Pharmacogn J. 2018; 10(1):64-70
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Phytochemical Composition and Evaluation of the Antioxidant Activity of the Ethanolic Extract of *Calendula suffruticosa subsp. suffruticosa Vahl*

Sofiane Ismahene[1]*, Seridi Ratiba[1], Cortes Martinez Diego Miguel[2], Cabedo Nuria[2]

ABSTRACT

Aim: *Calendula suffruticosa ssp. suffruticosa Vahl,* Medicinal and Aromatic plant, spontaneous and widespread in the Mediterranean region and in the Northeast of Algeria, is widely used by the local population for its medicinal properties. **Methods:** The chemical composition of the ethanolic extract of *Calendula suffruticosa* was established for the first time using GC / MS. The antioxidant activity of the ethanolic extract was evaluated *in vitro* by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test, the FRAP assay and β-carotene bleaching assay. **Results:** sixty-four compounds are identified by GC/MS analysis, six are predominant: α-linoleic acid (24.20%), linoleic acid (23.58%), Stigmasterol (8.53%), 2-Metyl-Z-4-tetradecene (6.62%) And α-Bisabolol (1.70%). The free radical scavenging by DPPH method indicated that the ethanolic extract of *C. suffruticosa* showed a very high antioxydant activity with an IC_{50} = 0.017 ± 1.00 mg / ml. This extract also showed an ability to reduce very high iron with a maximum optical density of 1.157 at a concentration of 0.5 mg / ml and an antioxidant activity compared to the β-carotene / linoleic acid test of order of 75 ± 1.02%. **Conclusion:** These results show for the first time the richness of phytochemicals and the strong antioxidant activity of the species *Calendula suffruticosa* endemic to north-east of Algeria.

Key words: *Calendula suffruticosa*, GC / MS, Phytochemical composition, Antioxidant activity, DPPH, FRAP.

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History
- Submission Date: 03-08-2017;
- Review completed: 31-08-2017;
- Accepted Date: 02-11-2017

DOI : 10.5530/pj.2018.1.13

Article Available online
http://www.phcogj.com/v10/i1

INTRODUCTION

Calendula is a genus of about 12 to 20 species originating from Macaronesia and the Mediterranean.[1] They are popular medicinal herbs well known throughout the world because of their vast areas of biological activities such as antimicrobial, anti-oxidant, anti-mutagenic, hepatoprotective, healing and anti-inflammatory.

The species *Calendula suffruticosa Vahl,* is a perennial and viscous herbaceous plant, belonging to the family Asteraceae and reaching about 40 cm in height; the stem is upright.[2] It is usually wooded at some distance above the base, simple or little branched.[3] The leaves are of a pale green color and lanceolate, slightly wavy and toothed. The flowers are united in capitules exceeding 2 cm in diameter and of yellow or orange color, the peripheral flowers are tied and united in two rows, while those in the center are tubular and toothed; the fruit is an achene often curved and equipped with peaks.[2]

The genus name of the "*Calendula*" dates back to the Middle Ages, it comes from the Latin "*Calendar*": The calends being in the Roman calendar on the first day of the month, probably with a sense analogous to calendar, metrological indicator because of its long period of flowering because the heads open and close with the appearance of the sun.[4] While *suffruticosa*, derived from the Latin "frutex" means bush which brings this woody species closer to the sub-shrubs.[2]

Numerous phytochemical investigations carried out on *Calendula* species such as *C. officinalis* and *C. arvensis* show that they constitute an enormous reservoir of potentially active natural molecules, the majority of which are essential oils,[1,2,3,4,5] Flavonoids,[6,7,8,9] the saponosides,[10] Carotenoids,[11,12,13] Organic acids, saccharides, sterols and lipids.[9]

While the species *Calendula suffruticosa subsp suffruticosa Vahl.* has not been the subject of any phytochemical or pharmacological studies to our knowledge.

