

Chemical Composition, Antimicrobial, and Antioxidant Activities of Medicinal Plants Nutsedge and Fenugreek

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Purple nutsedge roots (*Cyperus rotundus* L.) and fenugreek seeds (*Trigonella foenum-graecum* L.) have been traditionally used as food and to treat common ailments. After extraction by solid-phase microextraction (SPME), the chemical structure of the revealed volatile fractions was researched with gas chromatography with mass spectrometry (GC-MS). The determined substances of the *C. rotundus* were pentadecanolide (72.0%), palmitic acid (8.2%), 16-hydroxy-6-hexadecenoic acid omega lactone (4.4%), and (Z)-anethol (3.9%). Most of the identified compounds of the *T. foenum-graecum* were pentadecanolide (61.3%) and (Z)-anethol (16.5%). The *C. rotundus* showed good antifungal activity against the yeast strands of *Candida albicans* and *Candida krusei*. Minimum inhibitory concentration (MIC) numbers were 250 and 125 µg/mL, respectively. However, the *T. foenum-graecum* seeds did not show any effect against the test microorganisms. The *C. rotundus* roots in particular exhibited good 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with an IC₅₀ number of 0.91 mg/mL.

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INTRODUCTION

Medicinal herbs play an important role in the protection of human health and the beautification of human living conditions. Such plants are used in the food, cosmetics, fragrance, paint, and pharmaceutical industries, as well as valuable food ingredients such as spices and beverages. Almost seventy-four percent of the 121 therapeutically active plant-derived phytochemicals currently used in the world were created after further studies were conducted to demonstrate their ethnomedical use (Olaoluwa *et al.* 2022). The use of different medicinal and herbal plants and the presence of valuable natural compounds in these plants directly affect human life. Pharmaceutical research with medicinal plants containing biologically active groups, which is a good source for new powerful drug discovery process, supports the interest in phytochemicals (Mouthe Kemayou *et al.* 2021; Nalawade *et al.* 2022). Numerous extracts from herbs have been found to have antimicrobial properties *in-vitro* and *in-vivo*, which justifies traditional medicine studies focused on the nature of the biological activities of these plants (Martínez *et al.* 1996). These extracts, obtained directly or indirectly from plants, are used in the structure of many pharmacologically important drugs (Alsabah *et al.* 2018). Despite the

dependence on synthetic chemistry to produce and develop drugs today (Zainal *et al.* 2018), the use of herbs in disease control and treatment is still very high (Salih *et al.* 2017).

The number of drug-resistant bacteria across the world has increased, especially in recent years. One method to prevent antibiotic resistance in resistant bacteria is to use new substances different from existing synthetic antimicrobial compounds (Shah 2005). Systematic screening of herbs may result in the discovery of new effective compounds that will overcome the existing problem of drug resistance. It is hoped that plant components can effectively target drug-resistant bacterial pathogens (Yang *et al.* 2002).

Reactive oxygen species, which are present in cells and are random by-products of body metabolism, show cytotoxic properties and cause cardiovascular diseases, inflammation, and cancer, among other various diseases (Josh and Janardhanan 2000; Gomes *et al.* 2001). Antioxidants protect the body against damage produced by free radicals such as reactive oxygen species and act as a defense against toxicity caused by radicals (Manzi *et al.* 1999; Yang *et al.* 2002). Recently, interest in natural antioxidants obtained from various types of vegetables, fruits, plants, grains, and seeds has increased (Alara *et al.* 2020).

Cyperus rotundus L. (Cyperaceae family), commonly known as musta or motha, is one of the most widespread herbs in the world, and it is abundant in pan-India. *C. rotundus* is also known as tiger nut, purple nutsedge, red nutsedge, nutgrass, and java grass. *C. rotundus* grows naturally in the tropical, subtropical, and temperate zones throughout the world and is predominant in the south, center, and northeast of Tunisia (Sharma and Gupta 2007). *C. rotundus* is a conventional medicinal herb found within Indian, Japanese, and Chinese native medicines utilized for stomach illnesses, spasms, inflammatory bowel diseases, and menstrual irregularities (Dang *et al.* 2011). The extract of this plant has been used in the treatment of intestinal problems, dysentery, fever, diarrhea, and pain (Umerie and Ezeuzo 2000).

Trigonella foenum-graecum L., commonly known as methi, is a self-pollinating legume plant native to the eastern Mediterranean region and the Indian subcontinent (Petropoulos 2002). *T. foenum-graecum* is one of the traditional and most promising medicinal plants from the legume family.

