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Investigation into the biological activities and chemical composition of *Calendula officinalis* L. growing in Tunisia

[1,2*]Rigane, G., [3]Ben Younes, S., [3]Ghazghazi, H. and [1]Ben Salem, R.

[1]Laboratoire de Chimie Organique-Physique UR11ES74, Département de Chimie, Faculté des Sciences de Sfax, B.P « 1171 » 3038 Sfax, Université de Sfax, Tunisie
[2]Département de Physique-Chimie, Faculté des Sciences et Techniques de Sidi Bouzid, Université de Kairouan, Tunisie
[3]Institut Supérieur de Biotechnologie de Béja, B.P « 382 » 9000 Béja, Université de Jendouba, Tunisie

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Abstract

Calendula officinalis (Marigold) was characterized in respect to its chemical composition, antioxidant potential and antimicrobial activities. Five compounds were identified and quantified by LC/MS and HPLC in leaves and flowers of aqueous-methanolic extracts. Total flavonoids ranged between 44.91 and 76.44 mg QE/g dry weight in leaf and flower extracts, respectively. Rutin, quercetin-3-*O*-glucoside, scopoletin-7-*O*-glucoside, isorhamnetin-3-*O*-glucoside and gallic acid were tentatively identified in this plant. The highest antioxidant activities using two methods, DPPH and FRAP assays were obtained with aqueous-methanol flower extract from *C. officinalis* (0.35 mg.mL^{-1} and 28.37 mM of Trolox). The potential antimicrobial activity of the leaf and flower aqueous-methanol extracts from the *C. officinalis* was screened against three bacteria and two pathogenic fungi, using the cellulosic disc method. A strong inhibited activity against the five microorganisms is obtained. This study could provide useful information for industry to produce potentially bioactive plant extract.

Introduction

Chemical and pharmacological studies involving medicinal plants have increased in the last decades, not only related to the isolation of active principles, but also to the characterization of new components with therapeutic activity and nutraceutical characteristics, important for the use in food industries, as well as in cosmetology and pharmacology.

Calendula officinalis L. (English marigold, pot marigold) belongs to the Asteraceae (Compositae) family; is an annual herbaceous plant, native of Mediterranean countries (Danielski *et al.*, 2007). *C. officinalis* can be broadly applied as an antiseptic, anti-inflammatory and cicatrizing as well as a light antibacterial and antiviral agent (Khalid *et al.*, 2010). The plant has been reported to contain mainly polyphenols such as *p*-phydroxybenzoic, salicylic, vanillic, caffeic, gallic acids (Gora *et al*., 1979; Gong *et al*., 2012), acylated flavonoid-*O*-glycosides and methoxylated flavonoids, amino acids (Abasova *et al*., 1995), alkaloids, carotenoids, saponins, tannins (Duke, 1992), high molecular weight polysaccharides (Wagner *et al.*, 1984) and triterpenoid monoesters (Neukirch *et al.*, 2004). Alpha-cardinol (Chalchat *et al*., 1991), deltacadinol, delta-cadinine and gamma murolene (Marczal *et al*., 1987) have been identified in the essential oil.

Previous studies showed that different species of this plant, as well as different cultivars of the same species, were markedly different in the content of their phenolics, flavonoids and antioxidant activities as well as antioxidant properties were in correlation with the content of total phenolics and flavonoids (Ercetin *et al*., 2012).

Taking this information on *Calendula* into account, in this study, we aimed to examine, for the first time, the chemical composition of the methanolic extract of *Calendula officinalis* L. growing in North of Tunisia. The biological activities of the extract including antimicrobial and antioxidant activities which were tested by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays. Total phenol and flavonoid contents of the extracts were calculated spectrophotometrically whereas individual phenolic compounds were quantified by HPLC apparatus.