Due to the economic value of species of the genus *Calendula* as medicinal plants and their uses in cosmetics, pharmaceutical preparations and food industry, and the limited number of phytochemical and pharmacological studies of these species in Algeria.

In this study, we are interested in studying for the first time the chemical composition of the ethanolic extract of the endemic *Calendula suffruticosa subsp suffruticoa Vahl* species in northeastern Algeria, and

Cite this article: Sofiane I, Seridi R, Cortes DM, Cabedo N. Phytochemical Composition and Evaluation of the Antioxidant Activity of the Ethanolic Extract of *Calendula suffruticosa subsp. suffruticosa Vahl.* Pharmacog J. 2018;10(1):64-70.

the evaluation of the antioxidant activity of this species by three different methods. It should be noted that to date, no studies on the chemical composition and biological activity of *Calendula suffruticosa subsp suffruticoa Vahl.* has been made.

SUBJECTS AND METHODS

Chemical reagents

Ethanol,2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-tert-butyl-4-hydroxycarboxylic acid (BHT), butylated hydroxy anisole (BHA), Linoleic acid, aluminum chloride ($AlCl_3.6H_2O$) and quercetin are from Sigma-Aldrich (USA). Ascorbic acid and gallic acid are from BIOCHEM Chemo pharma (Monteral, Quebec).

Iron (III) chloride ($FeCl_3.6H_2O$) and Potassium permanganate ($KMnO_4$) are from PROLABO Chemical.

Plant material

The samples of *Calendula suffruticosa* were collected in full bloom and fruiting in March 2015 in the Edough region "Cap de Fer" (20 km north-east of the town of Annaba, Algeria).

The botanical identification of the species was made according to Quezel and Santa, (1962-1963)[14] and validated by Doctor HAMEL. T, Teacher-Researcher in Plant Physiology, Department of Biology, Annaba University, Algeria.

Preparation of the ethanolic extract of Calendula suffruticosa Vahl

The extraction was carried out according to the method of Rigane *et al.* (2013)[8] to which we added some modifications.

Different organs of *C. suffruticosa*, previously cleaned and ground, are macerated in 90% ethanol with agitation for 24h at room temperature. The ethanol extract is recovered after filtration of the mixture using a filter paper, the ethanol is removed from the filtrate by evaporation under reduced pressure in a rotary evaporator.

The extract obtained is characterized by a dark green color (blackish green), which is the crude extract. It is then stored in an amber flask at 4°C. until the analysis time.

GC / MS analysis

The constituents of the ethanol extract were identified by GC / MS at the Mass Spectrometry laboratory of the Institute of Chemical Technology (ITQ) of Valencia (Spain).

The analysis was performed by a gas chromatograph of the type Chimadzu coupled to mass spectrometer. The capillary column was of type QP 2010 S of 25 m length and the diameter of 0.25 mm. The flow rate of the carrier gas (helium) was 1 ml / min. 3 µl of the extract was injected using the split less mode. The column temperature was maintained at 90°C for 10 min and then increased to 20°C per min up to 220°C and finally 4°C per min up to 300°C for 20 min. The ionization mode was by electron impact and the scanning range was between 40 and 620 (m / z). The Mass spectra were obtained at 0-2-min intervals.

Identification of the compounds of the ethanol extract was carried out using the data base of the National Institute of Standards and Technology (NIST) and the Wiley spectra data base.

Antioxidant activity

The antioxidant activity *in vitro* of the ethanolic extract of *C. suffruticosa* was carried out according to three methods: scavenging of the free radical DPPH, the beta-carotene bleaching test and the reduction of iron.

Free radical scavenging DPPH

The free radical scavenging activity was measured using the stable free radical DPPH which is one of the main tests used to explore the use of herbal extracts as antioxidants, the experimental protocol followed is according to Benhammou *et al.* (2007).[15]

Briefly, DPPH is solubilized in methanol to have a solution of 0.3 mM. 1 ml of methanol and 1 ml of the extract (at different concentrations 1 mg / ml in methanol) are introduced into tubes and 2 ml of the methanol solution are added to the DPPH.