T. foenum-graecum has been widely used for more than 2,500 years due to its medicinal and food properties (Shi *et al.* 2017). The seeds and leaves of this herb are broadly used for medicinal objectives, such as an anti-microbial (Subhapiya and Gomathipriya 2018), and anti-cancer (El Bairi *et al.* 2017), anti-inflammatory (Tavakoly *et al.* 2018), anti-diabetic, and antioxidant agent (Bahmani *et al.* 2016).

Therefore, the objective of this paper was to determine the chemical substance of the volatile fractions extracted by solid-phase microextraction (SPME) from *C. rotundus* roots and *T. foenum-graecum* seeds and determine their antimicrobial and antioxidant properties *in-vitro*.

EXPERIMENTAL

Materials

Chemicals

2,2-diphenyl-1-picrylhydrazil (DPPH), methanol, 2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's phenol reagent, and 6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Aluminum nitrate nonahydrate ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), sodium carbonate (Na_2CO_3), ammonium acetate ($\text{C}_2\text{H}_3\text{NO}_2$), neocuproine (2,9-dimethyl-1,10-phenanthroline), and acetic acid (CH_3COOH) were obtained from Merck (Kenilworth, NJ, USA).

Plant material

The dry seeds of *T. foenum-graecum* and the roots of *C. rotundus* used in the study were acquired from the local herbalist (market) of Mardin, Turkey, and identified by Dr. Hayal Akyıldırım Begen, Department of Forest Botanical Science, Artvin Coruh University, Artvin, Turkey.

These studied plants have been used as medicinal plants for a long time by the people of Mardin, and they have are edible. The seeds and roots of the studied plants were cut into small pieces with the help of a pestle and an electric grinder was used to grind them into powder. Approximately 10 g samples (roots or seeds) were used to prepare the methanolic extract. The samples were then stirred on a shaker at room temperature for 24 h. The methanolic extracts obtained were filtered using ordinary filter paper. The recovered extracts were used to reveal the antioxidant properties and the analyses for the experiment were repeated three times. Spectrophotometric measurements were used for the antioxidant activity determination, total flavonoid content, and total polyphenol content analysis. Spectrophotometric methods are widely used in this kind of natural raw material analysis. Additional volatile fractions and antibacterial effects of plant extracts were also studied.

SPME procedure

Five-gram portions of *T. foenum-graecum* or *C. rotundus* plants were cut into small pieces, placed in a 10 mL sealed bottle, and sent to the SPME device (Supelco, Bellefonte, PA, USA). A divinylbenzene/carboxy/polydimethylsiloxane coating fiber was laid to expose the volatile substances. The SPME-treated fibers were placed in a gas-chromatography (GC) injector at 250 °C for 5 min. The extraction process was carried out at 80 °C, with a waiting time of 5 min and a total extraction time of 10 min by mixing in a magnetic stirrer. Then the fiber was sent to the GC injector. Helium at a flow rate of 1 mL/min was used as the carrier gas. The injection was carried out in split mode (1:30) and at 250 °C. The results of the sample analysis were reported. The extraction, incubation, and temperature values required for the experimental conditions were made according to the study by Renda *et al.* (2016).

Gas chromatography-mass spectrometry (GC-MS) analysis

A Shimadzu QP2010 plus gas chromatography device (Kyoto, Japan) was used for the GC analysis. A TRB-5MS capillary column (Teknokroma Analítica, Barcelona, Spain) with dimensions of 30 m × 0.25 mm and a film thickness of 0.25 μm was also used. The GC device was connected to a mass selective detector using the split mode with a split ratio of 1:30. The furnace dimensions for analysis were made as follows. Initially, it was increased to 40 °C in 2 min and to 240 °C in 3 min, and it was kept for 4 min with the final temperature at 250 °C. A total of 55 min was spent on this analysis. The temperature values of the mass transfer section and the injector were arranged as 200 and 250 °C, respectively. Helium (99.999%) with a flow rate of 1 mL/min was used as the carrier gas. The ionization voltage value was set to 70 eV. The sensing part was applied in electronic pulse mode (EI), and the scanning mode used for mass gain was 40 to 400 m/z (Renda *et al.* 2016).

Identification of constituents

The retention values of the substances were obtained by the Kováts method using the C7 to C30 alkane standards (Kováts 1958). Mass spectral values of volatile fraction components were determined using version 1.3 of the Flavors and Fragrance of Natural and Synthetic Compounds library (Shimadzu 2008).