Materials and Methods

Chemicals

The solvents used for extraction and chromatographic separation (methanol, acetic acid and acetonitrile) were purchased from Riedel-deHaën (Seelze, Germany). Formic acid was purchased from Panreac Quimica (Barcelona, Spain). Ethanol was obtained from Carlo Erba (Milan, Italy). The solvents were of appropriate purity. 2,2-Diphenyl-1-

*Corresponding author.
Email: *gaith.rigane@yahoo.fr*
Tel: (00216) 97 23 40 87/ (00216) 20 58 07 47

Table 1. Total phenol and flavonoids in *Calendula officinalis* flowers and leaves extracts

	Extract yield (w/w, %)	Total phenol (mg GAE/g of dry weight extract)	Total flavonoids (mg QE/g of dry weight extract)
Flowers	32.12	109.27 ± 0.23[a]	76.44 ± 0.01[A]
Leaves	18.24	58.68 ± 0.12[b]	44.91 ± 0.02[B]

Results are expressed as mean ± standard deviation of 3 determinations.
Means with different letters were significantly different at $p < 0.05$.

picrylhydrazyl (DPPH), 2,6-di-tert-butyl-4-hydroxy-boxylic acid (BHT), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and quercetin were purchased from Sigma-Aldrich (USA). Gallic acid was purchased from Extrasynthese (Geney, France). Iron (III) chloride ($FeCl_3.6H_2O$) and sodium acetate ($CH_3COONa.3H_2O$) were purchased from Merck (Darmstadt, Germany). Double distilled water was used in the HPLC mobile phase.

Plant material

The sample of *C. officinalis* was collected from Béja (Tunisia, 36°42'45" N; 9°10'53" E; 340 m) in March 2011. The plant samples were identified by Pr. Makki Boukhris from the Ecology vegetal laboratory, Department of sciences of life, Sfax University (Tunisia) the voucher specimens of *C. officinalis* are preserved (MBCA 2011) at the Sciences of Faculty of Sfax, Sfax University, Tunisia.

Preparation of marigold extracts

The leaf and flower parts of *C. officinalis* were separated by hand, air-dried at room temperature, and powdered in a mechanical grinder. Each plant part was weighed accurately in a digital balance (Shimadzu AW320), extracted sequentially with methanol/water (70:30, v/v) was left to stand under agitation for 24 h at room temperature. Then, the filtrated solvents were evaporated in vacuum until dryness using rotary evaporator (Büchi, Switzerland). The extract yields (w/w) are given in Table 1.

Total phenolics determination

The total phenol content of aqueous-methanol extract was determined using the phenol reagent (Rigane *et al*., 2011, 2013). Briefly, 50 µL aliquot of different extracts was assayed with 250 µL of Folin–Ciocalteau reagent and 500 µL of aqueous sodium carbonate (20%, w/v). The mixture was vortexed and diluted with water to a final volume of 5 mL. After incubation for 30 min at room temperature, the absorbance was measured at 765 nm. The total phenols were expressed as gallic acid equivalents (mg gallic acid equivalent/g of dry weight of extract), using a calibration curve of a freshly prepared gallic acid solution. For the gallic acid, the curve absorbance versus concentration is described by the equation $y = 0.0017x$ ($r^2 = 0.9023$).

Total flavonoids determination

The flavonoids content in the extracts was determined spectrophotometrically according to Rigane *et al*. (2011), using a method based on the formation of a complex flavonoid aluminium, having the maximum absorption at 510 nm. The flavonoids content was expressed in mg of quercetin equivalent per gram of dry weight extract (mg QE/g). For quercetin, the curve absorbance versus concentration was described by the equation $y = 0.043x$ ($r^2 = 0.997$).

Reverse-phase HPLC condition

The quantification of phenolics was carried out by HPLC. The assays were performed with a Schimadzu apparatus (Schimadzu, Japan) equipped with a LC-10ATvp pump, a UV-Vis detector SPD-10Avp and a Shim-pack C-18 column (250 mm x 4.6 mm, particle size of 5 µm, Shimadzu, Kyoto, Japan). A gradient of two solvents, A and B, was used. Formic acid (0.1 %, v/v) in water and 0.1 % of formic acid in acetonitrile served as solvents A and B, respectively. Analysis conditions were as described previously by Rigane *et al*. (2011, 2012a) and involved a four-step linear gradient analysis for a total time of 18 min was used as follows: starting from 95% A and 5% B for 1 min, followed by an 11 min step gradient from 5% B to 100% B. Then, elution was conducted in the isocratic mode with 100% B for 4 min. Finally, the elution was achieved with linear gradient from 100% B to 5% in 2 min. The flow rate was 200 µL/min. An aliquot (5 µL) of sample solution was injected.

