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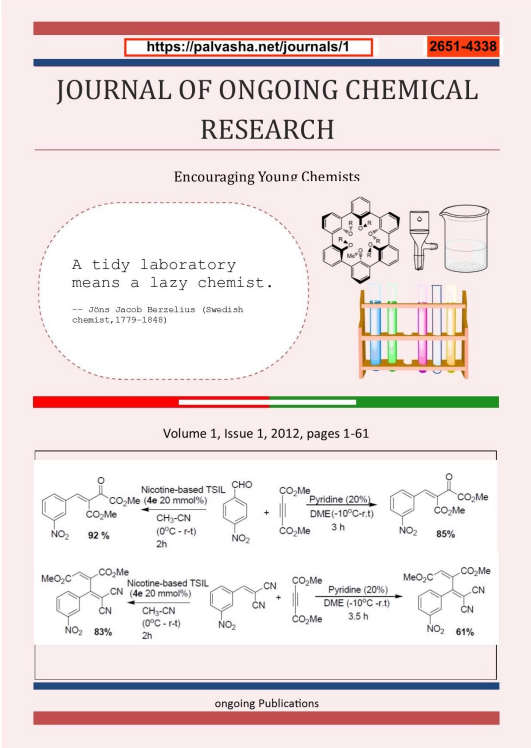

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Research Article

The Chemical Composition of *Centaurea Furfuracea* Coss. and Dur. Essential Oil with Antioxidant, Anticholinesterase and Antibiofilm Activities

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Abstract

The aim of this work was to conduct the chemical composition of essential oil (EO) of *Centaurea furfuracea*from Algerian Sahara and investigate the antioxidant, anticholinesterase, antimicrobial and antibiofilm activities of its essential oil and methanol extract. EO of *C. furfuracea* was analyzed by GC and GC-MS. Sixty-nine compounds identified, representing 96.94 % of the total oil. Caryophyllene oxide (12.01 %), Z-10-pentadecen-1-ol (8.11 %), farnesyl methylester (7.79 %) were identified as the main constituents. The antioxidant activity was determined using three complementary assays, in lipid peroxidation inhibition the methanol extract showed the best activity with an IC_{50} = 28.73 ± 0.29 µg/mL followed by EO with an IC_{50} = 95.95 ± 15.20 µg/mL. While in DPPH and CUPRAC assays, EO and methanol extract indicated a less to moderate activity. The *in vitro* anticholinesterase activity, the methanol extract showed moderate inhibitory activities against acetylcholinesterase (IC_{50}= 164.4 ± 5.69 µg/mL) and butyrylcholinesterase (IC_{50} = 82.4±1.75 µg/mL), while EO was inactive against both enzymes. Minimum inhibitory concentrations (MICs) were calculated by microtitre broth dilution method, and antibiofilm effect by microplate biofilm assay. EO inhibited the growth of all tested microorganisms, MIC values were between 6.25 and 25 µL/mL concentrations, better than methanol extract. The highest antibiofilm activity have reached to 87.90 % with methanol extract of *C. furfuracea* against *Staphylococcus aureus* ATCC 6538 Pat 50 mg/mL concentration.These results showed that *C. furfuracea* is a natural source of active compounds with antibiotic and antibiofilm effects against *S. aureus*and *B. subtilis*, and *Bacillus cereus*, respectively, and also presents antioxidant and anticholinesterase properties.

Keywords: Antioxidant, Antibiofilm, Anticholinesterase, Centaurea Furfuracea, Essential Oil

INTRODUCTION

Since ancient times, medicinal plants have been incorporated into food because of their phytochemical properties, nutritional content, antibacterial, preservative and flavoring properties. Apart from these unique qualities, medicinal plants have also been found to improve the shelf life of food, as they exhibit antioxidant activities which can mop up the free radicals, thereby inhibiting rancidity and decay [Shan et al, 2007]. Medicinal plants have been proven to be safe for consumption, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies [Smid et al, 1999]. After some microbes were found to be resistant to antibiotics, researchers found out that spices and essential oils (EOs) also possess antimicrobial actions alongside their flavoring and preservative properties. These properties have placed a lot of value on them, making them relevant for varied applications [Dorman et al, 2000]. These unique properties have been exploited and classified, forming the basis for the use of extracts of medicinal plants in the production of several food and pharmaceutical products, and their extensive application in many natural therapies, raw material and processed food preservation, alternative medicine, vaccine production, and pharmaceutical and biomedical researches [LisBalchin et al, 1997; Yildiztekin et al, 2016].

Due to its variety of geographical locations, Algeria has a rich flora of medicinal plants. There are about 3500 plant species reported in Algeria among which 500 are regarded of medicinal values [Quzel et al, 1963]. The genus *Centaurea*(Asteracea) comprises about 500 species, which are predominately distributed around the Mediteranean area and in West Asia

[Djeddi et al, 2008]. The genus *Centaurea*is represented by 42 species in the Algerian flora, which seven are localised in the Sahara [Quzel et al, 1963].

