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The Parasitic Plant *Cistanche violacea* (Desf.) Beck from Ghardaïa (Algeria): A Source of Biologically Active Compounds

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Abstract: The present study aims to determine the contents of total polyphenol (TPC) and total flavonoid (TFC) and evaluate the antioxidant and antimicrobial activities of *Cistanche violacea* (Desf.) Beck. The extraction of bioactive compounds from the whole plant of *C. violacea* (Desf.) Beck was carried out by three different methods, including maceration, Soxhlet, and ultrasound, with ethanol and water as solvents. The results showed that the ultrasound ethanolic extract recorded the greatest amounts of TPC with 188.95 ± 2.2µg gallic acid equivalent/mg dry extract, while for TFC, it was the maceration ethanolic extract with 40.26 ± 6.02µg quercetin equivalent/mg dry extract. The High-Performance Liquid Chromatography (HPLC) results showed the presence of phenolic compounds, with 32 identified compounds in the ethanolic and aqueous extracts. The different extracts showed antioxidant capacities for DPPH, ABTS, phenanthroline, and reducing power assays. The highest antioxidant ability was observed with the ethanolic extract obtained by the maceration method (IC_{50} 33.35 ± 1.4 and IC_{50} < 12.5 µg/mL for DPPH and ABTS tests). The antimicrobial investigation on seven microbial strains revealed that the ethanolic extract ultrasound showed moderate antibacterial activity (18.66 ± 1.1mm) against only *Staphylococcus aureus* ATCC25923, while the other extracts showed weak or no antimicrobial activity. This work suggests that *C. violacea* (Desf.) Beck has antioxidant properties of natural origin, which justify its traditional use in treating many conditions related to oxidative stress.

Keywords: *Cistanche violacea*, extracts, phenolic compounds, HPLC, antioxidant potential, antimicrobial activity.

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1. INTRODUCTION

The *Orobanchaceae* family comprises more than 2000 species in about 100 genera and contains the most significant number of parasitic angiosperm plants (1, 2). This family comprises facultative or obligate parasite plants distributed in all climatic zones and continents except Antarctica (2, 3).

The genus *Cistanche* Hoffmanns. & Link (1813) includes around 25 species of obligate parasite plants, meaning they cannot complete their life cycles without host plants[1] (4). As a result, they have lost their photosynthetic abilities and are thus called holoparasites (5). They are found in different environments, including halophytic habitats and semi-arid and arid zones across Eurasia and North Africa

[1] http://www.gbif.org

(4). Long underground stolons, fleshy stems and colored inflorescences characterize the *Cistanche* species. Generally, species belonging to the *Cistanche* genus grow on the roots of plants of the following families: *Plumbaginaceae*, *Zygophyllaceae*, *Chenopodiaceae,* and *Tamaricaceae* (6).

In the Algerian flora, three species of *Cistanche* are reported, namely *C. tubulosa* in Tassili N'Ajjer (Central Sahara of Algeria), *C. tinctoria* (Desf.) Beck a species with a Saharo-Mediterranean distribution and *C. violacea* (Desf.) Beck[2] (1893), the holoparasitic endemic species of North Africa (7). In Algerian popular medicine, the locally known plant Danoun (*C. tinctoria* (Desf.) Beck) is traditionally used to treat diabetes, diarrhea, and abdominal pains. In addition, this plant is used as an aphrodisiac, analeptic and, for muscle contractions, bruises and as a lactation stimulant (8). On the other hand, *C. phelypaea* (L.) synonym of *C. tinctoria* (Desf.) Beck is used in North African traditional medicine for diarrhea, diabetes, intestinal troubles, infection, and as a diuretic. In addition, several species of *Cistanche* are used in traditional Chinese medicine as food additives and tonics. Moreover, they are used to treat conditions like renal deficiency, impotence, infertility, profuse metrorrhagia and chronic constipation (9).

Information about the traditional use of *C. violacea* is lacking in Algeria, but a few studies have been conducted on its phytochemical and biological properties. Indeed, Bougandoura et al. (10) revealed the chemical constituents and the anti-inflammatory activity of the ethanolic extract of the aerial parts of *C. violacea* collected from southwest Algeria. Furthermore, Alia et al. (11) studied the extent to which host plant variation affected the natural product extracts of *C. violacea* (Desf.) Beck growing in the Oued Souf region (southeast Algeria). In Tunisia, Debouba et al. (12) have shown that the flowers and bulbs of *C. violacea* collected from the seashore of Hassi Jerbi village, located in the southeast of Tunisia, had important amounts of total phenolic compounds. In addition, Ben Attia et al. (13) highlighted the antioxidant and cytotoxic activities of *C. violacea* collected from Tataouine (southeast Tunisia). Finally, the recent study of Bouzayani et al. (14) for samples collected from Sfax (southeast of Tunisia) showed important phenolic content and antioxidant activities for polar extracts, while evaluating the antimicrobial activity indicated that the methanol extracts had the highest activities. However, the methanol and ethanol extracts of *C. violacea* collected from the Qassim region of Saudi Arabia exhibited no or weak antimicrobial activity, except for *Staphylococcus epidermidis*, which had good activity (15).

As part of our project to study the Algerian desert flora and search for plants with therapeutic value, we were interested in studying *C. violacea* (Desf.) Beck growing in a spontaneous state in Ghardaïa Province. This work aims to evaluate the chemical composition of different extracts (ethanolic and aqueous) obtained by three extraction methods: maceration, Soxhlet, and ultrasound of the whole plant of *C. violacea* (Desf.) Beck. We explored the phenolic and flavonoid contents, antioxidant properties and antimicrobial activities of the obtained extracts. In addition, we evaluated the relationship and correlation between the phytochemical contents of the different extracts and the tested antioxidant activity.

2. EXPERIMENTAL SECTION

2.1. Plant Material

Whole plants of *C. violacea* (Desf.) Beck were collected from Noumerate (32°23’30.7"N 3°46’03.3"E) at the campus of the University of Ghardaia. This region is characterized by a semi-arid climate and is located in the Septentrional Sahara (16). First, the freshly collected plants are washed with running tap water and put to dry in the laboratory. Then, the samples were grounded using a mechanical grinder (Sayona Electric, France) and finally stored pending subsequent analysis.

2.2. Extraction Methods

Bioactive compounds were extracted from the analyzed plants by three extraction methods. The conventional methods used were maceration and Soxhlet and the unconventional method was ultrasound-assisted extraction.

For the maceration method, 10 g of the plant powder was macerated in 100 mL of ethanol (70%) or water under magnetic stirring for 24 h. The extracts were filtered, and the ethanol was evaporated using the rotavaporator R-210 (Buchi, Switzerland), while water was eliminated by the lyophilizer (Telstar lyoQuest, Spain). For the Soxhlet extraction, 10 g of the plant powder was placed in a Soxhlet cartridge. The extraction was performed using a Soxhlet apparatus with a 500 mL flask containing 100 mL of ethanol (70%) or water and the extraction cycle lasted 4 h, and the temperature was fixed at 60 °C or 100 °C, respectively. Then, the resulting ethanolic solutions were evaporated using the rotavaporator R-210 and a lyophilizer was used for removal of water. The ultrasound-assisted extraction was carried out in a Sonorex Digetec DT 514 (Bandelin, Germany) using a 250 W power and 35 kHz frequency. Ten grams of plant powder was put separately in a beaker and put through a 1 h extraction with 100 mL of ethanol or water. Then, the obtained extracts were evaporated using the rotavaporator R-210 and a lyophilizer. Finally, the yield (%) of each extract was determined after weighing and then stored in a refrigerator at 4 °C pending further analysis.

