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Evaluation of Antidiabetic Activity of Two Plants *Berberis vulgaris* and *Zygophyllum geslini*

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Abstract

This work is part of the valorisation of the methanolic extract and aqueous solutions of *Berberis vulgaris* and *Zygophyllum geslini* as antidiabetic plants; widely used in Algeria and more specifically in the region of the West as a remedy for diabetes. The method applied to measure the antioxidant activity is that of trapping of free radicals to the help of DPPH after quantified the total polyphenols revealing an important content with a powerful antioxidant activity in which the percentage of inhibition radical is of (83.71%), (55.35%), (88.22%) and (75.89%) for 2 mg/l of the methanolic extracts and in aqueous of the two plants respectively. While it has been advocated to the test of the α-amylase *in vitro* for the assessment of the effect antihyperglycemic agent, the results obtained revealed a capacity of remarkable inhibition on the activity of the enzyme with a slight peak for the methanolic extracts of *Berberis vulgaris* (89.81%).

Keywords: *Berberis vulgaris; Zygophyllum geslini*; Antioxidant activity; α-amylase; Antidiabetic activity

Introduction

The past decades are marked by the interest in the development of medicinal plants as a source of bioactive substances including natural and the antioxidant substances in relationship with their therapeutic properties [1]. It is admitted that the oxidative stress is the result of an imbalance between the generation of reactive species oxygens and the antioxidant potential of the organization [2]. This may contribute to the emergence of various pathologies tumor cells, cardiovascular and metabolic even as the diabetes [3]. This last is associated on the one hand or another to the increase of the production of free radicals and the decrease in the antioxidant potential which causes of disorders and complications in favour of morbidities and significant mortalities [4-6], this fact Diabetes is a heavy pathology by its clinical consequences and even economic [7,8]. In view of the prohibitive costs of the support for the populations has the unfavorable socio-economic conditions guide for the subjects in question to the herbal medicine [9].

The herbal medicine antidiabetic known to this day is an important development in the fact of the discovery of more and more extracts from plants efficient in terms of prevention and healing [10]. The name of the *Berberis vulgaris* and *Zygophyllum geslini. Berberis vulgaris* apostrophized Ghriss locally is a medicinal plant belongs to the family of Berberaceae, is 2 to 3 meter in height, conducted of leaves frequently transformed into backbone of where the appellation "Epine-Vinette" [11,12]. The deciduous brown roots and bark which has a bitter taste and a slight odour, is located primarily in the area of the Northern hemisphere (temperate regions and Subtropics) providing in most of the regions of Central Europe and Southern Europe and in the regions of the North East United States, North Africa and the temperate Asia [11,13]. In Algeria, Berberis is located on the high mountains, above 1500 m, Djurdjura-Babors, Atlas of Blida, Aurès mountains, mountains of the Hodna and Saharian Atlas [14]. It is recognized for its virtues, pharmacological and therapeutical as an anti-inflammatory, an anticancer, an antipyretic [15], with a wide use by the local population as an antidiabetic agent according to a survey préétablit ethnobotany. *Zygophyllum geslini sp* is an endemic plant known as the vernacular name "El-Aggaya", belongs to the family of Zygollaceae. 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, pear shaped dilated regularly since the database upto the Summit. The stalk is fruitful, as long as the fruit. The free portion of the carpels is three to four times shorter than the welded portion, making barely protrusion [16]. It is figured in all continents on the arid and semi-arid regions [17], mainly on the northern Sahara Algeria [18]. The species of genus *Zygophyllum* are carried out of the biological properties including remarkable Antiseptic, a carminative [19], antibacterial, antifungique, anti-eczema [20], antispasmodic [21] and cytotoxic [22]. Also according to antidiabetic of *in vivo* studies conducted there above [23]. In the present study, we are interested in the evaluation of the *in vitro* effect of methanolic extracts and aqueous solutions of the bark of the Roots of *Berberis vulgaris* and the aerial part of *Zygophyllum geslini* on the antioxidant activity and the activity of α-amylase with a view to their recovery as antidiabetic medicinal plants.

Materials and Methods

Plant material

The *Berberis vulgaris* and *zygophyllum geslini* study was collected in March 2015 in the Algerian Sahara (wilaya of Adrar) where the parties used are the bark of the roots and the aerial part respectively (Figures 1 and 2). The identification of plants has been established thanks to the rural dwellers of the region, herbalists and some number of documents including Larousse Encyclopedia of Medicinal Plants. New Flores of Algeria and desert regions of southern quezel and Santana Volume I and II.

Extraction of polyphenols total

Several processes of extraction can be used because of the diversity of secondary metabolites in particular the polyphenols. For the

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Figure 1: *Berberis vulgaris* in the region of Adrar.

