Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 18(3): 1105-1114, June 2024

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Biochemical characterization, antioxidant and antidiabetic properties of *Zygophyllum geslini* Coss extracts

Meriem TOUAIBIA and Abdellali DHOHA

Biotechnology, Environment & Health Laboratory. SNV Faculty. Blida-1 University. Algeria.
**Corresponding author; E-mail : biomeriem@hotmail.com; Tel (00213)0666513160.*

Received: 16-05-2024 Accepted: 27-06-2024 Published: 30-06-2024

ABSTRACT

Zygophyllum geslini Coss. is a medicinal plant growing wild in the northern Sahara of Algeria, belonging to *Zygophyllaceae* family. The objective of this study was to evaluate some biochemical properties of the aqueous lyophilized extract and the methanol leaf extracts of this plant, their antioxidant and antidiabetic activities were also tested. The content of total phenols, flavonoids and tannins detected in the methanol extract (183.22 mg Gallic acid equivalent/g Dry Extract, 101.13±0.02 mg Quercetin equivalent /g Dry Extract, 54.07±0.98 mg Tannic acid equivalent/g Dry Extract respectively) was higher than those detected in aqueous lyophilized extract. The best DPPH scavenging activity was found in the methanol extract followed by the aqueous lyophilized extract (IC50=65.36 and 67.44 µg/ml, respectively), but still less effective than the positive controls. This two extracts showed also a very good ferric reducing activity. In addition, animals treated with the methanol extract showed a significant decrease in blood glucose level and glycosylated hemoglobin. A significant increase was observed in serum insulin and liver glycogen levels. This study reveals that the methanol extract of *Z. geslini* can be considered as excellent candidate for future studies on diabetes mellitus.

Keywords: *Zygophyllum geslini* Coss, Extracts, Biochemical, Antioxidant, Antidiabetic.

INTRODUCTION

The importance of medicinal plants has increased recently with the aim to find drugs against diabetes and cancer as well as finding new molecules that possess antioxidant activities for using them in the agri-food sector, pharmaceutics and cosmetic industries (Shinsembu, 2019; Arar and Ghouini, 2021).

Oxidative stress is responsible for the degradation of biomolecules (proteins, lipids, and nucleic acids) leading to cell death and physiological disorders (Subedi et al., 2014). The synthetic antioxidants have shown toxic effects on health, for that reason, many studies have been done to find new natural antioxidants as an alternative and to battle effectively the imbalance between the production of free radicals and the body's antioxidant defense system (Dermane et al, 2024) In addition, the antioxidant activity of bioactive compounds depends on their nature and the solvents used for extraction (Maguirgue et al., 2022). Diabetes is associated to the increase of the production of free radicals and the decrease in the antioxidant potential which causes disorders and complications in favour of morbidities and significant mortalities (Kolling et al, 2010), this pathology

DOI : https://dx.doi.org/10.4314/ijbcs.v18i3.30

is a serious disease in terms of its clinical and even economic consequences. As a result, populations with unfavorable socio-economic conditions are more likely to turn to the phytotherapy.

The genus *Zygophyllum*, is the largest genus in the *Zygophyllaceae* family, it consists of 285 species, which are subdivided into 25 genera and 240 species (Shawky et al, 2019). It is widely distributed in semi-arid, deserts and stepps from the Mediterranean to Central Asia, South Africa and Australia (Bier et al., 2003). Species belonging to genus *Zygophyllum* represent a group of succulent plants that are drought resistant and/or salt tolerant, living under severe climatic conditions (Shawky et al., 2019). *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 (Mohammedi, 2020; Hal et al., 2022; Medjdoub et al., 2023). Some of them are reported to be rich in triterpenoids (Iqbal et al., 2011), saponins, polyphenols and flavonoids (Ahmed et al., 2007).

Zygophyllum geslini Coss. (*Z. geslini*) Is a perennial plant in small bushes branched, to whitish twigs, small fleshy leaves and composed of two leaflets. The flowers are small and white and the fruit is extended in lobes (Kouadri Boudjelthia et al, 2017). In the Northern Sahara of Algeria, *Z. geslini* is called "*El-Aggaya*", It is used in traditional medicine for the treatment of dermatitis, diabetes, hypertension, rheumatism and asthma as other *Zygophyllum* species (Tigrine-Kordjani et al., 2011). Hence, the aim of the present study was to evaluate antioxidant and antidiabetic properties of the aqueous lyophilized extract of *Z. geslini* and its methanol faction, and a characterization of total phenols, flavonoids and tannins, which, to the best of our knowledge, have not yet been reported.

