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Zygophyllum Geslini Coss : Biochemicals and Antioxidant Activity

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Research Article

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Abstract

Zygophyllum geslini Coss. is an endemic plant growing wild in the northern Sahara of Algeria, belonging to Zygophyllaceae family. The antioxidant activities of the aqueous lyophilized extract and the methanolic extract of *Z. geslini* leaves were investigated by DPPH test and ferric reducing activity. Also, the content of polyphenols, flavonoids, anthocyanins and tannins was estimated by spectrophotometric methods. The content of total phenols, flavonoids and tannins is highly detected in the methanol extract (183,22 mg GAE/gDE, 101,13±0,02 QE mg/gDE, 54,07±0,98 mg TA/gDE respectively) was higher than those detected in aqueous lyophilized extract, it may be responsible for the good activities of this plant. The best DPPH scavenging activity was found in the methanol extract followed by the aqueous lyophilized extract (IC50=65,36 and 67,44µg/ml, respectively), but sties less effective than the positive controls. This two extracts showed a very good ferric reducing activity, better than positive controls (BHT and gallic acid).

Keywords: *Zygophyllum Geslini* Coss; Polyphenols; Flavonoids; Anthocyanins; Tannins; Antioxidant Activity

Introduction

Herbs are used in various domains, including medicine, nutrition, and cosmetics. Numerous species have been recognized to have medicinal properties. The importance of medicinal plants has increased recently with the aim to find drugs against diabetes, hypertension and cancer as well as finding new molecules that possess antioxidant activities for using them in agri-food sector, pharmaceutics and cosmetic industries [1-5]. The synthetic antioxidants have shown toxic effects on health, for that reason, many studies have occurred to find new natural antioxidants as an alternative.

The genus *Zygophyllum*, is the largest genus in the *Zygophyllaceae* family, it consists of 285 species, which are subdivided into five subfamilies and 27 genera [6]. It is widely distributed in semi-arid, deserts and stepps from the Mediterranean to Central Asia, South Africa and Australia [7-9]. Species belonging to genus *Zygophyllum* represent a group of succulent plants that are drought resistant and/or salt tolerant, living under severe, dry climatic conditions [10,11].

Zygophyllum species have been utilized in traditional medicine for various ailments, such as treatment of rheumatism, gout, diabetes, asthma, hypertension, dysmenorrhea, as well as fungal infections [12-21]. Some of them are reported to be rich in triterpenoids [22,23], saponins, polyphenols and flavonoids [19,24,25].

Zygophyllum geslini Coss. is a small perinnial shrub with fleshy leaves and flowers, It is an endemic xerophyte plant characterized by its dilated fruits on top in a free portion of carpels recurved into hooks as long as the welded portion [9]. In the Northern Sahar of Algeria, *Z. geslini* is called *"El-Aggaya"*, It is used in traditional medicine for the treatment of dermatitis, diabetes, hypertension, rheumatism, gout and asthma as other *Zygophyllum* species [20,26,27].

Many studies have confirmed the antidiabetic, anti-hypercholestolemic, anti-inflammatory and antidiarrheal activities of some *zygophylum* species [28-36]. The aim of our study was to evaluate the antioxidant properties of the aqueous lyophilized extract of *Z. geslini* and its methanolic faction and also to determine their contents on total phenols, flavonoids and specially on anthocyanins and tannins, which, to the best of our knowledge, have not yet been reported.

Experimental

Plant Material

The aerial parts of *Z. geslini* were collected in Jun 2021, from Ouargla (in the northeast of Algerian sahara). The plant material was stored at room temperature in a dry place before use. Fresh aerial parts (leaves) of the plant were dried at ambient temperature (24°C) for 10 days and reduced into a fine powder.

