African Journal of Microbiology Research Vol. 6(20), pp. 4388-4395, 30 May, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.259
ISSN 1996-0808 ©2012 Academic Journals

*Full Length Research Paper*

# Chemical composition, *in vitro* antifungal and antioxidant activities of essential oil from *Cotula coronopifolia* L. growing in Tunisia

**Fethi Bel Hadj Kether[1], Mohamed Ali Mahjoub[1], Samia Ammar Mahjoub[1], Karima Belhaj Salah[3], Ahmed Noureddine Helal[1] and Zine Mighri[2]***

[1]Université de Monastir, Laboratoire de Génome, Diagnostic Immunitaire et Valorisation, (03/UR /09-01) Institut Supérieur de Biotechnologie de Monastir, 5000 Monastir, Tunisie.
[2]Université de Monastir, Faculté des sciences-Département de chimie. UR 1204-Chimie Appliquée-Environnement 5000 Monastir, Tunisie.
[3]Laboratoire des Maladies transmissibles et substances biologiquement actives, Faculté de pharmacie 5000 Monastir Tunisie.

Accepted 23 February, 2012

**The chemical composition of *Cotula coronopifolia* L. essential oils obtained from the aerial parts (flowers, leaves, stems) and roots by hydro distillation was analyzed by gas chromatography/ mass spectrum (GC/MS). Fifty seven components were identified. The most important compound detected in leaves was Agarospirol (10.43%). Hexacosane was identified in flowers with an abundance percentage of (31.7%) and 1-Eicosanol (17.1%) in stem. The major constituents identified in the root oils were Heptacosane (28.4%), 1-Eicosanol (14.6%), Octacosane (5.4%), and γ Amorphen (5.2%). *In vitro* antioxidant activities of prepared essential oils were determined by using ABTS and DPPH methods and compared to those of the Trolox. Among the tested samples, flowers had the highest phenolic content with 22.02 mg equivalents Catechin/g of extract and roots presented the lowest phenolic content with 3.12 mg equivalents Catechin/g of extract. To evaluate *in vitro* antifungal activity, all volatile oils were tested against four fungi (two dermatophytes and two hyphamycets).**

**Keys words:** *Cotula coronopifolia* L, essential oils, chemical composition, by gas chromatography/ mass spectrum (GC/MS), antioxidant activity, antifungal activity.

## INTRODUCTION

The importance of aromatic plants is considerable owing on the one hand, to their applications in folk medicine and on the other hand, to their potential for commercial exploitation in various fields as aroma and flavour enhancers, cosmetic and drugs.

*Cotula coronopifolia* L. flowering from March to May is a mono specific genus belonging to the Asteraceae family. It is a South African endemic species, distributed in the north east and the center of Tunisia.

*C. coronopifolia* L. or Common brassbuttons is an aquatic plant, perennial or yearly herb with pinnatisect leaves, yellow and hermaphrodite flowers. The capitulum are homogames, with naked and conical receptacle (Pottier, 1981). Seeds germination carried out in the end of winter, (Van der Toorn, 1982).

Le floc'h (1983) and Boukef (1986) did not report about its possible use in popular medicine in Tunisia. However

*Corresponding author. E-mail: zinemighri@yahoo.fr. Tel: +21696941116.

**Abreviations: FID,** Flame ionization detectors; **GC/MS,** gas chromatography coupled with mass; **ABTS,** 2,2azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) di ammonium salt); **DPPH**, 1,1-diphenyl-1-picrylhydrazyl radical); **MIC**, minimal inhibitory concentration; **TEAC,** Trolox equivalent antioxidant capacity; Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid); **EtOH,** Ethanol; **PBS,** phosphate buffered saline; **KI,** Kovats' Indexes; **L,** leaves; **F,** flowers; **S,** stem; **R,** roots; **NI,** not identified; **TCP,** total phenolic content; **SGA,** Sabouraud glucose agar.

many species of the Genus Cotula have long been used in traditional medicine. *Cotula cinera* DEL synonym of *Brocchia cinera* VIS. has an antifungal, anti-inflammatory, analgesic antiseptic and anti-hadache activities (Markouk et al., 1999). *Matricaria aurea* is used for the treatment of diabetes (Yaniv et al., 1987).

In our present research, we report the chemical composition and the evaluation in vitro of the antifungal and the antioxidant properties of *C. coronopifolia* L. from the Tunisian littoral.

## MATERIALS AND METHODS

### Plant material

Plant material was gathered at the flowering stage in March 2008 in Monastir (Tunisian littoral). The fresh plant was separated in four parts: roots, leaves, stems and flowers. Each part was cut in to little pieces and weighed before the extraction of volatile compounds. Voucher specimens are deposited in Faculty of Sciences, Monastir-Tunisia.

### Essential oils extraction

Fresh quantity of each organ of the plant *C. coronopifolia* L. was cut in small pieces and submitted to hydrodistillation for 5-6 h. The oils were extracted with dichloromethane and dried over anhydrous $Na_2SO_4$ and essential oils samples were stored prior to the analysis. Yields based on fresh weights of the samples were calculated.

