

Nutritional Composition, Analysis of Secondary Metabolites and Antioxidative Effects of the Leaves of *Globularia alypum* L.

L. KHANTOUCHE*, F. GUESMI, S. MOTRI AND M. ABDERABB

Preparatory Institute for Scientific and Technical Studies, La Marsa 2075, Tunisia

Khantouche *et al.*: Nutritional antioxidative *Globularia alypum* L

This investigation dealt with the determination of the content of phenolic compounds, flavonoids, anthocyanins, tannins and *in vitro* antioxidant activity of the ethanol extract of the leaves of *Globularia alypum*. The physicochemical characteristics of the leaf powder of *Globularia alypum* such as metabolic energy, mineral element and organic matter were evaluated. Fatty acid content was determined using gas chromatography. This investigation appeared to be the first to report the analysis of the leaf powder, ethanol extracts of *Globularia alypum* L. In this study dried extracts, ashes, proteins, crude fat fibre, percent sugar, metabolic energy and minerals such as calcium, sodium and potassium were quantified. The ethanol extract of the leaf had shown antioxidant activity and a high percent of polyphenols and flavonoids (180.5 ± 2.1 mg *Globularia alypum* extracts/g and 20.7 mg QE/g). A high correlation was observed between the total phenolic and flavonoid content and the antioxidant activity ($R^2 = 0.943$ and $R^2 = 0.910$, respectively). Gas chromatographic analysis showed the presence of 13 acids in this extract, mainly linoleic acid (30.1 %) and linolenic acid (24.8 %). The leaf powder of this plant is considered as a source of mineral elements even though, it is full of fatty acids in comparison to proteins and sugar. The ethanol extract of the leaf of *Globularia alypum* could be considered as a source of polyphenols and as an extract with potential antioxidant activity.

Key words: *Globularia alypum* L, physicochemical characteristics, phenolic compounds, antioxidants

Globularia alypum L is a wild plant belonging to Globulariaceae family. It is a perennial shrub found throughout the Mediterranean area. The plant is used for various purposes^[1,2]. In the Tunisian traditional pharmacopoeia, *G. alypum* L. locally named as *Zrigua*, is one of the most used traditional plant remedies. Its leaves are traditionally used as hypoglycaemic, laxative, cholagogue, stomachic, purgative and sudorific agent^[3,4]. It is also used in the treatment of cardiovascular and renal diseases as displayed in ethnobotanical surveys. An infusion of *G. alypum* exhibited no toxicological effects but produced a significant hypoglycaemic activity in rats both by oral and intra-peritoneal administrations^[5,6]. The ethanol extract has been investigated for chemical composition and antioxidant activity.

This plant is widely used as a curative for many diseases in addition to the fact that only few studies have been reported on the Algerian and Moroccan *G. alypum* L strain^[7]. This has prompted us to investigate the ethanol extract of this plant and their major

compounds. So far, important chemical investigations of *G. alypum* were reported by Merghache *et al.*^[8] and Amessis-Ouchemoukh *et al.*^[9], where the presence of some glycosidic iridoids (globularin), phenolic acids, flavonoids and lignan diglucoside were reported. In addition, Touaibia *et al.*^[7] have reported the presence of secondary metabolites such as poly-phenols, flavonoids and anthocyanins. However, an analysis of primary metabolites and minerals such as Ca, Na, P, K has yet to be reported. Therefore, an attempt has been made to analyse the above components in this plant. In the present work, the physicochemical characteristics of the leaf extract powder, gas chromatographic analysis, estimation of the phenolic content and antioxidant activity of the ethanol extract of the leaf of *G. alypum* is reported.

