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Antioxidant properties of the endemic plant *Zygophyllum cornutum* (Zygophyllaceae) extracts

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Abstract: *Zygophyllum cornutum is a plant growing in the northern Sahara of Algeria, belonging to Zygophyllaceae family. The antioxidant activities of four organic extracts (aqueous lyophilized extract, methanol extract, butanol extract and cyclohexane extract) of Z. cornutum leaves were investigated by DPPH test, ferric reducing activity and scavenging of superoxide radical (NBT test). In addition, polyphenols, flavonoids, tannins and anthocyanins were estimated by spectrophotometric methods.*

The content of total phenols, flavonoids and tannins is highly detected in the methanol extract (183.22 mg Gallic acid equivalent/g, 101.13 Quercetin equivalent mg/g, 54.07 mg Tannic acid equivalent/g and 3.12 Cyanidin equivalent)/g, respectively) in comparison with the other extracts. The best DPPH scavenging activity was found in methanol extract followed by the cyclohexane extract (DPPH-IC50=15.36 µg/ml and 43.85 µg/ml, respectively), but remains less effective than the positive controls. These two extracts exhibited strong ferric reducing activity, with values of 16.96 mM and 16.33 mM, respectively. However, the best NBT-IC50 was obtained for the methanol extract (181.43 µg/ml).

Keywords: *Zygophyllum cornutum* Coss., Extracts, Polyphenol, Flavonoids, Anthocyanins, Tannins, Antioxidant activity

1. Introduction

Herbs are widely utilized in medicine, nutrition, and cosmetics, with numerous species recognized for their 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

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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 across semi-arid regions, deserts, and steppes, ranging 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 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, asthma, hypertension, dysmenorrhea, as well as fungal infections [12-21], due to their high content of saponins and polyphenols [19, 22-25].

Zygophyllum cornutum Coss. is an endemic xerophyte plant (Figure 1), characterized by its dilated fruits [9], it is commonly called *Aggaya* or *Bougriba* by local population [26]. In the Algerian Sahara, it is used in traditional medicine for the treatment of dermatitis, diabetes, hypertension, rheumatism and asthma [20, 27-29].

The objective of this study was to assess the antioxidant properties of the aqueous lyophilized extract of *Z. cornutum*, as well as its methanol, butanol, and cyclohexane extracts. Additionally, the study aimed to determine the extracts' total phenolic, flavonoid, anthocyanin, and tannin contents, which, to the best of our knowledge, have not been previously reported.

Figure 1. *Z. cornutum* in its natural biotope

2. Material and Methods

2.1. Plant material

The aerial parts of *Z. cornutum* were collected in March 2024, from Ouargla (in the North East of Algerian Sahara). The plant material was stored at room temperature in a dry place before use. Fresh aerial parts (leaves) were dried at ambient temperature (24°C) for 10 days and ground into a fine powder.

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2.2. Preparation of the extracts

Four extracts were prepared in order to be tested: for the first one, the aerial parts of *Z. cornutum* (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 methanol, butanol and cyclohexane extracts, 100 g of the powdered aerial parts was macerated at room temperature with the appropriate solvent 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.

2.3. Determination of total phenols content

The total phenolic content from the extracts was quantified using Folin-Ciocalteu's method [30]. 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 phenols content was expressed as gallic acid equivalent (GAE) in mg/g of dry extract (DE).

2.4. Determination of total flavonoid content

The total flavonoid content in the extracts was estimated by using aluminum chloride colorimetric method [31]. 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).

2.5. Determination of total tannin content

Total anthocyanin are water-soluble pigments. They were analyzed according to pH-differential method [32]. The absorbance was measured by *Shimidzu* spectrophotometer UV-visible 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 29.6.

The structural change associated with the modification of the chromophores determines the different colour of the anthocyanin solutions as a function of pH. The coloured form (oxonium) predominates at pH 1 and the colourless form (hemiacetal) at pH 4.5. The absorbance (A) of each extract is calculated according to the following formula:

$$A_{extract} = [A_{\lambda 520} - A_{\lambda 700}]_{pH=1} - [A_{\lambda 520} - A_{\lambda 700}]_{pH=4.5}$$

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2.6. Determination of total anthocyanin content

Anthocyanins are reversibly transformed under the influence of pH. The anthocyanins content of each extract was expressed as milligrams of cyanidin-3-glucoside equivalents per gram, after the application of the following formula:

$$A_{extract} \times MM \times DF \times 1000 \varepsilon \times d$$

Where: 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.