Total Phenolic Assay

The Folin-Ciocalteu method (Slinkard and Singleton 1977) was used to find the total phenolic content of the plants. Various concentrations of gallic acid were used as a standard for this experiment at doses of 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL. To summarize, 20 μ L of methanolic extracts (1 mg/mL), 680 μ L of distilled water, 20 μ L of gallic acid of varying concentrations, and 400 μ L of 0.5 N Folin-Ciocalteu reagents were mixed, and the resulting mixture was vortexed. After waiting for 3 min, 400 μ L of Na₂CO₃ (10%) was added to the mixture and vortexed, then the mixture was left for 2 h to incubate at room temperature. At the end of this period, the absorbance values of the mixtures were recorded at 760 nm. The total amounts of phenolic compounds were calculated as gallic acid equivalents per gram of the samples in dry form.

Total Flavonoid Assay

The aluminum chloride assay was used to determine the total flavonoid content (Chang *et al.* 2002). Various concentrations of quercetin (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL) were used as the standard. The mixture was vortexed by adding 4.3 mL of methanol, 0.5 mL of quercetin, 0.1 mL of 1 M NH₄CH₃COO, and 0.1 mL of 10% Al(NO₃)₃ into the tubes. The mixture was then allowed to stand at room temperature for 40 minutes. After this 40-minute waiting period, the absorbance of the plants at 415 nm was measured and recorded. The total flavonoid content values of the plants were calculated as mg quercetin equivalent per gram of dried samples.

Determination of Antioxidant Activity

The antioxidant contents of the plants were revealed by using the cupric reducing antioxidant capacity (CUPRAC), fluorescence recovery after photobleaching (FRAP), and DPPH methods.

In the FRAP experiment, the reduction of the yellow-colored Fe³⁺-TPTZ compound to the blue Fe²⁺-TPTZ compound was realized with the electron-donating substance in acidic conditions, and antioxidant capacity was determined with this principle (Benzie and Szeto 1999). The FRAP reagent (containing acetate buffer, TPTZ, and iron(III) chloride (FeCl₃)) was prepared for the experiment. Then, 3 mL of the FRAP reagent was added to the 100 μ L plant extract sample and vortexed. After 4 min of incubation, the absorbance values were measured at 593 nm. The obtained absorbances were compared with the standard curve (100 μ mol/L to 1,000 μ mol/L). The results were expressed as μ mol FeSO₄.7H₂O equivalent per gram of the samples in dry form.

The CUPRAC method consists of mixing a pH=7 ammonium acetate aqueous buffer with a solution of neocuproin in alcohol with a solution of copper (II) chloride. Then, after 60 min of incubation, absorbances at 450 nm were measured and recorded (Apak *et al.* 2004). Considering this information, 0.2 mL sample, 1 mL of 7.5 mM neocuproine, 0.9 mL of H₂O, 1 mL of 10 mM CuCl₂, and 1 mL of 1M NH₄Ac were placed in test tubes and the final mixtures were vortexed. The final volume was 4.1 mL. After 1 h at room

temperature, absorbances were recorded at 450 nm. The results obtained were explained as Trolox equivalent antioxidant capacity (TEAC).

The scavenging effect of the DPPH radical was revealed using the Molyneux trial (Molyneux 2004). According to this method, 750 microliters were taken from the samples prepared in different concentrations, and 750 microliters of DPPH dissolved in methanol was added on it and the mixture was vortexed. Thereupon, the DPPH dissolved in methanol was added and vortexed. Afterward, the test tubes were kept at room temperature and in the dark for 50 min. At the end of this period, the spectrophotometer absorbances were determined at 517 nm. Trolox was used as the standard in this experiment and the values obtained were expressed as IC₅₀ (mg sample per mL).

Biological Materials

A total of 13 microorganisms were utilized in this research. The microorganisms were obtained from Anadolu University Faculty of Pharmacy, commercial culture collections, the American Type Culture Collection (ATCC), United States Agricultural Research Service Culture Collection (NRRL). The bacteria samples were *Gordonia rubripertincta* NRRL B-3906, *Pseudomonas citronellois* NRRL B-2504, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Streptomyces griseolus* NRRL B-1062, *Proteus vulgaris* NRRL B-123, *Bacillus velezensis* NRRL B-14580, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* NRRL B-4378, and *Salmonella typhimurium* ATCC 13311. The yeast samples were *C. krusei* ATCC 6258, *Candida glabrata* ATCC 2001, and *C. Albicans* ATCC 90028.

