PHYTOCHEMICAL SCREENING AND BIOLOGICAL STUDY OF ETHANOL EXTRACTIVES OF *DIPCADI SEROTINUM (L.) MEDIK*

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ABSTRACT

The characterization of chemical constituents, from the aqueous extract of *Dipcadi serotinum (L.) Medik*, revealed the presence of flavonoids, alkaloids, sterols, tannins, reducing compounds and saponosides.The amount of total phenolics and total flavonoids were evaluated. The extract contained an amount of phenolics (65 mg/g), and flavonoids (2.34mg/g). Furthermore, the antioxidant activities, antiradical properties, and antimicrobial activity were also evaluated.

Keywords: *Dipcadi serotinum (L.) Medik*; Ethanol extracts; Phytochemical, Biological activity.

INTRODUCTION

Hyacinthaceae consist presently of approximately 70 genera and 1000 species of perennial herbs growing from bulbs, usually with a membraneous tunic and several bulb scales. A few species have succulent leaves. Flowers are usually radialy symmetrical with six petals arranged in two whorls of three each, but this may be different from a species to another. The flower stalk is leafless and the flowers are always arranged in racemes, which may be compact. Fruits are dry, dehiscent capsules, often tetrahedral or at least angular. (Aafi et al., 2005)

The Hyacinthaceae are widely distributed through the temperate, subtropical and tropical parts of the world. They are well represented in Morocco where half of the known species are found. The deciduous habit of many species reflects seasonal climates with seasonal fires, hot, dry or cold phases that are unfavorable for growth. The succulent bulb stores water and food until growing conditions become favorable (Pfosser et al., 2012).

In Moroccan traditional medicines, the species of the genera Squill, Urginea and even other *Liliaceae* like *Dipcadi sp.(D. serotinum, etc.)* are considered warmers and involved at very low doses, mixed with a meal as warming up in treating colds, bronchitis, influenza, etc.; They are also used in the treatment of jaundice, and the bulbs of these plants are also prescribed as a diuretic and anti-inflammatory treatment, and their poisonous properties as

insecticides and rat poison are well known by the aboriginal population (Moussaid et al., 2013).

METHODOLOGY

Plant materials

The botanical taxa studied in this work is detailed in Table (1), covering the scientific and common names, the parts used in their preparation, medicinal uses and yield of extracts of the plant. The research site (Tamaris) is located in the south of Casablanca, Morocco.
Field data were collected during the periods of November 2008 to March 2009. Prior informed consent was obtained for all interviews conducted. The most useful information came by old people, since most of young interviewed persons know very little about this aspect of local traditions.

The collected plants were generously authenticated by Pr. Laila RHAZI from Biology Department (faculty of science, Casablanca, Morocco).

Table 1: Ethno-botanical information of *Dipcadi serotinum (L.) Medik*

Scientific name (family)	Moroccan common name	Preparation	Used parts	Local traditional uses	Yield (%)
Dipcadi serotinum (L.) Medik (Hyacinthaceae)	Bssal Eddib	Raw	Bulbs	Toxic, cystitis, abortion, gastrointestinal disorders, bronchitis	14.4

Preparation of samples

The plant material was air dried at room temperature, cut into small pieces, then the extraction with 70% aqueous Ethanol (EtOH) was done through maceration (48 h for three times), again at room temperature. The total extracts were dried under reduced pressure to determine the yield as weight showed in Table (1).

Phytochemical screening

The purpose of this step was to characterize and to identify the main chemical groups of the plant extract. The protocol used for extractions and characterizations was advocated by Harborne (1998).

Antioxidant and free radical scavenging activity assays

- DPPH assay

The antioxidant activity of the *Dipcadi serotinum* extract was studied in vitro using the DPPH method (1, 1-Diphenyl-2-picrylhydrazyl), according to the methods used (Diaz et al,. 2004).
In this method, 2.95 ml of a methanolic solution of DPPH was added to 50 µl sample of different concentrations of the extracts (10 to100 mg/mL) in disposable cuvettes. The reaction mixtures were shaken vigorously and then kept in the dark for 30 min.

The absorbance of the resulting solutions was measured in 1 cm cuvettes, using a Perkin-Elmer Lambda 40 UV/VIS spectrophotometer at 517 nm, against blank without DPPH. The decrease of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity.
This activity is given as % DPPH radical scavenging that is calculated by the following equation:

$$\boldsymbol{Inhibition}(\%)(\boldsymbol{Reactive\ reaction\ rate}) = \frac{\boldsymbol{Abs.(DPPH\ solution) - Abs.(sample)}}{\boldsymbol{Abs.(DPPH\ solution)}} \times \mathbf{100}$$

The DPPH solution without sample solution was used as a blank. All tests were run in triplicate and averaged. Ascorbic acid was used as positive control.

