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Volatiles of Turkish *Cyperus rotundus* L. Roots[§]

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Abstract: Purple nutsedge, *Cyperus rotundus* L. (Cyperaceae) is an invasive plant grown in all around the world. *C. rotundus* rhizomes are called "topalak" and an analgesic plant for the treatment of stomach ache in infants, folk medicine in Turkey. The volatile compounds of *C. rotundus* rhizomes were investigated by Headspace-SPME. Cyperene (30.5% and 28.0%), α-copaene (10.6% and12%) and α-ylangene (7.7% and10.5%) were identified as main volatile components of rhizomes and analyzed at room temperature and 40ºC respectively. This study designed to support detailed studies on underground plant parts volatiles and it is the first time to identification of volatile compounds of raw *C. rotundus* rhizomes collected from Turkey by Headspace-SPME and GC and GC-MS.

Keywords: *Cyperus rotundus* L.; purple nutsedge; rhizome; volatile compounds; Headspace-SPME.

1. Introduction

Cyperus rotundus L. (Cyperaceae) called as purple nutsedge, is an invasive plant grows different parts of the world [1]. Purple nutsedge used in traditional medicine and landscaping in China and also used in India as a soil binder. Extracts and isolated compounds of purple nutsedge have healing properties such as the reduction of fever, inflammation, and pain. Tuber extracts reduce nausea and effect as muscle relaxant [2]. This plant is one of the most extensively researched plants due to both invasive characteristic and ethnobotanical usages. There are some reviews on its medicinal and traditional usages, phytochemistry, biological and pharmacological activities [3-7]. Rhizomes of the plant are used as an Ayurvedic drog (called musta) for anti-dypsic, itching destroyer and galacto-depurant [8] and used a hyperglycaemic herb in Sri Lankan Ayurveda [9]. After our ethnobotanical investigation in Eskisehir/Turkey, it was found that rhizomes known as "topalak" and used for asthma, dyspnea and boiled with quince leaf for intake to treat pain.

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2. Materials and Methods

2.1. Plant Material

Collected *C. rotundus* rhizomes from Eskisehir, Turkey (June 2014) used for the analyses. The identification of the plant done using Davis *et al*. (1985) [1]. The fresh rhizomes were grinded. The voucher specimens are deposited in Anadolu University, Faculty of Pharmacy Herbarium (ESSE 14690).

2.2. Headspace Solid Phase Microextraction (HS-SPME)

For extraction of the sample volatiles, the manual SPME device (Supelco, Bellafonte, PA, USA) precoated by a fibre of a 65 μm thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB-blue) was used. The grinded rhizomes were put into the vial and it was sealed with parafilm. The fiber was propelled through the film layer for exposure to the headspace of the extract for 15 min at room temperature and 40°C. And then the fiber insertion of the GC-MS injection port done immediately due to desorption of the adsorbed volatile compounds for analysis.

2.3. GC–MS Analysis

Agilent 5975 GC-MSD system was used for the analysis. Innowax FSC column (0.25 mm film thickness and 60 m x 0.25 mm) was used with helium as carrier gas (0.8 mL/min). The temperature of GC oven was kept at 60°C (for 10 min) and it was programmed to 220°C at a rate of 4°C/min, and it was kept constant at 220°C (for 10 min) and then it was programmed to 240°C at a rate of 1°C/min. The injector temperature was set at 250°C. Mass spectra were itemized at 70 eV. Mass ranges were 35 to 450 (*m/z*).

3.3. GC Analysis

An Agilent 6890N GC system was used for GC analysis. TIC detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column within the same operational conditions. TIC chromatograms were used for the relative percentage amounts of the separated compounds. To identification, the volatile components were analyzed by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder3 Library) [10,11] and in-house "Başer Library of Essential Oil Constituents" built up by authentic compounds and components of known oils. The MS literature data [12, 13] was also used for the distinguishing.

3. Results and Discussion

It is aimed to investigate the volatile compounds of emitted by *C. rotundus* raw rhizomes directly captured by HS-SPME at room temperature and to determine the possible changes of them at 40°C. It was identified forty compounds. The main volatile components of rhizomes were identified as *α*-ylangene (7.7% and 10.5%), *α*-copaene (10.6% and 12%) and cyperene (30.5% and 28.0%) (at room temperature and 40ºC respectively) (Table 1). HS-SPME results within two temperature consist of monoterpene hydrocarbons (*α*- and *β*-pinene, limonene, *p*-cymene etc.), oxygenated monoterpene derivatives (terpinen-4-ol, *trans*-pinocarveol, myrtenol and etc.), oxygenated sesquiterpene derivatives (calamenene), monoterpene ketones (pinocarvone, verbenone, carvone), sesquiterpene hydrocarbons (*α*-cubebene, *α*-ylangene, *α*-copaene, cyperene, *γ*-vetivenene etc.) were determined from the rhizomes. Depend on the temperature rising, the percentages of cyperene, rotundene, limonene, *α*- and *β*-pinene amounts were decreasing while *α*-copaene, *α*-ylangene, myrtenal and *trans*-pinocarveol amounts were increasing. The SPME technique is effective to trap volatiles and combinative with the GC and GC-

MS for identifying of them. To the best of our knowledge, raw and fresh grinded rhizomes has not been studied directly used by HS-SPME for volatiles.