Preparation of the Extracts

Two extracts were prepared in order to be tested: for the first one, the aerial parts of *Z. geslini* (100 g) were refluxed at 60-70°C in 500 ml distillate water for 30 minutes, and the decoction was filtered with cotton wool. The filtrate was concentrated at 65°C by a rotavapor (*Buchi Labortechnink AG, Postfach, Switzerland*) under reduced pressure and frozen at -70°C before lyophilization (*Christ, alpha 1-2 LD*). The aqueous lyophilized extract was stored at ambient temperature until further use.

For the preparation of the second extract, 100 g of the powdered aerial parts was macerated at room temperature with MeOH–H_2O (70:30, v/v) for 24h. After filtration, the filtrate was evaporated till dryness at 70°C the crude extracts were solubilized in ethanol (1:1 wt/v) and stored at 4°C until use.

Determination of Total Phenolic Content

The total phenolic content from the extracts was quantified using Folin-Ciocalteu's method [37]. 200 µl of plant extract was mixed with 1 ml of Folin Ciocalteu reagent (diluted 10%) and incubated at room temperature. After 4 min, 800 µl of sodium carbonate (7.5%) was added. The absorbance was measured after 2 h at 760 nm. The total phenol content was expressed as gallic acid equivalent (GAE) in mg/g of dry extract (DE).

Estimation of Total Flavonoid Content

The total flavonoid content in the extracts was estimated by using aluminum chloride colorimetric method [38]. Briefly, 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of extract. After 30 min incubation at room temperature, the absorbance was measured at 430 nm and the results were expressed as mg quercetin equivalent per gram of plant dry extract (mg QE/g DE).

Total Tannin Content

The total tannin content of the extracts was carried out according to the method [39]. Three milliliters of 4% ethanol vanillin solution and 1.5 ml of concentrated hydrochloric acid were added to 0.4 ml of extract. The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm. The results were expressed as mg tannic acid equivalent per gram of plant dry extract (mg TAE/g DE).

Total Anthocyanins Content

The total anthocyanins are water-soluble pigments. The total anthocyanins were estimated by a pH differential method [40]. The absorbance was measured in spectrophotometer at 520 nm and at 700 nm in buffers at pH 1 and 4.5, using a molar extinction coefficient of cyanidin-3-glucoside of 29,600.

The results of the equation were expressed as milligrams of cyanidin-3-glucoside equivalents per g of extract, after the calculation of the following formula:

$$A \times MM \times DF \times 1000 \varepsilon \times d$$

Where:
A is the absorbance calculated by the way: $A_{\lambda 520} - A_{\lambda 700})pH=1 - (A_{\lambda 520} - A_{\lambda 700})pH=4.5$;
MM is molecular mass of cyanidin-3-glucoside;
DF is the dilution factor;
ε is the extinction coefficient;
d is the thickness of a curve.

Determination of Antioxidant Activity

DPPH radical scavenging assay: The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging effect was evaluated following the procedure described by Bouaziz et al. [41]. In succinct terms, the aliquots (50 ml) of the extract were added to 5 ml of a methanol DPPH solution (0,004%). After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm. The inhibition of free radicals DPPH in percentage (IP%) was calculated in the following way:

$$IP\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where: IP is the inhibition percentage;
A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract);
A_{sample} is the absorbance of the test compound.

The results are expressed as IC50, the lower IC50 values indicate a higher antioxidant activity. The synthetic antioxidants butylated hydroxytoluene (BHT) and ascorbic acid were used as positive controls.

Determination of ferric reducing antioxidant power (FRAP assay): The FRAP assay measures the change in absorbance at 700nm due to the formation of a blue colored complex of ferrous ion (Fe^{2+}) and 2,4,6-tripyridiyl-striazine (TPTZ). Prior to this, colorless ferric ion (Fe^{3+}) was oxidized to ferrous ion (Fe^{2+}) by the action of the electron-donating antioxidants. This assay has been described by Oyaizu [42]. Different concentrations of the extract (1 ml) were mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The resulting solutions were incubated at 50ºC for 20 minutes. After incubation, the reaction mixture is added to 2.5 ml of 10% TCA and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was taken and 2.5 ml distilled water and 0.5 ml of ferric chloride (0.1%) were added to it. The absorbance was measured at 700 nm, using Gallic acid and BHT as a positive control, and the results were expressed as mM equivalent ascorbic acid.

