Antibacterial, antioxidant, anti-cholinesterase potential and flavonol glycosides of *Biscutella raphanifolia* (Brassicaceae)

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Abstract: Different extracts of the aerial parts of *Biscutella raphanifolia* (Brassicaceae), which has not been the subject of any study, were screened for the phytochemical content, anti-microbial, antioxidant and anti-cholinesterase activities. We used four methods to identify the antioxidant activity namely, $ABTS^{\bullet+}$, $DPPH^{\bullet}$ scavenging, CUPRAC and ferrous-ions chelating methods. Since there is a relationship between antioxidants and cholinesterase enzyme inhibitors, we used two methods to determine the *in vitro* anti-cholinesterase activity by the use of the basic enzymes that occur in causing Alzheimer's disease: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The extracts were also tested *in vitro* antimicrobial activity against various bacteria. The phytochemical study of *B. raphanifolia* afforded four flavonol glycosides; namely, quercetin-3-*O*-*β*-*D*-g1ucoside, quercetin-3-*O*-[*β*-*D*-glucosyl(1→2)-*O*-*β*-*D*-glucoside], quercetin-3-*O*-[*β*-*D*-glucosyl(1→3)-*O*-*β*-*D*-glucoside] and kaempferol-3-*O*-[*β*-*D*-glucosyl(1→2)-[(6‴*p*-coumaroyl)-*β*-*D*-glucoside], being isolated here for the first time from *Biscutella raphanifolia* and the genus. The ethyl acetate extract showed the highest activity in $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and CUPRAC assays, while the petroleum ether extract demonstrated optimum efficiency metal chelating activity. The diclorometh ane and petroleum ether extracts showed a mild inhibition against AChE and BChE. However, the petroleum ether extract showed a good antibacterial activity against the pathovars *Enteropathogenic E. coli* (EPEC), *Enterotoxigenic E. coli* (ETEC) and *Enterococcus feacalis*, whereas the *Enterohemorrhagic E. coli* (EHEC) strain was more sensitive to dichloromethane and *n*-butanol extracts.

Keywords: *Biscutella raphanifolia,* antioxidant, anti-cholinesterase, anti-bacterial, flavonols.

INTRODUCTION

For a long time, plants have been the subject of extensive research, heightened by the hope of therapeutically active molecules. The therapeutic use of plants is very old and is experiencing a revival. It is possible to use the whole plant or mining products they provide (Mark *et al*., 2001). According to the WHO, nearly 6377 species of plants are used in Africa; more than 400 are medicinal plants that constitute 90% of traditional medicine. In 2004, nearly 75% of Africa's population has used plants to treat and has no access to modern drugs. Today, it has been estimated that the active ingredients from plants represent 25% of prescription drugs for a total of 120 composed of natural origin from 90 different plants (Potterats, 1997). Algeria has a considerable floristic richness. There are about 3000 species of plants of which 15% are endemic (Quezel *et al*., 1963).

Plants products received great interest as safe antioxidants, since the synthetic antioxidants were reported that they may be accountant for carcinogenesis and damage to the liver (Grice, 1988). Natural compounds are also important where they prevent the oxidative stress damage (Scalbert *et al*., 2005). Moreover, the delay of evolution of the disease of Alzheimer and the reduction of neuronal degeneration can occur by using of antioxidants (Atta-ur-Rahman *et al*., 2001). There is a hypothesis that the lack of the necessary amount of neuromediator acetylcholine is the cause of Alzheimer's (Grossberg, 2003). Therefore, the treatment of Alzheimer's disease is effected by the acetylcholinesterase enhibitors drugs. The use of products of natural origin, possessing different biological activities such as anti-cholinesterase, anti-microbial and antioxidant, is more effective than the use of drugs, exhibiting some side effects.