*Centaurea furfuracea*Coss. & Dur. is an Annual or perennial herb, with short main stem and ending with a head (capitulum) in which the branches are born long branches and ending with heads (less than 15 mm diameter), lobed leaves, bracts with short woolly hairs and yellowish-white achene [Ozenda, 1977; Quzel et al, 1963].

Chemical investigations of various *Centaurea*species have revealed mainly flavanoids, alkaloids and lignans [Karamenderes et al, 2007; Shoeb et al, 2005] and sesquiterpene lactones lactones, which are guaiane, germacrane, elemane and eudesmane skeletons [Gonzalez et al, 1978; Grbz et al, 2007; Koca et al, 2009]. Many *Centaurea*species are reported in the literature to be used in folk medicine such us antidandruff, antidiarrhoic, antirhevmatic, anti-inflammatory, and antibacterial [BlentKse et al, 2007; Csupor et al, 2010; Zengin et al, 2010]. *C. furfuracea*was found to contain 13 flavonoid compounds. such as: hispidulin-7-*O*-methylglucuronide [Akkal et al, 1999], apigenin, hispidulin, cirsimaritin, 5,7,4'-trihydroxy-3-methoxyflavones, apigenin-7-*O*-glucoside, apigenin-7-*O*-methylglucuronide, hispidulin-7-*O*-glucoside, patelutin-7-*O*-glucoside [Akkal et al, 2003], acacetin, jaceosidin [Fakhfakh et al, 2005], isokaempferide-7-*O*-methylglucuronide, isokaempferide-7-*O*-glucuronide . The last two compounds have showed cytotoxic and antiparasitic activities [Akkal et al, 2007]. It was also reported that *C. furfuracea*contains lignans: (-) trachelogenin and tracheloside and sucrose acetat [Fakhfakh et al, 2005]. Two new sesquineolignans, furfuraceol A and furfuraceol B have been isolated from the flowers of *C. furfuracea*as a mixture of two isomers [Fakhfakh et al, 2007].

Oneof the motives that stimulate the interest in this subject, especially in the desert area, is the diversity of vegetation and their content in natural products that can have therapeutic benefit. In the study in hand, the biological effect through the study of antioxidant by using three methods (DPPH, CURAC and □-caroten/linoleic acid), antibiofilm and acetylcholineseterase activities of essential oil and methanol extract of *C. furfuracea* from Algerian Sahara. Moreover, polyphenols and falvonoids content and GC/MS analysis of essential oil were carried out.

RESULTS AND DISCUSSION

Chemical composition

The table 1 shows the chemical composition of the essential oil of *C. furfuracea* obtained by hydrodistillation method, the yield of essential oil was 0.52% (volume/dry-weight) and having a yellow color. A total of 69 constituents comprising 96.94% were characterized by the essential oil. The components listed in order of elution on a DB-1 column. Caryophyllene oxide, Z-10-Pentadecen-1-ol and Farnesyl methylester were major compounds representing 12.01 %, 8.11%, and 7.79% respectively.

Caryophyllene oxide (12.01%), the major component of the aerial part oil of *C. furfuracea* has been also identified as the major fraction (38.5%) of essential oil of aerial part of *C. pullata* growing in Blida, North Algeria (Djeddi et al, 2011); the chloroform extract of aerial parts of *C. ensiformis* (28.72%),*C. austro-anatolica*(21.32%)and*C. cariensis*subsp. *niveo-tomentosa*(20.79%) collected in Mugla, Turkey (Ugur et al, 2009; Ugur et al, 2010); aerial parts of *C. athoa* oils (17.1%) (Erel et al, 2013); *C. aucheri* (17.4%) (Asadipour et al, 2005); *C. raphanina* subsp. *mixta* (10.3%)(Lazari et al, 1999)and *C. thessala*. subsp. *Drakiensis* (7.8%) (Lazari et al, 2000). Aslo, presented in many *Centaurea* essential oils by a different percentage. Such as: Aerial parts of*C. solstitialis* (25.2%); *C. depressa* (4.0%) (Esmaeili et al, 2006); capitula of *C. deflexa* (12.8%); *C. aladaghensis* (7.5%); *C. cheirolepidoides* (6.1%); flower heads of *C. chrysantha* (9.5%) (Flamini et al, 2006); *C. eryngioides* (4.3%)(Senatore et al, 2005) and seeds of *C. huber-morathii* (3.3%) (Baser et al, 2006). Whereas, Farnesyl methylester 8.11%, Z-10-Pentadecen-1-ol 7.79%, and trans-2-hexadecenoic acid6.08% have not been identified in *Centaurea* essential oil previously.