Table 1. Volatile compounds of *C. rotundus* rhizomes obtained by HS-SPME-GC-MS at room temperature (A) and at 40ºC (B).

Peak no	RRI	Compounds	A (%)	B (%)	Identification Method
1	1032	*α*-Pinene	4.6	3.0	a,b
2	1118	*β*-Pinene	7.0	3.2	a,b
3	1174	Myrcene	-	1.2	a,b
4	1203	Limonene	0.5	0.8	a,b
5	1280	*p*-Cymene	0.5	0.6	a,b
6	1466	*α*-Cubebene	0.3	-	a
7	1493	*α*-Ylangene	7.7	10.5	a
8	1497	*α*-Copaene	10.6	12.0	a
9	1544	Cyperene	30.5	28.1	a
10	1586	Pinocarvone	2.7	2.3	a,b
11	1600	*β*-Elemene	0.3	0.4	a,b
12	1573	Nopinone	0.2	0.3	a
13	1611	Terpinen-4-ol	-	0.1	a,b
14	1648	Myrtenal	2.1	1.7	a
15	1661	Rotundene	9.4	8.7	a
16	1670	*trans*-Pinocarveol	2.0	1.9	a,b
17	1683	*trans*-Verbenol	0.7	0.7	a,b
18	1693	*β*-Acoradiene	t	0.1	a
19	1704	*γ*-Muurolene	2.3	2.9	a
20	1725	Verbenone	0.3	0.4	a,b
21	1740	*α*-Muurolene	-	0.2	a
22	1741	Isorotundene	0.8	0.7	a
23	1733	Carvone	0.1	0.2	a,b
24	1773	*δ*-Cadinene	0.2	0.2	a
25	1804	Myrtenol	0.4	0.6	a
26	1834	*γ*-Vetivenene	-	t	a
27	1845	*trans*-Carveol	-	t	a,b
28	1834	*cis*-Calamenene	0.1	0.1	a
29	1893	Cyperene epoxide	0.9	1.0	a
30	1900	*epi*-Cubebol	t	0.1	a
31	1941	*α*-Calacorene	t	0.1	a
32	2008	Caryophyllene oxide	2.2	2.3	a,b
33	2016	Perilla alcohol	-	0.1	a
34	2071	Humulene epoxide	0.9	0.9	a,b
35	2289	4-oxo-*α*-Ylangene	0.4	0.5	a
36	2291	Cyperenone	0.1	0.1	a
37	2304	*α*-Cyperone	0.8	0.9	a
38	2316	Caryophylladienol-I	0.3	0.2	a
39	2389	Caryophyllenol-I	0.5	0.4	a
40	2392	Caryophyllenol-II	0.2	0.1	a
		TOTAL	**89.6**	**87.6**	

RRI: Relative retention indices calculated against *n*-alkane; % calculated from TIC data;
t: Trace (<0.1 %); Identification Method; a= comparison of mass spectra with the Wiley and Mass Finder libraries and retention times; b= comparison with genuine compounds on the HP Innowax column.

Quite a few studies can be reached in case making a literature scan about *C. rotundus* aerial parts or roots solvent extraction or essential oil analysis and their biological activities. Kilani-Jaziri *et al.* [14] studied on aqueous, ethyl acetate, methanol and enriched extracts by total oligomer flavonoids