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*Address for correspondence

E-mail: khantouchelinda@yahoo.fr

March-April 2018

Indian Journal of Pharmaceutical Sciences

Accepted 21 January 2018
Revised 12 May 2017
Received 11 January 2017
Indian J Pharm Sci 2018;80(2):274-281

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MATERIALS AND METHODS

Fresh leaves of *G. alypum* were collected in March 2015 from the North East of Tunisia (Jbel Zaghouan). Specimens were identified in the National Institute of Applied Sciences and Technology (INSAT, Tunisia) and voucher specimens were deposited at the Herbarium of the Department of Molecular Physico-chemical in the Preparatory Institute of Scientific and Technical Studies.

Physicochemical analysis and mineral concentration of the leaves powder:

The whole plants were shade-dried in a well-ventilated area for 20 d. Thereafter, the leaves were isolated from the plant and crushed. The powder was stored in dark glass boxes. To determine water or moisture content^[10], a quantity of leaves (5 ± 0.01 g, M_0) dried at ambient temperature for 15 d was further dried in an oven at 105° for 24 h to constant weight (M_d). The mass of the dried leaves was determined using a precise balance to 0.01 g. The water content in dry and wet bases was determined by the following Eqns.: $X = 100 \times (M_0/M_d)/M_d$ (water/kg of the dry matter); $X_h = 100 \times (M_0/M_d)/M_0$ (water/kg of the moist matter). The ash content was determined by combustion of 500 mg of the sample in a muffle furnace at 550° for 4 h^[11].

The mineral content was determined by atomic absorption spectroscopy^[11] based on the concept of atoms in the state E0 can absorb photons of energy. The estimating number of absorbed photons can be related to the concentration of elements in the solution to be analysed. The concentrations of calcium (Ca), sodium (Na) and potassium (K) were determined by atomic absorption (atomic adsorption spectrophotometer 1100, Perkin Elmer Instruments, Bois d'Arcy, French), powered by an air acetylene flame (C_2H_2). Total phosphorus (P) content was determined using a molybdenum blue colorimetric method.

The total sugar content was measured by colorimetric assay according to the phenol-sulphuric acid method. This method relies on the formation of furfural compounds by heating the neutral monosaccharide in sulphuric acid. Associated with phenols, furfural derivatives formed yellow complexes whose absorbance is spectrophotometrically determined at 490 nm^[12]. To assess protein content in the leaves, the Kjeldahl method was followed. A factor of 6.25 was used for the conversion from total nitrogen into crude protein^[13]. All assays were carried out in triplicate,

and the results were calculated as the mean \pm standard deviation (SD).

Fifty grams of the leaf powder was macerated during 72 h at room temperature with 500 ml of ethanol under continuous magnetic stirring. The extract was filtered using a 0.45-1 μ m Millipore filter and evaporated under rotavap. The volatile fatty acids of the ethanol extract were analysed using a Hewlett Packard 5890 series II Gas chromatograph equipped with flame ionization detector (FID) and HP-5 MS capillary column (5 % phenyl; 95 % dimethylpolysiloxane: 30 m \times 0.25 mm id, film thickness 0.25 μ m). The injector and detector temperatures were set at 250 and 280° , respectively. Oven temperature was kept at 50° for 1 min then, gradually raised to 250° at $5^\circ/\text{min}$ and subsequently, held isothermal for 4 min. Nitrogen was the carrying gas at a flow rate of 1.2 ml/min. The percent of the constituents were calculated by electronic integration FID peak areas, without the use of response factor correction. The peak identification of various fatty acids on a chromatogram is made by comprising of their retention times to those of a mixture of fatty acids previously injected indicator. The percent of the various fatty acids were calculated from the peak area represented in the chromatogram. The peak area was large and the percent of acids was important. The percent of fatty acids was estimated using the Eqn. 3: fatty acids (%) = $S/\sum s \times 100$, where, S is the peak area corresponding to the considered fatty acid, $\sum s$ is the sum of the areas of all peaks of a chromatogram corresponding to the fatty acid mixture.

