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CHEMICAL ANALYSIS OF ESSENTIAL OIL FROM *ECHINOPS SPINOSUS* L. ROOTS: ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES

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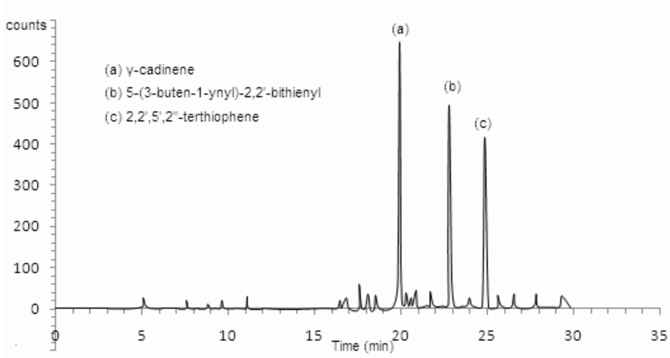
Echinops spinosus L. roots (ES) are traditional and popular medicinal plants in Tunisia. In this work, the essential oil obtained by hydrodistillation of roots of this species was analyzed by GC-FID and GC-MS. Twenty compounds were identified, representing 97.470% of the total oil. γ-cadinene (27.224%) was the main component followed by 5-(3-buten-1-ynyl)-2,2'-bithienyl (21.334%), 2,2',5',2''-terthiophene (18.024%), caryophyllene oxide (5.217%), agarospirol (1.627%), alpha caryophyllene (1.522%), thujone (0.328%) and 1,8-cineole (0.321%). The biological activities, including antioxidant and antimicrobial properties, of the *Echinops spinosus* roots essential oil, were investigated by different methods. The antimicrobial activity was evaluated against gram-positive and gram-negative bacteria. Results showed that the ES essential oil exhibited strong antimicrobial activity against the tested species. The results suggested that the ES essential oil prossesses antimicrobial and antioxidant activities, and therefore a potential source of active ingredients for food and pharmaceutical industry.



INTRODUCTION

In the recent past, an increasing interest in plant derivatives has been observed in academic society and food industry.[1,2] To discover new efficacious compounds, many research groups screen medicinal plants. This method has gained significant importance especially with the growing approval of herbal medicine as a different form of health care.

Essential oils are concentrated hydrophobic liquids containing volatile aroma compounds which are synthesized in several plant organs. These molecules are a group of terpenoids, sesquiterpenes and possibly diterpenes with different groups of aliphatic hydrocarbons, alcohols, acids, acyclic esters, aldehydes and lactones.[3] Essential oils of herbs and their components are gaining new attention as an alternative form of health care; they have many applications in folk medicine, food flavering and preservation as well as in cosmetic and pharmaceutical industries. The antimicrobial and antioxidant properties of essential oils have been

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known for a long time and a number of investigations have been conducted on antimicrobial activities using various bacteria, viruses and fungi.[4]

Consequently, in this study, we report, for the first time, the chemical composition and the antioxidant and antibacterial activity against several micro-organisms of the essential oils from roots of *Echinops spinosus* L., growing in Tunisia.

MATERIALS AND METHODS

Plant material

Echinops spinosus L. (Fig. 1) was collected in March 2017 in flowering season from Gafsa (south of Tunisia). The botanical identification was performed by G.Pottier–Alapetite[5] and Dr. Abdessatar Ghobtane. Voucher specimens were conserved at the herbarium of Montpellier 2 University France under the number MPU025131 for *Echinops spinosus* L. (Boiss.). The *Echinops spinosus* roots were washed and air-dried for four weeks in the dark and used for analyses.

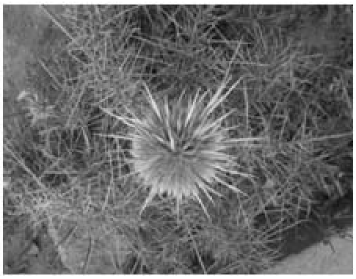

Fig. 1 – *Echinops spinosus*.

