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Original Research Article

Chemical composition and antimicrobial activities of essential oil and ethanol extract of *Cyperus fuscus* L burs from Turkey

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Abstract

Purpose: *To investigate the chemical composition of the essential oil of Cyperus fuscus burs as well as the antimicrobial activities of the ethanol extracts and essential oil.*
Methods: *Fresh burs roots of C. fuscus were collected at the flowering stage in an open area in Güdül (Ankara, Turkey). Preparation of the ethanol extract, hydrodistillation of the essential oil, GC-FID-MS analysis, and agar diffusion and MIC agar dilution assays were performed to determine various parameters for the oil and extracts.*
Results: *The major compounds of the essential oil were dehydroaromadendrene (10.7 %), azulenone (8.5 %), α-selinene (7.5 %), α-ylangene (6.0 %) and β-caryophyllene (5.6 %). The essential oil of Cyperus fuscus exhibited activity against Gram-negative bacteria with minimum ınhibitory concentration (MIC) values ranging from 1000 to 31.25 µl/mL. Similarly, the ethanol extract of the burs showed good antimicrobial activity with the MIC of the ethanol extracts on ranging from 1000 µg/mL (Escherichia coli) to 250 µg/mL (Pseudomonas. Aeruginosa, $p < 0.05$). However, the ethanol extract was inactive against yeast strains.*
Conclusion: *Thus, the essential oil and ethanol extract of the studied plant can potentially be used as antimicrobial agent.*

Keywords: *Cyperus fuscus, Antimicrobial activity, Chemical composition, Essential oil.*

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INTRODUCTION

For years, the plants have been utilized by many cultures as flavoring agents and as natural preservatives in food and medical therapeutic agents [1]. Some *Cyperus* species has health benefits and nutritional value. In recent years, they are used as functional foods since they possess high amount of oleic acid, glucose, phosphorus, potassium, vitamins C and E. These burs are also used as a food additive in the preparation of oil for cooking, salad preparation, production of caramel, milk and a refreshing drink [2,3]. However, scientific interest on these natural plants have increased especially for the

protection of food against antimicrobials. For this reason, the people demand natural foods long shelf-life. These prolong both the long shelf-life of food and prevent new foodborne disease outbreaks caused by pathogenic microorganisms resistant to antibiotics. Essential oil obtained from several plants has antimicrobial [4] as well as antifungal activity [5,6].

The genus *Cyperus* members has been used in pain, fever, diarrhoea, dysentery and other intestinal problems [7]. Characterized by the presence of quinines [8], flavonoids [9,10] and sesquiterpenes [11]. The chemical composition of essential oils of different *Cyperus* species have been reported, the major compounds identified in oil samples are mostly monoterpenes and sesquiterpenes [12-14].

There are some studies on the chemical composition of the essential oil of *Cyperus* species. Essential oils of some Syperus species including *C. rotundus* [13,15], *C. difformis* L. and *C. arenarius* [12] were studied regarding their chemical composition. In addition, chemical compositions of the three Egyptian *Cyperus* species; *C. articulatus*, *C. esculentus* and *C. papyrus* were showed by comparison [16]. In this study we showed the chemical composition of essential oil of *C. fuscus* and antimicrobial activity of ethanol extracts in the first time.

The chemical composition of the essential oil derived from the burs of *Cyperus fuscus* in Turkey as well as antimicrobial activities of this oil and ethanolic extract of this plant against human pathogenic bacteria were studied in this work.

EXPERIMENTAL

Collection of plant material

The fresh burs of *C. fuscus* were collected at the flowering stage from, 2009-2010 in July by Erdem, (1025) in open area on sandy loam, mudflats found in Yeşilöz Village- Kirmir valley, stream side, 750 m in Güdül (Ankara). The current study area is within the A4 square in the grid-square system adopted for the Flora of Turkey [17,18]. The research area lies between latitude N 40°14' 11.09" and longitude E 32° 15' 44.4".

Plant tissues were pulverized into fine powder. The plant materials were collected and positioned in plastic bags and transported to the laboratory. Voucher specimen was deposited in the Herbarium of Ahi Evran University, Kırşehir-Turkey (Ahi Herb. No 1025).