2.3. Total Phenols Content (TPC)

The determination of Total Phenols Content (TPC) was performed according to the Folin-Ciocalteu method described by Singleton et al. (17). First, 20 µL of each extract was added to 100 µL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water). Next, the mixtures were shaken and then added to

[2] http://www.gbif.org

75 µL of sodium carbonate (7.5% Na_2CO_3) and allowed to incubate in the dark at room temperature for 2 h. The absorbance was measured at 765 nm using a 96-well microplate reader (Thermo ScientificTM Multiskan Sky, France). Finally, the results are expressed in µg gallic acid equivalent/mg dry extract (µg GAE/mg DE) with reference to the calibration curve of different concentrations of gallic acid.

2.4. Total Flavonoids Content (TFC)

The Total Flavonoids Content (TFC) of the plant extracts was evaluated by the method described by Zhishen et al. (18). First, 50 µL of each extract was added to 50 µL of 2% aluminum chloride hexahydrate. Next, the mixtures were stirred and then 150 µL of 5% (w/v) sodium acetate was added and incubated in the dark at room temperature for 2.5 h. After incubation, absorbance was determined at 440 nm using a 96-well microplate reader. The results were expressed in µg quercetin equivalent/mg dry extract (µg QE/mg DE) by referring to the calibration curve of different concentrations of quercetin.

2.5. High-Performance Liquid Chromatography (HPLC) Analysis

In order to characterize the phenolic composition of the various extracts of *C. violacea* (Desf.) Beck, a high-performance liquid chromatography system (Agilent 1100) was used, as described in Meguellati et al. (19). For this, a Hypersil BDS C18 analytical column (4.6 X 250 mm and 5 µm particle size, a UV Diode Array Detector (DAD) ranging from 200 to 400 nm and equipped with a quaternary rapid separation pump were used. The injected sample volumes were 5 µL and the flow rate of the mobile phase was 1.5 mL/min. Acetic acid (0.2% in water) as solvent A and acetonitrile as solvent B in a linear gradient for 30 min were used as the mobile phase. The analysis started with 95% of solvent A and finished with 100% of solvent B. Comparing the obtained retention times of the compounds with the standards that were injected under the same conditions revealed the identity of the compounds in the extracts.

2.6. Antioxidant Assays

The evaluation of the antioxidant activity was carried out on the different extracts using four different methods.

2.6.1. DPPH radicals scavenging assay

The free radical scavenging activity was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Blois et al. (20). Forty microliters of each extract were diluted to 12.5, 25, 50, 100, 200, 400, and 800 µg/mL concentrations. Then, they were blended with 160 µL of a methanolic DPPH solution (0.1 mM). After 30 min of incubation in the dark, the absorbance was measured at 517 nm using a microplate reader. Butylated hydroxytoluene (BHT) and ascorbic acid were used as positive controls and the results were expressed as % Inhibition and IC_{50} values in µg/mL.

$$\% \text{ Inhibition} = [(A_c - A_s/A_c)] \times 100.$$

Where A_c and A_s were the absorbance of the negative control and the absorbance of the sample at 30 min, respectively. IC_{50} (µg/mL) corresponds to half the maximal inhibitory concentration.

2.6.2. $ABTS^{\bullet+}$ scavenging activity

ABTS radical-scavenging activities were carried out based on the method of Re et al. (21). $ABTS^{+}$ was prepared by combining 5 mL of a 7 mM ABTS solution with 5 mL of a 2.45 mM potassium persulfate solution. The resulting mixture was then stored in a dark environment in a refrigerator at 4 °C for 16 h. Subsequently, the mixture was modified using distilled water till achieving an absorbance of 0.700 ± 0.020 at a wavelength of 734 nm. Sample solutions at different concentrations were prepared with distilled water (12.5, 25, 50, 100, 200, 400, and 800 µg/mL). Then 40 µL of sample solutions were mixed with 160 µL of $ABTS^{\bullet+}$ solution. After 10 min of incubation, the absorbance was recorded at 734 nm using a 96-well microplate reader. Results were expressed as inhibition percentage (%) and as IC_{50} values (µg/mL). BHT and ascorbic acid were used as the positive controls and the inhibition of ABTS free radical was determined as below;

$$\% \text{ Inhibition} = [(A_0 - A_1/A_0)] \times 100.$$

Where A_0 is the absorbance of the negative control, A_1 is the absorbance of the sample at 10 min. IC_{50} (µg/mL) corresponds to half of the maximal inhibitory concentration.

2.6.3. Reducing antioxidant Power (RP) assay

The reducing antioxidant power (RP) assay was carried out according to the Oyaizu (22) method. Ten microliters of the different concentrations of the extracts (3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/mL) were added to 40 µL of 0.2 M phosphate buffer (pH 6.6) and 50 µL of 1% potassium ferricyanide. The plate was incubated at 50 °C for 20 min. Then, 50 µL of tricarboxylic acid (TCA, 10%), 40 µL of distilled water and 10 µL of ferric chloride ($FeCl_3$ 0.1%) were added to each mixture. The absorbance was measured in the microplate reader at 700 nm. BHT and ascorbic acid were used as positive controls. Results were expressed as absorbance against a reagent blank and $A_{0.5}$ values (µg/mL) corresponding to the concentration indicating 0.50 absorbance intensity.

2.6.4. Phenanthroline activity

Phenanthroline activity (Phen) was determined according to the method of Szydlowska-Czerniak et al. (23). Ten microliters (10 µL) of the extracts, 50 µL of $FeCl_3$ (0.2% in water) and 30 µL of phenanthroline (0.5% in methanol) were placed in a 10 mL volumetric flask and then 110 µL of methanol were added. The obtained solution was homogenized and put in the dark at room temperature. The absorbance of the resulting solution was measured at 510 nm after 20 min against a reagent blank that consisted of 1 mL of $FeCl_3$ (0.2%) and 0.5 mL of Phen (0.5%). BHT and ascorbic acid were used as positive controls. Results were expressed as absorbance against a reagent blank and $A_{0.5}$ values (µg/mL)

corresponding to the concentration indicating 0.50 absorbance intensity.

2.7. Antimicrobial Activity

2.7.1. Preparation of microbial suspensions

In order to evaluate the antibacterial activity of the extracts, six bacterial strains were used in this study. The Gram-negative strains were *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853) and the Gram-positive bacteria were *Listeria monocytogenes* (ATCC 35152), *Bacillus cereus* (ATCC 14579) and *Staphylococcus aureus* (ATCC 25923). One yeast, namely *Candida albicans* (ATCC 10231), was used to test the antifungal activity. Bacterial and fungal suspensions were prepared using a sterile saline solution.