Statistical Analysis

All tests were analyzed in triplicate. The results were expressed as means ± SD.

Results and Discussion

Extracts Yields and Polyphenolic Contents

The aqueous lyophilized and methanolic extracts from *Z. geslini* leaves yielded 28.09% and 25.64% respectively. Kouadri Boudjelthia et al. [43] reported that this plant presented a highest extraction yields for the aqueous extract (29.03%), however its methanolic extract led to a performance of (26.32%)

Generally, plant diversity is responsible for the wide variability of physico-chemical properties influencing the extraction yields [44,45]. Among other things, the solubility of phenolic compounds is affected by the polarity of the solvent used. Consequently, it is very difficult to develop an extraction process suitable for the extraction of all phenolic compounds from the plant [46,47]. The fluctuations and the variations in the yields can be attributed to several factors: the diversity interspecific, the nature of the bodies, the locality of harvest of samples, are as many parameters which may have an influence on the performance of plants [48].

In the other hand, the results for the quantitative determination of the total phenols, flavonoids, tannins and anthocyanins contents of the aqueous and methanolic extracts of *Z. geslini* leaves are recapitulated in Table 1. The Statistic analysis of phenolic content revealed a high significant difference between the aqueous (55.56 ± 0.04 mg GAE/g DE) and methanolic extract (101.22 ± 0.32 mg GAE/g DE). The total flavonoid content in the methanolic extract (101.13±0.02 mg QE/g DE) was higher than that in the aqueous extract (45.82±0.36 mg QE/g DE. Moreover, no significant differences (P 0.05) were found in the total anthocyanins, representing 0.12±0.05 mg Cyanidin/g DE for the ethanolic extract and 0.10±0.01mg/g DE for the aqueous lyophilized extract. According to Table 1, the tannins are present in higher amounts in methanol extract (ME) when compared with the aqueous lyophilized extract (ALE).

	Yield (%)	Total Phenols*	Flavonoids**	Tannins***	Anthocyanins****
ALE[a]	28.09 %	55.56±0.04	45.82±0.36	27.54±0.33	0.10±0.01
ME[b]	25.64%	183.22±0.32	101.13±0.02	54.07±0.98	0.12±0.05

Table 1: Yields and concentration of the major constituents of *Z. geslini* extracts.
[a]Aqueous lyophilized extract, [b]Methanol extract
Values are mean ± IC of 3 replications. *expressed as gallic acid equivalent (GAE) in mg/g DE; **expressed as Quercetin equivalent (QE) in mg/g DE; ***expressed as tannic acid equivalent (TA) in mg/g DE

According to Radjeh et al. Total phenolics and flavonoids content varied significantly as the function of stages, extraction solvents and species. For flavonoids, tannins and anthocyanins, the values obtained are generally low, but compared with other studies, we find that our results are higher than those of *Z. cornutum* recorded by Rouibi et al, [49] and Belguidoum et al. [50], but still close to the values recorded by shehab et al. [51] on ethanolic and methanolic extracts of *Z. hamiense* from the Sahara of Muhaisnah in Dubai.

However, it is difficult to compare our results with those of the bibliography, as several factors may influence the qualitative and quantitative distribution of phenolic compounds in our extracts, mainly climatic and environmental factors: geographical area, drought, soil [52]

and the harvesting period, stage of development and part of the plant used [53], and the method of extraction and quantification. The selectivity of the solvent used can also influence the total phenol and flavonoid content [40].

Antioxidant Activity

To screen the antioxidant properties of the extracts, two biochemical assays were performed: scavenging effect was measured by the DPPH assay and the ferric reducing effect mesaered by FRAP test.