Components of the essential oil of were separated into five classes, including monoterpenoids (1.15%), sesquiterpenes (19.83%), sesquiterpenoids (52.3%); fatty acids (6.97%) and others (16.69%). The essential oil consisted mainly of oxygenated sesquiterpenes (52.3%). caryophyllene oxide (12.01%) and Z-10-Pentadecen-1-ol (7.79%) were the prevailing oxygenated sesquiterpenes. Different parts of *C. zuvandica*; *C. cariensis* subsp. *niveo-tomentosa*; *C. ensiformis* and *C. athoa* were rich in oxygenated

sesquiterpenes (Erel et al, 2013; Salmanpour et al, 2009; Ugur et al, 2009; Ugur et al, 2010). The essential oil of *C. furfuracea* also resembled those oils from the classification side.

Total phenolic and total flavonoid contents and Antioxidant activity

There are many methods for the assessment of antioxidant potential, and we cannot reliance on a single universal method. Thus, we tested three antioxidant assays, that would give a better understanding of the true antioxidant potential of the essential oil and methanol extract. Table 2 summed the results of antioxidant activity of the extracts. In the □-carotene-linoleic acid assay, the methanol extract of *C. furfuracea* showed the best lipid peroxidation inhibition activity with an $IC_{50}$of 28.73 ± 0.29 µg/mL followed by its essential oil (95.95 ± 15.20 µg/mL). Some literature reported that the inhibition capacity of *Centaurea* species such as *C. mucronifera* (35.2%) (Tepe et al, 2006), *C. ensiformis* (85.15% of ethyl acetate, 72.51% of chloroform extract) (Ugur et al, 2009) and 63.60% of methanol extract in *C. pulchella* (Zengin et al, 2010). In contrast, in DPPH assay, the methanol extract and essential oil showed moderate to low activity with IC_{50}= 190.47 ± 0.99 µg/mL for Methanol extract and 1664.95 ± 32 µg/mL for essential oil. The results of cupric reducing antioxidant capacity were given as absorbances (Fig. 1). Higher absorbance exhibited higher activity. Generally, the extracts showed weak absorbances compared with BHA and α-Tocopherol. The values $DPPH^{\bullet}$ scavenging activity of *Centaurea* species which plants growing in Scotland was found as ranging from 0.018 mg/mL and 0.095 mg/mL (Kumarasamy et al, 2007).

From the results, it can be concluded that *C. furfuracea* has lower free radical scavenging activity than growing in Scotland. Total phenolic and flavonoids contents of the extracts were investigated spectrophotometrically. The methanol extract had 4.75 ± 0.009µg pyrocatechol equivalents as its phenolic content and demonstrated 3.17 ± 0.001 µg quercetin equivalents as its flavonoid content. The number of phenolics was very small with the results described in the literature for other *Centaurea* species. For example, the higher content was detected as 348.56 mg GAE $g^{-1}$for MeOH extract of *C. pulcherrima* var. *pulcherrima* growing in Turkey (Aktumsek et al, 2013).

Table 1: The Chemical composition (%) of essential oil of *C. furfuracea*

N°	Compounds	Composition (%)	RI[a]
1	Furan, 2-pentyl-	0.10	984
2	2-Nonen-1-ol	0.17	1088
3	Linalool	0.09	1100
4	Camphor	0.06	1141
5	p-cymen-8-ol	0.06	1186
6	Safranal	0.06	1189
7	(E)-2-Decenal	0.29	1202
8	Perilla aldehyde	0.43	1270
9	Chroman	0.15	1286
10	Carvacrol	0.07	1300
11	2,4-Decadienal, (E,E)-	0.10	1305
12	α-cubebene	0.13	1350
13	Alloaromadendrene	0.06	1372
14	β-Elemene	1.61	1389
15	7-tetradecene	0.09	1394
16	(2Z)-3,7-Dimethyl-2-octenyl-2-methylpropanoate	0.14	1405
17	Cedrene	0.23	1410
18	β-Caryophyllene	5.57	1420
19	Isopropenyl-2,3,4,5-tetra methyl benzene	0.81	1428
20	γ(tau)-Elemene	2.64	1436
21	di-epi-α-cedrene	0.22	1440
22	α-humulene	0.51	1445
23	Z-β-Farnesene	0.96	1448
24	α-Himachalene	0.14	1450
25	gamma-elemene	0.31	1456
26	α-Guaiene	1.33	1476
27	Epi-bicyclosesquiphellandrene	2.48	1479
28	Eremophilene	0.43	1483
29	Ledene	0.10	1487
30	γ-Gurjunene	0.66	1495
31	Z-10-pentadecen-1-ol	7.79	1498
32	Isocaryophyllene	0.50	1509
33	Aromadendrene oxide-2	0.12	1514
34	5,6-Decadien-3-yne, 5,7-diet	0.53	1519
35	β-Cadinene	0.33	1530
36	β-Guaiene	0.17	1532
37	Epiglobulol	0.23	1539
38	tau-Gurjunene	1.46	1550
39	aromadendrene oxide	0.62	1569
40	Spathulenol	1.76	1577
41	Caryophyllene oxide	12.01	1556
42	Z-α-bisabolene epoxide	2.52	1590
43	Longifolenaldehyde	0.31	1613
44	8-cedrene-13-ol	1.68	1624
45	β-Eudesmol	3.68	1649
46	Santalol	0.22	1657
47	5β-7β-10α-Eudesm-11-en-1α-ol	1.32	1663
48	7,10,13-Hexadecatrienoic acid methyl ester	5.51	1670
49	Z-9,17-Octadecadienal	2.10	1674
50	Murolan-3,9(11)-diene-10-peroxy	1.43	1693
51	(2Z, 13E)-Octadecadien-1-ol	0.87	1725
52	6,8,8-trimethyl-2-methylenetricylo(5,2,2,0)undecan-3-ol	1.22	1736
53	Geranyllinallol	1.52	1751
54	Eudesma-4,11-diene-2-ol	1.12	1773
55	γ-costol	0.90	1775
56	Farnesyl methylester	8.11	1780
57	3Z,15Z-Octadecadien-1-ol acetate	0.18	1805
58	Hexahydro farnesylacetone	0.88	1838
59	1-Eicosanol	3.05	1874
60	Methyl 9,10-epoxystearate	0.10	1955
61	Verticiol	0.14	2014
62	1,2,3,4-tetrahydrophenanthren-9-ol	0.21	2044
63	n-Hexadecanoic acid	0.47	2103
64	9-Hexadecenoic acid	0.42	2125
65	trans-2-hexadecenoic acid	6.08	2213
66	3,7,11,15-tetramethyl-2-hexadecen-1-ol	1.96	2235
67	2-Octadecoxyethanol	3.94	2475
68	3-ethyl-5(2-ethylbuthyl) Octadecane	0.49	2598
69	1-docosanol	1.00	984
Total identified: 96.94; Monoterpenoids: 1.15; Sesquiterpenes: 19.83; Sesquiterpenoids: 52.30; Fatty acids: 6.97; Other: 16.69			