Preparation of ethanol extract

The harvested plant bruses were dried in shadow, pulverized and mixed with 50 % ethanol in solvents using a Soxhlet apparatus and extracted for 72 hours. The solution then filtered through a Whatman no. 1 filter paper and the solvent were evaporated. Finally, the crude forms of the crystals were obtained and stored in a refrigerator until further use. The ethanol extract was diluted to concentrations ranging from 250 to 1000 µg/ml, with DMSO [19].

Extraction of essential oil

Hydro-distillation method was used to obtain the essential oil using a modified Clevenger apparatus. A total of 100 g burs parts of fresh plant material were used. The resulting essential oil (EO) was dried over anhydrous sodium sulphate prior to filtration, and kept at 4°C until the analysis.

Gas chromatography-mass spectometry (GC-FID/GC-MS) analysis

GC- FID / MS analysis of the essential oils were done in Plant Products and Biotechnology Research Laboratory (BUBAL), Firat University, by means of Hewlett Packard-Agilent 5973N GC-MS system with 6890 GC equipped with a flame ionization detector (FID). The study was performed by simultaneous injection in the same instrument. HP-5 MS column (30 m×0.25 mm i.d., film thickness (0.25 µm)) was used in both analysis with helium as the carrier gas in GC-MS. The injection volume was 1.0 µL of diluted solution (1/100) of oil in *n*-hexane. The temperature of the injector was 250°C, and the flow rate was 1.3 mL/min. (splitless mode). The GC oven temperature was preserved at 70°C for 2 min and automated to 150°C at a rate of 10 °C/min and then kept constant at 150°C for 15 min to 240°C at a rate of 5°C/min. A series of *n*-alkanes were used as reference points in the calculation of retention indices (RI). MS were occupied at 70 eV and a mass range of 35–425. The determination of the compounds was based on comparison of their retention indices (RI), and mass spectra with those acquired from authentic Wiley (7^{th} version) and Nist 98 libraries.

Evaluation of antimicrobial activity

Test microorganisms

The ethanol extract was tested against 11 microorganisms; *L. monocytogenes* (ATCC 7644), *S. aureus* (ATCC 29213), *E. feacalis* (ATCC 29212), *E. coli* (ATCC 25922), *S.*

typhimurium (ATCC 14028), *E. aerogenes* (ATCC 5402), *A. hydrophila* (ATCC 7966), *K. pneumoniae* (ATCC 21541), *P. aeruginosa* (ATCC 27853) and *C. albicans (*ATCC 10231) *C. glabrata* (ATCC 90030) yeasts. The bacterial cultures were provided by Department of Biology, Faculty *of* Arts *and* Science, University of Ahi Evran.

Agar diffusion assay

The agar diffusion test method applied by Aneja and Joshi [20] was used to observe the antimicrobial activity of both oil and ethanolic extract. The microbial suspension were swabbed and spread on nutrient agar. The plates were cultured in 0.1 mL (10^5-10^6 cells/mL) and subsequently 8 mm wide wells were bored within these agar plates using a sterile cork borer. The each wells were aseptically filled with 100 µL of ethanolic extract (250, 500 and 1000 µg/mL) and 20 µL of essential oil was placed on the inoculated agar. The plates were incubated overnight at 37 ^{o}C for bacteria and at 20-22 ^{o}C for 5 days for yeast cultures. Microbial growth was determined by measuring the diameter of zone of inhibition. For each strain, a negative control was maintained where DMSO was used in place of extract. In order to determine the sensitivity of gram negative and gram positive bacteria, Gentamycin (20 g/disc) was chosen as standard, while Nystatin (50 g/disc) was selected as Standard for yeasts (Table 2). Each experiment was carried out three times and mean values are presented.

Minimum ınhibitory concentration (MIC) assay

Tube dilution method was used to determine MIC of the extracts. The essential oil and extract of *Cyperus fuscus* were dissolved in 10 % dimethylsulfoxide (DMSO). The highest concentration (1000 µg/mL) was prepared at the beginning. Then serial two-fold dilutions were prepared resulting the concentrations ranging from 31.25 to 1000 µg/mL in 15 mL sterile test tubes containing nutrient broth and 100 µL of the bacterial suspension containing 10^8 CFU/mL of respective test organisms. The tubes were incubated at 37 ºC in an incubator for 24 h for bacteria and 22 ^{o}C for 48 h for yeast. A tube containing nutrient broth without extract was used as control. The least extract concentration inhibiting the growth of the test organisms was considered as MIC (Table 2).