Detection and quantification were performed at 280 and 335 nm. Each phenolic compound was quantified in comparison to its standard when it was available. Due to the absence of standard for some detected compounds, Isorhamnetin-3-*O*-glucoside, quercetin-3-*O*-glucoside and rutin were expressed as quercetin. Quantitative evaluation of individual phenolic was performed by means of a four-point regression curve ($r^2 = 0.989$) using authentic external standards.

LC-MS analysis

Samples were analyzed on Agilent 1100 series LC-MSD consisting of a degasser, a binary pump, an auto sampler, and a column heater. For chromatographic conditions, the compounds were separated with a Zorbax 300 Å Extend-C-18 column (150 x 2.1 mm, particle size 5 µm, Agilent Technology Inc., Wilmingtom, DE, USA). The column outlet was coupled with an Agilent MSD ion trap XCT mass spectrometer (Santa Clara, CA, USA) equipped with an ESI ion source. The capillary voltage was set to 3.5 KV, the temperature to 350°C, the nebulizer gas

to 40 p.s.i, and the drying gas flow to 10 L/min. The maximum accumulation time was 50 ms, the scan speed was 26.000 m/z s^{-1} (ultra scan mode) and the fragmentation time was 30 ms. The HPLC separation part was carried out as described in the Reverse-phase HPLC condition section (Rigane *et al*., 2011; 2012a).

Antioxidant activities

DPPH assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging effect was evaluated following the procedure described in a previous study (Rigane *et al*., 2011; 2012a). Aliquots (50 µl) of various concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg/mL) of the test extracts in methanol were added to 5 ml of methanolic solution containing DPPH radicals (6 x 10^{-6} M). After a 30 min incubation period at room temperature and in the dark, the absorbance was read in opposition to the control at 517 nm. The Inhibition (IC_{50}) of free radical DPPH (IC_{50} %) was calculated in percentage: IC_{50} % = $[(A_{blank} – A_{sample})/A_{blank}]$ x 100, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract. The concentration of the test extract providing 50% inhibition (IC_{50}, expressed in mg/mL) was calculated from the graph plotted with inhibition percentage against the extract concentration. The synthetic antioxidant reagent butylated hydroxytoluene (BHT) was used as positive control and all tests were carried out in triplicate.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out as described by Rigane *et al.* (2011; 2012a; b). Reagents included 300 mM acetate buffer (pH 3.6); 10 mM TPTZ; 20 mM $FeCl_3 \bullet 6H_2O$ in 1 M hydrochloric acid. The working FRAP reagent was prepared freshly by mixing 10 mL acetate buffer, 1 mL TPTZ solution and 1 mL ferric chloride hexahydrate solution. 100 µL sample was added into the 3 mL FRAP solution, 900 µL of 96% EtOH, and 300 µL H_2O was added. The reaction was maintained for 6 min, and the absorbance reading was constant at 593 nm.

The antioxidant activities of the extracts were calculated from the dose–response curves of Trolox (y (absorbance) = 0.2595 x (mM TE/g of extract) (r^2 = 0.9965), which were carried out respectively by the mentioned above three methods, and all of the results were expressed as Trolox equivalent antioxidant capacity (TEAC) values in this study.

Determination of antimicrobial and antifungal activities

The used micro-organisms to evaluate the antimicrobial activity are famous pathogens. Bacterial species were *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* and fungus were *Candida albicans* and *Aspergillus niger*. The antibacterial and antifungal activity of various methanolic extracts was performed by the modified cellulosic disc method (Bauer *et al*., 1966; Choi *et al.*, 2006). Agar LB and Malt Extract are inoculated with bacterial and fungal strains. The bacterial cultures (OD = 0.4 - 0.6) are homogenized with solid media. Thus, the tested quantities of each ethanolic extract are deposited on the cellulose paper disc (diameter 0.9 mm, previously sterilized (120°C for 15 min). After evaporating the solvent, these discs are applied directly to a petri dish previously inoculated with the tested-strain. Times and incubation temperatures were 24 and 48 h for bacterial and fungal strains, respectively at 30 and 37°C. Antimicrobial activity is observed by the presence of an inhibition zone around the disk impregnated with extract. A diameter greater than 1.5 cm of the inhibition zone represents the minimum inhibition quantity (MIQ). Standard chloramphenicol antimicrobial disk is used as control in tests of antibacterial activity. Amphotericin B is used as a control in testing the antifungal activity. The test was carried out by triplicate.