2.7. Evaluation of the antioxidant activity

2.7.1. Determination of DPPH radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging effect was evaluated following the procedure described by Bouaziz et al. [33]. 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 according to the following formula:

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

Where: *IP* is the inhibition percentage;

A_{blank} is the absorbance of the control reaction (containing all reagents except the tested extract);

A_{sample} is the absorbance of the tested extract.

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.

2.7.2. 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-s-triazine (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 [34]. 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

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rpm for 10 minutes. 2.5 ml of the supernatant was taken and completed with 2.5 ml distilled water + 0.5 ml of ferric chloride (0.1%). The absorbance was measured at 700 nm, using Gallic acid and BHT as a positive control.

2.7.3. Determination of scavenging of superoxide radical (NBT test)

The scavenging activity towards the superoxide radical ($O_2^{\bullet -}$) was measured in terms of inhibition of $O_2^{\bullet -}$ generation. The nitro-blue tetrazolium (NBT) reacts with the superoxide anion to give the oxidized NBT (tetrazolyl) which becomes formazan, water insoluble and purple [35].

The reaction mixture consisted of 100µl of samples, phosphate buffer, riboflavin, EDTA and NBT. The absorbance was read at 580 nm after illumination under UV lamp for 10 min against blank. The blank contained all the components except NBT. The percentage of inhibition was calculated using the following formula:

$$IP\% = [1 (OD_{sample}/OD_{100\%})] \times 100.$$

Where: *IP* is the inhibition percentage;

OD_{sample} is absorbance of the extract tested;

$OD_{100\%}$ is absorbance of the control reaction.

2.8. Statistical analysis

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

3. Results and Discussion

3.1. Extracts yields and polyphenolic contents

As shown in Table 1, the highest yields were obtained for the aqueous lyophilized and methanol extracts (28.09% and 25.64% respectively). Kouadri Boudjelthia et al. [36] reported that *Zygophyllum geslini* aqueous extract had highest yield than methanol extract (29.03% and 26.32% respectively).

Generally, plant diversity is responsible for the wide variability of physico-chemical properties influencing the extraction yields [37, 38]. Among other things, the solubility of phenolic compounds is affected by the polarity of the solvent used [39]. The fluctuations and the variations in the yields can be attributed to several factors such as: the interspecific diversity and the locality of harvested samples which may have an influence on the performance of plants [40].

Table 1. Yields of *Z. cornutum* extracts

	***AL-E*[a]**	***M-E*[b]**	***B-E*[c]**	***CH-E*[d]**
Yield (%)	**28.09**	**25.64**	13.54	12.85

[a]: Aqueous lyophilized extract, [b]: Methanol extract, [c]: Butanol extract, [d]: Cyclohexane extract

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On the other hand, the results for the quantitative determination of the total phenols, flavonoids, tannins and anthocyanin contents of *Z. cornutum* organic extracts are recapitulated in Figure 2. The Statistical analysis of phenolic content revealed a high significant difference between the aqueous (55.56 ± 0.04 mg GAE/g DE) and methanol extracts (183.22 ± 0.32 mg GAE/g DE). The total flavonoid, tannin and anthocyanin contents in methanol extract were higher than those of the other extracts. No statistically significant differences were observed in the total anthocyanin content among the extracts.

