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Research Article

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Antioxidant and anti-inflammatory activities of different extracts from aerial parts of *Zilla spinosa* (L.) Prantl

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ABSTRACT

Zilla spinosa L. is a medicinal plant widely used in traditional Algerian phytotherapy against urinary lithiasis. The present study aims to evaluate the antioxidant and anti-inflammatory effects of different extracts from the aerial part of this plant. The antioxidant activity of the extracts was examined by two different methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and cupric reducing antioxidant capacity (CUPRAC). The anti-inflammatory activity of Z. *spinosa* was determined by the protein denaturation method. The qualitative phytochemical screening shows the presence of the majority of secondary metabolites in the aerial parts except anthraquinones and steroids, on the other hand, the root is characterized by the absence of several metabolites except tannins and coumarins.The ethyl acetate fraction displayed the highest antioxidant capacity (IC_{50} value: 10.47 ± 0.18 µg/mL in DPPH assay, and $A_{0.50}$ value: 40.89 ± 0.86 µg/mL in CUPRAC). The percentage of inhibition of BSA denaturation (0.2%) is proportional to the concentration of the different plant extracts, where the highest percentage was recorded in the concentrations of ethyl acetate 500; 250 µg/mL compared to Diclofenac (75 mg/3 mL), in contrast to the aqueous extract which gave non-significant results compared to the standards (p≥5%). In comparison to the standards used in this study, the ethyl acetate extract demonstrated better DPPH inhibitory activity, while all organic extracts demonstrated lower CUPRAC inhibitory activity but higher anti-inflammatory activity.

KEYWORDS: *Zilla spinosa* L., Phytochemical screening, Antioxidant activity, Anti-inflammatory activity

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INTRODUCTION

The use of medicinal plants began instinctively, as it does with animals (Petrovska, 2012). Their therapeutic applications, vernacular names, methods of preparation, and routes of administration were orally transmitted to form a local ancestral knowledge that characterized each population or ethnic group living in a specific area. The global plant diversity is estimated to be greater than 500,000 species (Laurance *et al.*, 2012; Corlett, 2016) and the variety and complexity of plant metabolites present a challenge when exploring the chemical repertoire available. Plants contain active compounds with biological activities, making them very useful as ingredients in traditional and modern medicines. The role of free radical reactions in disease pathology is well established, and they are known to play a role in a wide range of acute and chronic human disorders, including atherosclerosis, diabetes, aging, neurodegeneration, and immunosuppression (Harman, 1992). Inflammation is known to be an evolutionarily conserved process of protection and a critical survival mechanism (Liu *et al.*, 2017). It is made up of a series of complex changes in the tissue that are meant to eliminate the source of the cell injury, which may have been caused by chemicals, infectious agents, toxins, or physical agents such as radiation, burn, and trauma (Jang *et al.*, 2016; Fialho *et al.*, 2018). Local redness, swelling, pain, heat, and loss of function are all symptoms of inflammation (Virshette *et al.*, 2019). In recent decades, a large number of medicinal have been investigated for their antioxidant properties (Zengin *et al.*, 2011) and hundreds of research papers have been published regarding the anti-inflammatory activities of plants (Azab *et al.*, 2016; Oguntibeju, 2019).

Algeria, with a total area of nearly 2.4 million km^2 and 1,600 kilometers of coastline, is the largest country in the Mediterranean basin, Africa, and the Arab region. It has rich florist diversity, with 3139 species distributed across nearly 150 botanical families, 653 of which are endemic (Tani *et al.*, 2010). Several studies have been conducted to document local

knowledge about the use of medicinal plants to treat various diseases (Benarba, 2015; Benarba *et al.*, 2016; Chelghoum *et al.*, 2021; Mechaala *et al.*, 2021). To this end, our interest fell on the study of a medicinal plant, *Zilla spinosa* L., used in Algeria against urolithiasis.

Z. spinosa L. is a member of the Brassicaceae family (WCSP, 2018), which comprises 321 genera and 3660 species distributed worldwide (Al-Shehbaz, 2012). Traditional medicine has widely used *Z. spinosa* to treat a variety of illnesses, including gastrointestinal disorders and diabetes (Sekkoum *et al.*, 2011, 2015), urinary tract pains (Bouchouka *et al.*, 2012; Berreghioua *et al.*, 2014), diarrhea, gall bladder, kidney stones, liver and pancreas pain, respiratory ailments, and rheumatism (Keffous *et al.*, 2016; Alghanem *et al.*, 2018).