### Analyze of the essential oils

#### *Gas chromatography*

HP 5890-series II equipped with: flame ionization detector (FID), HP -5, 30 m × 0.25 mm ID, 0.25 µm film thickness fused capillary colum and HP Innowax 30 m × 0.25 ID, 0.25 um film thickness fused capillary colum. The carrier gas was nitrogen (1.2 ml/min). The oven temperature program was 1 min isothermally at 50°C, then 50-280°C at rate of 5°C /min and held isothermally for 1 min. The injection port temperature was 250°C, detector: 280°C. Volume injected: 0.1 µL of 1% solution diluted in hexane. Percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction.

#### *GC/MS*

The analysis of the volatile constituents was run on a Hawlet-Packard GC: 5890 series II; MSD 5972). The fused-silica HP-5 MS capillary column (30 m × 0.25 µm) was directly coupled to the Mass spectrometer. The carrier gas was helium with a flow rate of 1.2 ml/min. Oven temperature was programmed (50°C for 1 min, then 50-280°C at a rate of 5°C /min) and subsequently, held isothermally for 20 min. Injector port: 250°C, detector: 280°C, split ratio 1:50. Volume injected: 0.1 µl of 1% solution (diluted in hexane).

#### *Mass spectrometer*

HP 5972 recording at 70 eV; scan time 1.5 s; mass range 40-300 amu. Software adopted to handle mass spectra and chromatograms was a ChemStation®.

#### *Identification of the compounds*

The components of the oils were identified by comparison of their mass spectra with those of a computer library (Wiley 275 Library). Further confirmation was done by referring to Kovats Index data generated from a series of alkanes (C9-C28) (Shibamoto, 1987; Adams, 1995).

### Antioxydant activity

#### *Chemicals and reagents*

2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu and catechine were obtained from Sigma-Aldrich; potassium persulphate, sodium acetate and sodium carbonate, ethanol and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were used for gradient purity.

#### *Cation radical $ABTS^{+\bullet}$ scavenging activity*

The standard method described by (Miller et al., 1993) has been adopted with minor modifications. This method based on the ability of a compound to scavenge the stable cation radical ($ABTS^{+\bullet}$). The blue $ABTS^{+\bullet}$ radical form was produced through the reaction between ABTS (20 mg), potassium persulfate ($K_2S_2O_8$) in deionized water. The solution was incubated at room temperature in the dark for minimum 12 h. A concentrated $ABTS^{+\bullet}$ stock solution was diluted with phosphate buffered saline (PBS), pH 7.4 to give a final absorbance of 0.7±0.02 at 734 nm at 37°C. Then, different concentrations of samples were prepared by dilution with ethanol for the volatile fractions. Sample absorbance was compared to a maximal absorbance of 990µl of the $ABTS^{+\bullet}$ solution and 10 µl of Ethanol. Ten micro liters of studied solution were added to 990 µL of $ABTS^{+\bullet}$ solution and the absorbance at 734 nm were measured at 5, 10, 15 and 20 min. All measurements were performed in triplicate. Results were expressed in inhibition percentage of different samples concentrations (mg/ml) at different times.

### DPPH scavenging activity

The electron donating ability of the extracts was measured from the bleaching of purple –coloured EtOH solutions of DPPH. This spectrophotometric assay was performed as described elsewhere with some modifications (Burits and Bucar, 2000; Cuendet et al., 1997). The DPPH radical solution was prepared in absolute ethanol to give a final a wavelength of 520 nm at 37°C. 0.5 ml of each sample concentration was mixed with a same volume of DPPH solution. The reaction for scavenging DPPH radicals was carried out at room temperature in the dark for 30 min, and then the reduction in absorbance was recorded at 520 nm. A mixture of 0.5 ml of DPPH solution and 0.5 ml of ethanol was taken as a reference. Decrease in absorption induced by the tested extracts was compared to that of the positive control Trolox. All measurements were performed in triplicate. Results were expressed in inhibition percentage of different sample concentrations (mg/ml) at 30 min.

#### *TEAC assay*

TEAC assay was performed with minor modifications according to the original method proposed by (Van Den Berg et al., 1999) with minor modifications**.** Solutions were prepared as the $ABTS^{+\bullet}$ scavenging activity section. Measurements were performed in

triplicate at four concentrations. The TEAC values of the antioxidant (expressed in mmol of trolox per milligrams of samples) were calculated at 5, 10, 15 and 20 min after the mixing of the reactants and by relating this decrease in absorbance to that of a trolox solution on a molar basis.

#### *Determination of total phenolic content (TPC)*

The amount of TPC was determined according to the method of Wojdylo et al. (2007) which used Folin-Ciocalteu reagent. Tested extracts were prepared at a concentration of 1 mg/ml. 100 µl of extract was transferred into a test tube and 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with deionized water) were added and mixed. The mixture was allowed to stand at a temperature of 25°C for 5 min. 0.75 ml of saturated sodium carbonate solution was added to the mixture and then mixed gently. After standing at 25°C for 90 min, the absorbance was red at 725 nm using an UV–Vis spectrophotometer. The standard calibration (0.01–0.05 mg/ml) curve was plotted using catechin. The TCP was expressed as catechin equivalents in milligrams per 1 g of vegetable extract.