MATERIALS AND METHODS

Plant material

The aerial parts of *Z. geslini* were harvested in Jun 2021, from Ouargla (in the North East of Algerian Sahara). The fresh leaves of this plant were selected and dried at ambient temperature (24°C) for 10 days, finely grounded and kept away from light and humidity for later analyses.

Animals

Healthy adult male and female Albino rats (weighing 200±2 g) were used throughout the experimental period. They were kept under standardized animal house conditions and fed with standard rat feed prepared by the Medical Research Institute, Pasteur-Algeria according to W.H.O. standards and water *Ad libitum*. All animal experiments were conducted in accordance with the internationally accepted laboratory animal guidelines and rules of the ethical committee, University of Blida, for animal experimentations.

Preparation of the extracts

Two extracts were prepared in order to be tested: for the first one, the grounded plant material (100 g) was refluxed at 60-70°C in 500 ml distilled water for 30 minutes, and the decoction was double-filtered. 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 (ALE) was stored at ambient temperature until further use.

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

Total phenolic content

The total phenolic content from the extracts was quantified using Folin-Ciocalteu's method (Wong et al., 2006). 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 mg Gallic acid equivalent (GAE)/g of dry extract (DE).

Total flavonoid content

The total flavonoid content in the extracts was estimated by using aluminum chloride colorimetric method (Djeridane et al., 2006). 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 described by Ali-Rachedi et al. (2018). Three milliliters of 4% vanillin ethanol 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).

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. (2008). In succinct terms, 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:

$$IP\% = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} X\ 100$$

IP: Inhibition percentage;

A blank: Absorbance of the control reaction (containing all reagents except the test extract);

A sample: Absorbance of the tested sample.

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 700 nm due to the formation of a blue colored complex of ferrous ion (Fe^{2+}) and 2,4,6-tripyridiyl-striazine (TPTZ). Prior to this, colourless 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 (1986). 1 ml of the extract 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 was added to 2.5 ml of 10% TCA (Trichlor acetic acid) 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 as a positive control, and the results were expressed as mM equivalent Gallic acid.

Determination of the antidiabetic activity

According to the protocol of Dheer and Bhatnagar (2010) with slight modifications, a single dose (100 mg/kg BW) of alloxan monohydrate (Sigma Ltd, USA) dissolved in normal saline was used for induction of Type II diabetes in rats after overnight fasting. After one hour of alloxan administration, the animals were fed standard pellets and water *Ad libitum*. The animals were stabilized for a week and animals showing blood glucose level (estimated by GOD-POD method) more than 100 mg/dl were selected for the study.

The fasted rats were divided into six groups of six animals each (three group of normal animals and three groups for induction of diabetes, each group includes 3 males and 3 females).

Group 1: Served as normal control rats and received distilled water.

Group 2: Diabetic rats served as diabetic control and received distilled water.

Group 3: Diabetic rats received methanol extract (100 mg/kg) using an intra gastric tube for 2

weeks.

Group 4: Normal rats received methanol extract (100 mg/kg) using an intra gastric tube for 2 weeks.

Group 5: Diabetic rats received aqueous lyophilized extract (100 mg/kg) using an intra gastric tube for 2 weeks.

Group 6: Normal rats received aqueous lyophilized extract (100 mg/kg) using an intra gastric tube for 2 weeks.

The drug treatment was carried out every day morning (100 mg/kg) and evening (100 mg/kg) with the help of intragastric tube for 2 weeks.

After 2 weeks, body weights were measured and the animals were sacrificed under ether anesthesia. The blood was collected by heart puncture and the liver was excised and chilled in ice cold 0.9% sodium chloride.

The blood samples withdrawn from the sacrificed animals were centrifuged at 3000 rpm for 10 min, glycosylated hemoglobin, Blood glucose and serum insulin were analysed on the 15th day. The excised liver tissues were processed and liver glycogen amounts were estimated.

Statistical analysis

All tests were analyzed in triplicate. The results were expressed as means ± SD. Statistical comparisons were made using one way ANOVA and Student test. A P value <0.05 was considered as significant.