2.7.2. Disk diffusion assay

The antimicrobial activity of *C. violacea* extracts was investigated using the disk diffusion method (24). The Mueller–Hinton and Sabouraud chloramphenicol agar plates were surface inoculated using the bacterial and fungal suspensions containing 10^6 and 10^8 CFU/mL, respectively. Then, 6 mm sterile discs (Whatman filter papers) were soaked with the extracts (dissolved in 2.5% DMSO and sterilized through 0.22 µm filters) and placed on the surface of the inoculated agar plates. DMSO was used as a negative control, while gentamicin and fluconazole were used as positive controls for antibacterial and antifungal activities, respectively. The plates were then incubated at 37 °C for 24 h for bacteria and 28 °C for 48 h for fungi and the diameters of the inhibition zones were measured using a Vernier caliper. The experiment was performed in triplicate.

2.8. Statistical Analysis

Results are expressed as mean values ($n = 3$) with standard deviation (SD). Data were analyzed by Statistica software. An analysis of variance (ANOVA) followed by testing the significance of differences using a Tukey LSD test ($p \leq 0.05$) was applied. Correlations between the variables were determined with a Hierarchical Cluster Analysis (HCA) and a Principal Component Analysis (PCA). Pearson correlation analysis was performed based on the results of the TPC, TFC, DPPH, ABTS, RP and Phen tests.

3. RESULTS

3.1. Phytochemical Composition

The present study shows the contents of total phenolics and flavonoids in the ethanolic and aqueous extracts of *C. violacea* (Desf.) Beck (Table 1). The content of total phenolics was the highest for the ultrasound extraction method, with a value of 188.95 ± 0.4 µg GAE/mg DE, followed by the maceration extraction (183.51 ± 2.2 µg GAE/mg DE) and the Soxhlet extraction (159.17 ± 3.3 µg GAE/mg DE) for ethanolic extracts. In contrast, the lowest results were obtained with the aqueous extraction for TPC. In the same manner, the highest results of the total flavonoids contents were obtained for ethanolic extracts, with contents of 40.26 ± 6.02 µg QE/mg DE for maceration, 27.73 ± 0.8 µg QE/mg DE for ultrasound and 19.76 ± 3.7 µg QE/mg DE for Soxhlet. In addition, we did not obtain a significant difference in the total flavonoids contents for the three types of extraction methods for the aqueous extract of *C. violacea* (Desf.) Beck.

Table 1: Quantitative analyses of total phenolics (µg GAE/mg DE) and total flavonoids (µg QE/mg DE) contents of the ethanolic and aqueous extracts from *Cistanche violacea* (Desf.) Beck.

Extract	Extraction method	TPC (µg GAE/mg DE)	TFC (µg QE/mg DE)
Ethanolic extract	Maceration	183.51 ± 2.2^{b}	40.26 ± 6.02^{c}
	Soxhlet	159.17 ± 3.3^{d}	19.76 ± 3.7^{ab}
	Ultrasound	188.95 ± 0.4^{b}	27.73 ± 0.8^{b}
Aqueous extract	Maceration	79.48 ± 2.4^{a}	17.05 ± 0.1^{a}
	Soxhlet	107.22 ± 5.7^{c}	17.45 ± 0.2^{a}
	Ultrasound	71.48 ± 5.3^{a}	18.35 ± 0.3^{a}

Values are the mean of three replicates ($n = 3$) ± SD. Results with different superscript letters are significantly different ($p \leq 0.05$).

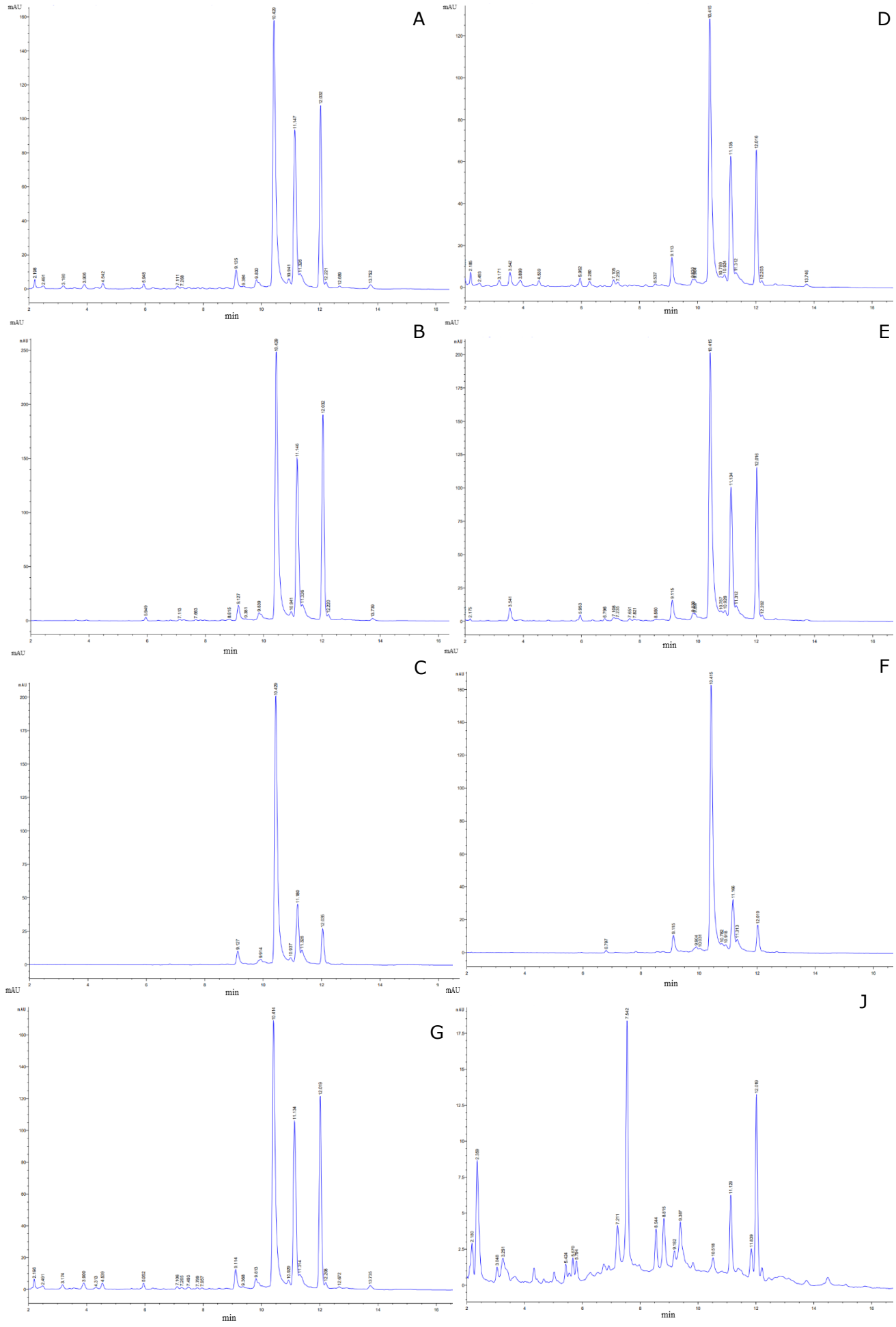
In the HPLC analysis, the retention times of the samples and standards were identified. The composition of the obtained extracts was recorded in Table 2 and the chromatograms (280, 300 and 355 nm) were presented in Figure 1. The different HPLC profiles showed many peaks corresponding to different phenolic compounds. A total of 32 compounds were identified in the six extracts, with 29 compounds each in maceration, Soxhlet and ultrasound for the ethanolic extracts, while we recorded 22 compounds in maceration and Soxhlet and 20 compounds in ultrasound for the obtained aqueous extracts. The phenolic acids included hydroxybenzoic acids like salicylic and gallic acids and hydroxycinnamic acid derivatives such as trans-cinnamic and caffeic acids. Some of the identified phenolic acids were ferulic, syringic, vanillin, m-anisic, cinnamic, and 3, 4, 5-trimethoxybenzoic acids. The flavonoids included, for example, luteolin-7-glycoside, apigenin-7-glycoside and rutin. Also, other phenolic compounds were identified, like resorcinol and hydroxyquinone.