After stirring by a vortex, the tubes are placed in the dark at room temperature for 30 min. The reading is performed by measuring the absorbance at 517 nm.

The negative control is composed of 1 ml of the DPPH methanol solution and 2.5 ml of methanol. BHT, BHA and ascorbic acid were used as synthetic antioxidants of reference.

The capacity of the antioxidant to scavenge the free radical is estimated as a percentage of discoloration of the DPPH in solution in methanol.

The percentage of antioxidant activity was determined according to the following equation:

% Antioxidant activity= (Abs control - Abs sample / Abs control) × 100

The results are the average of two separate measurements ± standard deviation

- Calculation of IC_{50}

IC_{50} or 50% inhibitory concentration is the concentration of the test sample needed to reduce 50% of the DPPH free radical.

Ferric reducing antioxidant power: FRAP assay

The reduction activity is determined according to the method of OYAIZU (1986),[16] based on the chemical reduction reaction of the iron (III) present in the complex K 3 Fe (CN) 6 in Iron (II). The absorbance is determined at 700 nm.

The ethanolic extract diluted (1 ml) at different concentrations was mixed with 2.5 ml of the phosphate buffer solution (0.2M, pH 6.6) and 2.5 ml of potassium ferricyanide ((K3Fe (CN) 6) at 1 %). All incubated at 50° C for 20 min. Then, 2.5ml of trichloroacetic acid (TCA) at 10% was added to the mixture to stop the reaction, Then the tubes are centrifuged for 10 min at 3000 rpm. Distilled water (2.5 ml) and ferric chloride (FeCl 3) (500 µl to 0.1%) were added to 2.5 ml of the supernatant. The absorbance was measured at 700 nm against a blank using a spectrophotometer.

Ascorbic acid was used as positive control at the same selected concentrations and in the same operating conditions as the samples.

B-carotene bleaching method

The experimental protocol followed is that of Ozsoy *et al.* (2008).[17] To prepare the β-carotene emulsion, 2 mg of that are dissolved in 10 ml of chloroform, then 1 ml of This Solution Is mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40. Then, the chloroform is evaporated under reduced pressure by a rotary evaporator at 40°C. and the residue obtained is taken up in 50 ml of oxygenated ultra-pure water.

Tubes containing 5 ml of this emulsion are prepared, for which 200 µl of extract and antioxidant (BHA) at different concentrations are added.

The mixture is well stirred and the absorbance is measured at 470 nm immediately at t0 against a blank that contains the emulsion without the β-carotene. The covered tubes are incubated at 50°C and the absorbance is reading after 120 min.

A negative control is realized in parallel, comprising 5 ml of the emulsion of β-carotene and 200.mu. l of ethanol.

The results obtained are expressed in terms of percentage inhibition of the decoloration of β-carotene using the following formula: **% Inhibition = $[1 - (A_0 - A_t / A^0_0 - A^0_t)] \times 100$**

A_0: Absorbance of the sample at t0.

A_t: Absorbance of the sample after 120 min of incubation.

A_0^0: Absorbance of the negative control at t0.

A_t^0: Absorbance of the negative control after 120 min of incubation.

RESULTS

GC / MS analysis

The phytochemicals compounds of the ethanolic extract of the species *Calendula suffruticosa*, harvested in the Edough region of Annaba Province (Algeria) were identified by GC / MS for the first time Figure 1.

From the data collected from the chromatographs and spectra of mass spectrometry and after treatment of the results, we could identify 64 chemical compounds in the ethanolic extract of the aerial part of the species *Calendula suffruticosa*.