The microorganisms used for the study were stored in 15% glycerol at -85 °C. Then, malt extract agar (1.05398, Merck) and nutrient agar (1.05450, Merck) were maintained at 4 °C with gradients.

The microorganisms used in this study were selected from important human and plant pathogens that have been the subject of antimicrobial activity studies by many recent researchers.

In-vitro antimicrobial activity

To determine the antifungal and antimicrobial activities of the plant extracts *in-vitro*, the broth microdilution assay used by the Clinical Laboratory Standards Institute (CLSI) was applied (Amsterdam 1996). Ketoconazole and amphotericin B were used as standards for the antifungal agent, while tetracycline and chloramphenicol were used as the antimicrobial agents. The antifungal and antimicrobial agents were obtained from Merck. All the applied tests were tested in duplicate in two separate independent assays.

Broth microdilution test for bacteria

The broth microdilution experiment was performed in accordance with the CLSI M100-S16 (CLSI 2006) requirements. The minimum inhibitory concentration (MIC) value of *Quercus infectoria* galls was investigated by the microdilution method using 96-well microtiter plates that were obtained from Sigma-Aldrich. Overnight cultivated bacterial mixes in double strength Mueller-Hinton broth (MHB) (Merck) were rendered uniform by 10⁸ CFU 1/mL, using the McFarland No. 0.5 standard. The test extracts were mixed in 10% dimethyl sulfoxide (DMSO) and diluted in MHB to obtain a concentration value of 15.6 to 4,000 µg/mL. The DMSO was utilized as a negative check. Then, the mixture was diluted twofold in 100 L of MHB and inoculated with the microorganism strains. It was then incubated at 37 °C for 24 h. Resazurin mixture obtained from Merck was added to confirm

the MIC values. The final MIC value was applied as the lowest concentration value with a full growth value (100%). The outcomes of antibacterial assaying were compared with the results of the chloramphenicol and ampicillin standards. The final concentrations were between 0.25 and 32 µg/mL. Dimethyl sulfoxide was tested as the negative check.

Broth microdilution test for yeasts

The CLSI Broth microdilution assay also used 0.5 cells/mL to 2.5 cells/mL × 10³ cells/mL (McFarland 0.5), with RPMI-1640 media (Sigma-Aldrich) and 96-well microtiter plates as mentioned in document M27-A2. The final concentration values of the *Q. infectoria* galls were found between 15.6 and 4000 µg/mL, and the MIC values were found at 37 °C for 24 h. The resazurin mixture was annexed to approve the MIC. The final MIC value was applied as the lowest concentration value with a full growth value (100%) (CLSI 2002).

RESULTS AND DISCUSSION

Chemical Composition

The outcomes of the SMPE and GC-MS analysis of the volatile fractions from the *C. rotundus* roots and *T. foenum-graecum* seeds are shown in Tables 1 and 2. In total, 32 and 30 compounds were identified and quantified for purple nutsedge roots and fenugreek seeds, respectively. The chemical ingredients were formulated based on literature data and library research (FFNSC 2008).

The test outcomes listed in Table 1 can be summarized as follows. Pentadecanolide and palmitic acid were the main substances of the *C. rotundus* root species. While ethyl pyruvate was eluted first from the column [Retention time (RT) of 1.331 min], 16-hydroxy-6-hexadecenoic acid omega lactone was held in the column for the longest time of all (RT of 53.940 min and Retention index (RI) of 2143). Pentadecanolide was the main substance of this volatile fraction of *C. rotundus*, with content of approximately 72%, followed by palmitic acid (8.2%), 16-hydroxy-6-hexadecenoic acid omega lactone (4.4%), and (*Z*)-anethol (3.9%). The other constituent, beta-patchoulene was detected with an average percentage of 1.3%. The rest of the ingredients generally had a content of less than 1%. The compounds were separated into 11 classes, which were ether, ketones, monoterpenes, fatty acids, monoterpenoids, alcohols, esters, sesquiterpenes, aldehydes, hydrocarbons, and others (Table 1). The research of Jirovetz *et al.* (2004) indicated that α-copaene (11.4%), valerenal (9.8%), caryophyllene oxide (9.7%), cyperene (8.4%), nootkatone (6.7%), and trans-pinocarveol (5.2%) are the major constituents of the volatile fraction of *C. rotundus* roots from south India. In another study, alpha-cyperone (11.0%), myrtenol (7.9%), caryophyllene oxide (5.4%), and β-pinene (5.3%) were identified as the main components of the *C. rotundus* tubers from south Africa (Dhar *et al.* 2017). It can therefore be concluded that the proportion of the substances or chemical combinations of the volatile fractions are different with respect to the environmental and climatic conditions. Other surveys have also demonstrated that the chemical combination of the volatile fractions of *C. rotundus* can be altered significantly with respect to the harvest period, the geographical zones, and the age of the herb (Kilani *et al.* 2008).