Despite the wide therapeutic potential presented by *Z. spinosa*, studies on the phytochemical profile, antioxidant, and anti-inflammatory activities of this plant are still lacking. Consequently, the present study focused on evaluating the antioxidant and anti-inflammatory activities of different extracts of the aerial parts and roots of Algerian *Z. spinosa* collected from the Tamnrast region. The present work also aims to provide an overview of this species' chemical and biological properties to develop sufficient background knowledge for continued research into the different extracts of this plant.

MATERIAL AND METHODS

Plant Material

Zilla spinosa plant was collected in December 2014 from Tamanrasset, an oasis city and capital of Tamanrasset Province in southern Algeria. Identification of the plant was confirmed by Dr. Rim Mecheri and her collaborators in the Cryptogamy and Medical Botany laboratory (Badji Mokhtar University, Annaba, Algeria) with herbarium specimens. The different parts of the plant were ground to a fine powder, after which they can be used in preparing different extracts.

Lyophilization

300 g of the powder of the aerial parts of *Z. spinosa* was infused in 3 liters of distilled water and boiled to 160 °C. After filtration, the filtrate is frozen with liquid Nitrogen and lyophilized using a freeze-dryer (Cryonext) for 24 h.

Extracts Preparation

The extracts were prepared from the lyophilized aerial parts of *Z.spinosa*. Then, 10 g of the lyophilized powder was extracted with 100mL boiling distilled water (160 °C); with hydro-alcoholic solution (Ethanol/Water: 60: 40: v/v); and butanol/water solvent solution (50V/50V) with the use of ultrasounds (Ultrasound, Branson 2800). After 30 min, the supernatants were decanted, and the lyophilisates were extracted twice more under the mentioned conditions. Finally, the supernatants collected for each lyophilisate were mixed together, filtered through syringe filters, and concentrated to dryness at 40 °C by applying rotary evaporation (Rotavapor, Buchi).

Phytochemical Screening

The aqueous extract of *Z. spinosa* plant sample was subjected to a qualitative phytochemical screening using the methods described by Mecheri *et al.* (2023). The results are given as the relative abundance of the respective compound (Bruneton, 1999).

Antioxidant Activity

DPPH radical trapping test

Blois (1958) method was used to calculate the DPPH% for each solvent extract (Blois, 1958). 40 µL of each extract at different concentrations was placed in the presence of 160 µL of a DPPH solution prepared in advance by dissolving 6 mg of DPPH in 100 mL of methanol were placed and gently mixed in micro plate 96-wells. The mixture was vortexed for 30 s and left for 30 min in the dark at room temperature (25 ± 2 °C). Prepared in the same conditions, Butylated hydroxytoluene (BHT) and butylated hydroxylanisole (BHA), were used as standard antioxidants. A spectrophotometer (Model UV 2005; Selecta, Barcelona, Spain) was used to estimate the absorbance at 517 nm against a blank (DPPH/methanol).

The results were expressed as a percentage of DPPH inhibition (IP %) according to the following Equation:

$$I(\%) = \frac{A0 - A}{A0} X\ 100$$

With
I(%): The percentage of inhibition
A0: Absorbance of DPPH solution without extract
A: Absorbance in the presence of extract or standard.

Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity assay was determined by the CUPRAC method that was developed by Apak *et al.* (2004). On 96-wells microplate, 40 µL of the dilutions of each extract are distributed, to which, 50 µL cupric chloride (10 mM), 50 µL of neocuprine solution (7.5 mM) and 60 µL of ammonium acetate buffer solution of pH 7 were added. These mixtures were incubated for half an hour at room temperature and measured against a blank at 450 nm. The reduction capacity of the extracts was compared with BHA and BHT as positive reference standards. The results were unveiled as A0 5 µg/mL corresponding to the concentration, indicating 0.50 absorbance. All methods were repeated in triplicate in order to get mean value.

Anti-inflammatory Activity

The anti-inflammatory activity of plant extracts was tested in vitro using the Bovine Serum Albumin Protein Denaturation

Assay (BSA) as described by Kandikattu *et al.* (2013), with minor modifications. For 1 mL of each extract concentration or standard (Diclofenac 75 mg/3 mL), 1 mL of BSA solution (0.2%) prepared in Tris Buffered (pH 6.6) was added. The samples were incubated in a 37 °C oven for 15 minutes before being immersed in a 72 °C water bath for 5 minutes. After cooling the tubes, the turbidity (level of protein precipitation) was measured at 660 nm in a spectrophotometer (HELIOS EPSILON), and the percentage inhibition of denaturation of the proteins was calculated using the following equation:

$$\%I = [\text{Control}-(\text{Sample}-\text{White}/\text{Control}] \times 100$$

With
Sample: 1mL extract + 1 mL BSA
White: 1 mL extract + 1mL Tris-phosphate (ph: 6.8)
Control: 1mL H2O + 1 mL BSA.