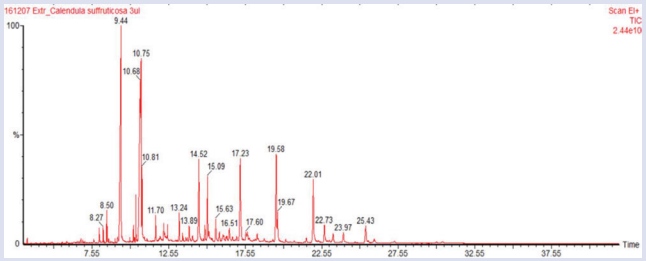

Figure 1: Chromatographic profile of the ethanolic extract of the aerial part of *Calendula suffruticosa*.

Table 1: Chemical compounds of the ethanolic extract of the aerial part of *C. suffruticosa* by GC-MS.

No	Retention time	Name of Compound	The molecular formula	The molecular weight	Percentage (%)
1	6.810	Undecanoic acid	$C_{11}H_{22}O_2$	186	-
2	6.830	2(4H) -Benzofuranone, 5, 6,7,7a-tetrahydro-4,4,7a-trimethyl	$C_{11}H_{16}O_2$	180	-
3	7.645	α-Bisabolol	$C_{15}H_{26}O$	222	1.70%
4	7.665	3-Buten-2-one,4-(4-hydroxy-2,2,6-trimethyl-7-axabicycol (4,1,0) hept-l-yl-	$C_{13}H_{20}O_3$	224	-
5	8.015	Myristic acid	$C_{14}H_{28}O_2$	228	-
6	8.070	1-cyclohexane,2-methyl-2-oxobutyl	$C_{12}H_{20}O_2$	196	-
7	8.185	7-Hexadecene, (Z)-	$C_{16}H_{32}$	224	-
8	8.270	Acetic acid,2-(2,2,6-trimethyl-7-axa-bicyclo (4,1,10) hept-1-yl propenylester	$C_{14}H_{22}O_3$	238	0.2 %
9	8.335	Bicycle (3,2,1) octan-3-one, 6-(2-hydroxyethyl, endo-	$C_{10}H_{16}O_2$	168	0.17%
10	8.496	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.22 %
11	8.546	2-Pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}O$	268	-
12	8.631	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	-
13	8.766	Phtalic acid, butyl undecylester	$C_{23}H_{36}O_4$	376	-
14	8.776	3-Octadecyne	$C_{18}H_{34}$	250	-
15	9.051	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270	-
16	9.221	Cis, cic, cis-7, 10,13-Hexadecatrienal	$C_{16}H_{26}O$	234	-
17	9.436	Palmitic acid	$C_{16}H_{32}O_2$	256	1.15%
18	10.011	Margaric acid	$C_{17}H_{34}O_2$	270	0.16%
19	10.241	Nonadecane	$C_{19}H_{40}$	268	-
20	10.271	Linolelaidic acid, methyl ester	$C_{19}H_{34}O_2$	294	-
21	10.316	Linolenic Acid, methyl ester	$C_{19}H_{32}O_2$	292	-
22	10.406	Phytol	$C_{20}H_{40}O$	296	1.20%
23	10.656	Linoleic acid	$C_{18}H_{32}O_2$	280	23.58 %
24	10.746	α-linolenic acid	$C_{18}H_{30}O_2$	278	24.20 %
25	10.811	Stearic acid	$C_{18}H_{36}O_2$	284	4.93 %
26	11.702	Tricosane	$C_{23}H_{48}$	324	0.27%
27	12.232	Arachic acid	$C_{20}H_{40}O_2$	312	0.15%
28	12.272	7-metyl-Z-tetradecen-1-olacetate	$C_{17}H_{32}O_2$	268	0.18%
29	12.437	Ocatdecane,6-methyl-	$C_{19}H_{40}$	268	1.13%
30	12.477	Adipic Acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370	1.46%
31	13.242	Octacosane	$C_{28}H_{58}$	394	0.22%
32	13.512	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	-
33	13.673	6βBicyclo [4,3,0] nonane,5β-iodomethyl-1β-isopropenyl-4α-dimethyl-	$C_{15}H_{25}$	332	-

Continued...