Figure 2: *Zygophyllum geslini* of the area of Adrar.

extraction of these we have opted for the use of the method of Pizzal et al. which is based on a maceration of plant material in the methanol and distilled water (10 g/100 ml) at ambient temperature with an agitation, a filtration of extracts, and then an evaporation of the filter to pure water under reduced pressure at 40°C in a rota- steam. After 72 h the fractions obtained are retrieved and retained at -4°C [24].

Phytochemical screening

The revelation of the main chemical constitutions was conducted by the tests of chemical detection described in the work of Benmehdi, N'Guessan Ilboualo and [25].

Dosage of total polyphenols

The estimate of the content of phenolic compounds total extractable has been achieved spectrophotometriquement by the method of Folin-cioclateu using a set of reagents and extracts. The samples of the extracts 200 µl and 800 µl of sodium carbonate (7,5 g/l) have been added after agitation and incubation of 2 min to 1 ml of 10% (v/v) of the Reagent Folin-ciocolteau (a mixture of phosphotungstic acid and phosphomolybdic acid). After 30 min of incubation in the dark at ambient temperature the reagent undergoes a reduction therefore a color change from yellow to blue as a result of the oxidation of polyphenols [26]. This blue coloration whose intensity is proportional to the rate of phenolic compounds present in the medium gives an absorption maximum at 760 nm. The content of phenolic compounds was calculated from the calibration curve of the Gallic acid. The results are expressed in mg equivalent of gallic acid per gram of dry matter (mg EGA/g DM).

Determination of total flavonoids

The determination of the rate of the total flavonoids of extracts is carried out by the method described by Bahroun [27] or 1 ml of a solution of 2% $ALCL_3$-methanol was added to 1 ml of sample. After incubation for 40 min at room temperature in the dark, the absorbance was measured at 420 nm.

The results are expressed in milligram equivalent catechin per gram of sample (mg EQC/g).

Evaluation of the antioxidant power of polyphenols totals

For this method of DPPH was adopted in which antioxidants reduce the DPPH with a purple color in a yellow compound [28]. After incubation period of 30 minutes of a mixture of 1 ml of solution of DPPH (4%) and 10 µl of the methanolic solution of each extract tested has different concentrations according to the principle described by Brand-Williams [29]. The intensity of the color is inversely proportional to the capacity of antioxidants present in the middle to give protons [30]. The antioxidant activity which expresses the ability to trap the free radical has been estimated by the percentage discoloration of the DPPH by report to a witness negative control containing only the solution DPPH and a positive control of reference represented by a methanolic solution of an antioxidant standard (ascorbic acid), after a reading streptophotométric to 517 nm. The antioxidant activity $\%=[A_1-A_2)/A_1] \times 100$ including the A_1, A_2 indicate the absorbance of the control and in the presence of the extract respectively.

Evaluation of the antidiabetic activity

Including the test of the *in vitro* effect of extracts on the activity of α-amylase is adapted and for this a range of reagents and solutions has been used including:

1. Reactive 3.5 dinitro-salicylic acid (DNSA) which is a solution of orange color prepared initially by an aqueous solution of DNSA (1 g/40ml) with 30 g of sodium tartrate and secondarily by the addition of 20 ml of NaOH (2N).

2. Solution of α-amylase conducted an activity of 260 IU and from this mother solution (1 g of α-amylase solubilized in 100 ml of phosphate buffer solution 0.02M, PH=6). We have prepared a solution which the enzymatic activity of the final α-amylase in the reaction medium of 1.34 IU/ml [31].

3. Solution of substrate to the basis of the potato starch and phosphate buffer solution (0.02M, PH=6) to 0.4 mg/ml.

4. Solution of extracts of plants

Different concentrations (1.6- 2.4- 3.2- 4.8- 6.4 mg /l) extracts of plants are prepared in the phosphate buffer solution in order to assess their effects on the enzymatic activity of α-amylase. The evaluation of the effect of the extracts on the activity of α-amylase is based on the reading streptophotométrique at 540 nm of a white and a solution containing 200 µl of the extract for each concentration, solution of a-amylase and substrate incubated at 25°C for 5 min. The reaction is stopped by the suite using 600 µl of DNSA and in order to stop the reaction between the maltose. The DNSA tubes are agitated and placed in a boiling water bath immediately cooled in an ice water bath [32]. The results are expressed as a percentage of inhibition according to the following formula:

Inhibition%=(Control absorbance - sample absorbance) × 100/ absorbance control.