In continuation of our works on plants growing at Constantine, possessing a high antioxidant potential (Bencharif-Betina *et al*., 2012; Benkiki *et al*., 2003; Do Rego *et al*., 2007: Kabouche *et al.*, 2005 and 2007; Kolak *et al*., 2009; Laggoune *et al.*, 2011; Lakhal *et al.,* 2011 and 2014; Touafek *et al.*, 2011 and 2012), we describe here the phytochemical study of the Algerian species *Biscutella raphanifolia* (Brassicaceae) (Quezel *et al*., 1963) and its anti-cholinesterase, antibacterial, and antioxidant activities. In this study, we compared the anti-cholinesterase activity with galantamine and the antioxidant activity with different commercial products.

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MATERIALS AND METHODS

General experimental procedures

UV spectra have been recorded on an Agilent 8453, UV-Visible spectrophotometer, in MeOH as blank. Anticholinesterase and antioxidant activities were performed on a Spectramax340PC^{384} 96 well plate microplate reader. Column chromatography (CC): Polyamide (ICN Biomedicals GMBH 09602 and ICN Biomedicals GMBH 09603), Merck silica gel 60 (Merck, Art. 9385), TLC: silica gel 60 F_{254} (Art. 5554). 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylatedhydroxyl anisole (BHA), α-tocopherol, 2,2'-Azinobis (3-ethylbenzo-thiazoline-6-suphonic acid) diammonium salt ($ABTS^{\cdot+}$), horse serum butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4U/mg, Sigma), 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB), electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 426U/mg, Sigma), acetyl-thiocholine iodide, Neocuproine, butyrylthiocholine chloride and galantamine were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany).

Plant material

Biscutella raphanifolia (Brassicaceae) is an endemic species (Quezel *et al*., 1963), collected in June 2010, locally at Djebel El Ouahch (800 m altitude), Constantine (Eastern Algerian) and identified at Badji-Mokhtar university (Annaba) by Pr. Gérard De Bélair.

Extraction procedures

1500g of the sprayed plant are macerated in a water-alcohol mixture (water/methanol; 80/20, v/v) for 24 hours at ambient temperature. The recovered extract was concentrated under reduced pressure at a moderate temperature (45°C). The method was repeated 3 times with replacement of the solvent in each case and lasts 24 to 48 hours. The three recovered alcoholic extracts were combined and concentrated. To the concentrated solution, 300ml of water were added. After filtration, a clear aqueous solution was obtained. This aqueous phase was extracted by liquid-liquid type using solvents of increasing polarity, beginning with petroleum ether, then dichloromethane, then ethyl acetate and finally with *n*-butanol leading, after evaporation, to corresponding extracts: petroleum extract of *Biscutella raphanipholia* (PEBR), dichloromethane extract (DEBR), ethylacetate extract (EEBR) and butanolic extract of (BEBR).

15g of the BEBR were fractionated by column chromatography on polyamide SC_6, eluted with a gradient of polarity of toluene/MeOH. A total of 368 fractions were achieved. Fractions of the similar composition were combined, 20 main fractions were obtained. Fraction F-2 was separated on column of silica gel, using an isocratic elution system of AcOEt/MeOH/H_2O (8/1/0.5:7/1/0.5) and TLC on polyamide DC6, eluted with H_2O/MeOH/metylethylketone/acetylacetone (13/3/3/1), affording four pure flavonoids which were identified by the use of spectral methods: UV, ^{1}H NMR and ^{13}C NMR and high multidimentional NMR experiment techniques.

Acid Hydrolysis

The acid hydrolysis of the pure products was achieved by the use of authentic samples of sugars (Lakhal *et al*., 2011).

Biological activities

Antibacterial activity

The study of the antibacterial activity (Bauer *et al*., 1966; NCCLS, 1993) of the PEBR, DEBR and BEBR was made against 5 pathovars of *Escherichia coli: Enterotoxigenic E. coli* (ETEC) ATCC 1493, *Enteropathogenic E. coli* (EPEC*)* ATCC 2348, *Enterohemorrhagic E. coli* (EHEC) ATCC HB 101, *Enteroinvasive E. coli* (EIEC) ATCC LT 10407, and *Enteroaggregative E. coli* (EAEC) and *Staphylococcus aureus* ATCC 25923 (*Gram-positive cocci*), *Pseudomonas aeruginosa* ATCC 27853 (*Gram-negative bacille*) and *Enterococcus feacalis* ATCC 29212 (*Gram-positive cocci*).