RESULTS

Extracts yields and polyphenolic contents

The aqueous lyophilized and methanol extracts from *Z. geslini* leaves yielded 28.09% and 25.64% respectively (Table 1). In the other hand, the results for the quantitative determination of the total phenols, flavonoids and tannins contents of the aqueous and methanol 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 methanol extract (101.22 ± 0.32 mg GAE/g DE). The total flavonoid content in the methanol 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). According to Table 1, the tannins are present in higher amounts in methanol extract (ME) when compared with the aqueous lyophilized extract (ALE).

Antioxidant activity

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

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

The free radical scavenging potency of the extracts is presented in Table 2. As illustrated, the methanol extract of *Z. geslini* was found to exhibit a very interesting radical scavenging activity (67.44 ± 0.91 µg/ml). The IC50 of BHT and ascorbic acid were 22.5 ± 0.62 µg/ml and 3.14 ± 0.33 µg/ml, respectively. N the other hand, ALE and ME showed a very interesting ferric reducing activity, especially for the aqueous lyophilized extract (15.47±0.57).

Antidiabetic activity

Methanol and aqueous lyophilized leaf extracts were assessed for their antidiabetic activity. ME exhibited a significant decrease in the blood glucose level in alloxan-induced diabetic animals. A non-significant decrease was seen with the aqueous lyophilized extract. But both treatments did not produce hypoglycemia in normal rats, which is a therapeutic advantage.

In the present study, alloxan was used as a diabetogen. It induces diabetes by destroying b-cells of the pancreas partially, through production of reactive oxygen species. Insulin level was found to be decreased in the alloxan-induced diabetic rats. Administration of ME

(P<0.05) and ALE (P<0.05) induce an increase in serum insulin level which was statistically significant (Table 3).

A statistically significant increase (P<0.05) was seen in the level of the glycosylated hemoglobin in the diabetic control group. The ME significantly decreased (P<0.05) the glycosylated hemoglobin level, but a moderate and non-significant decrease was seen with ALE.

Depletion of liver glycogen content was seen in the diabetic control group. A significant increase (P<0.05) in the glycogen content of liver was observed after administration of methanol leaf extract, but atill non significant with ALE.

Table 1: Yields and concentration of the major constituents of *Z. geslini* extracts.

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

[a] Aqueous lyophilized extract, [b] Methanol extract.
Values are mean ± IC of 3 replications. * expressed as mg gallic acid equivalent (GAE)/g DE; ** expressed as mg Quercetin equivalent (QE)/g DE; *** expressed as mg tannic acid equivalent (TA)/g DE.

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

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

[a] Aqueous lyophilized extract, [b] Methanol extract, [c] Butylated HydroxyToluene.

Table 3: Effect of methanolic and aqueous leaf extracts of *Z. geslini* on blood glucose level, serum insulin, liver glycogen, and glycosylated hemoglobin.

Parameters	NC	DC	ALE	ME
Blood glucose level (mg/dl)	82.05±0.12	556.2±0.32	488.50±0.05**	264.50±0.19
Serum insulin	43.45±0.21	11.23±0.45	26.25±0.77*	48.65±0.33*
Glycosylated hemoglobin	5.37±0.79	09.33±0.56*	08.64±0.86	07.45±0.75 **
Liver glycogen	4.32±0.26	01.94±0.65	3.05±0.51*	04.36±0.01**

NC: normal control; DC: Diabetic control; ALE: Aqueous lyophilized extract; ME: Methanol extract
Student's paired test:*P<0.05;* *P<0.01.

DISCUSSION

Studies were performed to assess the phenolic content of the aqueous and methanol extracts and also to determine their anti-oxidant and anti-diabetic effects. The ALE yield was higher than ME, these results are similar to those reported by Kouadri Boudjelthia et al. (2017) who noted that highest extraction yields was obtained for the aqueous extract (29.03%), however, methanol 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 (Belmimoum et al., 2017). 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 (Garcia-Salas et al., 2019). The fluctuations and the variations in the yields can be attributed to several environmental, pedoclimatic and genetic factors (Rodolfo et al., 2006; Ebrahimi et al., 2008). The selectivity of the solvent used can also influence the total phenol and flavonoid content (Lee et al., 2003).