### Antifungal activity

#### *Microbial strains*

The essential oil of *C. coronopifolia* were tested against four strains of fungi comprising: two dermatophytes (*Trichophyton rubrum* and *Microsporum canis*) and two hyphamycets (*Aspergilus fumigates* and *Scopulariopsis brevicaulis*). The microorganisms were obtained either from the Institut Pasteur, Paris, or from the Microbiology Laboratory, Faculty of Medicine, Besancon.

#### *Antifungal testing*

The antifungal activity of essential oils was assayed by the method of agar incorporation (dilution in a solid medium) including negative control as described previously (Griffin et al., 1999, 2000; Yang et al., 1996). The essential oil were mixed aseptically with 100 ml of Sabouraud glucose agar (SGA) to give final concentrations of 1000, 750 and 500 µl/ml. Oil were dissolved in 99% EtOH and this solvent was used as a negative control.

Dermatophyte myceliums were inoculated by disposition in center plates. The petri dishes were then incubated, 24 h at 37°C for *Scopulariopsis* and *Aspergillus* and 48 h at 37°C for *Trichophyton* and *Microsporum* These tests were carried out in triplicate.

The antifungal activity of the essential oils was evaluated by calculating the inhibition percentages (I%) according to the method of (Benjilali et al.,1986) and by determining the minimal inhibitory concentration (MIC: the lowest concentration which inhibits the visible growth of fungi during the incubation period).

### Statistical analysis

All analyses were performed in triplicate and the data were expressed as means ± standard deviations (SD). Analysis of variance was performed by ANOVA procedure and was performed by the PASW Statistics software.

## RESULTS

### Chemicals composition of the essential oil

Each part of *C. coronopifolia* (leaves, flowers, stem and roots) was subjected to a hydro distillation. The volatile fraction yield differed to the tested part. It was $11.34 \times 10^{-3}$% from the leaves, $39.35 \times 10^{-3}$% from the flowers, $1.23 \times 10^{-3}$% from the roots and $4.05 \times 10^{-3}$% from the stem.

The four oils were light yellow and liquid at room temperature. The composition of the volatile oils extracted by hydrodistillation from the different parts of the plant is reported in Table 1 together with the Kovats' Indexes (KI) calculated for each compound, the percentage composition and the identification methods. The constituents are arranged according to their elution on the polar KI (BP-1) column.

A total of 57 constituents were found from which 5 are not Identified (NI). The most important compound detected in leaves was Agarospirol (10.43%). Hexacosane was identified in flowers with an abundance percentage of (31.7%) and 1-Eicosanol (17.1%) in stem. The major constituents identified in the roots oil were Heptacosane (28.4%), gamma Amorphen (5.2%), 1-Eicosanol (14.6%), and Octacosane (5.4%).

Oils obtained from Leaves (L), Flowers (F) and Stem (S) was characterized by the predominance of oxygenated sesquiterpenes. On the contrary, oil obtained from roots (R), was rich in sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Monoterpene hydrocarbons are not representative in all the studied organs (Table 1).

Table 1 shows that Leaves are richer in oxygenated sesquiterpenes (24.64%), sesquiterpene hydrocarbons (3.49%), diterpenes hydrocarbons (2.49%) and in oxygenated Diterpenes (1.49%) than stem, flowers and roots. The latter contained more sesquiterpene hydrocarbons (5.2%) and oxygenated diterpenes (3.4%). Oil from Leaves showed as main constituents β-Agrofurane (n°6, 9.65%) and Agarosprol (n°16, 10.43%). Hydrodistilled oil of flowers (F) contained mainly Ethyl octadecanoate (n°44, 21.7%), Hexacosane (n°47, 31.7%). The main components found in the oil obtained from Stem were γ-Amorphen (n°8, 16.5%), benzyl benzoate (n°18, 10.6%), 1-Eicosanol (n°45, 17.1%) and Triacontane (n°54, 7.6).

### Radical cation $ABTS^{+\bullet}$ scavenging activity

Antioxidant activity screening method used to study this essential oil was ABTS radical cation decolorization assay. This latter is widely used to assess the total amount of radicals that can be scavenged by an antioxidant (Arts et al., 2004).

Results of our investigation on ABTS+• cation radicals scavenging activity of *Cotula coronopifolia* (L) essential oils, were represented by Table 2. Compared with Trolox, maximal inhibition percentage values calculated after 20 min of reaction indicated difference in the antioxidant activity for the four tested essential oils.

The essential oil of roots showed the highest inhibition of ABTS+•, with a value of 10.68% at a concentration of 1