DPPH free radical scavenging test

The free radical-scavenging activity has been performed according to Blois (Blois, 1958).

ABTS cation radical decolorization test

The $ABTS^{\cdot+}$ scavenging activity has been tested according to Re (Re *et al*., 1999).

Cupric reducing antioxidant capacity (CUPRAC)

The results of this method were compared from the absorbance values with the standards: BHA and α-tocopherol (Apak *et al*., 2004).

Metal chelating activity

The metal chelating activity by the ferrene-Fe^{2+} complexation assay has been carried on following the method of Decker (Decker *et al*., 1990), the standard used in this method is ethylenediamine tetra acetic acid (EDTA).

Determination of acetylcholinesterase- (AChE) and butyrylcholinesterase- (BChE)

AChE and BChE inhibitory activities were determined by Ellman (Ellman *et al*., 1961). The results were compared with galantamine.

STATISTICAL ANALYSIS

The tests were performed in triplicate for the antioxidant and anti-cholinesterase activities, recorded as mean ± standard deviation (Anova). Student's-*t* test procedures were used for determination of significant differences between means, *p.* values<0.05 were considered as significant.

Table 1: Antibacterial activity of the PEBR, DEBR and BEBR of *Biscutella raphanifolia*

Microorganism	Inhibition zone, mm		
	PEBR	DEBR	BEBR
Enterohemorrhagic E. coli	13.5	14.5	15.0
Enterotoxigenic E. coli	17.5	13.5	13.5
Enteroinvasive E. coli	12.5	14.0	13.5
Enteropathogenic E. coli	15.5	14.5	6.0
Enteroaggregative E. coli	12.0	6.0	11.5
Staphylococcus aureus	6.0	6.0	6.0
Pseudomonas aeruginosa	13.0	13.5	13.5
Enterococcus feacalis	15.5	14.5	14.0

Table 2: The inhibition (%) of ABTS and the free radical scavenging activity by DPPH assay of extracts of *Biscutella raphanifolia*

	ABTS				DPPH			
	12.5µg	25.0µg	50.0µg	100.0µg	12.5µg	25.0µg	50.0µg	100.0µg
BEBR	58.64±1.67	82.9±1.79	83.45±4.38	90.17±0.05	39.61±2.53	67.10±5.05	79.38±0.29	79.65±0.22
EEBR	69.71±0.94	83.61±0.42	89.09±1.39	90.34±0.15	35.35±0.59	53.97±2.65	73.05±1.56	80.59±0.16
DEBR	59.54±1.04	77.93±0.61	87.02±0.98	89.96±0.38	9.03±0.46	21.33±1.24	37.90±0.39	49.59±1.40
PEBR	14.07±1.09	22.48±0.20	38.66±0.39	60.99±0.23	4.03±0.32	7.26±0.22	13.88±0.46	22.93±0.16

Values expressed are means ± S.E.M. of three parallel measurements (p<0.05).

Table 3: The cupric reducing antioxidant capacity (CUPRAC) by Cu^{2+} Cu^{+} transformation and metal chelating activity (Inhibition %) by Ferrene-Fe^{2+} assays of the extracts of *Biscutella raphanifolia*

	% Inhibition				Absorbance			
	Metal chelating				CUPRAC			
	12.5µg	25.0µg	50.0µg	100.0µg	12.5µg	25.0µg	50.0µg	100.0µg
BEBR	na	na	na	5.00±0.55	0.30±0.01	0.54±0.00	0.93±0.06	1.63±0.07
EEBR	na	na	na	na	0.41±0.03	0.71±0.02	1.27±0.02	2.26±0.06
DEBR	1.49±0.23	4.69±1.22	5.18±0.73	6.33±1.22	0.23±0.00	0.41±0.01	0.66±0.02	1.21±0.02
PEBR	2.41±1.44	5.45±1.66	14.68±0.40	32.78±1.18	0.12±0.00	0.17±0.01	0.27±0.01	0.45±0.02