Determination of total phenolic content by the Folin-Ciocalteu method:

The amount of total phenolics of the sample was determined with Folin-Ciocalteu reagent using the method of Lister and Wilson^[14]. A curve of gallic acid (ranging from 0.005 to 0.05 mg/ml) was prepared, and the results determined by the regression equation of the calibration curve ($y = 6.94x - 0.67$, $R^2 = 0.99$). In short, 100 μ l of sample (diluted to obtain absorbance in the range of prepared calibration curve) were dissolved in 500 μ l (1/10 dilution) of the Folin-Ciocalteu reagent and 1 ml of distilled water. After 1 min of reaction at room temperature, 1.5 ml of 20 % sodium carbonate ($NaCO_3$) solution was added. The final mixture was shaken thoroughly and then incubated for 2 h in the dark at room temperature. Absorbance of all samples was measured at 760 nm using a HACH UV-Vis spectrophotometer^[15]. Tests were presented as

milligram gallic acid equivalents per gram dry weight of raw material (mg *G. alypum* extract/g dry weight).

Determination of total flavonoids:

The total flavonoids were determined according to the Dowd method adopted by Arvouet-Grand *et al.*^[16] In short, 1 ml of each sample or standard was mixed with 4 ml of aluminium trichloride solution (AlCl₃, 2 %). The absorbance was read at 415 nm after 15 min against a blank sample consisting of methanol (4 ml) and an extract (4 ml) AlCl₃ free. Quercetin was used as a standard for the construction of calibration curve. The results were expressed as milligram quercetin equivalent/gram of dry mass.

Determination of anthocyanins:

Total anthocyanins are water soluble pigments. The amount of anthocyanins contents was determined using the method of Pamino-Duran *et al.*^[17] The mixture composed with 1 ml of samples and 9 ml of HCl was incubated at room temperature for 5 min. The absorbance of samples was measured at 520 nm by UV/Vis spectrophotometer. The anthocyanins content is displayed in terms of milligram equivalent of cyanine per gram of dried mass (mg E Cy/g dried mass).

Determination of tannin content:

The total tannin content in the ethanol extract was determined according to the vanillin method^[18]. One milliliter of each extract solution (1 mg/ml) was placed in a test tube with vanillin (1 % in 7 M H₂SO₄, 2 ml) in an ice bath, and then incubated at 25°. The absorbance was read at 500 nm after 15 min. The concentration was calculated as microgram tannic acid equivalent per milligram of dry mass from a calibration curve.

Quantification of total antioxidant activity:

The antioxidant activities of *G. alypum* were investigated using three complementary colorimetric methods namely 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), β-carotene bleaching and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays and compared to those of butylated hydroxyl toluene (BHT), vitamin E and vitamin C. DPPH free radical scavenging activity was determined according to a slightly-modified version of the method reported by Barros *et al.*^[19]. In short, 1 ml of DPPH (0.1 mM) was dissolved in methanol and added to a 0.5 ml solution of *G. alypum* extract at different concentrations. Absorbance was measured at 517 nm after 30 min of incubation in the dark at room temperature. The blank

was prepared from the relation mixture without DPPH solution. BHT and vitamin E were used as positive controls. The decrease in the absorption of the DPPH solution was calculated by the following Eqn. 4: DPPH radical scavenging activity (%) = [(A₀-A₁)/A₀]×100, where A₀ refers to the absorbance of the control reaction containing all reagents except the tested compound, and A₁ to the absorbance of the test compound. Results were expressed as IC₅₀ (μg/ml) values, referring to the amount of a sample necessary to decrease by 50 % the absorbance of DPPH radicals.