The control represents 100% of the denatured proteins; and the results are compared with 75 mg Diclofenac.

Statistical Analysis

All tests were carried out in triplicate and the results were calculated by the mean ± SEM and analyzed by MINITAB 16. ANOVA procedures were used for variance analysis. Tukey test was used to determine significant differences between means; thus, P values of 0.05 were considered significant.

RESULTS AND DISCUSSION

Phytochemical Screening

Plants or plant-derived compounds are used in both traditional and modern medicinal systems (Deans & Ritchie, 1987; Kumar *et al.*, 2007). Many bioactive compounds in medicinal plants, including phenolic and polyphenolic compounds, play important roles in the detoxification of stress caused by free radicals (Hara-Kudo *et al.*, 2004). Table 1 shows the presence of the majority of secondary metabolites in the aerial parts except anthraquinones and steroids, on the other hand, the root is characterized by the absence of several metabolites except tannins and coumarins.

Table 1: Preliminary phytochemical profile of the aerial parts and roots of *Z. spinosa*

Phytochemical components	Aerial parts	Roots
Alkaloids	+	+
Anthraquinones	-	-
Coumarins	+	+
Flavonoids	+	-
Saponins	+	-
Triterpenes/sterols	+	-
Tannins	+	+

Antioxidant Activity

The results of the antioxidant activity determined using the DPPH and CUPRAC are resumed in Table 2.

Table 2: Antioxidant activity of different extract/fractions of *Z. spinosa* by DPPH and CUPRAC assays

Extract/ fraction	DPPH assay	CUPRAC
	IC_{50} µg/mL	$A_{0.5}$ µg/mL
Aqueous extract	196.83 ±1.05[b,c,d]	98.63 ±0.41[b,c]
Dichloromethane	6.91± 0.09[b,c]	81.08± 0.18[b,c]
Ethyl Acetate	10.47± 0.18	40.89± 0.86[b]
BHA[e,f]	6.14±0.41[b]	5.35 ±0.71[b]
BHT[e,g]	12.99±0.41[c]	8.97±3.94[c]
α-Tocopherole	13.02±5.17	-

a - IC_{50} and A0.5 values represent the means ± SEM of three measures Tukey test, b - $P < 0.05$ compared with BHT, c - $P < 0.05$ compared with BHA, d - $P < 0.05$ compared with α-Tocopherol, e - Reference compounds, f - Butylated hydroxylanisole, g - Butylated hydroxytoluene.

The DPPH assay

The IC_{50} value represents the amount of extract required to inhibit free radicals by 50%. This value is, however, inversely related to the antioxidant capacity of a compound and, as a result, the lower the IC_{50} value, the higher the antioxidant activity of a compound. The dichloromethane extract showed better antiradical activity (IC_{50}= 06.91±0.09 µg/mL), compared with the BHT standard (IC_{50}= 12.99±0.41 µg/mL). On the other hand, the aqueous extract showed very low antiradical activity compared with the values of the BHA and α-tocopherol standards. The ethyl acetate extract showed better DPPH inhibitory activity (10.47±0.18 µg/mL) than both standards.

The CUPRAC reduction test

All extracts showed lower CUPRAC inhibitory activity than the standards BHA ($A_{0.5}$ = 5.35±0.71 µg/mL) and BHT ($A_{0.5}$= 8.97±3.94 µg/mL) but to different degrees.

Anti-inflammatory Activity

Figure 1 shows the results of the in vitro anti-inflammatory activity of the organic extracts of the aerial parts of Z. *spinosa*. We find that the percentage of inhibition of BSA denaturation (0.2%) is proportional to the concentration of the different plant extracts, where the highest percentage was recorded in the concentrations of ethyl acetate 500; 250 µg/mL compared to Diclofenac (75 mg/3 mL), which is used as a standard; an anti-inflammatory medicine, and is completely inhibited the denaturation of BSA at the same concentration, in contrast to the aqueous extract which gave non-significant results compared to the standards (p≥5%). Z. *spinosa* is a herbal medicine used by the Algerian people as a remedy for urolithiasis. In the present study, we examined in vitro the antioxidant and anti-inflammatory effects of different extracts of Z. *spinosa* aerial parts. The different metabolites that exist in the aerial parts of our plant justify its therapeutic use. Indeed, the presence of steroids explains the use of Z. *spinosa* as an anti-inflammatory, while that of tannins makes clear its indication in gastric disorders and stomach aches, as well as the dissolution of kidney stones is related to the presence of flavonoids. The results of