Table 2: HPLC results of the ethanolic and aqueous extracts from *Cistanche violacea* (Desf.) Beck.

Compound number	Identified compound	Ethanolic extract			Aqueous extract		
		Maceration (retention time in min)	Soxhlet (retention time in min)	Ultrasound (retention time in min)	Maceration (retention time in min)	Soxhlet (retention time in min)	Ultrasound (retention time in min)
1	Ascorbic acid	+(2.197)	+(1.963)	+(2.197)	+(2.180)	+ (1.950)	+ (1.997)
2	Gallic acid	+(3.180)	+(3.171)	+(3.174)	+(3.251)	+ (3.209)	+ (3.220)
3	Hydroxyquinone	+(3.906)	+(3.542)	+(3.900)	/	+(3.553)	+(4.327)
4	Tannic acid	/	+(3.899)	/	+(3.048)	+(3.553)	+(3.046)
5	Resorcinol	+(4.542)	+(4.539)	+(4.539)	+(5.424)	/	+(5.031)
6	p-hydroxybenzoic acid	+(5.852)	+(6.280)	+(8.855)	+(5.545)	+(6.282)	+(5.546)
7	1,2 dihydroxybenzene	+(5.948)	+(6.280)	+(5.952)	+(5.794)	+(6.281)	+(5.672)
8	Dihydroxycinnamic acid	+(7.111)	+(7.105)	+(7.106)	+(7.211)	/	/
9	Caffeic acid	+(7.113)	+(7.108)	+(7.108)	+(7.210)	/	(7.653)
10	Syringic acid	+(7.268)	+(7.250)	+(7.265)	/	/	/
11	p-hydroxybenzaldehyde	/	/	+(7.799)	+(7.542)	/	+(7.531)
12	Isovanillic acid	+(7.276)	+(7.255)	/	+(7.539)	/	+(7.516)
13	Sinapic acid	+(8.815)	+(9.115)	+(9.116)	+(8.816)	+(9.115)	+ (9.118)
14	Vanillin	+(9.125)	+(8.537)	+(9.114)	+(8.544)	+(9.113)	+(8.543)
15	n-hydroxy cinnamic acid	+(9.127)	+(8.550)	/	/	/	+(8.532)
16	Rutin	+(9.127)	+(9.115)	+(9.116)	/	+(9.115)	/
17	Ferulic acid	(9.381)	/	+(9.367)	+(9.390)	+(9.832)	+(9.382)
18	Salicylic acid	+(9.830)	+(9.830)	+(9.813)	+(9.387)	+(9.832)	+(9.380)
19	3-hydroxy-4-methoxycinnamic acid	+(9.839)	+(9.830)	+(9.824)	/	+(9.833)	/
20	Luteolin-7-glycoside	+(9.914)	+(9.904)	+(9.901)	+(9.401)	+(9.912)	/
21	Quercetin-3-B-glucoside	/	+(10.031)	(10.024)	/	/	/
22	Rosmarinic acid	+(10.429)	+(10.415)	+(10.414)	+(10.526)	+(10.430)	/
23	Naringenin-7-glucoside	+(10.429)	+(10.415)	+(10.414)	+(10.518)	+(10.430)	+(10.493)
24	Apigenin-7-glycoside	+(10.429)	+(10.415)	+(10.414)	/	+(10.430)	/
25	Myricetin	+(10.937)	+(10.918)	+(10.924)	+(11.130)	+(11.184)	/

Compound number	Identified compound	Ethanolic extract			Aqueous extract		
		Maceration (retention time in min)	Soxhlet (retention time in min)	Ultrasound (retention time in min)	Maceration (retention time in min)	Soxhlet (retention time in min)	Ultrasound (retention time in min)
26	3, 4, 5 trimethoxybenzoic acid	+(10.946)	+(10.909)	/	/	+(11.009)	+(11.336)
27	Spiracoside	+(11.180)	+(10.782)	+(11.167)	/	+(11.328)	/
28	Coumarin	+(12.032)	+(12.016)	+(12.019)	+(11.839)	+(12.026)	+(11.848)
29	m-anisic acid	+(12.032)	+(12.016)	+(12.019)	+(12.019)	+(12.026)	+(11.846)
30	3,4,5-trimethoxy-trans cinnamic acid	+(12.220)	+(12.202)	+(12.666)	+(12.209)	/	+(12.223)
31	Rosmarinic acid	+(13.739)	/	+(13.720)	/	+(10.430)	/
32	Cinnamic acid	+(13.752)	+(13.746)	+(13.735)	/	/	/
Total		29	29	28	21	22	20