The antioxidant activity of ethanol extract of the leaf was evaluated according to a slightly-modified version of the β-carotene bleaching method^[15,20]. A stock of β-carotene linoleic acid mixture was prepared by dissolving 0.2 mg of β-carotene in 0.5 ml of chloroform; 20 μl of linoleic acid and 200 mg of Tween 40 were added, too. After the evaporation of the chloroform under vacuum, using a rotary evaporator (Heidolph, Germany) at 45°, the mixture was immediately diluted with 10 ml of triple-distilled water and was mixed well for 1-2 min. Then, 50 ml oxygenated distilled water was added and the mixture was vigorously shaken. Aliquots (4 ml) of this emulsion were transferred into different test tubes containing 0.2 ml of test samples. BHT was used for comparative purposes. A control, containing 0.2 ml of corresponding solvent and 4 ml of the above emulsion, was prepared. The tubes were placed, at 50°, in a water bath. Absorbance of all samples at 470 nm were taken at zero time (t₀). The measurement of absorbance was continued, until the colour of the β-carotene disappeared in the control reaction (t=180 min), at 15 min intervals. The mixture prepared as above mentioned, without β-carotene, served as blank. The antioxidant activity of the extracts was evaluated in terms of bleaching of the β-carotene using the following Eqn.: % inhibition = 100×[1-(A₀-A_t)/(A₀₀-A_{t0})], where A₀ and A₀₀ are the absorbance values measured at zero time of the incubation for test sample and controlled, respectively. A_t and A_{t0} are the absorbance measured in the test sample and controlled, respectively, after incubation for 180 min^[15].

ABTS⁺ radical scavenging activity of extracts was determined according to Re *et al.*^[21]. In this test, the relative capacity of antioxidants was measured to scavenge the ABTS⁺ radical compared to the antioxidant potency of L(+)-ascorbic acid (vitamin C) used as a standard. Thus, the ABTS⁺ radical was freshly prepared by adding 5 ml of a 4.9 mM potassium persulfate (K₂S₂O₈) solution to 5 ml of a 14 mM

ABTS solution and kept for 16 h in the dark. Before usage, this solution was diluted to get an absorbance of 7.00 ± 0.020 at 734 nm with PBS as a blank at pH 7.4 (5 mM NaH_2PO_4 , 5 mM NaHPO_4 and 154 mM NaCl). The final reaction mixture of standard group was made up to 1 ml with 950 μl of ABTS⁺ solution and 50 μl of vitamin C. Similarly, in the test group, 1 ml of reaction mixture comprised 950 μl of ABTS⁺ solution and 50 μl of the extract solution. The reaction mixture was vortexed for 10 s and the absorbance at 734 nm was recorded each minute after initial mixing. Appropriate solvent blanks were run in each assay, and all measurements were done within at least 6 min. The results, determined from regression equation of the calibration curve ($y = 0.0446x - 0.0076$, $R^2 = 0.987$), were suggested as milligram ascorbic acid per gram of dry weight of raw material (mg EVC/g dry weight). All the determinations were performed in triplicates. The statistical differences between phenols, flavonoids and antioxidant activity values of the extracts were calculated out using the Excel program. The differences between measurements were considered significant at $p < 0.05$. Correlations coefficients (R^2) to determine the relationship between two variables were calculated using MS Excel software.

RESULTS AND DISCUSSION

The physicochemical analysis of the leaves powder has never been realized (Table 1). Indeed, this first analysis showed that the leaves of *G. alypum* are rich in mineral elements such as calcium, sodium, potassium and phosphor (22.4; 302; 4.81 and 445 mg/kg of dry powder). In addition, the plant can supply an amount of energy of about 2405 kcal/kg of the dry powder. However, fat represents the lowest percent (3 %) in comparison with the organic material (protein: 6.2 % and sugar: 65.5 %). Due to its high energetic power and the presence of minerals in the *G. alypum* leaves, we can use it as food preparation or as additives in minerals and calories poor food. The fatty acid composition of *G. alypum* leaves was determined by GC. The results of this analysis are presented in Table 2. Indeed, Chromatographic analysis of the fat leaves of *G. alypum* identified 13 compounds that showed a total of 99.54 %.