[a]Kovats index on DB-1 fused silica column.

While lower content found with MeOH of *C. ammocyanus* as 10.9 mg GAE $g^{-1}$from Jordan (Alali et al, 2007).

Table 2: Antioxidant activity (%) of the essential oil and methanol extract of C. furfuracea by the DPPH and β-carotene/linoleic acid assays

	Concentration	MeOH	EO	BHA	a-Tocopherol
DPPH	12.5	3.98 ± 0.87	NA	31.15 ± 0.65	90.7 ± 0.23
	25	5.72 ± 0.97	NA	38.56 ± 0.81	91.16±0.17
	50	14.76 ± 0.90	2.59 ± 1.19	43.78 ± 0.21	92.03±0.55
	100	29.46 ± 0.45	3.80 ± 1.59	59.9 ± 0.35	93.77 ± 0,07
	200	51.16 ± 0.30	4.97 ± 0.64	79.83 ± 0.51	95.9 ± 0.05
	400	65.36 ± 0.57	5.98 ± 1.12	90.58 ± 0.24	96.1 ± 0.9
	800	68.73 ± 0.21	8.43 ± 1.22	94.16 ± 0.15	96.7 ± 0.21
	IC50	190.47 ± 0.99	1664.95 ± 32	45.4 ± 0.47	7.31 ± 0.17
β-carotene /linoleic acid	12.5	38.9 ± 6.51	18.27 ± 4.01	90.1 ± 0.2	89.15 ± 0.1
	25	51.56 ± 5.48	40.26 ± 2.41	91.56 ± 0.22	90.6 ± 0.3
	50	66.56 ± 1.18	46.23 ± 1.53	92.68±0.3	91.89±0.27
	100	72.09 ± 1.56	50.61 ± 0.36	93.6 ± 0.16	92.1 ± 0.51
	200	75.64 ± 1	52.99 ± 3.04	94.8 ± 0.21	93.32±0.33
	400	75.41 ± 1.98	57.59 ± 2.22	95.8 ± 0.15	94.22 ± 0,28
	800	77.12 ± 1.06	62.31 ± 0.24	97.7 ± 0.16	96.02 ± 0,30
	IC50	18.6 ± 4.11	91.25 ± 0.14	1.34 ± 0.04	2.10 ± 0.08

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

NA: not active

The same thing with total flavonoid content, the other *Centaurea* species showed high amount than *C. furfuracea*, ranging from 13.12 ± 1.01 mg Rutin Equivalent g^{-1}extract to 182.56 ± 2.13mg RE g^{-1}in MeOH extract of *C. babylonica* and *C. pulcherrima* var. *pulcherrima*, respectively (Aktumsek et al, 2013). These differences may due to genotype and growing location according to study carried out by Mpofu (Mpofu et al, 2006) on wheat. Also, Giorgi (Giorgi et al, 2010) reported that higher altitude may constitute a highly effective way to significantly enhance the levels of phenolic acids. The noticeable thing, the hight phenolic, and flavonoid contents showed with *C. pulcherrima* var. *pulcherrima* collected from Kardeştep village, Arpacay, Kars in Turkey at 2245 m altitude. whereas, *C. furfuracea* collected from Algerian Sahara at33 m altitude.