(TOF), antioxidative, antimicrobial and antigenotoxic activities of dried leaves of the plant due to its traditional usages in Indian, Chinese and Japanese against spasms, stomach and inflammatory bowel diseases. It was also detected some phytochemicals (polyphenols, flavonoids, tannins and sterols) of four extracts. TOF, ethyl acetate and methanol extracts were more active than the quercetin (positive control) for superoxide radical-scavenger activity. TOF enriched extract revealed significant antimicrobial activity of some gram-negative and gram-positive bacteria. Four extracts of leaves extracts showed no antigenotoxic effect on the indicator bacteria. Rabiei *et al.* [15] carried out the ethanolic extract of tubers of plant effects on learning and memory in rat model of Alzheimer. All nucleus basalis of Meynert-lesioned rats demonstrated significant cognitive deficits along with the Morris water maze swimming test. The tuber extract were standardized for total phenolic, flavonoid and flavonal compounds. Radical scavenging activity of extract, extract effects on the plasma levels of antioxidant power and malondialdehyde (MDA) were also analyzed. Standardized extract has some repairing effects on the memory and behavioral disorders on electric lesions of the in rats. Kumar *et al.* [6] investigated *in vitro* antioxidant and free radical scavenging activities, flavonoid and total phenolic contents of 70% ethanol, methanolic and water extracts of roots. Also 70% ethanol root extract was analyzed LC-ESI-MS/MS technique. Neuroprotective effects of the same extract studied with acetylcholinesterase (AChE) activity. The extract inhibited the activity in a dose dependent manner and showed maximum inhibition of AChE at the concentration of 500 µg. Aeganathan *et al.* [16] were studied the antioxidant and antimicrobial properties of roots chloroform fraction by GC-MS analysis. Chloroform extract showed high scavenging activity at four concentration. Also the extract showed better inhibitory activity against *Staphylococcus aureus*. Along with the GC-MS analysis some compounds (*trans*-(2-chlorovinyl) dimethylethoxysilane, 5-hydroxymethyl furfural, vanillin lactoside etc.) were identified from the chloroform extract of the *C. rotundus* rhizome. Nidugala *et al.* [17] were analyzed n-hexane extract from rhizomes using by GC and GC-MS technique. Twenty seven components were identified and major molecules were hentriacanthone (7.15%) (anti-inflammatory and cytotoxic on lymphoma cells), triacanthone (6.12%) (antimicrobial and anticancer activity), nonacosane (5%), octacosane (4.38%). Mentioned components are giving some idea to explaining the therapeutic potential of *C. rotundus* extract. Yenti *et al.* [18] studied on the penetration test of gel ethanol extract of *C. rotundus* rhizomes. Ethanol extract of the rhizomes was diluted with hexane and analyzed by GC and GC-MS technique. Isovelerenal (1.69%), 2,5-dimethyl dodecane (1.65%) and -(-) caryophyllene oxide (1.48%) were determined as major compounds. Gel formula consist of ethanol extract of rhizomes (7%), hydroxypropyl methylcellulose (5%), propilengylcol (10%), nipagin (0.1%) and aquadest. The gel were tested using a Franz diffusion cell and the membranes used were back skin of mice and Whatman® paper No. 1 with Sapangler liquid modification. Gel penetration of the extract through Sapangler and skin of mice membranes were also analyzed with GC-MS and some constituents of the essential oil that was equal to the extract. Sonwa and König [19] analyzed the essential oil of *C. rotundus* by silica chromatography into a hydrocarbon fraction and identification done by GC and GC-MS. It was identified minor sesquiterpene hydrocarbons of oil ((−)-isorotundene, (−)-cypera-2,4(15)-diene, (−)-norrotundene and (+)-cyperadione). Kilani *et al.* [20] were obtained essential oil of Tunisian tubers of *C. rotundus* by steam distillation and the analysis done by GC and GC-MS. Thirty-three compounds were identified and the oil was characterized by its high content of sesquiterpenes with cyperene (30.9%), cyperotundene (8.8%), rotundene (7.6%). Along with this study, the tubers' oil showed more important antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. Tam *et al*. [21] were obtained four group of *C. rotundus* rhizomes from China. Hydrodistilled, pressurized liquid extraction and supercritical fluid extraction methods were applied to the rhizomes. These three methods were compared with each other within the extractable contents (mg/g) of five volatile compounds (*α*-copaene, cyperene, *β*-selinene, *β*-cyperone and *α*-cyperone) of rhizomes. Pressurized liquid extraction has the highest extraction efficiency, while supercritical fluid extraction has the best selectivity for extraction of *β*-cyperone and α-cyperone Aghassi et al. [22] were investigated the essential oil chemical composition of aerial parts of *C. rotundus* grown in Iran by hydrodystillation and GC and GC-MS. Twenty-two compounds were identified from the oil. The oil showed the predominance of sesquiterpene compounds with the presence of monoterpenes. The major components were cyperene (37.9%) and cyperotundone (11.2%). Lawal and Oyedeji [23] studied from essential oils from rhizomes of *C. rotundus* collected from two locations Kwa-Zulu Natal Province of South Africa. After the hydrodistillation of rhizomes