/: non detected, +: present.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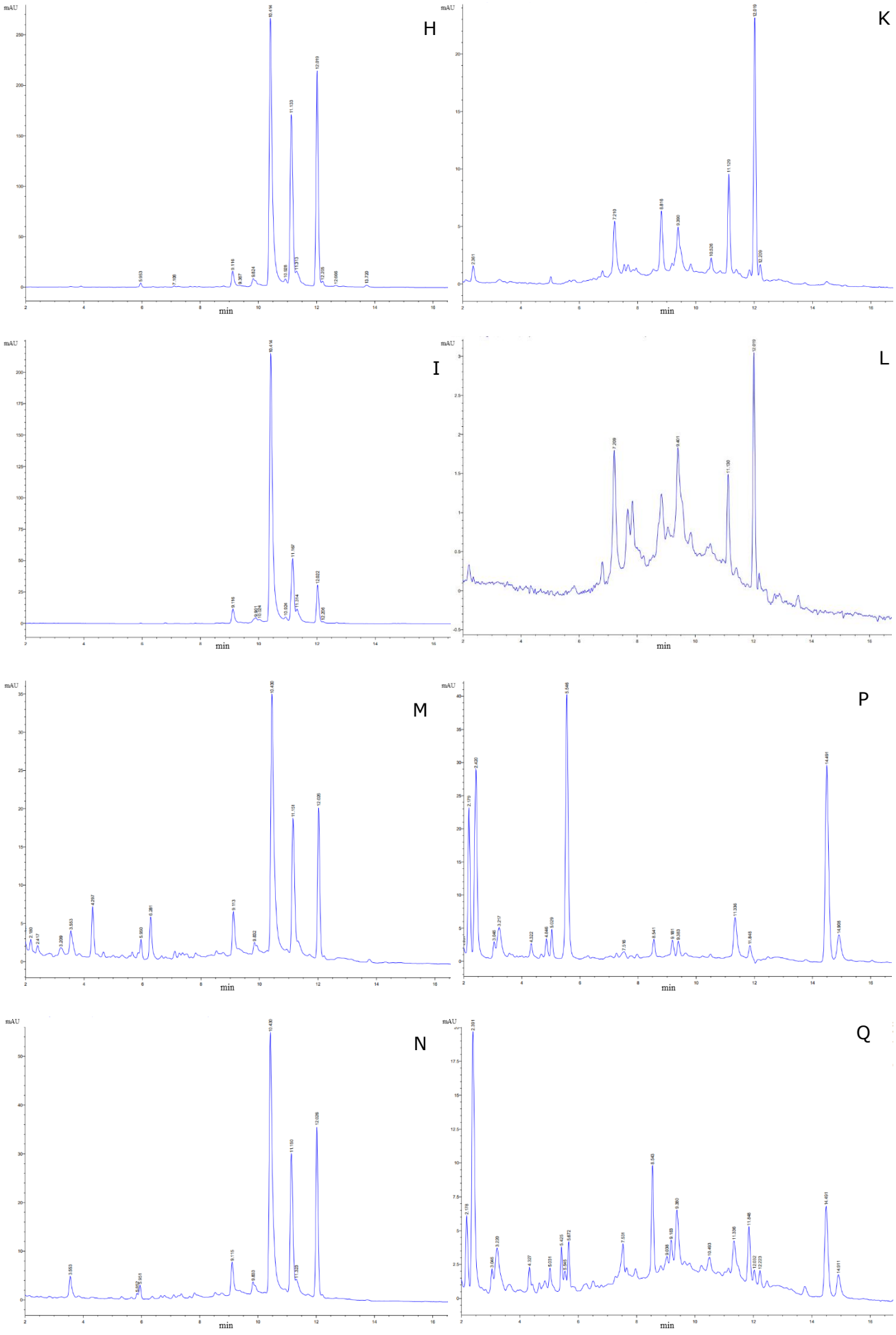
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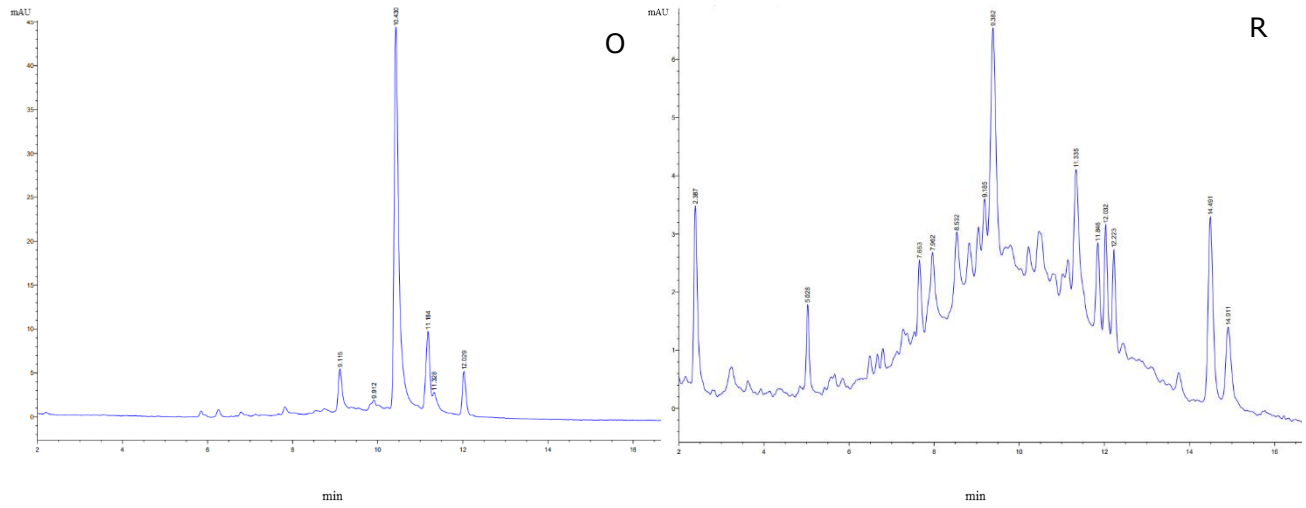


Figure 1: HPLC chromatograms of *Cistanche violacea* (Desf.) Beck extracts. A, B and C: Ethanolic extract. Maceration; D, E and F: Aqueous extract Maceration; G, H and I: Ethanolic extract Soxhlet; J, K and L: Aqueous extract Soxhlet; M, N and O: Ethanolic extract Ultrasound; P, Q and R: Aqueous extract Ultrasound at 280, 300 and 355nm.

3.2. Antioxidant Activity

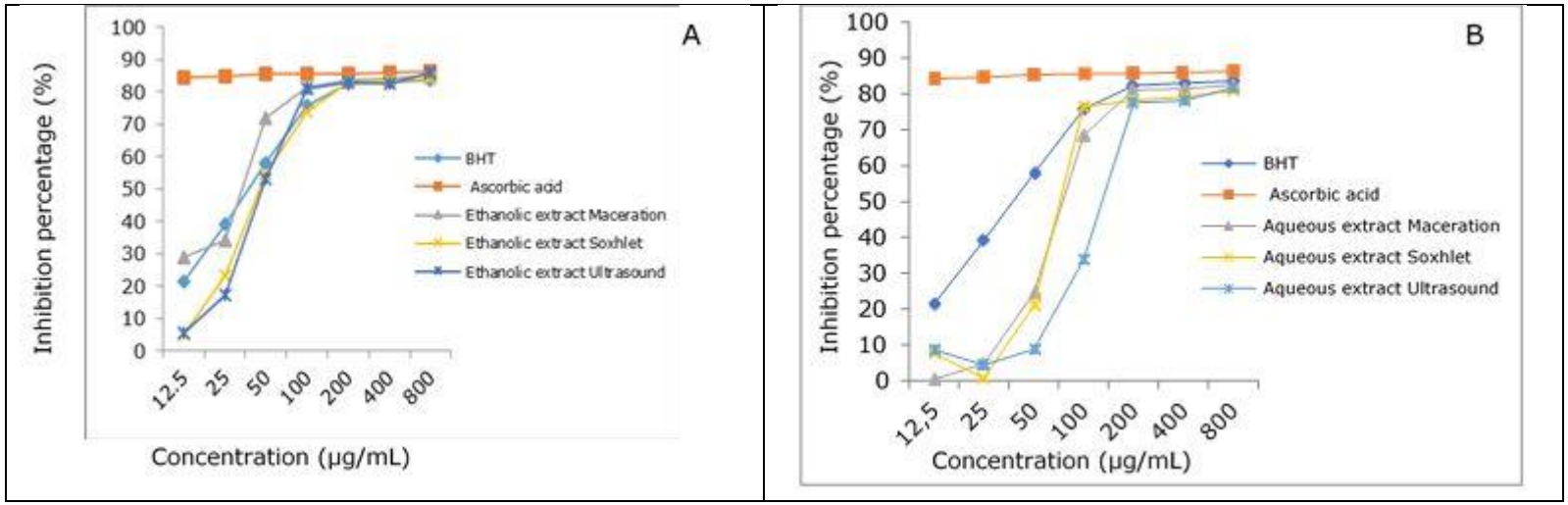
The results of the antioxidant activity of *C. violacea* (Desf.) Beck extracts are presented in Table 3 and Figure 2.