Results and Discussion

In order to characterize our different extracts (methanolic and aqueous) prepared from the bark of *Berberis vulgaris* and the aerial part of *Zygophyllum geslini*, analyzes have been carried out, first of

all a determination of performance, quantitative determination of polyphenols as well as an evaluation of the antioxidant and antidibetic activities *in vitro*. The objective of the extraction is to free the phenolic compounds present in the vacuolar structures. The preparation of extracts from the two plants was performed by maceration in the distilled water and in methanol. This extraction to permit to obtain four crude extracts whose performance was determined in relation to the weight of plant material. The results were expressed as percentage (Table 1). The performance parameter is a means not only to assess the total extracts from each species but also to consider the quantity of the organs to take in case of need, which rendered the rational use and therefore sustainable of the species referred [33]. From the results obtained it has proved that the performance in the extract varies from one species to another and depending on the solvent used. The results presented in Table 1 show that the highest yield was observed in the aqueous extract of *Zygophyllum geslini* (29.03%), however its methanolic extract led to a performance of (26.32%) followed by the methanolic extract and aqueous solutions of *Berberis vulgaris* (17.2 and 15.1%) respectively. As well as the extracts obtained are of colour and different aspects. The fluctuations and the variations in the yields observed in our results and those of the other species 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 [34].

Extract Character	*Berbéris vulgaris*		*Zygophyllum geslini*	
	Me-OH	Aqueous	Me-OH	Aqueous
Color	Brown	Pale yellow	Dark brown	Yellow
Performance %	17.2	15.1	26.32	29.03

Table 1: Color and performance of extracts of plants studied.

Extract Compounds	*Berberisvulgaris*		*Zygophyllum geslini*	
	Me-OH	Aqueous	Me-OH	Aqueous
Flavonoids	+	+	++	++
Alkaloids	+	+	+	+
Tannins	+++	+++	+	+
Saponines	++	++	++	++

Table 2: Photochemical analysis of different extracts.

Extract Content	*Berbéris vulgaris*		*Zygophyllum geslini*	
	Me-OH	Aqueous	Me-OH	Aqueous
Total polyphenols (mg EGA/g) (mg Eq AG /g) (mg Eq AG/ g MS)	10.23	9.25	7.5	6.3
Flavonoids (mg CEQ/g)	2.14	1.81	2.41	2.32

Table 3: Rates of total polyphenols and flavonoids in extracts of plants studied.

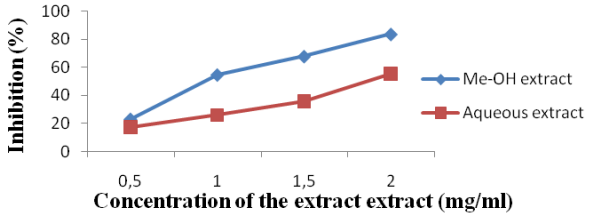

Figure 3: Percentage of inhibition of the free radical DPPH extracts in function of the different concentrations of *Berberis vulgaris*.

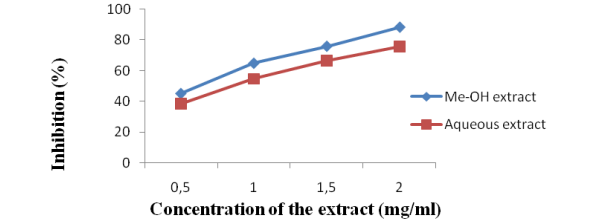

Figure 4: Percentage of inhibition of the free radical DPPH extracts in function of the different concentrations of *Zygophyllum geslini*.

Photochemical screening

The results of characterization of the chemical groups present in the four extracts have highlighted the presence of polyphones, flavonoids, alkaloids, tannins and saponins (Table 2).

Determination of total polyphenols and flavonoids

The choice to quantify the polyphenols among the different phytochemicals is the result of the fact that the polyphenols have biological activities very important like antioxidant and antidiabetic. The phenolic content in the extracts examined using the reagent Folin-Ciocalteu is expressed in terms of equivalent of gallic acid. It is calculated from the right of calibration established using a reference solution (gallic acid). The spectrophotometry has helped to quantify the rate of polyphenols in the extract methanolique aqueous and of the two plants (Table 3). The results of total polyphenols achieved and expressed in mg EGA/g of the dry matter vary between 6.3 and 10.23 as a function of solvent used whose extracts methanolic of two plants have recorded levels significantly higher than the aqueous extracts. The content of the higher phenols has been measured in the methanolic extract (10,23 mg EGA /g) followed by the aqueous extract (9.25 mg EGA/g) of *Berberis vulgaris*. While the methanolic extracts and aqueous solutions of *zygophyllum geslini* have been registered as 7.5 and 6.3 mg EGA/g respectively. This is due to the affinity of the solubilization of phenolic compounds in polar solvents. The flavonoids save a value of 2.41 mg CEQ/g of methanolic extract of *Zygophyllum geslini*. Any account fact these values comply the results conducted by Mezouar [35]. Finally several factors can influence the quantitative distribution of phenolic compounds in our extracts; among these factors we note the climatic factors and environmental [36]. The stage of development of the plant, the harvest period and conditions of conservation [37]. As it is important to note that the levels of total phenolics increase when the middle of life of the plant is not adequate which favors the synthesis of secondary metabolites in order to adapt of the same method of extraction and the selectivity of solvent used [38] including its polarity which allows to solubilize the compounds of similar polarity [39,40].