In Table 2, the identification of compounds has allowed classifying in saturated fatty acids (SFA) and unsaturated fatty acids (UFA). This analysis has showed that UFA (69.31 %) is more abundant than SFA (30.23 %). The majority of UFA is linoleic

TABLE 1: THE CONTENT OF THE ASH OF *G. ALYPUM* SAMPLES COLLECTED IN ZAGHOUAN

Analysis parameter	Values
Humidity (%)	9.46±0.001
Dry extract (%)	90.54±0.13
Ashes (%)	5.5±0.001
Protein (%)	6.20±0.02
Fat (%)	3.05±0.03
Crude fibre (%)	10.28±0.02
Non nitrogenous dry extract (sugar, %)	65.5±0.031
P (mg/Kg)	445±0.21
Ca (%)	2.24±0.01
Na (mg/Kg)	302±0.32
K (mg/Kg)	4810±1.02
Metabolic energy (Kcal/Kg)	2405±1.23

Determination of the content of humidity, total solids, total ash, raw protein, total lipids, crude fibre and quantification of minerals (mg/kg), from the ash of *G. alypum* samples collected in Zaghouan

TABLE 2: CHEMICAL COMPOSITION OF THE ETHANOL EXTRACT OF THE LEAF OF *G. ALYPUM*

	Fatty acid (%)	Ethanol extract
SFA	Lauric acid	3.54
	Myristic acid	2.94
	Palmitic acid	15.91
	Margaric acid	0.82
	Ginkgolic acid	0.84
	Stearic acid	3.62
	Arachidic acid	2.56
Total	-	30.23
UFA	Palmitoleic acid	1.26
	Gadoleic acid	0.9
	linolenic acid	24.8
	Oleic acid	11.3
	Cis vaccenic acid	0.95
Linoleic acid	30.1	
Total	-	69.31

SFA: saturated fatty acid, IFA: unsaturated fatty acid

(30.1 %) and linolenic (24.8 %). In other hand, the palmitic (15.9 %) and oleic (11.3 %) are the two major SFA. However, minor acids are palmitoleic, margaric (0.8 %), ginkgolic (0.84 %), cis vaccenic (0.95 %), gadoleic (0.9 %).

The analysis of the chemical composition has revealed that the major compounds (linoleic and linolenic acid) have important biological activities. Therefore, linolenic acid eliminates chronic inflammation^[22]. Other research studies showed the effectiveness of linolenic acid in the treatment of rheumatoid arthritis and other inflammatory disorders^[23]. Numerous studies^[22,24] have demonstrated that supplementation of linolenic acid tended to reduce pain, swelling and tenderness of the joints and reduce the need for antiinflammatory treatment.

It is worth noting that linoleic acid exhibits some interesting biological properties. According to Troegeler-Meynadier and Enjalbert^[25], linoleic acids are capable of limiting the indication and the extension of malignant tumours stimulating immune functions and promoting growth, particularly bone development. Furthermore, they amplify peripheral tissue utilization of lipids and carbohydrates and simultaneously lower the corresponding metabolic pathways of synthesis and storage, as well as they decrease cholesterolemia and eicosanoid production. Therefore, the conjugated linoleic acid would be used for treatment or prevention of obesity, atherosclerosis and diabetes mellitus.

Despite the low lipid compounds (3.05 %) in the leaves of *G. alypum*, they are rich of fatty acids with biological wealth. Linolenic and linoleic acids are the major's compounds of the leaves of *G. alypum* reveal the weightiness of the leaves with lipid fractions. Our study is an initial analysis of the lipid composition of *G. alypum*.

Total phenolics, flavonoids, anthocyanins and tannins present in the extract to the extent of 180.5±2.1 mg *G. alypum* extract/g of dry mass (Table 3). Our results are in agreement with those reported by Touaibia *et al.*^[7] in which the amount of total phenol is 139 mg *G. alypum* extract/g of dry mass from the macerated ethanol extract of *G. alypum* leaves. In addition, Taghzouti *et al.*^[26] have obtained 67.55 and 50.99 mg *G. alypum* extract/g of dry mass from macerated leaves methanol and ethyl acetate extracts, respectively. Djeridane *et al.*^[27] have shown 21.54 mg *G. alypum* extract/g dry mass from a hydroethanol extract of *G. alypum*. The variability in phenolic between our result and other results could be explained by the variation between the extracting solvents. Indeed, many studies have reported that polar fractions have more phenolic amounts^[28,29].