**Table 1.** Chemical composition of essential oils from different organs of *Cotula coronopifolia* L.

| No | KI (BP-1) | Compound | Identification | L | F | S | R |
|---|---|---|---|---|---|---|---|
| 1 | 1160 | Artemystyl acetate | MS, KI | 0.28 | 0.0 | 1.6 | 0.0 |
| 2 | 1195 | Benzothiazol | MS, KI | 0.94 | 0.0 | 0.1 | 0.0 |
| 3 | 1302 | Carvacrol | MS, KI | 0.12 | 0.0 | 0.0 | 0.0 |
| 4 | 1427 | Benzyl pentoate | MS, KI | 0.37 | 0.0 | 0.0 | 0.0 |
| 5 | 1445 | aromadendrene | MS, KI | 0.23 | 0.0 | 0.0 | 0.0 |
| 6 | 1480 | β-Agrofurane | MS, KI | 9.65 | 0.0 | 0.2 | 0.0 |
| 7 | 1495 | α-Amorphen | MS, KI | 0.53 | 0.0 | 1.7 | 5.2 |
| 8 | 1503 | γ-Amorphen | MS, KI | 0.94 | 0.4 | 5 | 0.0 |
| 9 | 1515 | γ-cadinene | MS, KI | 1.79 | 2.9 | 0.2 | 0.0 |
| 10 | 1524 | Cinnamyl caproate | MS, KI | 5.94 | 0.0 | 0.2 | 0.0 |
| 11 | 1549 | Acetovanillone | MS, KI | 0.44 | 0.0 | 0.7 | 0.5 |
| 12 | 1569 | Dodecanoic acid | MS, KI | 0.12 | 0.0 | 3.1 | 0.0 |
| 13 | 1586 | Acorenone | MS, KI | 0.51 | 1.7 | 0.4 | 0.0 |
| 14 | 1601 | Curzerenone | MS, KI | 0.80 | 0.0 | 0.1 | 0.0 |
| 15 | 1609 | Benzyl heptanoate | MS, KI | 0.73 | 1.8 | 0.0 | 0.3 |
| 16 | 1631 | Agarospirol | MS, KI | 10.4 | 0.0 | 0.2 | 0.0 |
| 17 | 1709 | α-bisabolone | MS, KI | 1.15 | 0.4 | 0.3 | 1.4 |
| 18 | 1728 | Benzyl benzoate | MS, KI | 0.29 | 0.0 | 10.6 | 0.0 |
| 19 | 1745 | α-oxo-Bisabolenne | MS, KI | 0.35 | 0.0 | 0.0 | 0.0 |
| 20 | 1773 | α-Atlantone | MS, KI | 2.30 | 0.0 | 0.0 | 0.0 |
| 21 | 1805 | Cinnamyl coproate | MS, KI | 1.15 | 2.5 | 0.6 | 0.5 |
| 22 | 1816 | Benzyl salicylate | MS, KI | 0.60 | 0.0 | 1.0 | 0.4 |
| 23 | 1856 | 2-Dodecenal | MS, KI | 0.55 | 0.0 | 0.0 | 0.0 |
| 24 | 1894 | 1-Nonadecene | MS, KI | 0.44 | 0.8 | 0.0 | 0.0 |
| 25 | 1900 | Nanadecane | MS, KI | 0.44 | 0.0 | 0.6 | 0.0 |
| 26 | 1916 | Beyerenne | MS, KI | 0.58 | 0.0 | 0.2 | 0.0 |
| 27 | 1934 | Ethyl hexadecanoate | MS, KI | 1.61 | 0.0 | 0.2 | 0.0 |
| 28 | 1951 | Dodecenal | MS, KI | 0.34 | 0.0 | 0.2 | 0.7 |
| 29 | 1958 | Hexadecanoic acid | MS, KI | 1.64 | 0.0 | 0.3 | 0.3 |
| 30 | 1963 | Isophytol | MS, KI | 0.34 | 0.0 | 0.6 | 0.0 |
| 31 | 1967 | Sclarene | MS, KI | 0.44 | 1.2 | 0.3 | 0.0 |
| 32 | 2000 | Eicosane | MS, KI | 0.59 | 0.0 | 0.0 | 0.0 |
| 33 | 2034 | Kaurene | MS, KI | 0.93 | 0.0 | 0.6 | 1.0 |
| 34 | 2054 | Manool | MS, KI | 0.42 | 0.0 | 0.7 | 0.0 |
| 35 | 2059 | Cinnamyle cinnamate | MS, KI | 1.13 | 1.0 | 3.0 | 0.0 |
| 36 | 2079 | Linoleic acid | MS, KI | 3.82 | 3.3 | 1.1 | 0.3 |
| 37 | 2100 | Heneicosane | MS, KI | 1.10 | 0.0 | 1.4 | 0.0 |
| 38 | 2110 | Methyl octadecanoate | MS, KI | 0.42 | 0.0 | 0.7 | 2.8 |
| 39 | 2128 | E-4-cinnamique acid | MS, KI | 3.07 | 0.0 | 0.4 | 0.0 |
| 40 | 2142 | Ethyl oleate | MS, KI | 7.22 | 4.1 | 4.4 | 3.0 |
| 41 | 2186 | 1-Docosene | MS, KI | 2.75 | 2.6 | 0.9 | 0.0 |
| 42 | 2203 | Sclareol | MS, KI | 0.42 | 0.0 | 0.5 | 3.4 |
| 43 | 2207 | Phytyl acetate | MS, KI | 7.63 | 0.0 | 2.9 | 0.8 |
| 44 | 2219 | Ethyl octadecanoate | MS, KI | 0.13 | 21.7 | 1.6 | 2.0 |
| 45 | 2252 | 1-Eicosanol | MS, KI | 1.86 | 0.0 | 17.1 | 14.6 |
| 46 | 2310 | Totarol | MS, KI | 0.31 | 2.7 | 0.0 | 0.0 |
| 47 | 2600 | Hexacosane | MS, KI | 0.30 | 31.7 | 0.9 | 3.7 |
| 48 | 2700 | Heptacosane | MS, KI | 6.19 | 3.3 | 2.2 | 28.4 |
| 49 | 2779 | NI | MS, KI | 2.06 | 0.1 | 2.1 | 0.0 |