Statistical Analysis

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

Results and Discussion

Phenolic and flavonoid content in Calendula officinalis L. extract

Based on the absorbance values after reaction with Folin–Ciocalteu reagent, results of the colorimetric analysis are given in Table 1. The amount of total polyphenols was higher in flowers (109.27 mg GAE/g dry weight extracts) than in leaves (58.68 mg GAE/g dry weight extracts). The same tendency was observed for flavonoid content (Table 1), being about two times more important in flowers than in leaves. These results were in agreement with the reports of Hertog *et al.* (1993) and Yen *et al.* (1996) and Boukhris *et al.* (2013), which proved

Table 2. Phenolic compounds detected in leaves[a] and flowers[b] of *Calendula officinalis* L. with their HPLC retention times, UV maxima and mass spectral data

N°	Flavonoid compound	Parts	Rt (min)	UV(λ_{max})	MW	Quasi-molecular ion [M-H]$^-$	Major ESI fragments
1	Gallic acid	a, b	9.21	216, 272	170	169	125
2	Scopoletin-7-*O*-glucoside	a	11.42	228, 345	354	353	191
3	Quercetin-3-*O*-glucoside	a, b	17.60	256, 350	464	463	301, 273, 179, 151
4	Rutin	b	18.00	218, 255, 355	610	609	463, 301
5	Unknown	a	18.20	258, 360	350	349	321, 117
6	Isorhamnetin-3-*O*-glucoside	b	18.60	255, 355	478	477	314, 285, 152
7	Unknown	b	25.10	242, 360	386	385	-------

Rt: Retention time; MW: Molecular weight

that the methanol is the most suitable solvent for the extraction of phenolic and flavonoids compounds. Such results were in accordance with those reported by Ercetin *et al.* (2012) who claimed that the richest total phenol (~80 µg/g of extract) and flavonoid (~20 µg/g of extract) contents were found in the flower methanolic extract from *C. officinalis* collected from Bartin province (Turkey) in 2008. In addition, Gong *et al*. (2012) reported that total phenolics content of defatted marigold residue extracts ranged from 8.50 to 62.36 mg GAE/g and the total flavonoids varied from 19.04 to 97.00 mg RE/g.

Identification and quantification of phenolic compounds in Calendula officinalis L. extract

Using LC-MS analysis, it was possible to reveal the presence of several phenolic acids, coumarin and flavonoid compounds. Table 2 lists each of the identified phenolic in elution order. The structure assignment of all phenolic compounds for which no standards were available was based on a systematic search for molecular ions using extracted ion mass chromatograms and comparing those with literature (Grayer *et al.*, 2000; Heneidak *et al.*, 2006; Gong *et al*., 2012; Rigane *et al*., 2012b; Boukhris *et al*., 2013). For example, the ESI mass spectrum in the negative mode of compound 2 exhibited a base peak [M - H]- at m/z 353 (Table 2) and an intermediate ion at m/z 191. The λ max of the UV spectrum at 228 and 345 nm suggests that compound 2 is a Scopoletin 7-*O*-sugar, and the combined results of the MS and UV spectra suggest that compound 2 could be Scopoletin 7-*O*-glucoside. This compound was detected, for the first time, in leaves of *Calendula officinalis* L. growing in Tunisia.

Compound 3 exhibited a base peak [M-H]- at m/z 463 and fragment ions at m/z 301, 273, 179 and 151 (Table 2). The fragmentation of the pseudomolecular ion [M-H]- of compound 3 at m/z 463 yielded a fragment at m/z 301 by neutral loss of 162 mass units. The ion m/z 273 was formed by the loss of CO from the fragment at m/z 301. On the other hand, the ion at m/z 179 was a glucoside moiety while m/z 151 was a result of a retro- Diels-Alder (RDA) fragmentation of the heterocyclic ring system. Those signals suggested that compound 3 was probable quercetin3-*O*-glucoside. This compound was detected in the flowers and leaves of *Calendula officinalis* L. growing in Tunisia, for the first time.