Table 1: Cont'd.

No	Retention time	Name of Compound	The molecular formula	The molecular weight	Percentage (%)
34	13.763	Diisoctyl phathalate	$C_{24}H_{38}O_4$	390	-
35	13.893	Docosanoic acid	$C_{22}H_{44}O_2$	340	1.30%
36	14.113	Tetradecane2,6,10-trimethyl-	$C_{17}H_{36}$	240	-
37	14.523	B-D-Mannofuranoside,farnesyl-	$C_{21}H_{36}O_6$	384	
38	14.923	A-Himachalene	$C_{15}H_{24}$	204	
39	15.173	Humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	222	0.11 %
40	15.873	Lignoceric acid	$C_{24}H_{48}O_2$	368	
41	16.149	Globulol	$C_{15}H_{26}O$	222	0.24 %
42	16.364	Thujopsene	$C_{15}H_{24}$	204	0.13 %
43	16.514	5-(7a-isopropenyl-4,5-dimethyl-octahydroinden-4-yl) -3-methyl-penta-2,4-dien-1-ol	$C_{20}H_{32}O$	288	1.61%
44	16.999	Ledol	$C_{15}H_{26}O$	222	0.17 %
45	17.224	Nonacosane	$C_{29}H_{60}$	408	4.89 %
46	17.704	4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl) -	$C_{20}H_{34}O_2$	306	1.59 %
47	18.334	Heptacosane	$C_{27}H_{56}$	380	-
48	19.670	2-Metyl-Z-4-tetradecene	$C_{15}H_{30}$	210	6.62 %
49	20.210	Vitamine E (α-tocopherol)	$C_{29}H_{50}O_2$	430	0.25 %
50	21.426	1,2-Pentanediol,5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a-tetramethyl-1-naphtanyl	$C_{20}H_{35}BrO_3$	402	-
51	21.551	Campesterol	$C_{28}H_{48}O$	400	0.10 %
52	22.006	Stigmasterol	$C_{29}H_{48}O$	412	8.53 %
53	22.341	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	$C_{13}H_{22}O_3$	226	-
54	22.731	β-sitosterol	$C_{29}H_{50}O$	414	3.50 %
55	22.941	Ergosta-7,22-dien-3β-ol,acetae	$C_{30}H_{84}O_2$		-
56	23.066	Oxalic acid,allyl pentadecyl ester	$C_{20}H_{36}O_4$	340	-
57	23.296	Urs-12-en-24-oic acid,3-oxo-, methyl ester	$C_{31}H_{48}O_3$	468	-
58	23.781	Kauran-18-al, 17-(acetyloxyl) -, (4β) -	$C_{22}H_{34}O_3$	346	-
59	23.967	α -Amyrin	$C_{30}H_{50}O$	426	2.49 %
60	24.112	Androstan-3-one, 17-hydroxy-2,4-dimethyl-, (2α,4α,5α,17β) -	$C_{21}H_{34}O_2$	318	-
61	24.727	Methanol, [6,8,9-trimethyl-4-(propenyl) -3-oxabicyclo[3,3,1] non-6-en-1-yl]	$C_{15}H_{24}O_2$	236	-
62	25.432	Lupeol	$C_{30}H_{50}O$	426	3.76 %
63	25.997	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	-
64	27.293	Pregn-16-en-20-one,3-hydroxy-, (3β,5β) -	$C_{21}H_{32}O_2$	316	-

The compounds identified with their retention time (RT), molecular formula (MF), molecular weight (MW) and peak area as a percentage are presented in Table 1.

This extract consists mainly of α linoleic acid (24.20%), linoleic acid (23.58%) Stigma sterol (8.53%), 2-metyl-4-Z-tetradecene (6.62%) and α-Bisabolol (1.70 %).

Antioxidant activity

Free radical DPPH scavenging

The antioxidant activity of the ethanol extract of *Calendula suffruticosa* and the standard antioxidants (ascorbic acid, BHA and BHT) against the DPPH radical was evaluated using a spectrophotometer.