Concerning the content of flavonoids, the *G. alypum* exhibited 20.7 mg QE/g of dry mass. This result

coincides with that of Touaibia *et al.*^[7], (19.26 mg QE/g of dry mass) for the same sample using the same procedure. Taghzouti *et al.*^[26], obtained 25.9 and 8.96 mg QE/g of dry mass from methanol and ethyl acetate extracts of leaves, respectively. However, the results of Ben Mansour *et al.*^[30], showed that the amount of flavonoids in a leaves water-ethanol extract (20:80) was 78.82 mg QE/g of dry mass. This increase in concentration could be explained by the higher polarity of this extract.

The content of anthocyanins in *G. alypum* is 30.5 mg Cy/g dry mass. This concentration is lower than Touaibia *et al.*^[7], (35.10 mg Cy/g dry mass). However, Ben Mansour *et al.*^[30], obtained 0.53 mg Cy/g dry mass of hydroethanolic leave extracts. The amount of tannins in the *G. alypum* was higher than the results of Touaibia *et al.*^[7], (8.68 and 5.64 mg TA/g of dry mass, respectively).

The variability in phenolic, flavonoid, tannin and anthocyanin amounts for our results and other studies^[7,26,27,30] could be attributed to several biological factors, including genotypic differences, as well as other edaphical and environmental parameters, such as maturation stages, salinity, temperature, water stress and light intensity conditions.

The total antioxidant activities of vegetables cannot be evaluated by any single method, due to the complex nature of phytochemicals^[31]. Two or more methods should always be used in order to evaluate the total antioxidative effects of vegetables. Accordingly, the antioxidant activities of the *G. alypum* were determined by three different methods: DPPH, ABTS and β -carotene bleaching test. The half maximal inhibitory concentrations (IC₅₀) of the *G. alypum*, the BHT and the vitamin E are determined graphically at the concentration-dependent part of each plot. Results are presented in Table 4.

The presence of different antioxidant components in the plant tissues makes it relatively hard to quantify each

TABLE 3: COMPARISON OF SECONDARY METABOLITES OF LEAVES OF *G. ALYPUM*

Type of extract	Total phenolic mg GAE/g	Flavonoids mg EQ/g	Anthocyanins mg Cy/g	Tannins mg TA/g	Reference
Hydroethanol	247.24	78.82	0.53	-	[30]
Soxhlet water ethanol	21.54	-	-	-	[27]
Macerated methanol	67.55	25.9	-	-	[26]
Macerated hydroacetate	50.99	8.96	-	-	[26]
Macerated ethanol	139	19.26	35.10	5.64	[7]
Macerated ethanol	180.5±2.1	20.7±0.7	30.5±0.8	8.68±0.18	

Milligram of gallic acid equivalent per g of dry plant extract (mg GAE/g); mg QE/g: mg of quercetin equivalent per g of dry plant extract; mg Cy/g: mg of cyanine equivalent per g of dry plant extract; mg TA/g: mg of tannic acid per g of dry plant extract

TABLE 4: THE ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF THE LEAF OF TUNISIAN *G. ALYPUM*

	IC ₅₀ (µg/ml) ^a		mg EVC/g dry weight
	DPPH	β-carotene ^a	ABTS ^a
GAp	29.8±0.20*	42.53±0.24*	89.5±0.32*
BHT ^b	31.7±0.32	35.87±0.21*	-
Vitamin E ^b	22.66±0.21*	-	-
Vitamin C ^b	-	-	25.76±0.23*

The antioxidant activity was determined using DPPH, β-carotene bleaching and ABTS methods. Milligram equivalent of vitamin C/g dry weight: mg EVC/g dry weight. ^aValues were expressed as mean±SE (n=3), ^bPositive controls. Only the statistical differences between ethanol extract and BHT (DPPH) are shown as non-significant: p<0.05

antioxidant one separately. Therefore, in many studies, several intermediate extractions were used to ensure a maximum extraction of the available antioxidants^[32]. The antioxidant activity of phenolics is mainly due to their redox properties, which make them act as reducing agents, as hydrogen donors and singlet oxygen. They also may have a metallic chelating potential^[33].