**Table 1**. Contd.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 50 | 2800 | Octacosane | MS, KI | 0.63 | 0.2 | 1.8 | 5.4 |
| 51 | 2821 | NI | MS, KI | 0.54 | 0.1 | 0.0 | 2.8 |
| 52 | 2881 | NI | MS, KI | 0.25 | 0.5 | 3.6 | 0.0 |
| 53 | 2900 | Nanocosane | MS, KI | 3.14 | 3.1 | 0.0 | 1.4 |
| 54 | 3000 | Triacontane | MS, KI | 3.11 | 2.7 | 7.6 | 3.3 |
| 55 | 3036 | NI | MS, KI | 1.79 | 4.0 | 0.9 | 3.7 |
| 56 | 3061 | NI | MS, KI | 2.36 | 1.5 | 0.8 | 4.6 |
| 57 | 3090 | Acorenone | MS, KI | 0.60 | 4.5 | 3.3 | 1.8 |
| | | Total | | 98.81 | 98.9 | 98.3 | 92.3 |
| | | Monoterpens hydrocarbons | | 0.0 | 0.0 | 0.0 | 0.0 |
| | | Oxygenated monoterpenes | | 0.12 | 0.0 | 0.0 | 0.0 |
| | | Sesquiterpenehydrocarbons | | 3.49 | 0.4 | 18.4 | 5.2 |
| | | Oxygenated sesquiterpenes | | 24.64 | 6.6 | 15.1 | 3.2 |
| | | Diterpenes hydrocarbons | | 2.93 | 1.2 | 1.1 | 1 |
| | | Oxygenated diterpenes | | 1.49 | 2.7 | 1.8 | 3.4 |
| | | Others | | 38.58 | 39.3 | 60.3 | 31.8 |

Abbreviations: L: leaves, F: flowers, S: stem, R: roots, NI: not identified.

**Table 2.** $ABTS^{+\cdot}$ inhibition percentage in the presence of different concentrations of essential oils of *cotula coronopifolia.*

| Concentration (%µg/ml) | | $ABTS^{+\bullet}$ % inhibition | | | |
|---|---|---|---|---|---|
| | | Time (min) | | | |
| | | 5 | 10 | 15 | 20 |
| L | 1.00 | -8.9±1.7* | -16.1±0.0* | -18.4±1.0* | -20±4.8* |
| | 0.50 | -16.3±1.3* | -18.6.±0.0* | -24,8±0.3* | -24.3±0.1* |
| | 0.25 | -8.4±1.4* | -14.5±1.7* | -18.5±0.7* | -21.9±0.6* |
| | 0.12 | -10.2±0.8* | -15.3±0.0* | -20.3±0.2* | -20.3±1.4* |
| | 0.06 | -9.7±1.1* | -15.3±0.0* | -20.3±0.2* | -20.0±1.3* |
| | 0.03 | -9.0.±2.4* | -15.3±0.0* | -20.7±0.8* | -20,6±1.4* |
| S | 1.00 | 1.7±2.7* | 0.0±3.1* | 3.4±1.1* | 1.6±0.4* |
| | 0.50 | 2.7±0.0* | -0.4±0.0* | 3.4±1.1* | 1.6±0.4* |
| | 0.25 | 3.7±4.0* | 1.1±1.7* | 3.4±1.1* | 1.6±0.4* |
| | 0.12 | -1.7±2.9* | -3.6±1.3* | 3.4±1.1* | -0.8±0.8* |
| | 0.06 | -1.7±2.9* | 4.2±0.9* | 3.4±1.1* | -0.8±0.8* |
| | 0.03 | -1.7±2.9* | -4.2±0.9* | 3.4±1.1* | -0.8±0.8* |
| F | 1.00 | 4.5±0.0* | -1.1±0.3* | -5.5±0.4* | -10.3±0.9* |
| | 0.50 | 2.4±0.0* | -6.4±0.4* | -13.3±2.1* | -19.9±1.4* |
| | 0.25 | 1.2±1.2* | -7.5±1.5* | -13.1.±1.2* | -21.8±1.0* |
| | 0.12 | -3.3±1.9* | -8.3±0.2* | -16.1±0.9* | -22.3±0.0* |
| | 0.06 | -10.9±0.4* | -13.1±2.1* | -23.9±0.8* | -26.5±0.9* |
| | 0.03 | -12.5±1.2* | -13.1±0.4* | -25.8±1.8* | -34.2±0.2* |
| R | 1.00 | 9.8±0.0* | 9.0±0.0* | 11.4±1.0* | 10.6±1.0* |
| | 0.50 | 8.8±0.3* | 8.1±0.5* | 9.5±1.4* | 9.9±1.0* |
| | 0.25 | 8.5±0.3* | 7.8±2.2* | 8.3±1.2* | 8.9±1.0* |
| | 0.12 | 8.6±0.0* | 4.5±2.2* | 2.8±3.2* | 4.0±1.2* |
| | 0.06 | -2.3±1.4* | -1.6±0.9* | -3.8±0.3* | -3.4±1.9* |
| | 0.03 | -1.0±1.9* | -2.0±1.0* | -3.8±2.1* | -4.5±0.6* |

L : Leaves ; S : Stems ; F : Flowers ; R : Roots, Values are expressed as mean ± M.S.E. (N=3); *≤0.001: significant from control.