The Figure 2 presents the inhibition percentages obtained from the ethanolic extract of *C. suffruticosa* compared with that of the positive controls used (BHA, BHT and ascorbic acid) as a function of the different concentrations used for scavenging of the free radical DPPH.

The values obtained have enabled us to trace curves having an exponential shape with the presence of a stationary phase which means the almost total reduction of DPPH in its non-radical form. This is reflected by the strong antioxidant activity of the standards which recorded high inhibition percentages of 97.72 ± 0.13% for ascorbic acid, 96.92 ± 0.04% for BHT, and 96.74 ± 0.05% for BHA. The ethanolic extract of the aerial part of *C. suffruticosa* has also showed a strong antioxidant activity with a percentage reduction of DPPH of 92.07 ± 0.05% Figure 2.

By comparing the IC_{50}s of the standards with those of the extract tested, we note that the three positive controls used possess a powerful antioxidant activity which is superior to that of the extract studied.

BHA is the most active with a IC_{50} = 0.0058 ± 1.45 mg / ml, followed by ascorbic acid and BHT (0.0071 ± 0.27 mg / ml and 0.0078 ± 6.52 mg / M1), respectively. The ethanolic extract of *C. suffruticosa* also showed a very high antioxidant activity with an IC_{50} = 0.017 ± 1.00 mg / ml.

Antioxidant activity:
Free radical DPPH scavenging:

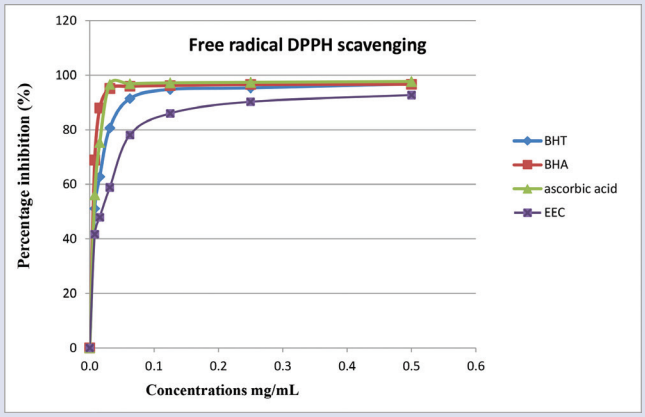

Figure 2: Anti-radical activity of the ethanol extract of *Calendula suffruticosa*. (Each value represents the mean of three trials ± SD).

Antioxidant activity:
β-carotene bleaching assay:

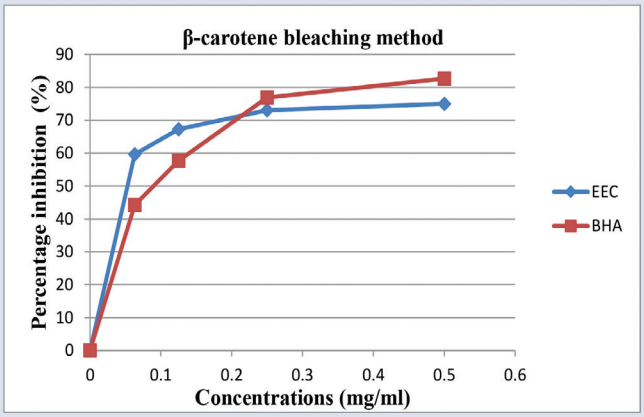

Figure 4: Antioxidant power of the ethanol extract of *C. suffruticosa* tested by the method of bleaching of β-carotene.