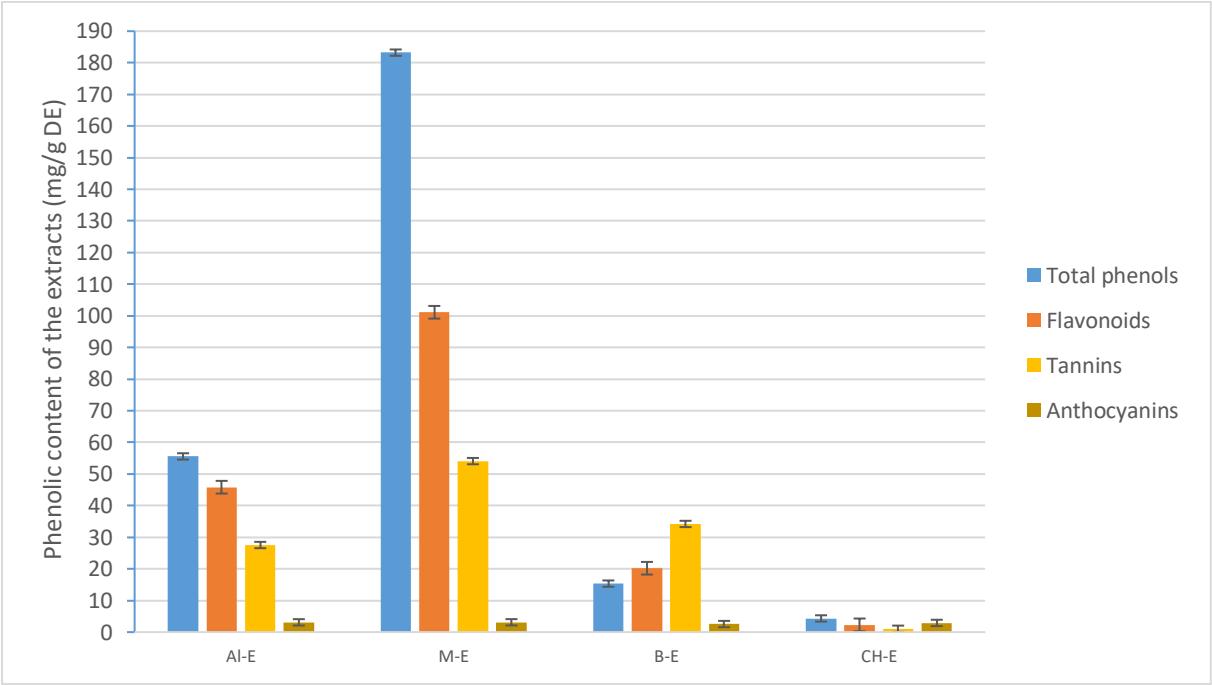

Figure 2. Total Phenols (mg GAE/g DE), Flavonoids (mg QE/g DE), Tannins (mg TAE/g DE), and Anthocyanins (mg C-3-GE/g DE) of *Z. cornutum* extracts

Al-E: Aqueous lyophilized extract, M-E: Methanol extract, B-E: Butanol extract, CH-E: Cyclohexane extract

Radjeh et al. [41] reported that the content of total phenols and flavonoids varied significantly depending on the stage of the plant's development. In comparison with other studies, flavonoids and tannins were higher than those reported for *Z. cornutum* by Rouibi et al. [42] and Belguidoum et al. [27]. However, they were comparable to the values observed by Shehab et al. [43] for ethanol and methanol extracts of *Z. hamiense* from the Muhaisnah Sahara in Dubai. Several factors may influence the qualitative and quantitative distribution of phenolic compounds, including genetic, climatic and environmental conditions (e.g., geographical area, drought, and soil), as well as the harvesting period, stage of development, plant parts used, and extraction method [44, 45]. The selectivity of the solvents used can also influence the total phenols and flavonoid contents [32].

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3.2. Antioxidant activity

To screen the antioxidant properties of the extracts, three biochemical assays were performed: scavenging effect measured by the DPPH assay, the ferric reducing effect measured by FRAP test and scavenging of superoxide radical evaluated by NBT test (Figure 3).