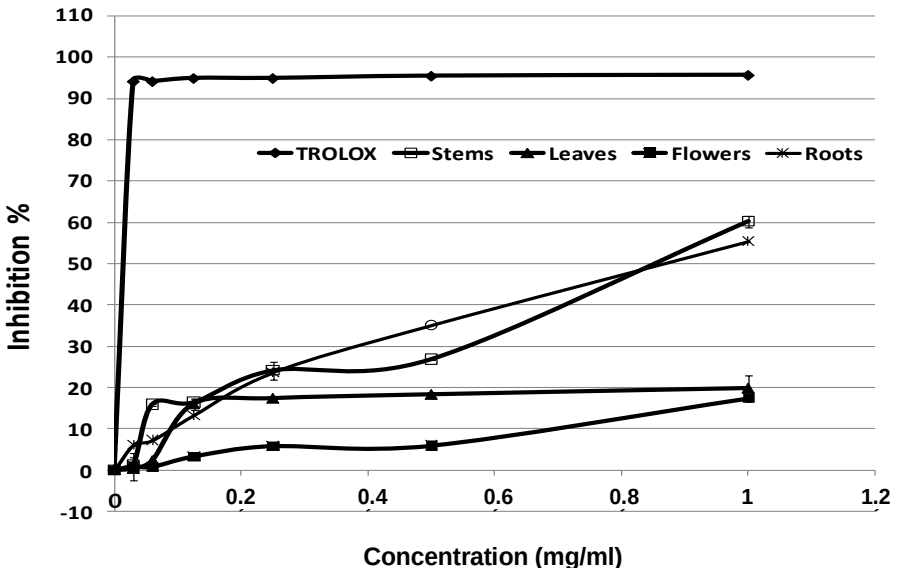


**Figure 1.** The percentage inhibition of DPPH radical in the presence of different concentrations of *Cotula coronopifolia* (L) essential oils roots, stems, leaves and flowers.

mg/ml. Stem essential oil showed a feeble $ABTS^{+\bullet}$ inhibition with a value of 1.65% at a concentration of 1 mg/ml and after 20 min. While the antioxidant oil activity against $ABTS^{+}$ cation radicals shows a pro oxidant activities with values of -10.36 and -20%, respectively, for flowers and leaves essential oils at a concentration of 1 mg/ml (Table 2). Essential oils of roots and stems were relatively active against $ABTS^{+\bullet}$ while the other two oils of flowers and leaves were inactive in comparison with the Trolox (95.59%) (Table 2).

## DPPH scavenging capacity

Free radical scavenging capacities of the essential oil the presence of DPPH radicals are shown in Figure 1. Compared with Trolox ® as a standard reference product, we note that all tested essential oils were relatively active against DPPH Radicals. We note that stems essential oil is the most active with inhibition percentage of 60.14% at a concentration of 1 mg/ml. Furthermore, we note that flowers seem to be the least inhibitor ones of free radicals DPPH in comparison to roots and leaves with a maximal inhibition percentage of 10 and 19.74%, respectively (Figure 1).

In the present investigation, we note that this method showed quite different results compared to those obtained in DPPH reaction (Thaiponga et al., 2006).

## TEAC assay

The values of the TEAC analysis measured by ABTS method at 20 min show that roots possessed the highest antioxidant capacity with a value of 0.26 mM of trolox/g of extract. Followed by stem which showed average value of 0.04 mM of trolox/g of extract thus shows pro-oxidant activity.

## Determination of total phenolic content (TPC)

The total phenolic content of the different essential oils were evaluated, using the Folin ciocalteux method (Figure 2). Among the tested samples flowers had the highest phenolic content with an amount of 22.02mg equivalents Catechin/g extract. Roots presented the lowest phenolic content with a value of 3.12 mg equivalents Catechin/g extract. Stems and leaves volatile fractions have a moderate phenolic content with respective values of 6.15 and 14.93 mg of Catechin per g of sample (Figure 2).

## Correlation between antioxidant activity and TCP

To evaluate the antioxidant activity and total ICP, the coefficient of ($R^2$) determination was calculated. We note that a negative linear correlation between the total antioxidant activity, determined by the TEAC assays, and phenolic content was reported. To correlate the observed activity with the chemical composition of the oils, we can cite the work of Ruberto and Baratta (2000) who studied the antioxidant activity of 98 pure essential oils chemical components and show that monoterpene hydrocarbons had a significant protective effect, with several variants