Antioxidant activity:
FRAP assay:

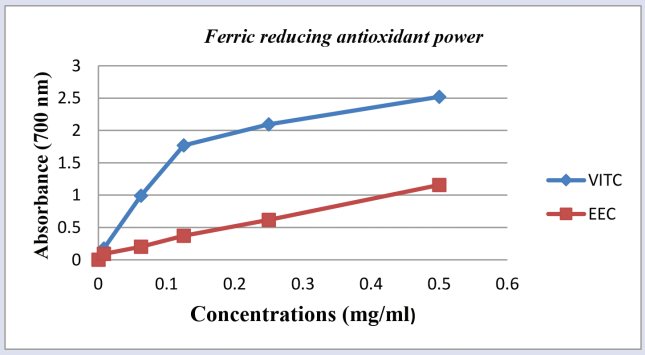

Figure 3: Reductive power of the ethanolic extracts of the studied plant and of the ascorbic acid tested by the FRAP assay.

FRAP assay

This method was described for the first time in 1996 by Benize and Strain. The FRAP method is a simple, inexpensive and robust spectrophotometric technique. It is based on the capacity of polyphenols to reduce ferric iron Fe^3 into ferrous iron Fe^2.[18]

The results of the reducing power of the ethanolic extract of *C. suffruticosa* and of ascorbic acid are represented in Figure 3.

The results obtained in FIG. (03) show that the capacity to reduce the iron of the ethanolic extract of the aerial part of *C. suffruticosa* is very high with a maximum optical density of 1.157 at a concentration of 0.5 mg / ml. But it is clearly lower to that of ascorbic acid which represent a DO of 1.77 mg / ml at the concentration 0.1 mg / ml only.

B-carotene bleaching assay

Figure 4 presents the antioxidant power tested by the method of bleaching of β-carotene of the ethanol extract of *C. suffruticosa*.

According to our results, we clearly note that BHA exerts a strong antioxidant effect, with inhibition percentages up to 82.69 ± 0.03%.

The extract tested also shows a strong inhibiting power of the coupled oxidation of linoleic acid and β-carotene with a percentage of 75 ± 1.02% at the concentration 0.5 mg / ml.

DISCUSSION

The ethanolic extract of the species *Calendula suffruticosa*, was identified by GC / MS for the first time. This extract consists mainly of α linoleic acid (24.20%), linoleic acid (23.58%) Stigma sterol (8.53%), 2-metyl-4-Z-tetradecene (6.62%) and α-Bisabolol (1.70 %).

We have not found in the literature information on the chemical composition of the ethanolic extract of the species *Calendula suffruticosa*. While the species *Calendula officinalis* has been the subject of numerous phytochemical investigations.

The results we have obtained of the phytochemical analysis agree with other scientific work reported on the species *Calendula officinalis.*

Various terpenoids were detected in the petroleum ether extract of *C. officinalis* flowers. They include sitosterol's, Stigma sterols [19] Lupeol.[20-21]

In another study [22], the petroleum ether extract of *C. officinalis* is rich in lipids such as: monol fatty acids, sterol esters, 3-monoesters, lauric acid, myristic acid, palmitic, stearic, oleic, linoleic and linolenic acid.

The existence of linoleic acid and stearic acid in the methanolic extract of the stem and the leaf of *C. officinalis* is also demonstrated with a percentage of 27.56 and 6.41% respectively.[23]

According to the results of GC / MS, the strong antioxidant activity of the ethanolic extract of *C. suffruticosa* is probably due to its richness of active ingredients which possess an antioxidant effect such as α tocopherol, campesterol, stegmasterol, β-sitosterol, Lupeol, α-Bisabolol and linoleic α-acid.

The scavenging effect of the hydro-methanol extracts of *C. officinalis* and of the standard on the DPPH radical expressed in IC_{50} values were of the order: flowers (0.35 mg.ml-1), leaves (0.57 mg. Ml-1) and BHT (8.11 mg.ml -1).[8] These data reveal that the extracts of the different parts probably have a stronger effect of trapping free radicals than the positive control (BHT).

The potentiality of the ethanolic extract of *C. suffruticosa* to reduce ferric iron Fe^{3+} into ferrous iron $Fe^{2+}$is related to the nature of the reducing substances existing in this extract.