The determination of antioxidant activity *in vitro* with DPPH samples to donate hydrogen is checked by using the free radical DPPH. It is one of the known mechanisms by which antioxidants inhibit lipid peroxidation. The amount of sample needed to decrease the initial DPPH concentration by 50 % (IC₅₀) is a parameter widely used to measure the antioxidant activity. A lower value of IC₅₀ indicates a higher antioxidant power.

The investigated extract displays an ability to scavenge the stable DPPH free radical reaching 50 % of reduction with an IC₅₀ value of 29.8 µg/ml, which is comparable to the IC₅₀ determined by the BHT as a positive control (31.7 µg/ml). However, the scavenging activity of this extract is low when compared to the vitamin E reference standard (22.66 µg/ml) used at the same dose. Phytochemical analysis of the ethanolic extract (Table 3) reveals the presence of phenolic compounds and flavonoids to which are attributed the antioxidant properties, due to their hydrogen donation ability and their structural requirement considered to be essential for effective radical scavenging.

It has been shown by Ben Mansour *et al.*^[30] that hydroethanol extract of *G. alypum* exhibited remarkable antioxidant effect, evaluated using the DPPH assay. In fact, the total phenolic and flavonoid contents in the hydroethanol extract of *G. alypum* were significantly higher (247.24 mg *G. alypum* extract/g dry matter and 78.82, respectively) than those of the ethanol extract

(180.5 mg *G. alypum* extract/g dry matter and 20.7, respectively).

The variability observed in the antioxidant activities of *G. alypum* shown by many researchers could presumably be attributed to the differences in the methodology and the experimental conditions used, as well as to the use of vegetables from different geographical regions, grown in different climatic and storing conditions, differences in extraction procedures, sample processing or drying. During the processing of extracts, some active volatile compounds may have been destroyed or evaporated^[34].

The potential of *G. alypum* to inhibit lipid peroxidation was evaluated using the β-carotene/linoleic acid bleaching test, which measures the extracts capacity for inhibiting the conjugated diene hydroperoxide formation upon linoleic acid oxidation. Results presented in Table 2 and 4 showed that *G. alypum* and BHT inhibit the linoleic acid oxidation. *G. alypum* is efficient to inhibit the linoleic acid oxidation with IC₅₀ value of 42.53 µg/ml. Concentrations required for *G. alypum* to provide 50 % inhibition are compared to BHT as a reference standard. The results have indicated that *G. alypum* exhibits lower antioxidant activity than BHT. Many researchers have reported that BHT is more potent than the plant extracts^[35-37].

The determination of antioxidant activity *in vitro* with ABTS method of *G. alypum* to scavenge free radicals is also assessed by their ability to quench ABTS^{•+}. The antioxidant activity measurement of ethanol extract, expressed as mg equivalent of vitamin C/g dry weight is 89.5. This high value would imply greater antioxidant activity capacity to scavenge the ABTS^{•+} radical cation. Using the ABTS assay, our extract has shown a higher antioxidant activity compared to the aqueous ethanol extract of *G. alypum* (70:30, v/v)^[27].

The analysis of the antioxidant activity by different methods can explain the strongly radical potential of *G. alypum*. For this reason, it has been reported those antioxidants possessed diverse biological activities such as anticarcinogenic, antidiabetic and antiinflammatory. On the other hand, the antiradical properties and the presence of secondary metabolites suggested that *G. alypum* might have several applications in human health care.