Statistical analysis

A completely randomized experimental design was used with three replications in factorial arrangements. One-way analysis of variance was also used. Tukey HSD multiple comparison tests were used to find out which group originated the difference between the groups. The normality assumption in the analyses was tested by Kolmogorov-Smirnov test. Statistical analyses were performed using SPSS (Version 20.0, SPSS Inc, USA) statistical package program. In the analyses, significance level was set at $p < 0.05$.

RESULTS

Chemical composition of essential oil

The yield of essential oil belonging to the aerial parts of *C. fuscus* was obtained as 0.4 % (v/w) yield. The composition of essential oil is presented in Table 1. It is determined that seventy three compounds comprised the 96.4 % of the oil. The major compounds in essential oil studied were dehydroaromadendrene (10.7 %), azulenone (8.5 %), a-selinene (7.5 %), a-ylangene (6.0 %) and b-caryophyllene (5.6 %) respectively. Many other compounds in minor amounts were also determined. Complex mixtures of monoterpenes and sesquiterpenes were observed in the oil. Sesquiterpenes in *C. fuscus* oil were found as the main class of terpenoids. The compounds, a-gurjunene, spathulenol, azulene, b-caryophyllene, a-selinene, a-ylangene, were comprised the high percentage of sesquiterpenes in the essential oil. Monoterpenes were comprised a little content of the essential oil (Table 1).

Antimicrobial activity

Antimicrobial activities of *C. fuscus* essential oil are presented in Table 2. This study clearly indicated that *C. fuscus* essential oil has showed different activities on the bacteria and yeast tested. About the tested bacteria, the oil could not inhibit the growth of some bacteria such as *S. typhimurium, E. aerogenes, A. hydrophila, K. pneumoni*ae, *L. monocytogenes* and *E. feacalis.* On the other hand, ethanolic extract the burs of *C. fuscus* showed antimicrobial activity on 4 bacteria (Table 2).

For *E. coli,* ethanol extract of *C. fuscus* exhibited the strongest antibacterial activity (18 mm), followed by *A. hydrophila* (17 mm). For *K. pneumoniae* and *P. aeruginosa*, ethanol extract of *C. fuscus* exhibited high antibacterial activity (15 mm), while the other bacteria and yeasts showed no activity. The MIC of the ethanol extracts showed that MIC ranged between 1000 and 250 µg/mL. The results showed inhibition of

growth of some of the tested microorganisms with various degrees. *E. coli* (1000 µg/mL), *A. hydrophila* (500 µg/mL), *K. pneumonia*e (250 µg/mL) and *P. aeruginosa* (250 µg/mL) was susceptible whereas other bacteria resistant against ethanol extract. Ethanol extract was not exhibited antifungal activity against *C. albicans* and *C. glabrata* strains (Table 2).

DISCUSSION

The usage of this plant for some medicinal treatment has given more importance on this plant group. The many species of the genus *Cyperus* are within the medicinal plants used in China, Japan, India and the Mediterranean region in order to treat spasms and stomach disorders [21]. In the present study, *C. fuscus* L. burs ethanol extract and essential oil were evaluated against important human pathogens by using agar well diffusion method and minimum inhibitory concentration (MIC) and the data reported in Table 2. In the study of Sonwa and Konig [15] three new compounds, (-)-eudesma-2, 4(15)-11-triene, (-)-eudesma-3,11-dien-5-ol and diterpene hydrocarbon(-)-dolabella-3,7,18-triene were isolated from the essential oil of *C. alopecuroides*. The analysis showed similarity with that study since identification of eudesma 2,4,11 triene (2.5 %), eudesma 3,5,11 triene (0.3 %), eudesma-4[14],11-diene (1.9 %) and eudesma-3,11-dien-2-one (0.4 %) in *C. fuscus* essential oil.