Anticholinesterase activity

AChE inhibitors are used for the treatment of mild to the moderate AD (Birks, 2006). Currently, the three cholinesterase inhibitors licensed Donepezil, Rivastigmine, and Galantamine are widely recommended for clinical use (Kaduszkiewicz et al, 2005). Recently, there is a growing interest from the researchers about finding new AChE inhibitors from the plant resources. Anticholinesterase activity of some *Centaurea* species has been studied by Aktumsen et al (Aktumsek et al, 2013). This study inspected the anticholinesterase activity of *C. furfuracea* for the first time. Table 3 shows the results of AChE and BChE inhibitory activities of the extracts compared with that of Galantamine used as a standard drug. The tests were screened for AChE inhibitory activity using Ellman's colorimetric method in a 96-well plate by a microplate reader. As shown in table 3 the methanol extract indicated a noticeable inhibition against AChE and BChE at all concentrations, the IC_{50}values were found be 164.4 ± 5.69 and 82.4 ± 1.75 µg/mL, respectively. These values are comparable to the value reported in the literature for other *Centaurea* species such as *C. polypodiifolia* var. *pseudobehen* (24.54% and 45.50% for AChE and BChE at 2 mg/mL) (Aktumsek et al, 2013). while the essential oil showed almost no activity against AChE and BChE. Whereas, essential oils of other *Centaurea* species showed moderate activity (Ertas et al, 2014). Values expressed as absorbance at 450 nm is means ± standard deviation of three parallel measurements. ($p < 0.05$).

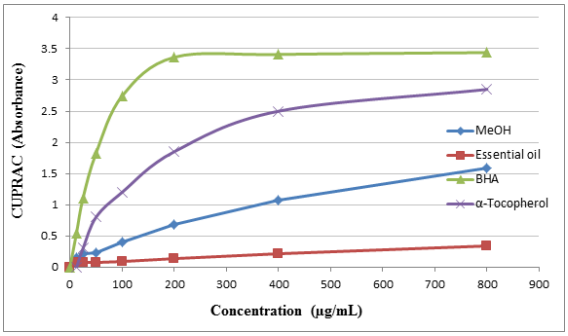

Figure 1. Cupric reducing antioxidant capacity of the essential oil and methanol extract of *C. furfuracea*

Minimum inhibitory concentrations and antibiofilm activity

Biofilms can provide a protective environment for pathogenic bacteria and reduce the effectiveness of inhibitory agents, Which leads to cause diseases in humans and animals (HallStoodley et al, 2004). The antibiofilm activity and MIC of the essential oil and methanol extract of *C. furfuracea* were studied in this work. The results are shown in Table 4. Statistically, the essential oil inhibited the growth of all tested microorganisms between 6.25 and 25 µL/mL concentrations, better than methanol extract. MIC values determined for methanol extract between 6.25 to 50 mg/mL. The extract has low activity on the growth of *S. aureus* ATCC 6538 P, *S. epidermidis* MU 30, *B. cereus*RSKK 863 and *M. luteus* NRRL B-4375 which are only inhibited at high concentration (50 mg/mL). The antimicrobial activity of essential oils and extracts of some *Centaurca*species have been investigated before. Ethyl acetate, acetone, chloroform, and ethanol extracts from *C. ptosomipappoides*, *C. odyssei*, *C. ptosomipappa*, *C. amonicola* and *C. kurdica* were investigated by agar-well diffusion assay, and all of the extracts exhibited an antimicrobial effect against *Staphylococcus aureus*, *Micrococ cusluteus*, *Bacillus cereus*, *Salmonella typhimurium*, *P seudomonas aeruginosa*, *Klebsiella pneumoniae*, *Cand ida albicans* and *Candida globrata*. When the high antimicrobial activity is shown in ethyl acetate extract with MIC values between 62.5 and 250 mg/mL (Gven et al, 2005).

Table 3: Acetylcholinesterase and butyrylcholinesterase inhibitory activities (%) of the essential oil and methanol extract of *C. furfuracea*.[a]

	Concentration µg/mL	MeOH	EO	Galantamine
AChE	3.125	9.59 ± 2.79	*NA*	41.75 ± 0.65
	6.25	10.67 ± 0.53	*NA*	52.32 ± 1.20
	12.5	18.62 ± 0.3	*NA*	62.21 ± 0.32
	25	21.22 ± 1.6	*NA*	68.36 ± 1.10
	50	22.92 ± 2.31	*NA*	74.38 ± 0.65
	100	41.27 ± 0.26	*NA*	78.59 ± 0.47
	200	55.9 ± 2.42	*NA*	80.4 ± 0.9
	IC_{50}	**164.4 ± 5.69**	*NA*	**5.01 ± 0.09**
BChE	3.125	*NA*	*NA*	17.44 ± 1.08
	6.25	*NA*	*NA*	21.35 ± 0.66
	12.5	*NA*	*NA*	29.62 ± 1.30
	25	13.56 ± 1.78	*NA*	40.59 ± 2.88
	50	34.52 ± 1.07	*NA*	48.73 ± 0.90
	100	59.14 ± 1.73	*NA*	65.02 ± 0.44
	200	-[b]	-	82.2 ± 1.6
	IC_{50}	**82.4 ± 1.75**	-	**53.9 ± 0.56**

NA: not active

[a] IC_{50} values represent the means ± SD. of three parallel measurements ($p < 0.05$).