In food industry, antioxidants can also be used to retard the oxidative degradation of fats by inhibiting the formation of free radicals. Leaf powder *G. alypum* can replace synthetic antioxidants such as BHA and

BHT. Therefore, the use of natural antioxidants as preservatives in food would have great potential due to the requirement of consumers to additive-free, fresher and more natural food.

Furthermore, the content of phenols in *G. alypum* correlated well with the antioxidant activity leading to a possibility that phenols are likely to contribute to the radical scavenging activity of this plant extract. The correlation between the total phenolics, flavonoids and antioxidant activity ranges between $R^2= 0.943$ and $R^2= 0.91$, respectively (fig. 1A and B). This result suggested that between 94.3 and 91 % of the antioxidant capacity of extract is due to the contribution of total phenolics and flavonoids. The antioxidant effects, evaluated by the DPPH, correlate more with phenol contents than flavonoids.

It has been shown by Broadhurst *et al.*^[38] that polyphenols, which are present in medicinal herbs, spices, vegetables, fruits and beverages have been used to treat many human diseases such as diabetes, cancer and coronary heart diseases. In other hand, flavonoids have been shown to exhibit the antioxidative, antiviral, antimicrobial and antiplatelet activities^[39]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which made them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also have a metallic chelating potential^[33]. Therefore, the high contents of phenolic

compounds and significant linear correlation between the value of phenolic concentration and flavonoid compounds and antioxidant activity indicated that these compounds contribute to the strong antioxidant activity.

In this study, the analysis of the leaf powder has shown it's the richness in mineral elements such as calcium, sodium and phosphor. In addition, the ethanol extract of *G. alypum* leaves has a powerful ability to inhibit oxidation. The protective effect of this extract is higher with widely used synthetic antioxidants BHT, vitamin C and E. Thus, ethanol extract from *G. alypum* leaves could be prepared and added in food preparations, marketed in cosmetic and pharmaceutical industries as a natural antioxidant and a suitable alternative for some synthetic antioxidant.

Acknowledgements:

The authors acknowledge the support of Dr. Boussaid Mohammed, National Institute of Applied Sciences and Technology (INSAT, Tunisia) in identifying the leaves of *G. alypum*.

Conflicts of interest:

There are no conflicts of interest.

Financial support and sponsorship:

Nil.

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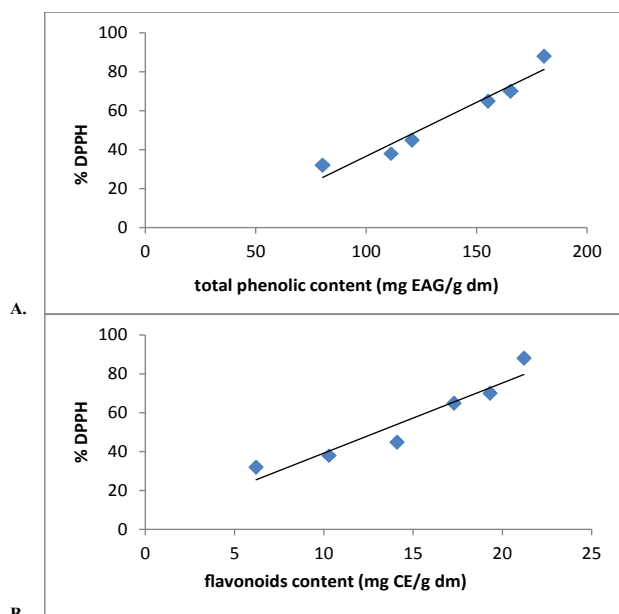


Fig. 1: Linear correlation between the total phenolic content or flavonoid content and antioxidant activity
(A) Total phenolic content and antioxidant activity, DPPH method, $y = 0.553x - 18.70$, $R^2 = 0.943$; (B) total flavonoid content and antioxidant capacity, DPPH method, $y = 3.611x + 3.124$, $R^2 = 0.910$

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