Essential oil isolation and analysis

The *Echinops spinosus* roots (*E. spinosus*) were subjected to hydro-distillation in a Clevenger's type apparatus for 3 h for isolation of essential oil (EO).[6] The essential oils were measured directly in the extraction burette and the amount of oil obtained (%) was calculated as volume (mL) of essential oil per 100 g of dry plant material. The oils were dehydrated over anhydrous Na_2SO_4 and kept in a cool and dark place prior to analysis.[7] The essential oil was analyzed as previously described.[8] Additionally, confirmation of identified compounds was done by determination of Kovats indices (RI), determined after injection of a series of *n*-alkane. The concentration of the identified compound was computed based on the percentage of the relative peak area (%).

Antioxidant activities

DPPH radical scavenging activity: radical-scavenging activity was determined by use of stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the procedure reported by Rigane *et al.*[8] with some modifications. The total antioxidant capacity of essential oil of *Echinops spinosus* was expressed as mg of ascorbic acid quivalent (AAeq) per 1 g of essential oil (mg AAeq/ g EO).[9,10]

Ferric reducing antioxidant power (FRAP) assay: the FRAP assay was carried as described in previous studies.[8,11,12] The FRAP values were expressed as mg BHT (2,6-Di-*tert*-butyl-4-methylphenol) eq/g EO. All of the samples were analysed in triplicate.

Antimicrobial activity

The bacterial strains used in this study were *Staphylococcus aureus* ATCC 29213 (Gram-positive), *Enterococcus faecalis* ATCC 29212 (Gram-positive), *Escherichia coli* ATCC 25922 (Gram-negative), *Klebsiella pneumoniae* ATCC 9997 (Gram-negative). The isolates were obtained from the laboratory of Houcine Bouzaienne hospital, Gafsa Tunisia. Pastor Institute in Tunis (Tunisia) confirmed the identity of the micro-organisms. Antibacterial activity was measured using the disc-diffusion assay by inhibition zone diameter and minimal inhibitory concentration (MIC) values described by S.T. Yildirim *et al.*[13] and CLCI 2012.[14] The experiment was carried out in triplicate. Results given were the means ± SD of three experiments.

Statistical analysis

Results of the analytical determinations were expressed as mean ± standard deviation (SD) of 3 measurements. Statistical differences were calculated using a one-way analysis of variance (ANOVA) employing the Student's t-test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Essential oil yields and chemical composition

The yield of yellow essential oil of ES roots growing in South of Tunisia (Gafsa) was 0.19 ± 0.21% (v/w based on dry weight). The value was in the same order as those reported in literature data for the same species of Echinops (E). In fact, the yield of yellow essential oil of E. grijsii and E. latifolius was, respectively, 0.46% and 0.15%.15,16

A total of twenty components of *E. spinosus* roots essential oil were identified, accounting for 97.47% of the total oil. The constituents identified by GC-MS, GC-FID analysis (Fig. 2), retention times and area percentages are summarized in Table 1. The essential oil of *E. spinosus* roots was dominated by sesquiterpenoids compounds which represent 42.245% of the total essential oil. γ-Cadinene (27.224 %) was the main component of this fraction followed by caryophyllene oxide (5.217%) and β-caryophyllene (2.736%). The monoterpenoids compounds constitute only 4.005 %. α- Pinene was the main compound in this series followed by camphor (0.862%). In addition, two thiophenes compounds were also identified and account for 39.358% (Table 1). These results showed some differences in composition with respect to data in literature. As regards to previous investigations on *E. grijsii*, *E. gigantus*, *E. bannaticus*, *E. phaerocephalus*, *E. graecus* and *E. rito*, many researchers mentioned the presence of sesquiterpene lactone (α and β-caryophyllene) and thiophenes such as 2,2',5',2''-terthiophene and 5-(3-buten-1-ynyl)-2,2'-bithienyl. In addition, Chinese *E. grijsii* roots essential oil was characterised by the presence of (Z)- β-farnesene (25.18%), 5-(but-3-en-1-ynyl)-2,2 -bithiophene (19.67%), β-bisabolene (12.11%), and α-terthienyl (8.36%), while, essential oils from the roots of *E. bannaticus* and *E. sphaerocephalus* harvested from South Serbia mainly contained 5-(3-buten-1-ynyl)-2,2 -bithienyl (47.3% and 48.9%, resp.) and α-terthienyl (15.5% and 13.7%, resp.).[17-21] The changes in the essential oil composition might arise from several environmental such as climatic, geographic origin, harvesting time of the samples as well as genetic factors.[22,23]