[b] Could not be determined due to turbidity in the well.

In another study, The essential oils of *C. sessilis* and *C. armena* showed antibacterial activity against *Yersinia pseudotuberculosis*ATCC 911, *Enterococcus faecalis*ATCC 29212, *Staphylococcus aureus*ATCC 25923, and *Bacillus subtilis* ATCC 6633 with inhibition zones between 5.5 and 10 mm (Yayli et al, 2005). Whereas, essential oils of *C.appendicigera*and *C. helenioides* showed antimicrobial activity against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10231 with MIC between 80 and 330 µg/mL (Yayl et al, 2009). In another study by (BlentKse et al, 2007). The high antimicrobial activity of *Centaurea aladagensis* essential oil has shown against *Staphylococcus epidermidis* ATCC 1228 with MIC = 0.11 mg/mL. However, extracts and essential oils of *Centaurea* species showed noticeable antimicrobial activity against some microorganism with various percentages. In the presence of 25 µL/mL essential oil (MIC), the mean biofilm formation values were equal to 40.75% for *B. subtilis* ATCC 6633 and 39.80% for *C. albicans* ATCC 10239. Essential oil did not exhibit inhibitory effect against *S. aureus* ATCC 6538-P biofilm formation. The highest antibiofilm activity has been found 87.90% with MeOH extract against *S. aureus* ATCC 6538 P and 87.53% against *B. subtilis* ATCC6633 at 50 mg/mL and 25 mg/mL concentrations (MIC), respectively. *C. furfuracea* essential oil has shown a good antimicrobial activity against test bacteria while extract has shown good antibiofilm activity.

Table 4: MIC and antibiofilm activities of the essential oil and methanol extract of C. furfuracea

Microorganism	Essential oil						Methanol extract					
	Planktonic	% inhibition on biofilms					Planktonic	% inhibition on biofilms				
	MIC µL/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16	MIC mg/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16
Staphylococcus aureus ATCC 25923	12.5	11.82	NI	NI	NI	NI	6.25	14.51	NI	NI	NI	NI
Staphylococcus aureus ATCC 6538 P	6.25	NI	NI	NI	NI	NI	50	87.90	34.41	18.60	NI	NI
Staphylococcus epidermidis MU 30	12.5	28.09	21.22	NI	NI	NI	50	80.13	38.35	18.49	NI	NI
Bacillus subtilis ATCC 6633	25	40.75	16.28	NI	NI	NI	25	87.53	56.74	34.01	10.09	NI
Bacillus cereus RSKK 863	6.25	6.94	NI	NI	NI	NI	50	83.03	72.34	47.94	15.60	7.47
Micrococcus luteus NRRL B-4375	25	10.40	NI	NI	NI	NI	50	79.07	58.85	51.20	22.91	13.47
Streptococcus mutans CNCTC 8/77	6.25	18.08	NI	NI	NI	NI	25	31.09	19.57	6.42	NI	NI
Candida albicans ATCC 10239	25	39.80	9.01	NI	NI	NI	12.5	36.11	14.44	9.51	NI	NI

NI: no inhibation

EXPERIMENTAL

Plant material

The aerial parts of *C. furfuracea* were collected in the North fringe of the Algerian Sahara during the flowering period in April 2012 near Stile, El-Oued, Algeria (34°18'N, 5°54'E) at 33 m altitude and taxonomic identification of plant was confirmed by Dr. Youcef Halice in the Scientific and Technical Research Centre for Arid Areas. The collected plant material was air-dried in darkness at room temperature for three weeks and a voucher sample (CAK 5) was deposited in the Laboratory of Biology, University of El Oued, Algeria.

Extraction of the essential oil

The essential oil of dried aerial parts (300g) of *C. furfuracea* was obtained via hydrodistillation by using a Clevenger type apparatus for 4 h. The oil was dried over anhydrous sodium sulfate and stored under nitrogen until required.

Gas chromatography (GC)

GC analyses of the oil were performed using a Shimadzu GC-17 AAF, V3, 230V LV Series (Kyoto, Japan) gas chromatography, equipped with an FID and a DB-1 fused silica column [30m x 0.25 mm (i.d.), film thickness 0.25 µm]; the oven temperature was held at 60 °C for 5 min, then programmed to 240 °C at 4 °C min^{-1} and held isothermal for 10 min; injector and detector temperatures were 250 and 270 °C respectively; carrier gas was He at a flow rate of 1.3 mL min^{-1}; Sample size, 1.0 µL; split ratio, 50:1. The percentage composition of the essential oil was determined with a Class-GC 10 computer program.