Values expressed are means ± S.E.M. of three parallel measurements (p<0.05). na: Not active

Table 4: Acetyl- and butyryl-cholinesterase inhibitory activities of extracts of *Biscutella raphanifolia*[a]

	AChE				BChE			
	25µg	50µg	100µg	200µg	25µg	50µg	100µg	200µg
BEBR	na	na	na	na	na	na	na	na
EEBR	na	na	na	na	na	na	na	na
DEBR	na	na	6.04±1.30	8.10±1.26	na	na	10.54±1.45	25.71±2.80
PEBR	2.40±0.78	3.14±1.79	5.54±1.81	7.85±0.53	na	na	1.19±0.42	7.91±1.50
Galantamine[b]	68.4±1.1	74.4±0.7	78.6±0.5	81.4±1.0	40.6±2.9	48.7±0.9	65.0±0.4	75.5±1.1

[a]Values expressed are means ± S.E.M. of three parallel measurements (p<0.05). [b]Reference compounds. na Not active.

RESULTS

Identification of isolated compounds

The phytochemical study of *Biscutella raphanifolia*, after successive splitoffs on different chromatographic supports, led to the isolation and identification of four flavonoids:

Quercetin-3-O-β-D-g1ucoside (1) - $C_{21}H_{20}O_{12}$, UV (MeOH, λ_{max}, nm): 269, 354; +NaOH: 267, 324, 402; +$AlCl_3$: 272, 407; +$AlCl_3$/HCl: 274, 377. ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm, *J*/Hz): 7.74 (lH, *d, J*=2, H-2'), 7.49 (lH, *dd, J*=8.4, 2.0, H-6'), 6.77 (1H, *d, J*=8.4, H-5'), 6.30 (1H, *d, J*=2.0, H-8), 6.10 (lH, *d, J*=2.0, H-6), 5.06 (lH, *d, J*=7.6, H-1"G1c), 3-4 (sugar protons). ^{13}C NMR (125 MHZ, DMSO-d_6, δ, ppm)) 178.9 (C-4), 165.1 (C-7),

161.8 (C-5), 158.5 (C-9), 156.2 (C-4'), 149.1 (C-2), 145.3 (C-3'), 136.4 (C-3), 123.1 (C-6'), 122.7 (C-1'), 116.3 (C-5'), 116.2 (C-2'), 114.1 (C-10), 101.2 (C-1"), 98.5 (C-6), 94.6 (C-8), 78.1 (C-3"), 76.3 (C-5"), 75.0 (C-2"), 70.4 (C-4"), 61.9 (C-6"). Acid hydrolysis of this compound produced quercetin and *D*-g1ucose. Spectral data were compared with previously published data (Touafek *et al.*, 2011).

Quercetin-3-O-[β-D-glucosyl(1→2)-O-β-D-glucoside] (2) - $C_{27}H_{30}O_{17}$ (Mohamed et al., 2001).