Table 1. Identified Substances in the Volatile Constituents of *C. rotundus*

No.	Compounds	RT (min)	Area (%)	RI (Cal) ^a
	Monoterpene			
1	Limonene	12.285	0.1	1031
	Monoterpenoids			
2	Carvone	20.484	0.1	1249
3	Piperitone	20.865	0.3	1259
4	Alpha-curcumene	26.192	0.8	1413
	Total		1.2	
	Sesquiterpenes			
5	Spathulenol	31.777	0.2	1589
6	Caryophyllene oxide	31.886	0.6	1593
7	Nootkatone	34.706	0.6	1689
	Total		1.4	
	Aldehydes			
8	Pelargonaldehyde	15.257	0.4	1708
9	Tetradecanal	51.100	0.4	2355
	Total		0.8	
	Ketones			
10	Pyruvophenone	1.525	0.2	
11	Acetoin	1.652	0.3	
12	Menthallactone	27.348	0.2	1448
13	Delta-undecalactone	33.351	0.4	1642
	Total		1.1	
	Alcohols			
14	2-ethylhexanol	12.408	0.2	1035
15	Tridecyl alcohol	47.265	0.4	2180
	Total		0.6	
	Fatty Acids			
16	2-methyl butyric acid	6.155	0.1	858
17	Valeric acid	10.669	0.2	989
18	Myristic acid	36.894	0.1	1767
19	Palmitic acid	47.045	8.2	2171
20	16-hydroxy-6-hexadecenoic acid omega lactone	46.398	4.4	2143
	Total		13	
	Ether			
21	(Z)-anethol	21.991	3.9	1290
	Esters			
22	Ethyl Piruvate	1.331	0.3	
23	Diethyl phthalate	32.161	0.5	1602
24	Methyl palmitate	41.327	0.3	1925
25	Pentadecanolide	46.572	72.0	2143
26	Citronellyl acetate	46.940	0.5	2166
27	Methyl epi-jasmonate	49.849	0.4	2296
28	Hexyl caprylate	51.064	0.8	2353
	Total		74.8	
	Hydrocarbons			
29	Beta-patchoulene	35.224	1.3	1707
30	1,4-cineole	36.063	0.3	1737
31	Eicosane	50.133	0.2	2309
	Total		1.8	
	Others			
32	Unknown	53.324	1.6	
	Total percentages		100	

^aRI calculated from retention times relative to that of alkanes (C7 to C30) on the non-polar TRB-5MS column. Bold values represent the major components.

Table 2. Identified Substances in the Volatile Constituents the of *T. foenum-graecum*

No.	Compounds	RT (min)	Area (%)	RI (Cal) ^a
Monoterpens				
1	Limonene	12.262	0.3	1032
2	Menthone	17.077	0.4	1157
		Total	0.7	
Monoterpenoids				
3	Isosopulegol	17.947	0.6	1180
4	Carvone	20.453	0.2	1248
5	Piperitone	20.841	0.8	1259
6	Carvacrol	22.565	0.2	1306
7	Dihydro-(Z)-carvone	46.369	0.8	2142
		Total	2.6	
Sesquiterpenoid				
8	Alpha-himachalene	28.572	0.3	1455
Aldehydes				
9	Pelargonaldehyde	15.229	0.1	1108
10	Lauric aldehyde	45.773	1.7	2116
11	2-Isopropyl-5-methyl-2-hexenal	49.865	0.8	2297
		Total	2.6	
Ketones				
12	Menthylactone	27.326	2.9	1448
13	Ethylene brassylate	33.335	0.4	1642
14	Civetone	53.912	0.3	2490
		Total	3.6	
Alcohols				
15	Propylene glycol	1.115	0.2	
16	2-Ethylhexanol	12.389	0.3	1034
17	Pelargol	17.825	0.1	1177
		Total	0.6	
Fatty Acids				
18	Valeric acid	10.566	0.2	986
19	Caproic acid	31.850	0.2	1592
20	Palmitic acid	42.163	2.8	1948
21	16-hydroxy-6-hexadecenoic acid	45.467	0.9	2103
	omega lactone			
		Total	4.1	
Ethers				
22	(Z)-Anethol	21.967	16.5	1290
Esters				
23	Ethyl Piruvate	1337	0.2	
24	Ethyl Lactate	1648	0.8	
25	Diethyl phthalate	32.143	1.6	1601
26	Guaiacwood acetate	35.206	0.2	1706
27	Methyl palmitate	41.314	0.3	1924
28	Pentadecanolide	46.390	61.3	2143
29	Butyl undec-10-enoate	52.005	0.1	2397
		Total	64.5	
Others				
30	Unknowns	5.182 to 51.580	0.09 to 0.96	
		Total	4.5	
Total percentages			100	