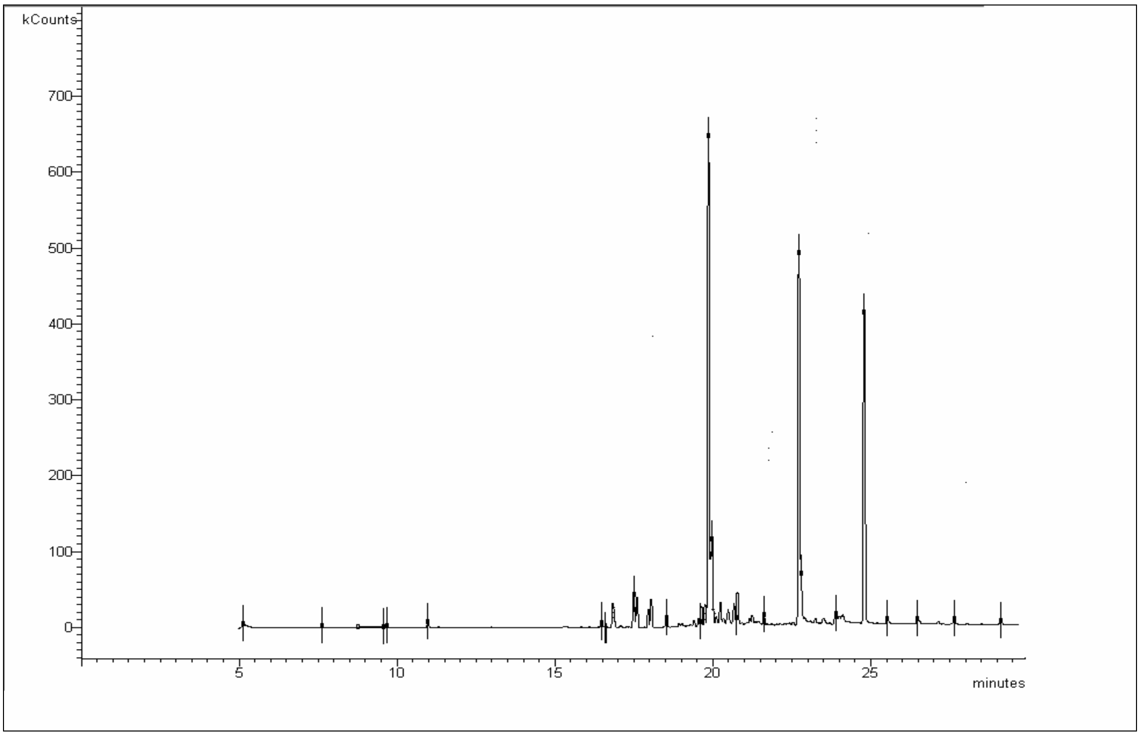

Fig. 2 – GC chromatographic profile *Echinops spinosus* roots essential oil.