The DPPH method is based on the reduction of the stable radical DPPH with a violet color to non-radical DPPH-H with a yellow color. The disappearance of the violet color can be monitored spectrophotometrically at 517 nm.

The free radical scavenging potency of the extracts is presented in Table 2. The DPPH antioxidant activity of the extracts indicates their ability to dispose of hydrogen atoms. As illustrated, the methanolic extract of *Z. geslini* was found to exhibit a very interesting radical scavenging activity (67.44 ± 0.91µg/ml). The IC50 of BHT and ascorbic acid was 22.5 ± 0.62µg/ml and 3.14±0.33µg/ml, respectively. The DPPH radical scavenging activity of the tested samples was in the order: Ascorbic acid >BHT> Methanol extract > Aqueous lyophilized extract. Therefore, there is a high difference between the standards and the methanol extract and a very high difference between the standards and the aqueous lyophilized extract.

Samples	DPPH IC50 (µg/ml)	FRAP assay (mM equivalent Gallic acid)
ALE	67.44±0.91	15.47±0.57
ME	65.36±0.55	6.96±0.36
BHT	22.50±0.62	0.76±0.07
Ascorbic acid	3.14±0.33	-
Gallic acid	-	1.14±0.11

Table 2: Antioxidant capacity of *Z. geslini* extracts.

The antioxidant activity of plant extracts is usually linked to their phenolic content. For that reason, several research studies have evaluated the relationships between the antioxidant activity of plant products and their phenolic content [54,55]. In some studies, a correlation between them was found [56,57]. In this study, the findings have shown a relationship between the antioxidant activity and total phenolic contents. This agrees well with the idea that the phenolic compounds have a key role in free radical scavenging and/or reducing systems. Nevertheless, these results must be interpreted with caution as the method used for estimating the total phenolic content has weak selectivity because the Folin–Ciocalteu reagent reacts positively with different antioxidant compounds (phenolic and no phenolic substances) [58]. The methanol extract has been shown to have higher total polyphenolic contents and antioxidant capacity than aqueous lyophilized extract, probably due to the polarity and good solubility for phenolic components in methanol [59].

It is generally assumed that the ability to act as a hydrogen donor and the inhibition of oxidation are due to the synergism between the antioxidants in the extracts, which makes the antioxidant capacity dependant not only on the concentration of phenols, but also on their structure and the interaction between them [60]. Thus, it is possible to deduce that the antioxidant power of the aqueous extract is lower than the methanolic extract due to its low content of phenols compared to ethanol extract.

The ability of the different extracts of *Z. geslini* to reduce the ferricyanide complex (Fe^{3+}) to the ferrous form (Fe^{2+}) was recorded by measuring the formation of Perl's Prussian blue at 700 nm. The lowest reducing activity was recorded for BHT. All extracts showed a very good ferric reducing activity, better than positive controls (BHT and gallic acid) : ALE>ME>Gallic Ac>BHT.

The mechanism for the reaction of DPPH with phenols depend on the reactivity as hydrogen or electron donator. The high activity is due to the number of hydroxyl groups available [61]. Flavonoids and tannins are among the main groups of polyphenols. Flavonoids have a high redox potential, permitting them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating potential [62]. Flavonoids are known to scavenger various oxidizing species and to have an ability to stabilize membranes by decreasing membrane fluidity [63]. Tannins are metal ion chelators, protein precipitating agents and biological antioxidants [62].

Conclusion

The extracts of *Z. geslini* were found to have very good antioxidant activities and to contain a considerable content

in polyphenolic compounds. These results may confirm the traditional use of this plant. Further work must be done like biological, anti-hyperglycemic and anti-hypercholesterolemic activities. These results have established that *Z. geslini* extracts is a true source of secondary metabolites such as: tannins and flavonoids with beneficial proprieties, and a promising source of health products for functional food or pharmaceutical industries. However, further research would be required.

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