In addition, the ESI-mass spectrum in negative mode of compound 4 (retention time 18.00 min) exhibited a base peak at m/z 609 in negative ion mode and strong peaks at m/z 301. This observation can be a diagnosis of quercetin derivatives, and a fragment at m/z 463, due to the loss of 146 Da corresponding to rhamnose. These results confirm the presence of rutin, having a molecular mass of 610 Da and already reported in *Calendula officinalis* cultivated in Italy (Bilia *et al*., 2002). On the other hand, the fragmentation of the pseudomolecular ion [M-H]- of compound 6 at m/z 477 yielded a fragment at m/z 315 by neutral loss of 162 mass units. The ion m/z 285 was formed by the loss of CO unit. While, the ion at m/z 152 was obtained by the elimination of $C_8H_7O_3$ molecule from the ion at 315. This suggested that 6 might be an isorhamnetin-3-*O*-glucoside. This compound was detected for the first time in the flower extract of *Calendula officinalis* L. For a lack of enough signals, compounds 5 and 7 were not identified in this study.

Further investigation should be done in the future. The analysis of phenolic substances using reversed phase-HPLC from *Calendula officinalis* aerial parts extracts, as described in the experimental section, allowed to the quantification of the phenolic compounds previously identified by LC-MS apparatus. The results obtained revealed a flower phenolic fingerprint composed of one phenolic acid (gallic acid), one coumarin (Scopoletin-7-*O*-glucoside) and three flavonoids (quercetin-3-*O*-glucoside,

Table 3. Quantification of phenolic compounds of aqueous-methanolic *Calendula officinalis* L. extract

Phenolic compound	*Flowers*	*Leaves*
Gallic acid [A]	10.15 ± 0.02[†]	5.08 ± 0.01[‡]
Scopoletin glucoside [B]	ND	7.78 ± 0.02[*]
Quercetin-3-*O*-glucoside [B]	12.31 ± 0.01[£]	23.42 ± 0.04[#]
Rutin [B]	34.33 ± 0.02[‡‡]	ND
Isorhamnetin-3-*O*-glucoside[B]	8.86± 0.02[!!]	ND

Results are expressed as mean ± standard deviation of 3 determinations.
[*], [†], [‡], [£], [#], [‡‡], [!!] Different symbols for the same phenolic compound indicate significant differences among aerial parts ($p < 0.05$).
[A] Phenolic compound was expressed as its standard expressed as mg/g.
[B] Phenolic compounds were expressed as quercetin expressed as mg/g.

Table 4. Antioxidant activities of *Calendula officinalis* extracts

	DPPH (IC_{50} mg.mL^{-1})	FRAP (mM of Trolox)
Flowers	0.35 ± 0.02[a]	28.37 ± 0.12[A]
Leaves	0.57 ± 0.03[b]	17.68 ± 0.02[B]
BHT	8.11 ± 0.01[c]	1.22 ± 0.03[C]

Results are expressed as mean ± standard deviation of 3 determinations.
Means with different letters were significantly different at $p < 0.05$.

isorhamnetin-3-*O*-glucoside and rutin) while rutin was absent in leaves extract Chromatogram analysis depicts important differences between flower and leaf phenolic composition. In fact, flavonoids were more largely represented in the flowers than in leaves (Table 3).

Antioxidant activity

The flower and leaf extracts of *Calendula officinalis* L. were subjected to *in vitro* tests to evaluate their antioxidant activities. In particular, we carried out two tests: the DPPH radical-scavenging and FRAP assays. The standard reference compound for all tests was BHT.

DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997). The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants. The scavenging effect of aqueous-methanol extracts and standard on the DPPH radical expressed as IC_{50} values was in the following order: flowers (0.35 mg.mL^{-1}), leaves (0.57 mg.mL^{-1}) and BHT (8.11 mg.mL^{-1}) (Table 4). The experimental data of *C. officinalis* reveal that extracts from different parts likely have a stronger effect of scavenging free radical than positive control (BHT).