Quercetin-3-O-[β-D-glucosyl(1→3)-O-β-D-glucoside] (3) - $C_{27}H_{30}O_{17}$, UV (MeOH, λ_{max}, nm): 257, 358; +NaOH: 271, 332, 406, +$AlCl_3$: 274, 433; +$AlCl_3$/HCl: 275, 424; NaOAc: 270, 368. +H_3BO_3: 264, 380. ^{1}H NMR (400 MHz, MeOD, δ, ppm, *J*/Hz): 7.72 (1H, *d, J*=2.2, H2'), 7.6 (1H, *d-d, J*=10.6-2.2, H-6'), 6.89 (1H, *d, J*=8.5, H-5'), 6.41 (1H, *d, J*=2.1, H-8), 6.22 (1H, *d, J*=2.2, H-6), 5.32 (1H, *d, J*=7.7, H-1'' glucose), 4.62 (1H, *d, J*=7.8, H-1''' glucose), 3.20-4.00 (sugar protons). ^{13}C NMR (400 MHZ, MeOD, δ) 177.9 (C-4), 165.2 (C-7), 161.6 (C-5), 157.4 (C-9), 157.1 (C-4'), 148.5 (C-2), 144.5 (C-3'), 134.1 (C-3), 121.8 (C-1'), 121.6 (C-6'), 116.1 (C-2', C-5'), 114.6 (C-10), 104.1 (C-6), 103.8 (C-8), 100- 60 (sugar carbons). HMBC experiment established a correlation between C-3 with H-1'' and C-3'' with H-1''', Acid hydrolysis of this compound produced quercetin and *D*-g1ucose which permitted the characterization of compound 3 (Imperatto, 1995).

Kaempferol-3-O-[β-D-glucosyl(1→2)-[(6'''p-coumaroyl)-β-D-glucoside] (4) - $C_{36}H_{36}O_{18}$, UV (MeOH, λ_{max}, nm): 269, 314; +NaOH: 276, 321, 365, +$AlCl_3$: 276, 399; +$AlCl_3$/HCl: 277, 399; NaOAc: 275, 374. +H_3BO_3: 275, 372. ^{1}H NMR (400 MHz, MeOD, δ, ppm, *J*/Hz): 8.02 (2H, *d, J*=8.8, H-2', H-6'), 7.35 (2H, *d, J*=15.9, H-7''''), 7.27 (2H, *d, J*=8.5, H-2'''', H-6''''), 6.89 (2H, *d, J*=8.8, H-3', H-5'), 6.7 (2H, *d, J*=8.6, H-3'''', H-5''''), 6.32 (2H, *d, J*=2.0, H-8), 6.16 (2H, *d, J*=2.0, H-6), 6.04 (1H, *d, J*=16.0, H-8''''), 5.21 (1H, *d, J*=5.2, H-1'' glucose), 4.66 (1H, *d, J*=7.6, H-1'''glucose), 3.20-4.40 (sugar protons). Acid hydrolysis of 4 produced keampferol and D-g1ucose. assignment of glucosidic protons system was achieved by analysis 1H-1H COSY and heteronuclear single quantum coherence (HSQC) experiments, the HMBC experiment which correlation between C-3 and H-1'', C-2'' and H-1''', C-9'''' and H-6''' were observed (Wang *et al.,* 2010).

Anti-bacterial activity

Antibacterial tests were carried on EEBR, DEBR and BEBR against several strains (reference strains and isolated from pathological germs samples). As reported in table 1.

Anti-oxidant activity

In their radicalic forms, DPPH free and ABTS cation radicals absorb at 517 nm and 734 nm, respectively. $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities of the extracts are shown in table 2.

Table 3 shows: the cupric reducing antioxidant capacity when the absorbance in this method is measured at 450nm, the results obtained are compared with the standards BHA and α-tocopherol at 0.1mg/ml (Absorbance 3.51 ± 0.01 and 1.85 ± 0.01 respectively) and the chelating effects were compared with standard on ferrous ions EDTA at 0.1 mg/ml (92.5±1.4%).

Anti-cholinesterase activity

Table 4 shows AChE and BChE inhibitory activities of BEBR, EEBR, DEBR and PEBR, compared with that of the standard (galantamine), used to treat mild Alzheimer's disease. The tests were carried on spectrophotometrically in a 96 well plate microplate reader at 25, 50, 100 and 200 µg/mL concentrations.

DISCUSSION

Anti-bacterial activity

The PEBR exhibited the best antibacterial activity against ETEC (17.5mm), EPEC (15.5mm) and *Enterococcus feacalis* (15.5 mm), whereas the EHEC strain was more sensitive to the DEBR (14.5) and BEBR (15 mm) with respective inhibition zone diameters.