Table 1

Constituents identified from the essential oil of *Echinops spinosus* roots

Peak No.	Compound	RT min	Kovats RI	% relative peak area
	Monoterpenoids			**4.005**
1	α- Pinene	5.115	939	1.532
2	β- Phellandrene	7.601	1012	0.413
3	1,8-Cineole	8.745	1050	0.321

Table 1 (continued)

4	Linalool	9.541	1082	0.549
5	Thujone	9.767	1090	0.328
6	Camphor	11.070	1146	0.862
	Sesquiterpenoids			**42.245**
7	β-Caryophyllene	17.564	1418	2.736
8	Longifolene	17.933	1440	1.988
9	alpha Caryophyllene	18.487	1460	1.522
10	γ-Cadinene	19.852	1526	27.224
11	Caryophyllene oxide	20.295	1578	5.217
12	Agarospirol	20.553	1635	1.627
13	α –Cadinol	21.693	1652	1.931
	Thiophenes			**39,358**
14	5-(3-buten-1-ynyl)-2,2'-bithienyl	22.730	1943	21.334
15	2,2',5',2''-Terthiophene	24.686	2240	18.024
	Others			**11.862**
16	Eugenol	16.494	1360	2.774
17	methyl-Eugenol	16.568	1403	2.265
18	9-octadecenoic Acid, 1,2,3-propanetriyl ester	23.837	1970	2.356
19	2-hexyl-1-decanol	27.822	2760	2.956
20	tetracontane,3,5,24-trimethyl- 3,5,24-trimethyltetracontane	29.335	3995	1.511
	Total			**97.470**

Antioxidant activities

Total antioxidant activities of plant essential oils cannot be evaluated by any single method, due to the complex nature of phytochemicals. Therefore, it is well known that the chemical complexity of essential oil, often a mixture of compounds with different functional groups, polarity and chemical behaviors, could lead to scattered results, depending on the test employed. Therefore, two or more methods should always be used in order to evaluate the total antioxidative effects of plant essential oils.[24,25] Moreover, antioxidant activities of the studied oil were tested by the DPPH radical scavenging and FRAP methods. The results obtained from the preliminary analysis of antioxidant activity were shown in Table 2. According to the data obtained for *E. spinosus* roots essential oil has a good antioxidant activity, 2.560 mg AAeq / g EO and 2.814 mg BHT eq/ g EO, for DPPH and FRAP assays respectively. These minor differences in the antioxidant activities may be attributed to the intrinsic mechanisms of the antioxidant reactions in the different assays, or to factors such as stereo-selectivity of the radicals and the solubility of antioxidant components.[8] Our data are in accordance with those previously reported for the genus *Echinops* where a noticeable antioxidant activity is shown, and confirm that genus *Echinops is* a natural source of several antioxidant substances to be used as efficient antioxidant agents comparable to commercially used antioxidants.[26] Rigane *et al.* [8] and Kiyekbayeva *et al.* [26] mentioned that antioxidant activity of essential oil largely depends on its chemical composition and is especially attributed to their major compounds. In addition, the level of the antioxidant activity for the studied essential oil may be attributed to the high concentration of γ-cadinene and thiophenes, *i.e*,5-(3-buten-1-ynyl)-2,2'-bithienyl and 2,2',5',2''-terthiophene.[27,28] On the other hand, many scientific works mentioned that monoterpenes as well as eugenol could also be taken into account due to their powerful antioxidant activity.[29-34]

Table 2

Antioxidant activities of *Echinops spinosus* roots essential oil

	DPPH assay (mg AAeq/g EO)	FRAP assay (mg BHT eq/g EO)
Essential oil of ES (EO)	2.560 ± 0.03	2.814 ± 0.01

Table 3

Antimicrobial activity of essential oil of *Echinops spinosus* roots

Strains	**Inhibition zone diameter (mm)**		**Minimal inhibitory concentration MIC (μg/mL)**	
	Essential Oil	**Gentamycin**	**Essential oil**	**Gentamycin**
Bacterial strains				
Gram-positive				
Staphylococcus aureus ATCC 29213	13.5±0.3	26±0.5	20±0.5	8±0.1
Enterococcus faecalis ATCC 29212	17.5±0.3	12±0.2	20±0.5	4±0.1
Gram-negative				
Escherichia coli ATCC 25922	8.0±0.0	25±0.2	-	1±0.1
Klebsiella pneumoniae ATCC 9997	8.0±0.0	12±0.5	-	15±0.1

(-): resistant microorganisms.
(*): inhibition around the disc with 20 μl of extract. (Diameter of disc is included)
The values are average of 3 assays ± Standard deviation (SD).