^aRI calculated from retention times relative to that of alkanes (C7 to C30) on the non-polar TRB-5MS column. Bold values represent the major components.

The assay results shown in Table 2 indicate that in the volatile fractions of the *T. foenum-graecum* seeds, pentadecanolide, and (Z)-anethol were the main substances of the seed species. While propylene glycol initially appeared from the column (RT of 1.115 min), civetone remained in the column the longest (RT of 53.912 min). Pentadecanolide is the main substance of this volatile fractions of *T. foenum-graecum*, with content of approximately (61.3%), followed by (Z)-anethol (16.5%). The other constituents of menthalactone (2.9%), palmitic acid (2.8%), lauric aldehyde (1.7%), and diethyl phthalate (1.6%) were detected as average percentages. The rest of the ingredients were generally less than 1% in content. The compounds were separated into 10 classes, which were alcohols, esters, ketones, monoterpenes, alcohols, monoterpenoids, ether, fatty acids, sesquiterpenoids, aldehydes, ketones, and others (Table 2). In previous studies, large different compounds were found in the GC-MS analyses of *T. foenum-graecum* seeds for the plant growing in Bangladesh and Malaysia (Moniruzzaman *et al.* 2015; Akbari *et al.* 2019). These differences may result from the kind of extraction process and the kind of solvent utilized to herb extract the oil. Furthermore, it can be concluded that the chemical combination of the herb as a whole depends on the time the plant is harvested, as well as the place of growth and harvest (de Barros Fernandes *et al.* 2014).

There are not many published studies that report on the definition of volatile fractions ingredients, antioxidant properties, and antibacterial properties in a single study. Therefore, it is important to explore the novel biological properties of *C. rotundus* roots and *T. foenum-graecum* seeds which have many medicinal properties (Umerie and Ezeuzo 2000; Dang *et al.* 2011; Bahmani *et al.* 2016; El Bairi *et al.* 2017; Shi *et al.* 2017; Subhapiya and Gomathipriya 2018; Tavakoly *et al.* 2018) to contribute to the activities noted in the literature.

Antioxidant Activity

In this paper, three primary processes, DPPH radical scavenging activity, ferric reducing power (FRAP), and CUPRAC were utilized to determine the antioxidant activity. The properties of the total flavonoids and the phenolics were also determined for the herb extracts. The total flavonoid values, total phenolic values, DPPH, CUPRAC, and FRAP results are given in Table 3. The IC₅₀ outcomes calculated from test of the DPPH are showed in Fig. 1.

Table 3. Phenolic Contents, Flavonoid Contents, FRAP, CUPRAC, and DPPH Results for the Plant Samples

Sample	Total Phenolics Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)	CUPRAC (mmol TEAC/g)	FRAP (μmol Fe/g)	DPPH IC ₅₀ (mg/mL)
<i>C. rotundus</i>	4.07 ± 0.26	0.23 ± 0.15	0.18 ± 0.05	0.06 ± 0.00	0.91
<i>T. foenum-graecum</i>	0.82 ± 0.52	0.22 ± 0.01	0.02 ± 0.00	0.01 ± 0.01	25.67
Trolox	-	-	-	-	0.008