- β-Carotene bleaching test

Antioxidant activity was determined using β-carotene bleaching test (Diaz et al., 2004). Briefly, 1 ml of β-carotene solution (0.2 mg/ml in chloroform) was added to 0.02 ml of linoleic acid, and 0.2 ml of 100% Tween 20.

The mixture was then evaporated at 40˚C for 10min, by means of a rotary evaporator to remove chloroform, and immediately diluted with 100 ml of distilled water. The water was added slowly to the mixture and agitated vigorously to form an emulsion. Five milliliters of the emulsion was transferred into different test tubes containing 0.2 ml of samples in 70% ethanol at different concentrations (100, 50, 25, 10, 5 and 1 µg/ml). As control, 5ml from the above emulsion was added to 0.2 ml of 70% ethanol.

For comparison, the "propyl gallate" standard was used at the same concentration as samples. The tubes were then gently shaken and placed at 45˚C in a water bath for 60 min.

The absorbance of the samples, standard and control was measured at 470 nm using a Perkin-Elmer Lambda 40 UV/VIS spectrophotometer, against a blank consisting of an emulsion without β-carotene. The measurement was carried out at initial time (t = 0) and successively at 30 and 60 min. All samples were assayed in triplicate and averaged.

The antioxidant activity (AA) was measured in terms of successful bleaching β-carotene by using the following equation:

$$\mathbf{AA} = \left(\mathbf{1} - \frac{\mathbf{A0\ - At}}{\mathbf{A0° - At°}}\right) \times \mathbf{100}$$

Where A_0 and A_0° are the absorbance values measured at the initial incubation time for samples/standard and control, respectively, while A_t and A_t° are the absorbance values measured in the samples/standard and control, respectively at t = 30 and 60 min.

Determination of total phenolic content

Total phenolic content of the total extract was determined using Folin-Ciocalteu reagent and chlorogenic acid as standard (Kim et al., 2003). Fifty milligrams of the extract were weighed into 50 ml plastic extraction tube and vortexed with 25 ml of the extraction solvent (40 ml acetone: 40 ml methanol: 20 ml water: 0.1 ml acetic acid).

Then, the samples with the extraction solvent were heated at 60˚C (water bath) for 1 h, allowed to cool to room temperature, and homogenized for 30 sec with a sonicator. Two hundred microliters (three replicates) were introduced into screw cap test tubes; 1.0 ml of Folin Ciocalteu's reagent and 1.0 ml of sodium carbonate (7.5%) were added. The tubes were vortexed and allowed to stand for 2 h. The absorption at 726 nm was measured (Perkin-Elmer Lambda 40 UV/VIS) and the total phenolic content was expressed as milligram of chlorogenic acid equivalents per gram of dry material.

Determination of total flavonoid content

Total flavonoids were estimated in the plant extract using a colorimetric method based on the formation of a complex flavonoid-aluminum, having the maximum absorbance at 430 nm (Quettier-Deleu et al., 2000). All determinations were made in triplicate and values were calculated from a calibration curve obtained with quercetin. Final results were expressed as milligram of quercetin equivalent per gram of dried weight.

Antimicrobial screening

Ethanolic crude extract is tested against *Candida albicans* ATCC 28367, *Fusarium solani* ATCC 36031, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 23467, *Salmonella enteritidis* ATCC 13312 and *Streptococcus pneumoniae* ATCC 49619. (Origin ATCC Gene Bank, *National Institute of Hygiene*, Rabat, Morocco). The bacterial strains were cultured overnight at 37ºC in Mueller-Hinton Agar (MHA), and the tow fungi *Candida albicans and Fusarium solani* were cultured overnight at 30ºC in Sabouraud Dextrose Agar (SDA).

The antimicrobial activity was studied using the well diffusion method. The degree of growth inhibition was evaluated after 48h for bacteria and 12h for fungi and compared with the growth inhibition results obtained from the controls (Tetracycline for bacteria and Nystatin for fungi) (Vlietinck and Vanden Berghe, 1991).

Statistical analysis

Data were expressed as Mean ±SE of at least three independent experiments. The differences between control and treated groups were determined by one-way ANOVA followed by the least significant difference (LSD) (Armitage, 1971).

RESULTS

The finding of the preliminary phytochemical investigations was depicted in Table (2).

Table 2: Qualitative phytochemical evaluation.

Chemical groups	Reagents and positive results
Alkaloids	Mayer (potassium iodomercurate) ⟶ yellowish precipitate
Flavonoid	Cyanidin reaction ⟶ Coloration to orange, red or purple
Tannins	$FeCl_3$ ⟶ Darkening blue, green or black
Saponosides	Determination of Foam Index (IM*): positive if IM>100
Steroids and terpenes	Anhydride acetiq- H_2SO_4 (50:1) ⟶ violet-blue or green colouring
Oses and holosides	H_2SO_4 - saturated alcohol with thymol ⟶ red colouring
*: the degree of dilution of an aqueous decoction of the herbal drug which, in certain conditions, provides a persistent foam	

The results for the free radical scavenging activity are shown in Table (3).