Antioxidant activity of the extracts

We have noted that the antioxidant power of a plant extract is an important parameter that can have a strong relationship with the antidiabetic activity, this property is assessed by different techniques including that of the radical DPPH is the most used to see its stability and its simplicity [41]. The results illustrated in Figures 3 and 4 show that the four extracts are equipped with an important antioxidant activity proportional to the concentration leading to curves of exponential growth corresponding to a kinetic reflecting the effective inhibition of the radical DPPH. An antioxidant effect of maximum 88.22% is noted for a concentration of 2 mg/ml of the methanolic extract of *Zygophyllum geslini*.

Effect of extracts on the activity of α-amylase *in vitro*

The one of the therapeutic strategies of diabetes is based on the reduction of hyperglycemia post prandial as a result of the downturn in the intestinal absorption by the inhibition of certain enzymes

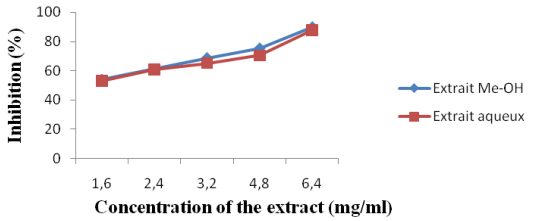

Figure 5: Percentage of inhibition of α-amylase by extracts of *Berberis vulgaris*.

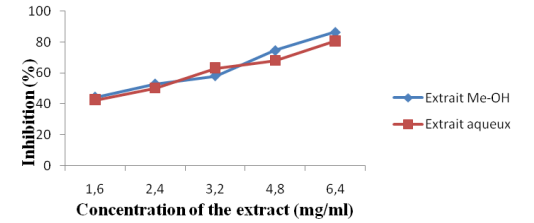

Figure 6: Percentage of inhibition of α-amylase by extracts of *Zygophyllum geslini*.

Extract	*Berbéris vulgaris*		*Zygophyllum geslini*	
	Me-OH	Aqueous	Me-OH	Aqueous
IC_{50} (mg/ml)	0.68	0.77	1.72	1.84

Table 4: Values of the IC_{50} extracts.

responsible for the hydrolysis of polyose including the α-glucosidase and α-amylase at the level of the digestive tube [42]. The mechanism of action of these inhibitors are variable, they act in combination with the enzyme, the substrate or the enzyme-substrate complex [43]. In order to determine the effect of extracts of two plants on the activity of α-amylase *in vitro*, we tested the effect of different concentration of everybody A on the activity of the enzyme with Fixing of substrate concentration to 2.5 g/l. The results are expressed in Figures 5 and 6. The overall results obtained shows that the totality of the aqueous and methanol extracts can inhibit the activity of α-amylase in proportion to the concentration of the extracts. The methanolic extract of *Berberis vulgaris* has the percentage of inhibition, the higher which is of the order of 90% to high concentration (6.4 mg/ml). In addition, we determined for each extract the concentration of antidiabetic medicinal product required to reduce 50% of the initial concentration of α-amylase, from equations regressions. The values of IC50 are shown in Table 4. According to these results the *Berberis vulgaris* as well as that of the *Zygophyllum geslini* have an effect of antidiabetic suggesting that this is linked to the wealth of its extracts in tannins and saponins.

Conclusion

Diabetes remains a systemic disease well answered and in continuous expansion constituting a public health problem in view of the cost of their support especially for the developing countries that requires a recovery and an exploration of resources natural phyto-genetics to antidiabetic interest. Two species widely used by the local population and that grow spontaneously in the south of Algeria have been the target of our study. The methanolic extracts and aqueous solutions of the bark of the roots of *Berberis vulgaris* and the aerial part of *Zygophyllum gesliné* have been tested. The assessment of the content of polyphenols totals, the antioxidant activity and antidiabetic activity including the whole of the results obtained supports the therapeutic potential of the two plants and its active components in the prevention and treatment of diabetes or it has been revealed an antiradical power important and an inhibitory effect interesting on the activity of α-amylase.

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