Table 3: Antioxidant activity of *Cistanche violacea* (Desf.) Beck extracts.

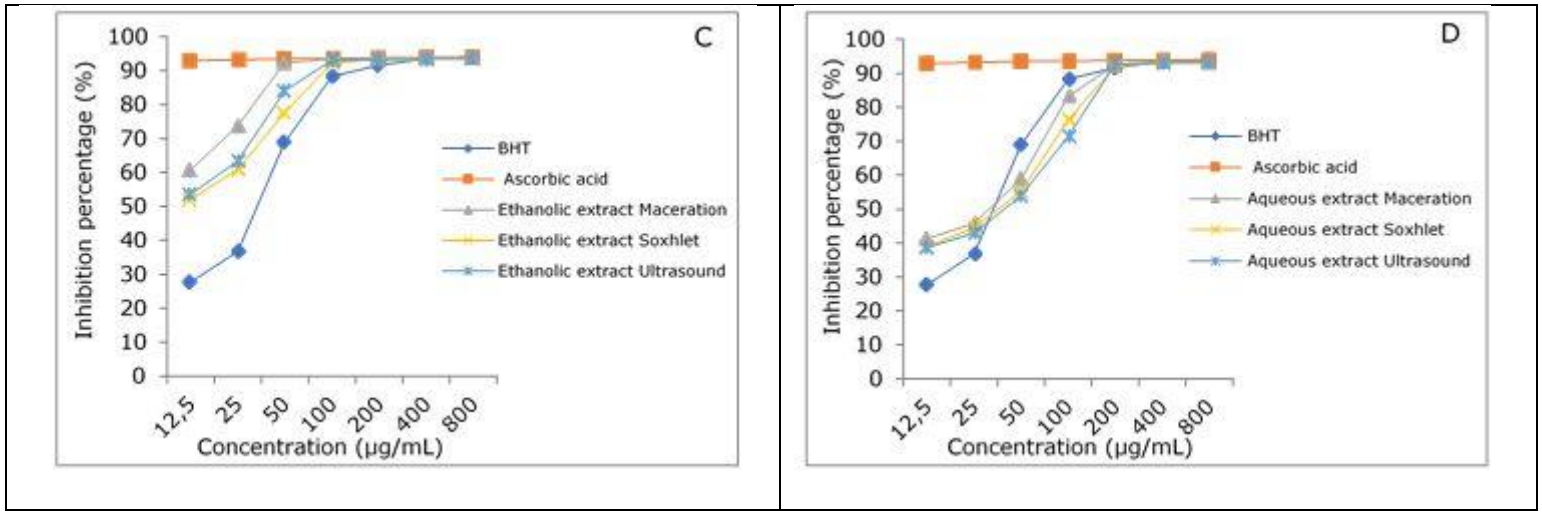
Standard/ extract	Extraction technique	IC_{50}		$A_{0.5}$	
		DPPH (µg/mL)	ABTS (µg/mL)	Phen (µg/mL)	RP (µg/mL)
BHT	/	42.60 ± 2.3^{e}	35.52 ± 1.05^{c}	8.84 ± 0.7^{f}	10.73 ± 0.7^{f}
Ascorbic acid		<12.5^{g}	<12.5^{e}	<3.125^{g}	1.48 ± 0.5^{g}
Ethanolic extract	Maceration	33.35 ± 1.4^{f}	<12.5^{e}	24.79 ± 1.9^{e}	38.67 ± 1.2^{f}
	Soxhlet	46.29 ± 0.7^{de}	<12.5^{e}	36.34 ± 0.7^{cd}	103.73 ± 0.5^{b}
	Ultrasound	48.39 ± 1.1^{d}	<12.5^{e}	32.71 ± 2.9^{d}	60.61 ± 3.3^{e}
Aqueous extract	Maceration	78.73 ± 1.4^{c}	32.91 ± 0.6^{d}	42.71 ± 1.9^{c}	69.35 ± 2.7^{d}
	Soxhlet	90.62 ± 2.5^{b}	38.09 ± 1.1^{b}	53.41 ± 4.88^{b}	84.26 ± 2.1^{c}
	Ultrasound	138.43 ± 1.5^{a}	42.82 ± 0.3^{a}	62.23 ± 2.38^{a}	142.63 ± 3.8^{a}

Values are the mean of three replicates ($n = 3$) ± SD. Results with different superscript letters are significantly different ($p \leq 0.05$). BHT: Butylated hydroxytoluene, DPPH: 2,2-diphenyl-1-picryl-hydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothia-zoline-6-sulfonic acid), Phen: phenanthroline, RP: reducing power. IC_{50} (mg/mL): concentration at which 50% is inhibited. $A_{0.5}$ (mg/mL): concentration indicating 0.50 absorbance intensity.