Much information can be attained by conducting a literature study concerning purple nutsedge (*C. rotundus*) roots or aerial parts solvent extraction or volatile fraction analysis and their biological properties. A study was carried out on the *C. rotundus* root in India (Kandikattu *et al.* 2015). The total polyphenol and total flavonoid values were

calculated as $149 \pm 7.5 \mu\text{g GAE/mg}$ and $390 \pm 22 \mu\text{g CE/mg}$, respectively. In addition, the DPPH activity was determined to be an IC_{50} value of $23.7 \mu\text{g/mL}$. Kumar *et al.* (2014) reported that the total polyphenol and total flavonoid contents *C. rotundus* root were $192.77 \pm 5.48 \mu\text{g GAE/mg}$ and $138.01 \pm 5.24 \mu\text{g CE/mg}$. The DPPH activity was determined to be an IC_{50} value of $86.2 \mu\text{g/mL}$. However, this study found that the total polyphenol and total flavonoid contents were equal to $4.07 \pm 0.26 \text{ mg GAE/g}$ and $0.23 \pm 0.15 \text{ mg QE/g}$, respectively. In addition, the DPPH activity was determined to be an IC_{50} value of 0.91 mg/mL . Therefore, the values found in the literature were higher than the values found in this study. The differences can be attributed to the extraction process and the contents of the herb from various zones.

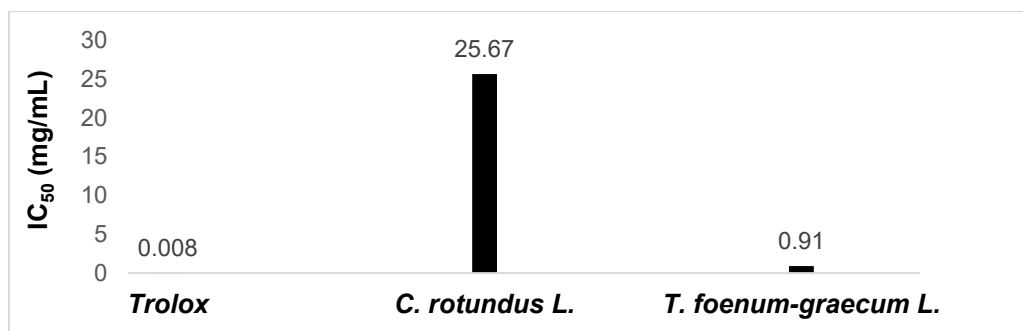


Fig. 1. DPPH values for the *C. rotundus* and *T. foenum-graecum* plant extracts

According to the CUPRAC method results, the antioxidant activity was $0.18 \pm 0.05 \text{ mmol TEAC/g}$, and according to the FRAP process outcomes, the antioxidant activity was $0.06 \pm 0.00 \mu\text{mol Fe/g}$. While the DPPH radical property determination process, which is one of the antioxidant ingredient definition processes for the *C. rotundus* herb, is described in the literature, there has been no reported research on the CUPRAC and FRAP processes. This study was the first known research on the antioxidant properties of *C. rotundus* roots analyzed by the CUPRAC and FRAP methods.

The antioxidant capacity seeds of the *T. foenum-graecum* were determined using a total polyphenol ($38.97 \pm 0.34 \text{ mg GAE/g sample}$), total flavonoid ($14.417 \pm 0.23 \text{ mg QE/g sample}$), and DPPH ($172.6 \pm 3.1 \mu\text{g/mL}$) process, as outlined by Akbari *et al.* (2019). In an article by Premanath *et al.* (2011), the antioxidant properties of *T. foenum-graecum* seeds were investigated using the total polyphenol, total flavonoid, FRAP, and DPPH processes. The total polyphenol and total flavonoid contents were found to be $4.2 \pm 0.15 \text{ mg GAE/g}$ and $0.32 \pm 0.02 \text{ mg QE/g}$, respectively. In addition, the DPPH activity and FRAP activity values were found to be $53.2 \pm 0.33\%$ and $31.0 \pm 1.0 \mu\text{mol Fe/g}$, respectively. However, this study determined total polyphenol, total flavonoid, DPPH, and FRAP values of $0.82 \pm 0.52 \text{ mg GAE/g}$, $0.22 \pm 0.01 \text{ mg QE/g}$, 25.67 mg/mL , and $0.01 \pm 0.01 \mu\text{mol Fe/g}$, respectively. Unlike other published studies, the CUPRAC method was used in this study, and according to this method, the antioxidant activity of the seeds of the *T. foenum-graecum* plant was determined as $0.02 \pm 0.00 \text{ mmol TEAC/g}$. This study was the first to use such methods to define the antioxidant properties of *T. foenum-graecum* seeds by the CUPRAC method.