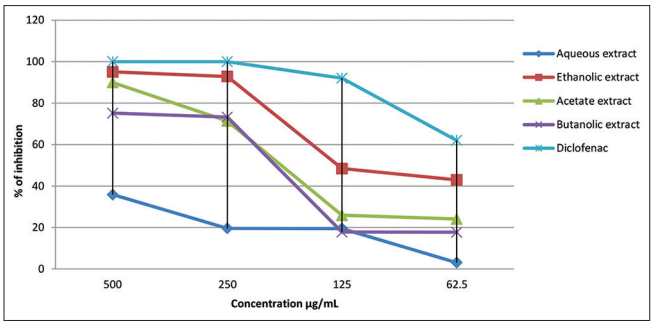

Figure 1: Effect of *Z.spinosa* extracts on albumin denaturation

El-Sawi *et al.* (2018), show that the aerial parts of Z. *spinosa* contain large amounts of carbohydrates, flavonoids, alkaloids, and anthraquinones, and moderate amounts of: sterols and/or terpenes, coumarins, and tannins, while only saponins are absent which is in discord with our results.

The experimental results of phytochemical screening, performed by Berrouigha (2016) on the species *Zilla macropetra*, revealed the presence of flavonoids, saponosides, and tannins. On the other hand, the same study indicated the absence of sterols and unsaturated cardenolides in the aerial part and the presence of steroids and alkaloids in lesser quantities. The DPPH assay was evaluated using a microplate reader where radical reduction is revealed by the color change from purple (DPPH) to yellow (DPPH-H), this was measured at the wavelength of 515 nm. The radical reduction ability is characterized by a decrease in absorbance induced by inhibitory free radicals (Keffous *et al.*, 2016). According to the recorded results, the aqueous extract has a relatively low antioxidant capacity compared to BHA, BHT, and tocopherol. It was only found that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids, and tannins reduce and decolorize DPPH due to their ability to give up hydrogen (Majhenic *et al.*, 2007). The polyphenols in the acetate extracts and chloroform extract of Z. *spinosa* are probably responsible for the antioxidant activity of these extracts. The CUPRAC reduction test was negative for all extracts.

Inhibition of protein denaturation, especially albumin, is a process in which proteins lose their tertiary and secondary structures by application of external stress or compound, such as strong acid or base, concentrated inorganic salt, organic solvent, or heat. Protein denaturation is a well-documented cause of inflammation (Kapewangolo *et al.*, 2015). As a test of the mechanism of anti-inflammatory activity, the ability of the plant extract to prevent protein denaturation has been studied. It was found that ethanolic, acetate, and butanolic extracts effectively inhibit heat-induced albumin denaturation: a maximum inhibition percentage (almost equal to 100%) was observed for the ethanolic extract, followed by that of the acetate and then butanolic extract, in contrary to the aqueous extract, whose inhibition percentage was very low 35.84%. All solvent extracts prevented albumin denaturation, the ethanol extract held first compared with acetate and butanol extracts. Diclofenac showed a maximum inhibitory activity of 73.6% at the concentration of 500 µg/mL. Several anti-inflammatory drugs have shown the dose-dependent ability to prevent thermally induced protein denaturation (Berreghioua, 2016). The ability of Z. *spinosa* extract to reduce the thermal denaturation of protein is likely a contributing factor its anti-inflammatory activity and may be due to the presence of therapeutically active flavonoids (Kandikattu *et al.*, 2013). In the genus *Zilla*, a study has highlighted the presence of two types of flavonoids 8,4'-dimethoxy, 5,7- dihydroxyflavone, and 7-(6''-methylglucuronyl) apigenin. Thus, our chemical screening and two phytochemical screenings carried out on the genus Z. *macropetra* from the region of Bechar, Algeria, revealed the presence of flavonoids (Kandikattu *et al.*, 2013).

CONCLUSION

The current study results suggest that Z. *spinosa* exhibited significant anti-inflammatory activity as revealed by *in vitro* methods. Further studies involving the isolation and purification of the chemical constituents of the ethanolic extract of Z. *spinosa* may result in the development of an effective anti-inflammatory agent with low toxicity and a better therapeutic index.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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