Radjeh et al. (2019) reported that total phenolics and flavonoids content varied significantly according to the species, the maturity stage, the extraction method and the nature of the solvents used for the preparation of the extracts. For flavonoids and tannins, the amounts obtained are generally moderate, but still close to those recorded by Shehab et al. (2015) on ethanol and methanol extracts of *Zygophyllum hamiense* from the Sahara of Muhaisnah in Dubai.

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 extracts and their phenolic content (Abdallah and Esmat, 2017). In some studies, a correlation between them was found (Miliauskas et al., 2004; Ahmed et al., 2020). 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 non-phenolic substances) (Que et al., 2006). The ME has shown the highest total polyphenolic contents and antioxidant capacity than ALE, it probably due to the polarity and good solubility of phenolic components in methanol.

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 (Hmid et al., 2013). Thus, it is possible to deduce that the lower antioxidant power of the ALE is to its low content of phenols compared to ME. The DPPH radical scavenging activity of the tested samples was in the order: *Ascorbic acid >BHT> ME > ALE*. Therefore, there is a high significant difference between the standards and ME and a very high significative difference between the standards and the ALE.

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 Gallic acid (positive control). All extracts showed a very good ferric reducing activity, better than positive control: *ALE>ME>Gallic Ac.*

The mechanism of phenols depends on their reactivity as hydrogen or electron donator. The high activity is due to the number of hydroxyl groups available (Villano et al., 2007). polyphenolics have a high redox potential, permitting them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating potential (Ignat et al., 2011). These metabolites can interact with free radicals generated by the human body in response to the aggressions of the environment (Miwonouko et al., 2024;

Ouedraogo et al., 2023). They are also known for their antidiabetic virtues by stimulating the regeneration of the damaged β-cells in the alloxan diabetic rats (Arar et Ghouini, 2021).

Insulin level was found very low in alloxan-induced diabetic rats. The opposite effect was seen on treatment by the leaf extracts, it may be indicative of the islet cells regeneration, and possibly, attenuation of the alloxan initiated degenerative changes more prominently in the ME-treated group as compared to the ALE-treated group. The same treatment, however, did not increase the serum insulin level in normoglycemic rats, so it can be concluded that the extract has the potential to enhance the glucose-dependent insulin release from the pancreatic β-cells and thereby decrease the blood glucose level only in alloxan-induced diabetic rats. This effect is very similar to biguanides. This class of medicines regulates blood sugar level to its normal value and do not produce hypoglycemia, it also exhibits a favorable effect on lipid profile.

The estimation of glycosylated hemoglobin (HbA_{1c}) gives an accurate reflection of mean plasma glucose concentration over this period and correlates best with the degree of the glycemia. A significant decrease with leaf extracts ($P<0.01$) was observed in both treated groups as compared to alloxan-induced diabetic rats. On ALE-treated group, the decrease was moderate.

The liver glycogen was found depleted in the diabetic control group. A significant increase in the liver glycogen level was observed for the ME-treated group, which may be due to its ability to increase insulin level. A high amount of liver glycogen indicates attenuation of diabetes severity and can be considered as an index of the presence of antidiabetic activity of the tested extracts. All the above observations suggest that the ME would be a promising antidiabetic drug (Arar et Ghouini, 2021).

Much attention has been focused on the role of oxidative stress in diabetes, and it has been suggested that oxidative stress may mediate common events in the pathogenesis of different diabetic complications (Sepici Dincel et al., 2007). Human diabetics and experimental diabetic animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia that may result in depletion of the antioxidant defense system and thus lead to an enhanced de novo free radical generation, and the interaction of these free radicals with membrane lipids would result in an increased lipo-peroxidation that can be prevented by antioxidants including plant phenolic compounds (Arawwawala et al, 2009).

Conclusion

In this paper, we have reported in vitro antioxidant and antidiabetic activities of *Z. geslini* aqueous and methanol leaf extracts, and reveal their richness in phenolic, flavonoids and tannins. This study has further revealed that the Increasing intake of natural antioxidants may help to maintain a tolerable antioxidant status, thus preventing the oxidative stress that could lead to the diabetes mellitus. These results should be encouraged by further studies, which could ultimately lead to the development of a new formulation that retain substantial antidiabetic capacity with minimal side effects, it would be also interesting to elucidate its mechanisms in detail.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS:

This work was carried out with the collaboration of all the authors. TM conducted the work, analyzed the data and drafted the manuscript, and AD contributed to the interpretation of the data and the critical revision of the article. The final manuscript was approved by all authors.

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