Anti-oxidant activity

For this study, we hypothesized that plants are a potential source of natural antioxidants. The results observed in antioxidant tests have confirmed this hypothesis.

The absorbance decreases by the reduction effect of antioxidants. Because of the steric hindrance, $ABTS^{\bullet+}$ assay has a superior effect than the $DPPH^{\bullet}$ one.

The BEBR (IC_{50}=18.80±0.33 µg/ml), and EEBR (IC_{50}=15.76±0.83 µg/ml) showed better activities compared to the reference BHA (IC_{50}=45.37±0.47 µg/ml), and close to the reference α-tocopherol (IC_{50}=7.31±0.17 µg/ml). In the ABTS assay, however, the BEBR (IC_{50}=10.10±0.2 µg/ml), EEBR (IC_{50}=7.21±0.16 µg/ml) and DEBR (IC_{50}=10.24±0.32 µg/ml) demonstrated good activities compared to references BHA (IC_{50}=4.10±0.06 µg/ml) and α-tocopherol (IC_{50} 4.31±0.10µg/ml).

In the metal chelating method, the EEBR did not exhibit antioxidant activity but the BEBR and DEBR have a very small percentage of inhibition. The PEBR showed the best antioxidant activity in this method at 0.1 mg/ml (32.78±1.18%). However, none of the latter antioxidant effects were close to that of EDTA.

In the cupric reducing activity, the BEBR showed a better or close activity to the reference α-tocophérol.

Anti-cholinesterase activity

The BEBR and EEBR were unable to indicate anti-cholinesterase activity. The DEBR and EEBR, however showed low activity against acetylcholinesterase and butyrylcholinesterase.

CONCLUSION

Secondary metabolites and biological effects of *Biscutella raphanifolia* are reported here for the first time. The phytochemical study of the aerial parts of this endemic species has led to the separation and identification of four flavonol glycosides. The PEBR exhibited the best antibacterial activity against various micro-organisms and a good antioxidant activity by metal chelating method.

The four studied extracts showed excellent antioxidant and antibacterial activities. However, the PEBR and DEBR showed a very weak anti-cholinesterase activity.

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REFERENCES

Apak R, Güçlü K, Özyürek M and Karademir SE (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E. using their cupricion reducing capability in the presence of neocuproine: CUPRAC method. *J. Agric. Food Chem*., **52**: 7970-7981.

Atta-ur-Rahman and Choudhary MI (2001). Bioactive natural products as a potential source of new pharmacophores. A Theory of memory. *Pure & Applied Chem.,***73**: 555-560.

Bauer AW, Kirby WM, Sherries JC and Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., **45**: 493-496.

Bencharif-Betina S, Miyamoto T, Tanaka C, Kabouche Z, Mitaine-Offer AC and Lacailles Dubois MA (2012). Two new triterpene saponins from *Cyclamen africanum* Boiss. & Reut. *Helv. Chim. Acta,* **95**(9): 1573-1580.

Benkiki N, Kabouche Z, Tillequin F, Vérité P and Seguin S (2003). A new polyisoprenylated phloroglucinol derivative from *Hypericum perfoliatum* (Clusiaceae). *Z. Naturforsch.,* **58c**(9-10): 665-668.

Blois MS (1958). Anti-oxidant determinations by the use of a stable free radical. *Nature*, **81**: 1199-1200.

Decker EA and Welch B (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem*., **38**: 674-677.

Do Rego JC, Benkiki N, Chosson E, Kabouche Z, Seguin E and Costentin J (2007). Anti-depressant-like effect of hyperfoliatin. a polyisoprenylated phloroglucinol derivative from *Hypericum perfoliatum* (Clusiaceae) is associated with an inhibition of neuronal monoamines uptake. *Eur. J. Pharmacol.,* **569**: 197-203.

Ellman GL, Courtney KD, Andres V and Featherston RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.,* **7**: 88-95.