As it was reported by Wang *et al.*,[35] it is difficult to attribute the antioxidant effect of a total essential oil to one or a few active compounds. Both minor and major compounds should make a significant contribution to the oil's activity which is the interaction result of their chemical composition.

Our results highlight the interest of *E. spinosus* roots essential oil which promotes its potential source of active ingredients for food and pharmaceutical industry.

Antimicrobial activity

The *in vitro* antimicrobial activity of *Echinops spinosus* roots essential oil against the tested microorganisms was qualitatively and quantitatively assessed by the presence or absence of inhibition zone diameters and minimal inhibitory concentration (MIC) values. As shown in Table 3, the essential oil exhibited varying degrees of antibacterial activity against all tested strains. The inhibition zones were in the range of 8-17.5 mm. The MIC values of Essential oil showed an antibacterial activity against tested Gram-positive more than negative bacteria (20 μg/mL for Gram-positive and nothing for Gram-negative). We found that the activity of the essential oil depends on its concentration and the strain of tested bacteria. The Gram-positive bacteria were more susceptible to the antimicrobial properties of essential oil than the Gram-negative ones. These differences could be attributed to the great complexity of the double membrane-containing cell envelope in Gram-negative bacteria compared to the single membrane structure of positive ones. The *Echinops spinosus* roots essential oil was highly active against *Staphylococcus aureus* ATCC 29213 (Gram-positive) and *Enterococcus faecalis* ATCC 29212 (Gram-positive) showing an important growth inhibition at lower concentrations. The *Echinops spinosus* roots essential oil was also found to inhibit moderately the growth of clinically important Gram-negative bacteria, *Klebsiella pneumoniae* ATCC 9997 (Gram-negative) and *Escherichia coli* ATCC 25922 (Gram-negative), with inhibition zone of 8 mm for both. The microorganisms tested in the present investigation are large and cover the most important human pathogens known as opportunists for men and animals and cause food contamination and deterioration. Therefore, the obtained results are of a great importance.

It is necessary to signal that some compounds in low content may also contribute to improve this high activity. In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration to account for their biological activity. In this context, minor constituents in the oil under study α-pinene (1.536%), linalool (0.548%) and thujone (0.328%) are monoterpenes reported to show activity against these organisms.[36] Another minor constituent, eugenol (2.774%), is known to have very efficient antibacterial properties.[36] Although these compounds are not abundant in the essential oil but their activity is important. For thiophenes, a study shows that 5-(3-buten-1-ynyl)-2,2-bithienyl has antibacterial activity against *Streptococcus pneumoniae.*[37] From these results, *Echinops spinosus* roots essential oil may be considered as a natural preservative against pathogens for food production industry and human being.

CONCLUSION

In the present work, we reported the chemical composition of Tunisian essential oil of *Echinops spinosus* roots. Twenty components were identified and the main components are γ-cadinene, 5-(3-buten-1-ynyl)-2,2'-bithienyl,2,2',5',2''-terthiophene. For the first time, we demonstrate that the essential oil of *Echinops spinosus* roots exhibits antioxidant activity and successfully inhibits the growth of different pathogens that can cause food spoiling as well as health problems. The results obtained in this study show that the essential oil of *Echinops spinosus* roots may be a new potential source of natural antioxidants and antimicrobial agents for the food industry. However, further studies need to be conducted to understand the mechanism of the activity and obtain more information on the safety and toxicity of the oil.

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