Antimicrobial Activity

The antimicrobial activity of the *C. rotundus* and *T. foenum-graecum* extracts that were tested against the microorganisms in this paper were quantitatively and qualitatively

appraised by evaluating the existence of inhibition zones, zone diameter, and MIC ranges. Three yeast strains and 10 bacteria were used to determine the antibacterial properties of the plant extract. The microorganisms that were selected for antibacterial activity properties are among the important human pathogens and plant and biofilm-producing microorganisms. These studied microorganisms have been used in many recent studies. Table 4 demonstrates the MIC values of the *C. rotundus* and *T. foenum-graecum* by broth microdilution for isolates.

Table 4. MIC Values of *Q. infectoria* Against the Bacterial Strains Tested

Sample	Minimal Inhibition Concentration Values ($\mu\text{g/mL}$)												
	Sa	Pv	St	Se	Bs	Sg	Pc	Bv	Gr	Ec	Ca	Cg	Ck
<i>C. rotundus</i>	4000	4000	4000	4000	4000	2000	>	4000	2000	>	250	1000	125
<i>T. foenum-graecum</i>	>	>	>	>	>	>	>	>	>	>	>	>	>
Chlor.	0.5	0.5	0.125	1	0.125	0.125	1	4	0.125	0.25			
Tet.	0.125	8	0.125	8	0.125	0.125	0.25	4	0.125	1			
AmfB.											0.25	0.125	0.125
Ket.											0.0625	0.0625	0.125

Sa: *S. aureus* ATCC 6538, Pv: *P. vulgaris* NRRL B-123, St: *S. typhimurium* ATCC 13311, Se: *S. epidermidis* ATCC 12228, Bs: *B. subtilis* NRRL B-4378, Sg: *S. griseolus* NRRL B-1062, Pc: *P. citronellosis* NRRL B-2504, Bv: *B. velezensis* NRRL B-14580, Gr: *G. rubripertincta* NRRL B-3906, Ec: *E. coli* ATCC 8739, as yeast; Ca: *C. albicans* ATCC 90028, Cg: *C. glabrata* ATCC 2001, Ck: *C. krusei* ATCC 6258, Chlor: Chloramphenicol, Tet: Tetracycline, AmfB: Amphotericin B, Ket: Ketoconazole, >: Not applicable as microorganisms are not susceptible to these samples.

At the tested concentrations, the *C. rotundus* exhibited good antifungal properties against the *C. albicans* and *C. krusei* with MIC values of 250 and 125 $\mu\text{g/mL}$, respectively. However, *C. rotundus* is not found to be very effective on gram-negative and gram-positive bacteria. Antimicrobial property research is available in the literature on the *C. rotundus*. Previous research (Parekh and Chanda 2006; Kabbashi *et al.* 2015) has found that herb extracts are more effective against gram-positive bacteria than gram-negative bacteria. There are not many studies in the literature that report on plants with antifungal effects.

The *T. foenum-graecum* had no effect against any of the test microorganisms at the tested concentrations in this study. However, it has been noted in the literature that the seeds of *T. foenum-graecum* show a great and broad effect on test microorganisms that can be used against gram-positive bacteria, gram-negative bacteria, and fungi (Premanath *et al.* 2011; Moniruzzaman *et al.* 2015).

Volatile fractions and herb extracts are potential resources of novel antimicrobial constituents, especially against bacterial agents. These natural substances may also be implemented for the treatment of diseases, which gives them effective antimicrobial properties (Torbaty *et al.* 2013).

CONCLUSIONS

1. This paper evaluated the chemical contents of the volatile fractions acquired from *C. rotundus* roots and *T. foenum-graecum* seeds and evaluated their antioxidant and antibacterial capacities. It was determined that the volatile fractions of *C. rotundus* and *T. foenum-graecum* contain numerous basic substances in great quantities. Under experimental conditions, the SPME-GCMS technique serves as a good method for the

study of volatile fractions from different parts of plants and can produce significant qualitative and quantitative results.

2. The outcomes of this study support the traditional usage of the studied plants. The herbal antioxidants from the studied species offer a safer alternative to synthetic antioxidants, and the volatile fractions are potential resources for novel antimicrobial constituents.
3. In conclusion, significant antioxidant and antibacterial activities of the methanolic extracts of *C. rotundus* roots and *T. foenum-graecum* provide a scientific validation for the traditional use of this plant as a novel source of natural antioxidants and antimicrobial with consequent health benefits.

Conflict of Interest

The authors declare no conflicts of interest.

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