Table 1: Constituents of the essential oil from *C. fuscus**

S/no.	Compound	RI*	%	S/no.	Compound	RI	%
1	α-Pinene	1022	0.5	38	Ledol	1512	0.6
2	Camphene	1034	0.1	39	α-Selinene	1514	**7.5**
3	β-Pinene	1056	0.6	40	β-Selinene	1516	1.5
4	p-Cymene	1091	0.1	41	Caryophyllene oxide	1518	0.5
5	Limonene	1095	0.2	42	trans-Calamenene	1519	**2.0**
6	Eucalyptol	1097	0.5	43	γ-Cadinene	1521	**3.0**
7	Linalool-L	1147	0.3	44	Isoaromadendrene epoxide	1524	0.8
8	Trans-Pinecarveol	1178	1.1	45	Eudesma 2,4,11 triene	1527	**2.5**
9	Camphor	1182	0.6	46	Eudesma 3,5,11 triene	1529	0.3
10	Pinocarvone	1193	0.4	47	Eudesma-4[14],11-diene	1533	1.9
11	Borneol-L	1200	0.4	48	α-Cubebene	1540	0.9
12	Bicyclo[3.1.1]heptan-3-one	1202	0.1	49	Valeranone	1542	1.5
13	δ-Terpineole	1205	0.1	50	α-Bisabolol	1544	0.8
14	Myrtenol	1215	1.2	51	β-Eudesmol	1547	1.1
15	β-Fenchyl-Alchohol	1217	0.3	52	α-Ylangene	1549	**6.0**
16	Endo-Isofenchol	1224	0.3	53	cis-Jasmone	1551	0.6
17	trans-Carveol	1230	0.1	54	Vulgarol-B	1554	0.5
18	Linalyl acetate	1248	0.1	55	Dehydroaromadendrene	1557	**10.7**
19	Isobornyl acetate	1282	0.1	56	Azulenone	1562	**8.5**
20	Phenol	1317	0.2	57	Isospathulenol	1564	**2.5**
21	α-Copaene	1360	0.9	58	Valerenol	1568	**2.0**
22	Germacrene-D	1368	0.1	59	Eudesma-3,11-dien-2-one	1570	0.4
23	β-Elemene	1370	0.1	60	α-Cadinol	1571	0.3
24	α-Gurjunene	1381	**3.5**	61	Thujopsene	1572	0.4
25	Epibicyclosesquiphellandrene	1422	1.7	62	t-Muurolol	1575	**2.0**
26	α-Humulene	1430	0.6	63	Aromadendrene oxide	1579	1.5
27	Naphthalene	1445	0.5	64	β-Humulene	1582	0.3
28	cis-Calamenene	1458	1.5	65	α-Eudesmol	1587	1.1
29	Alloaromadendrene	1470	0.9	66	Benzyl benzoate	1592	0.1
30	Cyclohexanemethanol	1474	0.9	67	Ledene oxide	1595	0.2
31	Bicyclogermacrene	1487	0.2	68	Ethanone	1596	0.2
32	Germacrene B	1489	0.6	69	Aplopenone	1599	0.1
33	Sphathulenol	1492	**2.5**	70	δ-Fenchene	1604	0.8
34	Nerolidol	1493	0.6	71	Benzoic acid	1611	0.4
35	Azulene	1496	**3.0**	72	1,2Benzenedicarboxyclic acid	1640	0.8
36	β-Caryophyllene	1498	**5.6**	73	Cycloprop[e]azulene	1670	1.1
37	Veridifloral	1510	0.5		**Total**		**96.4**

*RI = retention index

Table 2: Antimicrobial activities of ethanol extract and essential oil of *C. fuscus* burs against microorganisms (MIC and DD (diameter of the inhibition zones))