Up to date, several studies describing antioxidant activity in different tests have been reported for *Calendula* species, *C. officinalis* in particular. For instance; the butanol extract of *C. officinalis* was found to scavenge super oxide and hydroxyl radicals strongly (Cordova *et al*., 2002). In another study (Kaurinovic *et al*., 2003), the ethyl acetate extract of *C. officinalis* flowers exhibited the highest scavenging

Table 5. Antimicrobial and antifungal activities of aqueous-methanolic leaf and flower extracts from *Calendula officinalis*

Organisms	**MIQ (µg)**	
	Calendula officinalis (leaf extract)	*Calendula officinalis* (flower extract)
Escherichia coli	77 <MIQ	MIQ> 243
Salmonelle typhimurium	146 <MIQ	27<MIQ> 54
Staphylococcus aureus	29 <MIQ< 48	54<MIQ> 81
Candida albicans	MIQ> 436	MIQ> 243
Aspergillus niger	MIQ> 436	MIQ> 243

effect against hydroxyl radical. However, in our study, the aqueous-methanol flower extract from *C. officinalis* displayed the highest scavenging effect against DPPH when compared to the value of BHT. In a similar study on *C. arvensis* and *C. officinalis* growing in Serbia and Montenegro (Cetkovic *et al*., 2004), their scavenging potential was measured comparatively against DPPH, hydroxyl, and peroxyl radicals. All of the extracts obtained from both species scavenged all radicals in concentration dependant manner, although the best antioxidant activity was shown by the water extract from *C. officinalis* at 0.75 mg.mL^{-1}. In a similar study, Danila *et al.* (2011) screened the water and alcoholic extracts of several plant species from Romania including *C. officinalis* for their antioxidant activity. Dall'Acqua *et al.* (2008) investigated antioxidant activity of eleven plant species (including *C. arvensis*) used in Sardinian folk medicine using DPPH radical scavenging assay and *C. arvensis* was found to rank 7th among the tested plant species in terms of radical scavenging effect towards DPPH (Ercetin *et al*., 2012).

The radical scavenging properties of natural products are frequently linked with their aptitude to shape stable radicals. For instance, the aqueous-methanol flower extract from *C. officinalis* was the most effective in this test as well as FRAP assay (Table 4). It seems that there is a correlation between total phenol and flavonoid contents and antioxidant activity (Rice-Evans *et al.*, 1996; Rigane *et al.*, 2011; 2012 b).

Antimicrobial and antifungal activities

Aqueous-methanolic leaf and flower extracts from *Calendula officinalis* showed varying degrees of antimicrobial activities in regards to the five tested microorganisms (Table 5). Aqueous-methanolic leaf extract was more effective than flower ones to combat the studied pathogenic microorganisms. Likewise, *C. officinalis* (leaf extract) exhibited a better MIQ against *Escherichia coli* and *Staphylococcus aureus* than aqueous-methanolic flower extract having strong activity against *Salmonelle typhimurium* at lower

quantities. The phenolic compounds and flavonoids found in *C. officinalis* could be responsible for its antimicrobial activity against *Escherichia coli*, *Salmonelle typhimurium*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus nigeri.* Similarly, the aqueous-methanolic extracts from *Calendula officinalis* also showed antimicrobial activity against tested germs but required quantities ranged between 27 to 436 µg. Following these results, aqueous-methanolic leaf extract from *Calendula officinalis* has very interesting antibacterial and antifungal activities against the tested microorganisms. This agrees with the investigations have shown that *Calendula officinalis* allows an increase in antibacterial activity and a decrease of bacterial resistance, *in vitro*, and a very interesting and high antibacterial and antioxidant activities (Dumenil *et al.*, 1980; Janssen *et al*., 1986; De Tommasi *et al*., 1991). In fact, Calendula extract has activity against *Candida albicans* as was demonstrated by Janssen *et al.* (1986), but Riso *et al.* (1987) showed that *C. arvensis* has no activity against the same germ. In our knowledge, few detailed phytochemicals and/or biological studies were done dealing with this medicinal plant.

Conclusions

C. officinalis is perfectly growing to a very humid climate. This shrub is not yet appropriately exploited, except as wild dry pastures or in some folk medicine remedies. It showed a wealth of powerful antioxidant and antimicrobial activities. This study clearly indicated that it is important to consider both the associated antioxidant activity and phenolic content. Indeed, methanolic extract of flower containing higher level of phenolics possessed more powerful antioxidant potential. This plant, mainly its flower, constitutes an excellent source of flavonoids. Indeed, its elevated phenolic compound content makes it a potential source of dietary regime and a protection against numerous diseases and infections. Both methanolic flower and leaf extracts from *Calendula officinalis* showed interesting antimicrobial activities.

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