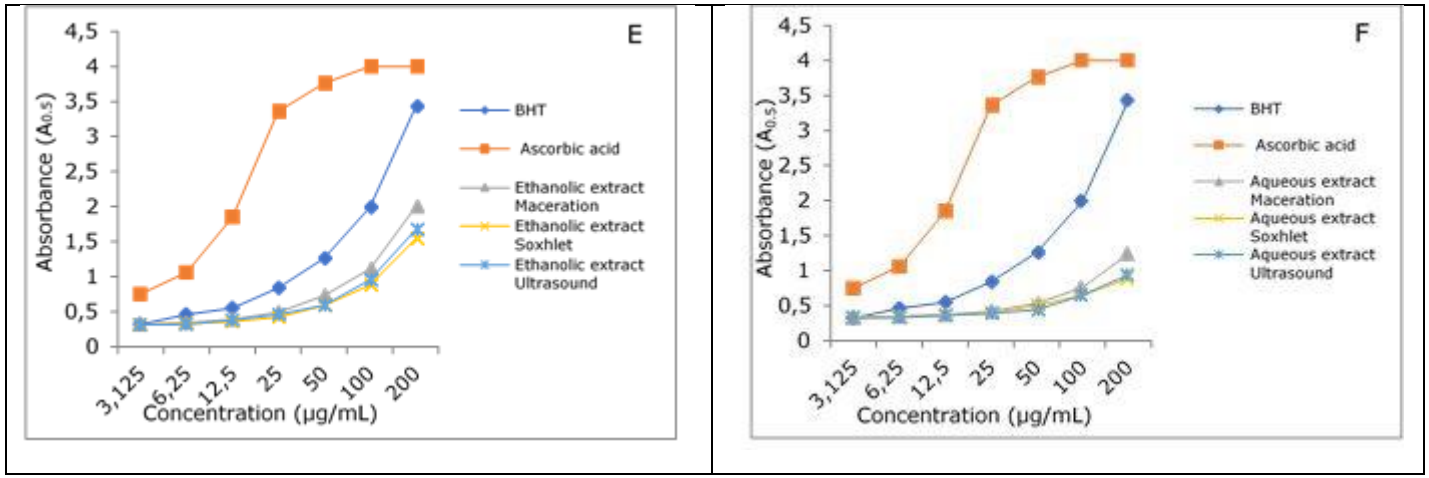
DPPH


A
Inhibition percentage (%)
Concentration (µg/mL)
BHT
Ascorbic acid
Ethanolic extract Maceration
Ethanolic extract Soxhlet
Ethanolic extract Ultrasound
B
Inhibition percentage (%)
Concentration (µg/mL)
BHT
Ascorbic acid
Aqueous extract Maceration
Aqueous extract Soxhlet
Aqueous extract Ultrasound

ABTS


C
Inhibition percentage (%)
Concentration (µg/mL)
BHT
Ascorbic acid
Ethanolic extract Maceration
Ethanolic extract Soxhlet
Ethanolic extract Ultrasound
D
Inhibition percentage (%)
Concentration (µg/mL)
BHT
Ascorbic acid
Aqueous extract Maceration
Aqueous extract Soxhlet
Aqueous extract Ultrasound

Phen



RP

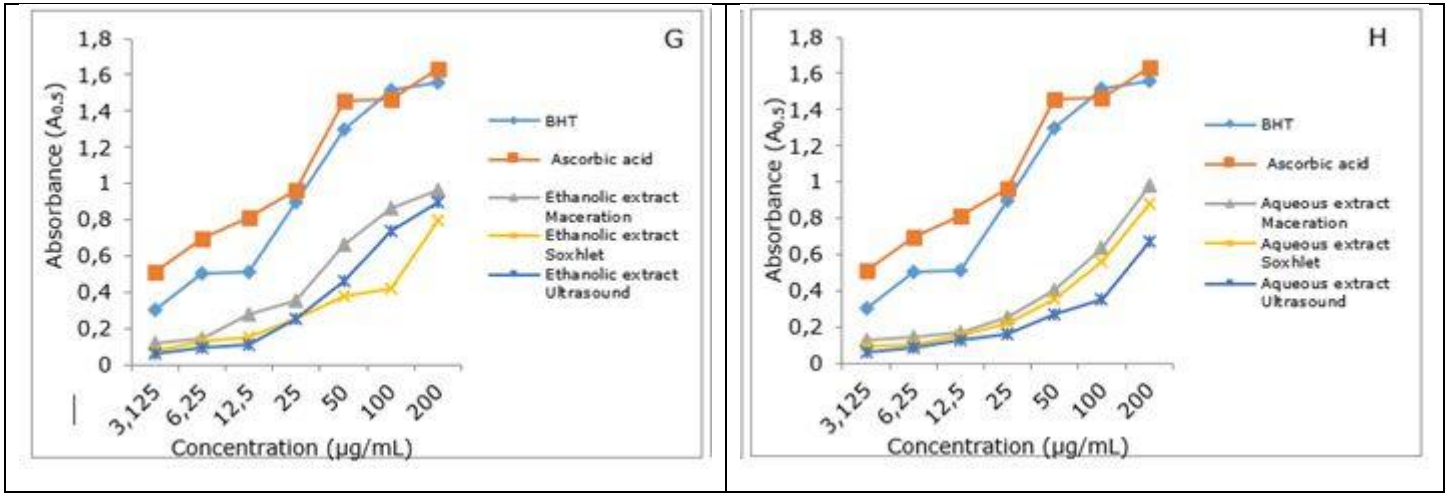


Figure 2: DPPH and ABTS scavenging effects and absorbance values in phenanthroline and reducing power assays of different extracts of *Cistanche violacea* (Desf.) Beck with BHT and ascorbic acid were used as the positive controls. BHT: Butylated hydroxytoluene, A and B: DPPH (2,2-diphenyl-1-picrylhydrazyl) for ethanolic and aqueous extracts, C and D: ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) for ethanolic and aqueous extracts, E and F: Phen (phenanthroline assay) for ethanolic and aqueous extracts and G and H: RP (reducing power assay) for ethanolic and aqueous extracts.

The DPPH and ABTS scavenging effects of *C. violacea* (Desf.) Beck extracts were evaluated. From Table 3, the obtained results show that the IC_{50} values of the DPPH assay were variable for the different extracts. Furthermore, we noted that the IC_{50} value was found to be 33.35 ± 1.4 µg/mL for the maceration ethanolic extract, which was higher than the ascorbic acid (IC_{50} < 12.5 µg/mL) and lower than BHT (IC_{50} = 42.60 ± 2.3 µg/mL). So, we conclude that the maceration ethanolic extract had an important antioxidant activity than BHT. For ABTS results, the IC_{50} values were < 12.5 µg/mL for the three extraction methods of the ethanolic extract. Thus, these different extracts had important antioxidant capacities when compared to BHT (IC_{50} = 35.52 ± 1.05 µg/mL).

The phenanthroline and reducing power assays were used to determine the potential of the different obtained extracts of *C. violacea* (Desf.) Beck to reduce metallic ions. However, all the tested extracts were less efficient than BHT ($A_{0.5}$ = 8.84 ± 0.7 µg/mL and $A_{0.5}$ = 10.73 ± 0.7 µg/mL) and the ascorbic acid ($A_{0.5}$ = < 3.125 µg/mL and $A_{0.5}$ = 1.48 ± 0.5 µg/mL) for the phenanthroline as well as the reducing power assays, respectively. Hence, BHT and ascorbic acid had more important antioxidant activities than our six analyzed extracts.

The antioxidant activity of *C. violacea* (Desf.) Beck is also presented in Figure 2 for the ethanolic and aqueous extracts. On one hand, the obtained results showed that the inhibition percentage increased with increasing fraction concentrations for the six extracts for DPPH and ABTS scavenging effects. On the other hand, the results of the phenanthroline and reducing power assay showed that the absorbance values of various extracts of *C. violacea* (Desf.) Beck increased with increasing fractions.

Overall, the results showed that the ethanolic extracts obtained by the three extraction methods from *C. violacea* (Desf.) Beck exhibited interesting high scavenging activities against DPPH and ABTS free radicals, while all the extracts showed weaker antioxidant properties than the used standards.

3.3. Antimicrobial Activity

The results of the antimicrobial activity of *C. violacea* (Desf.) Beck extracts are summarized in Table 4 and shown in Figure 3.