Microorg.	Plant extract (EtOH)					Essential oil		Gentamicin 20 µg/ml (Mean)	Nystatin 50µg/disc (Mean)	Control DMSO
	DD (Mean±s)				MIC (Mean±s)	DD(Mean±s)	MIC (Mean±s)			
	1000 mg/ml	500 mg/ml	250 mg/ml	Total	1000-31.25 µg/ml	20 µg/ml	1000-31.25 µg/ml			
E. coli ATCC 25922	18±0	16±1	14±1	6.75±7.08d	1000±0b	14±1b	125±0b	17	0	0
S. typhimurium ATCC 14028	0	0	0	0	0	0	0	14	0	0
E. aerogenes ATCC 5402	0	0	0	0	0	0	0	14	0	0
A.hydrophila ATCC 7966	17±1	14±1	0	11±6.72b	583.33±381.88ab	0	0	14	0	0
*K. pneumonia*e ATCC 21541	15±1	0	0	16.25±1.54a	291.66±190.94a	0	0	13	0	0
P. aeruginosa ATCC 27853	15±1	14±1	13±1	11.3±6.8c	291.66±190.94a	12±1ab	72.91±47.73ab	15	0	0
L.monocytogenes ATCC 7644	0	0	0	0	0	0	0	17	0	0
S. aureus ATCC 29213	0	0	0	0	0	10±1a	36.45±23.86a	16	0	0
E. feacalis ATCC 29212	0	0	0	0	0	0	0	15	0	0
C. albicans ATCC 10231	0	0	0	0	0	0	0	0	20	0
C. glabrata ATCC 90030	0	0	0	0	0	0	0	0	18	0

The differences between means with ±_are significant level ($p<0.05$). DD= diameter of Inhibition zone(mm); MIC=Minimum Inhibitory Concentrations (µg/ml); 0= No inhibition zone and/or MIC value measured

C. rotundus has been reported to comprise therapeutic oils (a-copaene, cyperene, b-selinene, a-cyperone and b-cyperone), mono and sesquiterpenes, norsesquiterpene, alkaloids, steroid glycosides, saponins, flavonoids, tannins, fructose-amino acid conjugate, isocurcumenol. Several biological activities of *C. rotundus* essential oil has been reported including anti-bacterial, anti-mutagenic, anti-oxidant, cytotoxic, apoptotic, analgesic, anti-inflammatory, anti-pyretic and anti-fungal activities [22].

Several studies on the antimicrobial activity of essential oils or extracts of other *Cyperus* species have been reported. It is clear from the previous studies that many species of *Cyperus* possess high antimicrobial activities [14,23,24]. Chemical composition and antimicrobial activity of the *Cyperus* species were also reported by many researchers. The chemical composition of essential oil and antimicrobial activity of *C. leavigatus* [14], *C. rotundus* [24,25], *C. esculentus* [26] were also studied. It is observed that *C. esculentus* and *C. rotundus* have possessed medicinal properties which have been harnessed by traditional medicine practitioners, but only a few of these properties have been proven scientifically [24,26].

The lowest MIC value (250 µg/mL) observed with the ethanol extract on *K. pneumoni*ae and *P. aeruginosa.* The ethanol extract of *C. fuscus* has shown the most antibacterial activity against *E. coli* (Table 2) and this probably supports the reported use of the plant by traditional medicine practitioners in treating diarrhoea and other stomach troubles.

Nowadays, some people are not aware of the use *C. fuscus* treating stomach or diarrhoea. This plant may be a potential source of a new type of antibiotic with the enlarged studies. Isolating and purifying the bioactive compounds may lead to the development of suitable antibiotic against *E. coli, A. hydrophila, K. pneumoni*ae and *P. aeruginosa.* As a result it is said that the antimicrobial activity of the ethanolic extract of the *C. fuscus* were high than the essential oil. In general, it is possible to say that both extracts of the *C. fuscus* have antimicrobial activities on some bacteria (Table 2).

CONCLUSION

The findings of this study show that while the ethanol extract of the *C. fuscus* exerts moderate antimicrobial activity against some bacteria (*E. coli, A. hydrophila, K. pneumoniae, P. aeruginosa*), it, however, possesses no activity on the other bacteria studied. On the other hand, the essential oil of *C. fuscus* has low antimicrobial activity against *E. coli, P. aeruginosa* and *S. aureus* and none at all against other microorganisms. Both extracts of the *C. fuscus* do not have any antifungal activity against the test microorganisms. Thus, the essential oil and ethanol extract may be suitable as natural antimicrobial agents.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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