Gas chromatography–mass spectrometry (GC–MS)

The analysis of the essential oil was performed using a Varian Saturn 2100 (Old York Rd., Ringoes, NJ, USA), ion trap machine, equipped with a DB-1 MS fused silica non-polar capillary column [30 m x 0.25 mm (i.d.), film thickness 0.25 µm]. The carrier gas was helium at a flow rate of 1.4 mL min^{-1}. The oven temperature was held at 60 °C for 5 min, then increased up to 240 °C with 4 °C min^{-1} increments and held at this temperature for 10 min. Injector and transfer line temperatures were set at 250 and 180 °C, respectively. Ion trap temperature was 200 °C. The injection volume was 0.2 µL and the split ratio was 1:30. EI-MS measurements were taken at 70 eV ionization energy. Mass range was from *m/z*28 to 650 amu. Scan time was 0.5 s with 0.1 s interscan delays.

Identification of components of the essential oils was based on GC retention indices and computer matching with the Wiley, NIST-2005 and TRLIB Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [Adams, 2007] and, whenever possible, by co-injection with authentic compounds.

Antioxidant activity

Free radical-scavenging activity (DPPH assay)

To determine the free radical scavenging activity of EO and methanol extract, an assay was carried out using the DPPH method as described by Blois, with slight modification [Blois, 1958; ztrk et al, 2011]. The assay principle states that; In its radical form DPPH absorbs at 517 nm, but on reduction by an antioxidant or a radical species its absorption decreases. Briefly, a 0.1 mmol L^{-1} solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 ml of samples solution in methanol at different concentrations. It was allowed to stand for thirty minutes, after which the absorbance was read at 517 nm. Lower absorbance indicates higher free radical scavenging activity.

The capability to scavenge the DPPH radical of an antioxidant was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

A graph of DPPH scavenging effect was plotted against sample concentration, and from the graph, the sample concentration providing 50% free radical activity (IC_{50}) was calculated. BHA and α-tocopherol served as antioxidant standards which were used as reference points.

-Carotene-linoleic acid assay

The β-Carotene-linoleic acid test system was used to investigate the antioxidant effect of EO and methanol extract of *C. furfuracea* [Miller, 1971; ztrk et al, 2011] with slight modifications. 0.5 mg of β-Carotene in one ml chloroform was added to 25 µL of linoleic acid, and 200 mg of Tween-40 emulsifier mixture. Chloroform was evaporated from the mixture under vacuum, and 100 mL of distilled water saturated with oxygen was added and mixed properly by shaking

vigorously. 4 mL of this mixture was added into test tubes containing different concentrations of EO and MeOH extract. At the point of mixing, the zero time absorbance was measured in a 96-well microplate reader (SpectraMax 340PC, Molecular Devices, USA) at 470 nm. The emulsion system was incubated for 2 hours at 50 ºC. A blank, devoid of β-carotene, was prepared for background subtraction. BHA and α-tocopherol served as test standards.

The bleaching rate (R) of β-Carotene was calculated according to the following equation:

$R = \ln(a/b) / t$

Where: ln = natural logarithm, a = absorbance at time zero, b = absorbance at time t (120 minutes).

The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using the following equation:

$AA\ (\text{inhibition } \%) = [(R_{control} - R_{sample}) / R_{control}] \times 100$

Cupric reducing antioxidant capacity (CUPRAC)

The method of Apak et al., was used to determine the cupric reducing antioxidant activity of EO and MeOH extracts with slight modifications [Apak et al, 2004; ztrk et al, 2011]. 50µL 10 mM Cu (II), 50 µL 7.5 mM necuproine, and 60 µL NH_4Ac buffer (1M pH 7.0) solutions were added to each well in a 96 well plate. Different concentrations of 40 µL EO and MeOH extract were added to the different mixtures on the plates to make the final volume 200 µL. After 1 hour, the absorbance at 450 nm was recorded against a reagent blank by using a 96-well microplate reader. BHT and α-tocopherol were used as antioxidant standards for comparison of the activity.

Determination of total phenolic compound

The content of phenolic compound was determined using Folin–Ciocalteu reagent, and expressed as microgramme of pyrocatechol equivalents [Slinkard et al, 1977]. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from the standard pyrocatechol graph:

Absorbance = 0.006 µg pyrocatechol + 0.035 (r^2 = 0.978)

Determination of total □avonoid concentration

Total flavonoid content was determined according to the aluminum method. The results were expressed as quercetin equivalents [Park et al, 1997]. The concentration of flavonoid compounds was calculated according to following equation that was obtained from the standard quercetin graph.