essential oils analyzed by GC and GC-MS technique. Forty-one compounds were identified. Essential oil of the locality Empangeni was rich for α-cyperone (11.0%), myrtenol (7.9%), caryophyllene oxide (5.4%) and β-pinene (5.3%). The main constituents of essential oil of locality KwaDlangezwa were β-pinene (11.3%), α-pinene (10.8%), α-cyperone (7.9%), myrtenol (7.1%) and α-selinene (6.6%). Jirovets *et al.* [24] studied the composition of essential oil which prepared by steam distilled fresh grounded pasta of the roots from South-India and SPME headspace volatiles of the essential oil by GC-FID and GC-MS and olfactoric methods. According to GC-FID analyses of essential oil, sesquiterpenes ((α-copaene (11.4%), cyperene (8.4%), valerenal (9.8%), caryophyllene oxide (9.7%), *trans*-pinocarveol (5.2%)) were found to be ascendent constituents of the essential oil of roots. About seventy volatiles were analyzed by SPME-headspace and GC/GC-MS of essential oil as follows: valencene (5.1%), *trans*-pinocarveol (7.4%), caryophyllene oxide (7.8%), valerenal (8.7%), cyperene (11.7%) and α-copaene (12.1%). Essential oil studies of aerial parts or roots of *C. rotundus* obtained by hydrodistillation show the dominance of the sesquiterpenoids [20-24].

There are some investigations on essential oils of other *Cyperus* species. Zoghbi *et al*. [25] studied the volatiles of cultivated rhizomes of *C. giganteus* by hydrodistillation and analyzed by GC and GC-MS. The major components identified as cyperotundone (25.9%) and cyperene (10.4%). Khamsan *et al.* [26] were analyzed the essential oil of aerial parts of *C. kyllingia* by GC, GC-MS. Twenty-three compounds were identified. The most representative compounds were α-cadinol (19.32%), caryophyllene oxide (12.17%), α-muurolol (11.58%), α-humulene (9.85%), and α-atlantone (6.07%). Rameshkumar *et al*. [27] investigated the Indian *C. compressus* hydrodistilled roots for essential oil and analyzed by GC and GC/MS. Cyperene (25.6%) and caryophyllene oxide (34.0%) were the major constituents and the oil has been characterized by the predominance of sesquiterpenoids (89.9%). Sesquiterpenoids dominance has also been observed within different *Cyperus* species' hydrodistilled aerial parts or roots.

Essential oil sesquiterpenoid compositions of *C. rotundus* classified basically as H-, K-, M-, O- chemotypes known from different parts of the Asia. Bicyclic sesquiterpenoids α-cyperone and β-selinene are H-type and known from Japan. Tricyclic sesquiterpenoids cyperotundene and cyperene are called O-type and known from Hawaii, Japan, Taiwan, the Philippines and Thailand. Type M contains all four of these sesquiterpenoids and studied from China, Vietnam, Japan and Taiwan. K-type known from Hawai and contain a large amount of sesquiterpene acetate [17, 28-30]. Essential oil of South Indian *C. rotundus* rhizomes were rich from valerenal (8.7%), cyperene (11.7%) and α-copaene (12.1%) [24]. Tunisian tubers of essential oil were rich from cyperene (30.9%), cyperotundene (8.8%) and rotundene (7.6%) [20]. Chinese roots were identified within the presence of α-copaene, α-cyperene and cyperene, β-selinene and β-cyperone [21]. And along with the present study, Turkish *C. rotundus* raw rhizomes volatiles are contain cyperene, α-copaene, rotundene and α-ylangene. Although the chemotypical difference can be recognized within the *C. rotundus* rhizomes' essential oils, South Indian, Tunisian, Chinese and Turkish ones have a similarity the predominance of sesquiterpenoid hydrocarbons as cyperene and α-copoene. Sesquiterpenoid content of *Cyperus* rhizomes is possibly related to allelopathic activity [22, 26].

Volatile organic compounds are emitted by plant's flowers, barks, roots and other specific structures. The roots emit volatile organic compounds when they exposed to any mechanical obstacles, allelopathy or root competition [31]. *C. rotundus* is known as "the world's worst weed" [32]. This species has an extensive underground parts of basal bulbs, thin wiry rhizomes. Within 2-3 weeks after shoot emergence, basal bulbs send out new rhizomes form additional bulbs and daughter plants [31]. *C. rotundus* rhizomes have an extensively ethnobotanical usages in different parts of the world. Volatiles are possibly responsible phytochemicals for healing properties of rhizomes and also rising up the fast vegetative reproduction of the plant.

4. Conclusion

With this study, emitting volatiles of raw *C. rotundus* rhizomes at room temperature and 40°C were trapped with HS-SPME and the volatiles identified by GC and GC-MS and chemotaxonomic place of the Turkish *C. rotundus* rhizomes volatiles are clarified. Turkish *C. rotundus* raw rhizomes volatiles are contain cyperene, α-copaene, rotundene and α-ylangene. Although the chemotypical difference can be recognized within the *C. rotundus* rhizomes' essential oils, South Indian, Tunisian,

Chinese and Turkish ones have a similarity the predominance of sesquiterpenoid hydrocarbons as cyperene and α-copaene. Sesquiterpenoid content of *C. rotundus* rhizomes is possibly related to allelopathic activity.

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