The antioxidant activity by the FRAP assay of methanolic extracts of the species *C. officinalis* collected in the Béja region of Tunisia was studied. The authors obtained values of 28.37 ± 0.12 and 17.68 ± 0.02 mM Trolox for the flowers and leaves respectively.[8]

In addition, the species *Calendula arvensis* of western Algeria, reported an antioxidant capacity of the essential oil of this species tested by FRAP test where it recorded the concentration of 90.7 µg / mL a reducing power of the HE of *C. arvensis* much higher with a value of (DO = 1.698).[24]

In a similar study, the aqueous extract of *C. officinalis* at a concentration of 100 µg / ml showed anti-radical and antioxidant activities, by an inhibition rate of 74.6% of the lipid peroxidation of linoleic acid, this activity is higher than that of α-tocopherol (63%) which has been used as a standard.[25]

CONCLUSION

In this study, we studied for the first time the chemical composition and the antioxidant activity of the ethanolic extract of the species *Calendula suffruticosa subsp suffruticoa Vahl* endemic to north-east of Algeria. The analysis of the ethanol extract by GC-MS showed the presence of various bioactive compounds confirming the medicinal interest of this plant. The antioxidant activity of this extract is very important and comparable to some extent to synthetic antioxidants (BHA, BHT and ascorbic acid).

This extract could therefore constitute as alternative to certain synthetic additives. It is necessary to attract attention to the fact that these results are obtained *in vitro*.

Their interest resides in the fact that they allow to directly search the antioxidant activity of this extract or pure compounds *in vivo* to correlate the results observed in the two cases.

CONFLICT OF INTEREST

The authors are declared no conflict of interest.

ABBREVIATION USED

GC-MS: Gas chromatography mass spectrometry; FRAP: Ferric reducing antioxidant power; DPPH: 2,2-diphenyl-1-picrylhydrazyl; BHT: 2,6-di-tert-butyl-4-hydroxycarboxylic acid; BHA : butylated hydroxy anisole; **EEC:** Ethanolic extract of *Calendula suffruticosa*.

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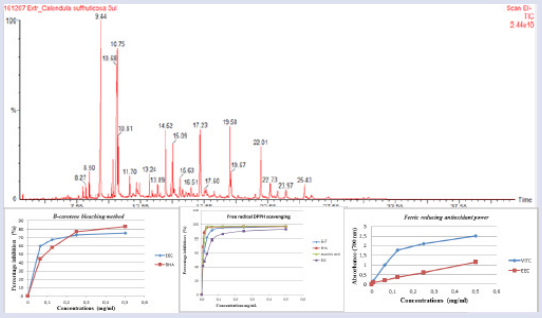
GRAPHICAL ABSTRACT



SUMMARY

- *Calendula suffruticosa ssp. suffruticosa Vahl*, Medicinal and Aromatic plant, spontaneous and widespread in the Mediterranean region and in the north-east of Algeria.
- Sixty-four compounds are identified by GC/MS analysis of the ethanolic extract , six are predominant : α-linoleic acid (24.20%), linoleic acid (23.58%), Stigmasterol (8.53%), 2-Metyl-Z-4-tetradecene (6.62%) And α-Bisabolol (1.70%).
- The free radical scavenging by DPPH method indicated that the ethanolic extract of C. suffruticosa showed a very high antioxydant activity with an IC_{50} = 0.017 ± 1.00 mg / ml.
- This extract also showed an ability to reduce very high iron with a maximum optical density of 1.157 at a concentration of 0.5 mg / ml and an antioxidant activity compared to the β-carotene / linoleic acid test of order of 75 ± 1.02%.

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Cite this article: Sofiane I, Seridi R, Cortes DM, Cabedo N. Phytochemical Composition and Evaluation of the Antioxidant Activity of the Ethanolic Extract of *Calendula suffruticosa subsp. suffruticosa Vahl.* Pharmacog J. 2018;10(1):64-70.