Grice HP (1988). Enhanced tumour development by butylated hydroxyanisole (BHA). from the prospective of effect on forestomach and oesophageal squamous epithelium. *Food & Chem. Toxicol*., **26**: 717-723.

Grossberg GT (2003). Cholinesterase inhibitors for the treatment of Alzheimer's disease: Getting on and staying on. *Curr. Ther. Res*., **64**: 216-235.

Imperato F (1995). Flavonol glycosides from *Pteridium aquilinum*. *Phytochemistry*, **40**(6): 1801-1802.

Kabouche A, Kabouche Z, Seguin E, Tillequin F and Bruneau C (2005). A phenylethanoid glycoside and flavonoids from *Phlomis crinita* (Cav.) Lamiaceae. *Biochem. Syst. & Ecol.,* **33**: 813-816.

Kabouche A, Kabouche Z, Oztürk M, Kolak U and Topçu G (2007). Anti-oxidant abietane diterpenoids from *Salvia barrelieri*. *Food Chem.*, **102**: 1281-1287.

Kolak U, Kabouche A, Ozturk M, Kabouche Z, Topçu G, Ulubelen U (2009). Anti-oxidant diterpenoids from the roots of *Salvia barrelieri*. *Phytochem. Analysis*, **20**: 320-327

Laggoune S, Brouard I, Leon L, Calliste CA, Duroux JL, Bermejo JB, Kabouche A and Kabouche Z (2011a). Lignans and an abundant flavone glycoside with free-radical scavenging activity from the roots of the endemic species *Stachys mialhesi* de Noé. *Rec. Nat. Prod.*, **5**(3): 238-241.

Lakhal H, Kabouche A and Kabouche Z (2011). Flavonoids and anti-oxidant activity of *Stachys ocymastrum*. *Chem. Nat. Comp.,* **46**(6): 964-965.

Lakhal H, Kabouche A, Alabdul Magid A, Voutquenne-Nazabadioko L, Harakat D and Kabouche Z (2014). Triterpenoids from *Salvia argentea* var. *aurasiaca* (Pomel) Batt. & Trab. and their chemotaxonomic significance. *Phytochemistry*, **102**: 145-151

Marc T, Gerard W and Denis L (2001). Classification des anti-inflammatoires. Guide pharmacologie. Etudiants et professionnels paramédicaux. 4ème Edition. p.426.

Mohamed SK, Khaled MM, Hashim AH, Kazuhiro O, Ryoji K and Kazuo Y (2001). Acylated flavonoid glycosides from *Bassia muricata*. *Phytochemistry*, **57**(8): 1259-1262.

NCCLS (1993). Performance Standards for anti-microbial Disk Susceptibilities Tests. Villionova. PA. USA: Approach Standard NCCLS Publication M2-A5.

Potterat O (1997). Antioxidants and free radical scavengers of natural origin. *Curr. Org. Chem.,* **1**: 415-440.

Quezel P and Santa S (1963). Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II. Editions CNRS, Paris, p.405.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C (1999). Anti-oxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med*., **26**: 1231-1237.

Scalbert A, Manach C, Morand C and Remesy C (2005). Dietary polyphenols and the prevention of diseases. Critical Rev. *Food Sci. & Nutrition*, **45**: 287-306.

Touafek O, Kabouche Z, Brouard I and Bermejo JB (2011). Flavonoids of *Campanula alata* Desf. and their antioxidant activity. *Chem. Nat. Comp.,* **45**(6): 968-969.

Touafek O, Kabouche Z, Boustie J and Bruneau C (2012). A new long-chain unsaturated ester and other constituents of *Hypericum tomentosum* L. (Clusiaceae). *Nat. Prod. Commun.,* **7**(1): 63-64.

Wang K, Ming-Ming L, Xuan-Qin C, Li-Yan P, Xiao C, Yan L and Qin-Shi Z (2010). Phenolic constituents from *Brainea insignis*. *Chem. Pharm. Bull*., **58**(6): 868-871.