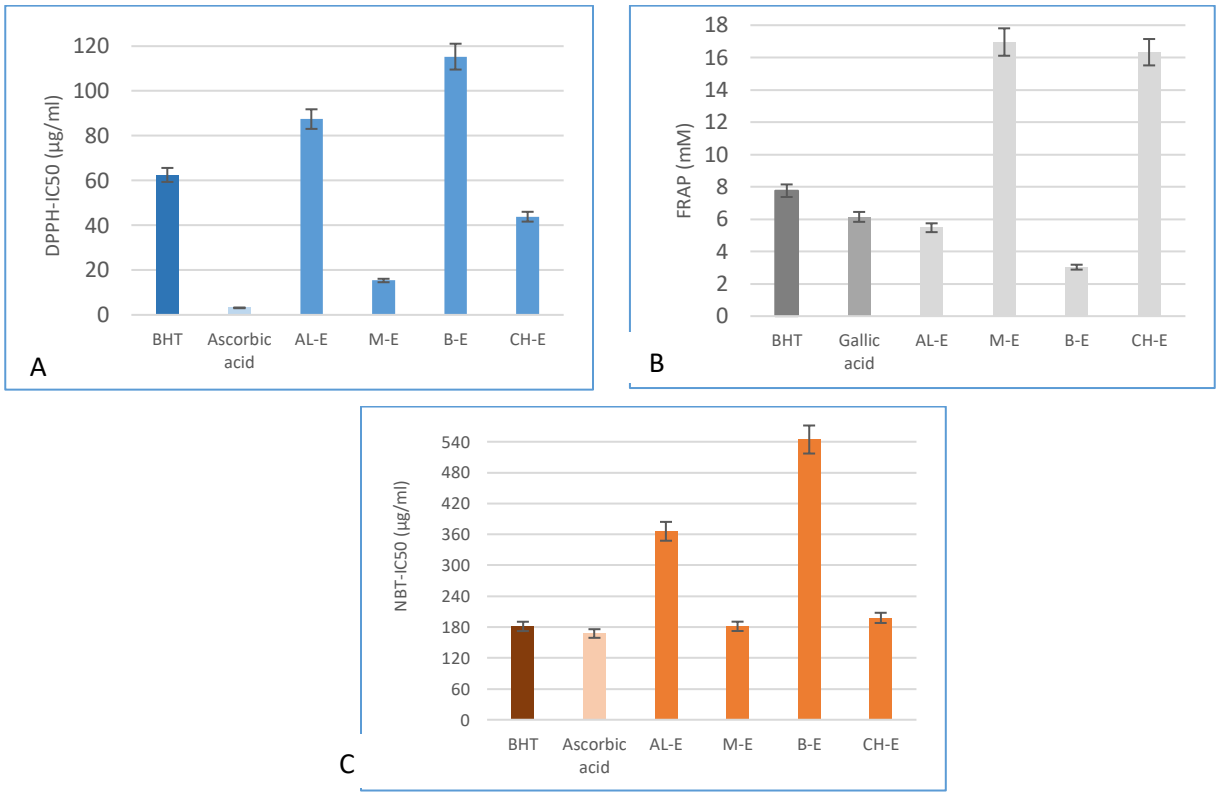

Figure 3. Antioxidant capacity of *Z. cornutum* extracts

A. DPPH-IC50 **(**µg/ml); B. FRAP (mM); C. NBT-IC50 (µg/ml)

The DPPH antioxidant activity of the extracts reflects their hydrogen-donating ability. As illustrated, methanol extract was found to exhibit a very interesting radical scavenging activity.

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.

The ability of the extracts to reduce ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}) was evaluated by measuring the absorbance of Prussian blue formation at 700 nm.

All extracts showed a very good ferric reducing activity, better than positive controls: *M-E>CH-E>BHT>Gallic Ac>AL-E>B-E*. Neverthless, the best NBT activity was obtained for methanol and cyclohexane extracts corresponding to 181.43 and 197.86 µg/ml, respectively.

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Several studies have evaluated a strong correlation between the antioxidant activity of plant products and their phenolic content [46-48]. Ignat et al. [49] reported that flavonoids have a high redox potential, enabling them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators, they can also scavenge various oxidizing species and have the ability to stabilize membranes, on the other hand, Tannins act as metal ion chelators, protein precipitating agents, and biological antioxidants [50].

In this study, cyclohexane extract demonstrated notably effective scavenging activity in spite of the low content of polyphenols, that could be explained by the interference of non-phenolic compounds like terpenoids and alkaloids of this plant, which are perfectly soluble in apolar solvents [51, 52]. This biological activity must be taken into consideration while utilizing raw plant materials for industrial applications and traditional therapies [53].

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 dependent not only on the concentration of phenols, but also on their structure and the interaction between the metabolites [54].

This difference between our results and those obtained by other researchers may be due to harvest seasons, climatic conditions, as well as extraction and assay conditions, which could play a critical role in the composition and structural characteristics of the main bioactive compounds and can affect the antioxidant properties of this plant [55-57].

Belguidoum et al. [29] reported that crude extract of *Z. cornutum* and its fractions have significant antioxidant activities; they reported that the EC50 (µg/ml) registered are (67.059 ± 4.727); (38.478 ± 2.085); (47.767 ± 1.571); (42.159 ± 3.029); (24.935 ± 1.983) for crude extract and fractions (chloroform, ethyl acetate, butanol and water) respectively.

4. Conclusion

The results gathered from this study show, on one hand, that *Z. cornutum* extracts contain interesting active compounds (phenolic acids, flavonoids, tannins and anthocyanins). On the other hand, they highlight its significant antioxidant potential compared to the well-known antioxidant activity of BHT, Gallic and Ascorbic acids, used as references. This observed property opens a promising opportunity, positioning this plant matrix as a potential source of natural antioxidants. Ultimately, studies on the evaluation of the effects of *Z. cornutum* extracts on the inhibition of acetylcholinesterase, an enzyme linked to Alzheimer's disease, are currently underway to better understand the plant’s effect in treating cerebral disorders.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

Authors' Contributions

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Data availability

All data generated or analyzed during this study are included in this article.

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