Absorbance = 0.051 µg quercetin + 0.001 (r^2 = 0.999)

Anticholinesterase activity

The inhibition activity of Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were measured by the method developed by Elman et al., in 1961, with slight modification [Ellman et al, 1961; ztrk et al, 2011], using 96-well microplate reader (SpectraMax PC340, Molecular Devices, USA). The substrates of the reaction of both enzymes were acetylthiocholine iodide (0.71 mM) and butyrylthiocholine chloride (0.2 mM). In a 96 well plate, 10 µL of samples (MeOH and EO) were mixed with 150µL sodium phosphate buffer 100mM (pH = 8) and 20 µL of enzymes of enzymes solution [AChE (5.32×10^{-3}U) or BChE (6.85×10^{-3}U)]. After 15 minutes incubation at 25 °C, 10 µL of Ellman's Reagent (DTNB 0.5 mM) and 10 µL of substrates were added, so as to make the final volume 200 µL. The absorbance was measured at 412 nm. Percentage of inhibition of AChE or BChE was determined by comparison of reaction rates of samples relative to control using the formula:

$(E - S)/E \times 100$

Where:

E: the activity of the enzyme with control.

S: the activity of the enzyme with the sample.

The experiments were carried out in triplicate. Galantamine was used as the standard.

MeOH and EtOH were used as solvents to dissolve MeOH extract and essential oil and controls.

Determination of minimum inhibitory concentrations and antibiofilm activity

Strains and growth conditions

In the present study, the microorganisms used in the experiments were: Gram-positive bacteria (*Staphylococcus aureus*(ATCC 25923, ATCC 6538-P), *Staphylococcus epidermidis*MU 30, *Bacillus subtilis*ATCC 6633, *Bacillus cereus*RSKK 863, *Streptococcus mutans*CNCTC 8/77 and, *Micrococcus luteus*NRRL B-4375) and yeast

(*Candida albicans*ATCC 10239). The above-mentioned bacteria except *C. albicans* were grown in nutrient broth (NB, Difco); *C. albicans* was grown in sabouraud dextrose broth (SDB, Difco).

Minimal inhibitory concentration assay

Minimal inhibitory concentration (MIC) is defined as the lowest EO and extract concentration which yielded no visible growth. This was determined using the microtitre broth dilution method recommended by the CLSI (Clinical Laboratory Standards Institute, 2006). In this method, 100 µL suspension of bacterial cells with cell concentration of 5×10^5colony-forming units (CFU)/mLin MHB test medium was inoculated into the wells of a 96-well microtitre plate containing final concentrations 6.25, 12.5, 25, 50, 80, 160µL $mL^{-1}$of EO in the presence of 1.56, 3.12, 6.25, 12.5, 25, 50 mg/mL final concentrations of methanol extract. After the inoculated microplates were incubated for 24 hours at a temperature of 37ºC, the absorbance was measured at 630 nm.

Effect of essential oil and methanol extract on bacterial biofilm formation

Microplate biofilm assay was used to test the effect of 1, ½, ¼ and 1/8 MIC concentrations of *C. furfuracea* essential oil and extract on the biofilm-forming activity of test microorganisms (Merritt et al, 2005). Briefly, 1% of overnight cultures of isolates were added into 200 µL of fresh Tryptose-Soy Broth (TSB) which was supplemented with 0.25% glucose and grown in the presence and absence of *C. furfuracea* essential oil/extract without agitation for 48 h at 37 ºC. The wells were incubated, and planktonic bacteria were removed by washing the wells with water. The wells containing TBS and cells served as control. After removal of the planktons, 0.1% crystal violet solution was used to stain the remaining bacteria at room temperature for a period of 10 minutes, after which the wells were washed again to get rid of the crystal violet solution. 200 µL of 33% glacial acetic acid was poured into the wells, and the wells were shaken to disperse the acid. Thereafter, a pipette was used to measure 125 µL of the solution from each well into a sterile test tube, and distilled water was used to make it up to the 1mL mark. The Optical Density (OD) of each well was measured at 500 nm (Thermo Scientific Multiskan FC, Vantaa, Finland). The following formula was used to calculate the percentage inhibition of the tested extracts:

Biofilm inhibition (%) = $[(OD_{550control} - OD_{550sample}) / OD_{550control})] \times 100$

Statistical analysis

The antioxidant and the anticholinesterase activity assays were in triplicate analyses. The data were recorded as means ± standard error meaning. Student's *t*-test was used to determine the significant differences between means; $p< 0.05$ were regarded as significant.

CONCLUSION

The results of antioxidant activity indicate that the essential oil and extract have moderate to good activity. Anticholinesterase activity screening leads to the conclusion that extract has noticeable activity against AChE and BChE. The studied oil was almost inactive. In results of antimicrobial activity, methanol extract and essential oil showed a certain inhibition of growth attributed to all tested microoganisms with different percentages. In antibiofilm activity, methanol extract showed better activity than essential oil and highest antibiofilm activity have reached 87.90 % with methanol extract of *C. furfuracea* against *Staphylococcus aureus* ATCC 6538 Pat 50 mg/mL. these results indicate that the essential oil and methanol extract of *C. furfuracea*have a potential to be exploit for the development of a anti-biofilm, as well as antimicrobial and anticholinesteraseagents, and demonstrate the importance of such medicinal plant in pharmaceutical production.

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