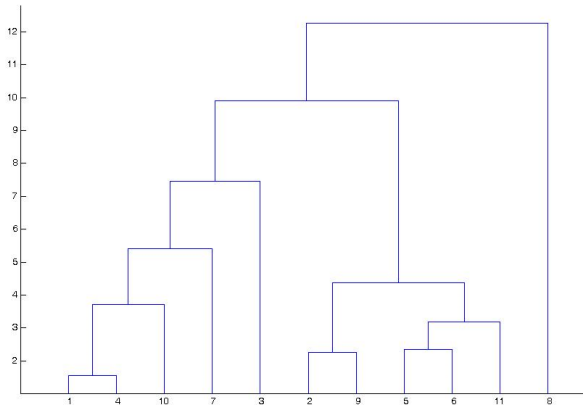

Figure 1: Hierarchical Cluster Analysis (HCA) method for different hydrosols (1-10; different collected samples, 11: standard hydrosol derived from hydrodistillation of TA seeds)

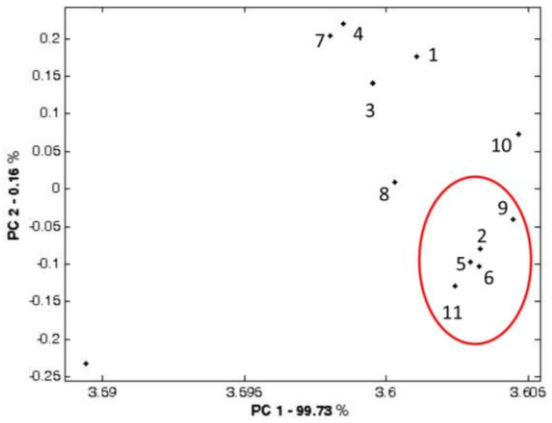

Figure 2: Principal Component Analysis (PCA) method for different hydrosols (1-10; different collected samples, 11: standard hydrosol derived from hydrodistillation of TA seeds)

mass spectra data of the components with those mentioned in the respective literatures[15,16].

Samples cluster analysis

In order to cluster the 11 samples based on the composition of essential oils, Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) methods were carried out. For this purpose the percentage composition of essential oil was considered as variable and a vector was accordingly generated for each sample. The resulted matrix was thereafter subjected to MATLAB (Mathworks Inc.) in order to perform HCA. Cluster definition was done by means of Euclidean distance as a measure of similarity using unweighted pair group method (UPGMA). In another study PCA was performed on the whole data set using singular value decomposition as a data reduction technique. The plot of the first two principal components was used to represent the dataset in two dimensional spaces.

RESULTS AND DISCUSSION

In the present study, essential oil components of ten different TA hydrosol samples were recovered by a liquid-liquid extractor and subsequently subjected to CG/FID and GC/MS for analysis. For more evident conclusion, a standard and pure hydrosol sample of TA seeds was also prepared. GC and GC/MS analysis of those samples resulted in the identification of totally 14 different components (Table 1). Thymol was found as the main constituent in all samples. The thymol content ranged from 84.33 to 95.62% in collected samples. The standard sample contained thymol in highest amount (97.77%). Compared to the major constituents of TA seeds essential oil, aromatic water is richer in thymol. Most of the previous studies on TA seeds essential oil have reported the abundance of thymol up to nearly half of the total oil (15, 17-18). The very high presence of thymol in TA hydrosols can introduce this type of preparation as an almost pure natural, but cheap and accessible source of pure thymol for pharmaceutical and pharmacological investigations.

In regard of the cluster analysis, two main groups (clade) were identified for those 10 samples and the standard one (Figure 1). These two groups involved samples 2,9,5,6 and 11 in the first and 1,4,10,7 and 3 in the second one. HCA analysis showed that sample 8 is less similar to other samples in regard of the components. Therefore, this sample was not included in any of those two groups. Using PCA, the resulted data were confirmed (Figure 2). According to the Table 1 and cluster analysis, similarity of samples 5 and 6 are very close to the standard sample.

There are numerous reports on commercial, pharmacological and clinical properties of pure thymol. Researchers have explored and proved the potent antimicrobial[19], antioxidant[20], anti-inflammatory[21], nociceptive, and local anaesthetic activities of this constituent as well as antileishmanial and effects on the morphological development of oocytes[22-24]. It is also revealed that thymol can be used to inhibit the growth of food borne pathogens in nutritional preparations[25]. Moreover, the effectiveness of thymol has been assessed on the olfactory memory and gene expression levels in honeybee brain[26]. As this compound is extensively applied in food and pharmaceutical sciences, TA hydrosol with rich amount of thymol can be considered as a natural source of this medicament.

CONCLUSION

As a by-product in the extraction of plants' essential oil, TA hydrosol can be considered as a cheap and easy producible resource for the extraction of natural thymol. In accordance with the pharmacological properties of thymol, the hydrosol of this herbal medicine can be applied in numerous relevant clinical and phytopharmaceutical approaches.

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