Table 3: IC_{50} values for antioxidant activities of extract of *Dipcadi serotinum (L.) Medik.*

Extract	IC $_{50}$ (µg/ml)[a]		
	DPPH	β-Carotene bleaching test	
		30 min of incubation	60 min of incubation
Dipcadi serotinum (L.) Medik	58 ± 0.17	2 ± 0.05	4 ± 0.05
Ascorbic acid[b]	2 ± 0.03	—	—
Propyl gallate[b]	—	1 ± 0.01	1 ± 0.01
[a] ± S.E.M. (n=3). [b] Ascorbic acid and propyl gallate were used as positive control.			

Table (4) reports the results of the total phenolic and total flavonoids analyses

Table 4: Total phenolic content of the extract using Folin-Ciocalteu method.

Extract	Total phenolic content (mg/g)[a]	Total flavonoid content (mg/g)[a]
Dipcadi serotinum (L.) Medik	65±1.0	2.34 ± 0.07
[a] Values expressed as chlorogenic acid equivalents/ g of extract.		

The results of antimicrobial activity are recorded in Table (5).

Table 5: Antibacterial and antifungal screening of ethanol extract of *D. serotinum (L.) Medik* bulbs.

Microorganisms	Zone of inhibition in mm*+	Standards
Fungi	Crude extract	Nystatin (50 IU)
Candida albicans	10.5	30
Fusarium solani	9.7	38
Bacteria		Tetracycline (30 IU)
Escherichia coli	11.5	24
Klebsiella pneumoniae	<5	<5
Salmonella enteritidis	<5	25
Staphylococcus aureus	12.5	<5
Streptococcus pneumoniae	9.4	26
Pseudomonas aeruginosa	7.0	<5
* Including the diameter of the well (4 mm); + Mean value of three independent experiments		

DISCUSSION

The preliminary phytochemical tests performed were qualitative. The phytochemical analysis showed that alkaloids, tannins, flavonoids, steroids, saponosides and reducing compounds were present in the extract. The reactions were positive with virtually all target compounds. Although there is the wealth of our plant saponins, and tannins, the presence of saponins is illustrated by a marked foam index; we also note the presence of alkaloids and reducing compounds.

This abundance of active ingredients with diverse pharmacological properties gives the plant remarkable properties, which could justify its multiple therapeutic indications and its use in traditional medicine (Bellakhdar, 2006; Bellakhdar and Younos, 1993). The preparations were able to reduce the stable free radical DPPH to the yellow-colored 1, 1-diphenyl-2 picrylhydrazyl. Thereby,the *Dipcadi serotinum (L.) Medik* extracts showed an IC_{50} = 58 µg/mL, as a reference, value of ascorbic acid was an IC_{50} = 2 µg/mL.

In the β-carotene bleaching test (after 30 min incubation) the *D. serotinum (L.) Medik* extracts showed the highest inhibition of linoleic acid oxidation (IC_{50} = 2 µg/ml). So, the antioxidant activity of the extracts decreased with reaction time, and after 60 min incubation, the IC_{50} values of the most active ones was 4µg/ml. In table (4), the results of the total phenolic and total flavonoids analyses; it is found that the bulbs of *D. serotinum (L.) Medik* contained phenolic compounds (65 mg/g), and flavonoids (2.34 mg/g).

Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables (Vanden Berghe and Vlietinck, 1991). However, we have found no correlation between antioxidant activity and total phenol/flavonoid content as determined by the square regression coefficient (r^2 = 0.34). The plant has high phenol/flavonoid contents but low antioxidant activity (Moussaid et al., 2011). The ethanol extract showed considerable activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, with a lesser activity than the standard Nystatin against *Candida albicans* and *Fusarium solani*.

We consider that the ethanol extract has low antimicrobial activity compared to the standard used, but the results of the sensitivity test have made it possible to prove a significant antibacterial activity for almost all studied pathogens.

CONCLUSION

The phenolic and flavonoid composition and antioxidant activity were evaluated in the ethanolic extract from bulbs of *Dipcadi serotinum (L.) Medik*. The qualitative test confirmed the presence of alkaloids, tannins, flavonoids, steroids, saponosides and reducing compounds in the extract. Regarding the antioxidant activity, the results have shown no correlation with the polyphenol content, in contrast with other studies that showed a linear relationship between total phenolic compounds and antioxidant activity (Saleem et al., 2002; Geronikaki and Gavalas, 2006). Nevertheless, the crud extract proved a significant antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*, and to a lesser extend *Escherichia coli* and *Streptococcus pneumonia*. Whereas the intifungal activity against *Candida albicans* and *Fusarium solani* was significantly less than the standards used.

This study is the milestone in the complete understanding of the ancient use of the medical properties of this marvellous plant, which could be completed by further studies on the antibacterial activities of the flavonoids and the phenolic compounds, richly found in the bulbs of the Moroccan *Dipcadi serotinum (L.) Medik.*

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