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# COMPONENTS AND ANTIOXIDANT ACTIVITY OF THE POLAR EXTRACTS OF *Chrysanthemum trifurcatum*

**H. Mokaddem-Daroui,[1] O. Touafek,[1] A. Kabouche,[1]* Z. Kabouche,[1] C. A. Calliste,[2] and J. L. Duroux[2]**

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The *Chrysanthemum* genus (Asteraceae) includes nearly 200 species of which 15 are distributed in Algeria [1, 2]. This genus is known to contain flavonoids and terpenoids, which are responsible for various activities, e.g., antimicrobial, antitumor, antiviral, anti-inflammatory, antioxidant, and hepatoprotective [3–8].

The species *Chrysanthemum trifurcatum*, endemic to the Septentrional Sahara [1], has not been the subject of any phytochemical study. This plant, named "gartoufa," is traditionally used to treat constipation and hepatic desorders [9]. It is also locally used in food such as "chorba" and "couscous."

Aerial parts of *Chrysanthemum trifurcatum* were collected during the flowering period in March 2008, at Ghardaia. A voucher specimen was deposited at the Herbarium of the Laboratory under the code number LOST.Ct.04.08.

Air-dried and powdered aerial parts (1 kg) of *Chrysanthemum trifurcatum* were macerated in a methanolic solution (70%). The extract was successively concentrated to dryness (under low pressure); the residue was dissolved in boiling water and extracted with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol, successively.

TLC tests showed the similarity of both the EtOAc and *n*-BuOH extracts.

The butanolic extract (15 g) was subjected to column chromatography on polyamid SC6 with a gradient of toluene–MeOH of increasing polarity. The major fraction was treated with preparative TLC on polyamid DC6 using the system toluene–MeOH–methylethylketone (4:3:3), leading to six compounds (**1–6**) which were identified by the use of $^1$H NMR and $^{13}$C NMR, DEPT, and high-technique experiments, COSY, HMQC, and HMBC, in addition to UV spectroscopy.

**Compound 1**, identified as caffeic acid [10].

**Compound 2**, identified as apigenin [10–12].

**Compound 3**, identified as luteolin [10–12].

**Compound 4**, identified as apigenin 7-glucoside [10–12].

**Compound 5**, identified as luteolin 7-glucoside [10–12].

**Compound 6**, identified as quercetin [10–12].

Flavonoids **2–6** have already been isolated from *Chrysanthemum* genus while caffeic acid (**1**) is reported for the first time from the genus.

**Acid Hydrolysis**.The pure compounds **4** and **6** were treated with 2 M HCl at 100°C for 1 h.

The hydrolysates were extracted with EtOAc, and the aglycones were identified by their UV spectra in methanol and by comparison of their $R_f$ with authentic samples. Sugars were identified in the aqueous residue by comparison with authentic samples on silica gel TLC impregnated with 0.2 M $NaH_2PO_4$, solvent $Me_2CO$–$H_2O$ (9:1), and revealed with aniline malonate.

**Antioxidant Activity**. The antioxidant activity of the butanolic extract of *Chrysanthemum trifurcatum* (BECT) was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH, from Sigma) free radical [13] using electron spin resonance (ESR) [14]. The reaction mixtures contained 100 mL test samples and 100 mL DPPH ethanolic solution ($5 \times 10^{-4}$ M). The inhibition ratio was determined by comparison with a water-treated control group. ESR spectra were obtained with a Bruker ESP300E spectrometer using micro-sampling pipettes at room temperature under the following conditions: modulation frequency, 100 kHz; modulation amplitude, 0.197 mT; scanning field, 349.7 mT; receiver

1) Universite Mentouri-Constantine, Departement de Chimie, Laboratoire d'Obtention de Substances Therapeutiques, 25000, Constantine, Algeria, e-mail: ahkabouche@yahoo.fr; 2) Laboratoire de Biophysique, UPRES EA-1085, Biomolecules et Cibles Cellulaires Tumorales Faculte de Pharmacie, 2 rue du Dr. Marcland, 87025, Limoges Cedex, France. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2012, pp. 446–447. Original article submitted August 28, 2010.

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gain, $1.25 \times 10^5$; sweep time, 11 s; microwave power, 4 mW; microwave frequency, 9.78 GHz. All spectra were recorded at 3 min after homogenization by agitation. The inhibition percentage was calculated using the double integral of the signal:

$$\text{Inhibition ratio} = \frac{\text{Ref-Ext}}{\text{Ref-Bg}}$$

where Ref is the reference signal (DPPH + water), Ext is the test signal, and Bg is the background signal. The data were the means of five measurements.

By comparison, the value of $IC_{50}$ of vitamin E was, under the same experimental conditions, 0.025 mg/mL. Quercetin $IC_{50}$ values were used as a reference. In the DPPH test, the quercetin $IC_{50}$ was 0.012 mg/mL. An $IC_{50}$ value of $0.199 \pm 0.0023$ mg/mL was obtained for BECT.

These results showed the ability of BECT to scavenge DPPH radical. This may be explained by the presence of quercetin.


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