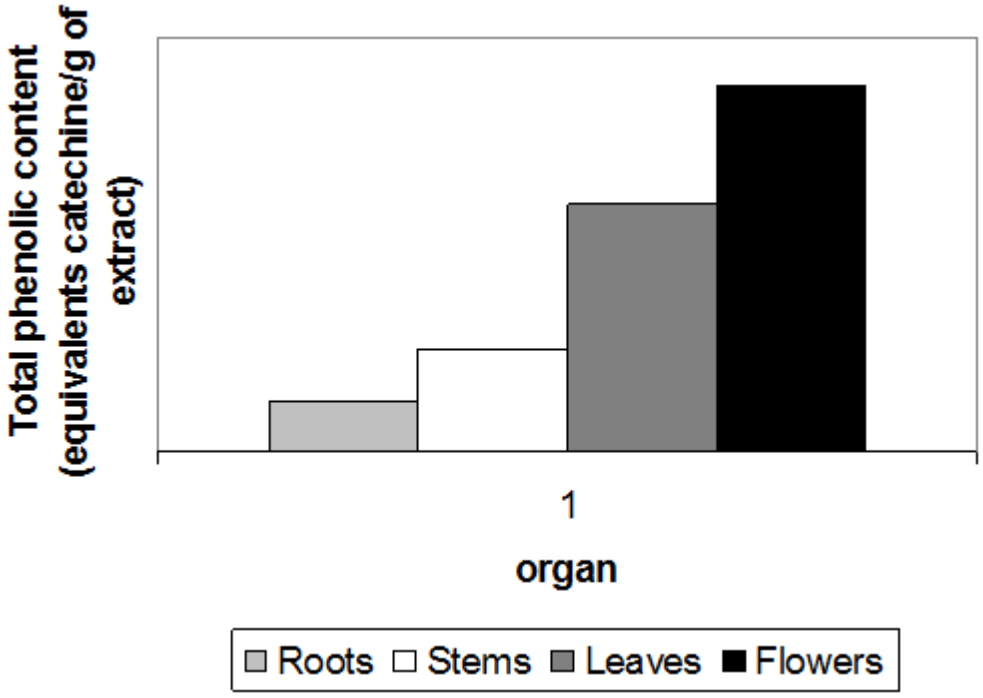


**Figure 2.** Total phenolic content of essential oils of *C. coronopifolia* L.

due to the different functional groups. Furthermore, some researchers show that some essential oils rich in non-phenolic compounds also have antioxidant potentials (El-Massry et al., 2002).The unclear relationship between the antioxidant activity and the total phenolics may be explained in numerous ways. In fact, the TCP does not incorporate all the antioxidants. Their redox properties allow them to act as reducing agents, hydrogen donators, and singlet and triplet oxygen quenchers (Pietta, 2000).

In addition, the synergism between the antioxidants in the mixture makes the antioxidant, not only dependent on the concentration, but also on the structure and interaction between antioxidants (Rice-Evans et al., 1997).

### Antifungal activity

The results of antifungal activity assays represented in Table 3 showed that essential oils of *C. coronopifolia* had inhibitory effects on the growth of fungi. In fact the inhibition varied from 0 to 85%. For the fourth strains tested. The essential oil of leaves showed a strong inhibition (85%) against *A. fumigates*. *S. brevicaulis* was resistant for leave, flower, root and stem essential oil with IP of 9, 0, 18 and 9%, respectively. *S. brevicaulis* seems to be more resistant to the flower essential oil than Aspergillus.

The MIC varied from 800 to 950 µgml$^{-1}$ for the essential oil of leaves and from 850 to 1000 µgml$^{-1}$ for flowers and roots. For stems the MIC varied from 900 to 1000 µg/ml$^{-1}$ (Table 3).

The MIC results confirm those obtained using the percentage inhibition method: *A. fumigatus* (Hyphamycets) is the most resistant strain in the presence of the different essential oils from different organs (MIC>1000 µgml$^{-1}$).

## DISCUSSION

Essential oil from leaves appeared to be more active than others essential oil organs. The screening of the chemical groups in the leaves essential oil of *C. coonopifolia* showed the presence of oxygenated sesquiterpens as Agarospirol which may play a role in the observed antifungal activity. According to (Griffin and Wyllie, 1999) the fungicidal effect of essential oils would be due to the oxygenated compounds.

Possible synergistic and antagonistic effects of compounds also play an important role in fungi inhibition. Previous papers on the antifungal activities of essential oils of some species of various genera have shown that they have varied degrees of growth inhibition effects against some agricultural pathogenic fungal species (Alvarez-Castellanos et al., 2001). Although the different compounds exhibited varied degrees of antifungal activity. These activities may be attributed to the presence of agarospirol, 1-eicosanol, hexacosane and heptacosane found in *C. coronopifolia* essential oils.

**Table 3.** Antifungal activity of *C. coronopifolia* L. essential oils: Percentage inhibition and minimum inhibitory concentration evaluations.

| Microorganisme (a) | Leaves | | Flowers | | Roots | | Stems | |
|---|---|---|---|---|---|---|---|---|
| | I[b]% | MIC | I% | MIC | I% | MIC | I% | MIC |
| *Trichophyton rubrum* (B) | 61 | 900 | 38 | >1000 | 52 | 950 | 23 | >1000 |
| *Microsporum canis* (IP) | 60 | 950 | 69 | 850 | 33 | 950 | 55 | 900 |
| *Aspergilus fumigatus* (B) | 85 | 800 | 33 | >1000 | 68 | 850 | 18 | >1000 |
| *Scopulariopsis brevicaulis* (B) | 9 | 950 | 0 | >1000 | 18 | >1000 | 9 | >1000 |

[a]IP: Institut Pasteur, Paris; B: Microbiological Laboratory, Besançon; [b]Percentage inhibition of microorganisms in the presence of 500 µg $ml^{-1}$ of sample; [c]Minimum inhibitory concentration (µg $ml^{-1}$).

This work is the first report on the essential oils composition, antioxidant and antifungal activity of *C. coronopifolia* L. growing in Tunisia. The essential oils from *C. coronopifolia L.* are moderately active in quenching the stable free radical DPPH and the ABTS+• radical cation. They represent a moderate source of phenolic compounds and show interesting antifungal activity against four pathogenic fungi. They may, therefore, have potential as natural preservative ingredients in food and/or pharmaceutical industries.

## ACKNOWLEDGMENT

The authors are grateful to the minister of High education and scientific research of Tunisia for funding this work.

### REFERENCES

Adams RP (1995). Identification of Essential Oil Components by Gas Chromatography–Mass Spectrometry. Allured Carol. Stream, pp. 1–15.

Alvarez-Castellanos PP, Bishop CD Pascual-Villalobos MJ (2001). Antifungal activity of the essential oils of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. Phytochemistry, 57: 99–102.

Arts MJTJ, Dallinga JS, Voss HP, Haenen GRMM, Bast A (2004). A new approach to assess the total antioxidant capacity using the TEAC assay. Food Chem., 88: 567-570.

Benjilali B, Tantaoui-Elaraki A, Ismaïlou-Alaoui M, Ayadi A (1986). Méthode d'étude des propriétés antiseptiques des huiles essentielles par contact direct en milieu gélosé. Plant Méd. Phytother., 20: 155–67.

Boukef MK (1986). Médecine traditionnelle et pharmacopée. Les plantes dans la médecine traditionnelle tunisienne. Ed. Agence de coopération culturelle et technique, Paris, p.165.

Burits M, Bucar F (2000). Antioxidant activity of Nigella sativa essential oil. Phytother. Res., 14: 323-3280.

Cuendet M, Hostettmann K, Potterat Helv O (1997). Iridoid Glucosides with Free Radical Scavenging Properties from Fagraea blumei. Chim. Acta, 80: 1144.

El-Massry K F, El-Ghorab AH, Farouk A (2002). Antioxidant Activity and volatile components of Egyptian Artemisia judaica L. Food Chem., 79: 331–336.

Griffin SG, Wyllie SG (1999). The role of structure and molecular properties of terpenoids indetermining their antimicrobial activity. Flavour Frag. J., 14: 322-332.

Griffin SG, Markham JL, Leach DN (2000). An Agar dilution method for the minimum inhibitory concentration of essential oils. JEOR,12: 249 -255.

Le floc'h HE (1983). Contribution à l'étude ethnobotanique de la flore Tunisienne : La république Tunisienne. Ed. Publications scientifiques Tunisiennes, p. 1-8.

Markouk M, Lazrek HB, Jana M (1999). Analgesic effect of extracts from *Cotula cinerea* **(**L**).** Phytother. res., 13(3): 229-306.

Miller NJ, Rice-Evans CA, Davies MJ, Gopinathan V, Milner A (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci.,84: 407–412.

Pietta PG (2000). Flavonoids as antioxidants. J. Nat. Products., 63: 1035-1042.

Pottier AG (1981). Flore de la Tunisie. Angiospermes-Dicotylydones – Gamopetales : La république Tunisienne : Publication scientifiques Tunisiennes, p. 1008.

Rice-Evans CA, Sampson J, Bramley PM., Holloway DE (1997). Why do we expect carotenoids to be antioxidants in vivo? Free Radical Res., 26: 381–398.

Ruberto G, Baratta MT (2000). Antioxidant activity of selected essential oils components in two lipid model systems. Food Chem., 69: 167–174.

Shibamoto T (1987). Retention indices in essential oil analysis. In: Sandra P, Bicchi C, editors. Cappilary gas Chromatography in essential oil. Dr. Alfred Heuthing, 259-357.

Thaiponga K, Boonprakoba U, Crosbyb K, Cisneros-Zevallosc L, Hawkins Byrnec D (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Comp. Anal., 19: 669–675.

Van den Berg R, Haenen Guido RMM, van den Berg Bast HA (1999). Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chem., 66: 511-517.

Van der Toorn J, Ten Hove HJ (1982). On the ecology of *Cotula coronopifolia* L. and *Ranunculus sceleratus* L. II. – Experiment on germination, seed longevity, and seedling survival. Acta Oecol., 3(17): 409-418.

Wojdylo A, Oszmianski J, Czemerys R (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem., 105: 940–949.

Yang D, Michel D, Mandin D, Andriamboavonjy H, Poitry P, Chaumont JP (1996) Antifungal and antibacterial properties in vitro, of three Patchouli essential oils of different origins. Acta Bot. Gall., 143(1): 29-35.

Yaniv Z, Dafni A, Friedman J, Palevitch D (1987). Plant used for the treatment of diabetes in Israël, J. Ethnopharmacol., 19(2): 145-151.