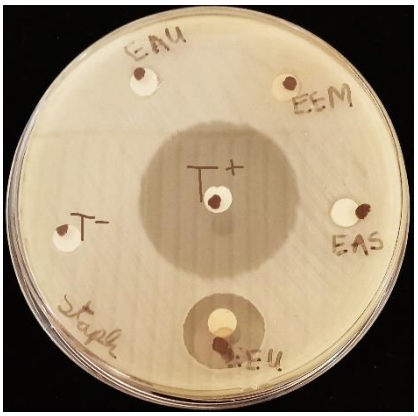


Figure 3: Representative photo showing *S. aureus* ATCC25923 (Staph) susceptibility to *Cistanche violacea* (Desf.) Beck extracts with negative and positive controls. EEM: Ethanolic extract Maceration, EEU: Ethanolic extract Ultrasound, EAU: Aqueous extract Ultrasound, EAS: Aqueous extract Soxhlet. T^-: negative control, T^+: positive control.

The antimicrobial activity of *C. violacea* (Desf.) Beck extracts revealed that the aqueous extract Soxhlet had mild antibacterial activity against *S. aureus* ATCC25923 with an inhibition zone of 18.66 ± 1.1 mm. In addition, a weak antifungal activity against *C. albicans* ATCC10231 was obtained by the ethanolic extract Soxhlet and the aqueous extract ultrasound with inhibition zones of 7.33 ± 0.5 mm. The other tested microorganisms did not show any susceptibility to the tested extracts.

Table 4: Antimicrobial activity of *Cistanche violacea* (Desf.) Beck extracts.

Control /Extract	**Extraction technique**	*E. coli* ATCC25922	*P. aeruginosa* ATCC27853	*S. typhimurium* ATCC14028	*S. aureus* ATCC25923	*B. cereus* ATCC14579	*L. monocytogenes* ATCC35152	*C. albicans* ATCC10231
Positive control	/	19.1 ± 0.7	31.44 ± 1.1	22 ± 0.9	30.11 ± 0.9	33.22 ± 0.8	33 ± 0.7	19.55 ± 0.7
Ethanolic extract	Maceration	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*
	Soxhlet	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	7.33 ± 0.5*
	Ultrasound	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*
Aqueous extract	Maceration	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6.66 ± 0.5*
	Soxhlet	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	**18.66 ± 1.1***	6 ± 0.00*	6 ± 0.00*	6.66 ± 0.5*
	Ultrasound	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	6 ± 0.00*	7.33 ± 0.5*

Values are the mean of three replicates ($n = 3$) ± SD. *$p < 0.001$ is significant compared to the positive control (one-way ANOVA followed by Tukey's test). Zone diameter equals 6 mm = no inhibition. In **bold** represents the highest antibacterial activity.

Table 5: Matrix of correlation between TPC, TFC and antioxidant assays.

	TFC	**DPPH**	**ABTS**	**Phen**	**RP**
TPC	0.74*	-0.87*	-0.94*	-0.84*	-0.62*
TFC	1.00	-0.65*	-0.65*	-0.73*	-0.69*
DPPH		1.00	0.93*	0.95*	0.79*
ABTS			1.00	0.92*	0.59*
Phen				1.00	0.81*

*Significant correlation with $p < 0.05$. TPC: Total Phenolics Content, TFC: Total Flavonoids Content, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), Phen: phenanthroline, RP: reducing power.

3.4. Correlation between total phenolics and flavonoids contents and antioxidant activity

Pearson correlation was used to analyze the relationship between the total phenolics and flavonoid contents and the antioxidant activity of *C. violacea* (Desf.) Beck extracts (Table 5).

The obtained results showed strong correlations between the DPPH, ABTS and Phen antioxidant assays, indicating the antioxidant properties of the tested extracts ($r = 1$, $p < 0.05$). Significant negative correlations were also found between the TPC of the extracts and their ABTS radical scavenging ($r = -0.94$ $p < 0.05$), DPPH radical scavenging ($r = -0.87$, $p < 0.05$), Phen ($r = -0.84$, $p < 0.05$) and RP ($r = -0.62$, $p < 0.05$) activities. Also, TFC showed a negative correlation with all the antioxidant activities measured by ABTS ($r = -0.65$, $p < 0.05$), DPPH ($r = -0.65$, $p < 0.05$), RP ($r = -0.69$, $p < 0.05$) and Phen ($r = -0.73$, $p < 0.05$) assays. On the other hand, a positive correlation was found between the DPPH and RP ($r = 0.79$), ABTS ($r = 0.93$) and Phen ($r = 0.92$). These findings indicated that TFC and TPC are not the key determinants associated with the antioxidant activity of the different extracts of *C. violacea* (Desf.) Beck.

3.5. Multivariate Analyses

Based on TFC and TPC as well as the antioxidant activity results from *C. violacea* (Desf.) Beck, multivariate analyses, including HCA and PCA, were conducted on the different extracts used in this study. These analyses were used to differentiate the different extraction methods and the used solvents on the TFC, TPC and antioxidant activities of the studied plant. HCA is a classification method that is frequently used to identify homogeneous groups based on the assessed variables. The dendrogram that results from the HCA analysis is based on Ward's linkage algorithm and Euclidean distances.

Based on the extraction method and the solvents used, HCA dendrogram (Figure 4) showed that *C. violacea* (Desf.) Beck extracts were clearly divided into three main clusters. The first cluster contained the ethanol extracts obtained by the three extraction methods. The second cluster contained the aqueous extracts after maceration and Soxhlet extractions, while the third cluster was the aqueous extract obtained by the ultrasound extraction method.

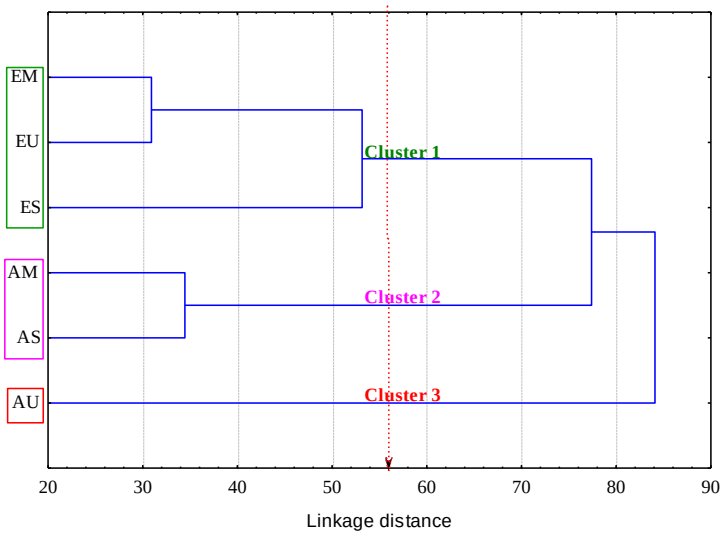


Figure 4: Hierarchical clustering analysis for the different extracts of *Cistanche violacea* (Desf.) Beck based on TFC, TPC as well as ABTS, DPPH, RP and Phen antioxidant activities. EM: Ethanolic extract Maceration; AM: Aqueous extract Maceration; ES: Ethanolic extract Soxhlet; AS: Aqueous extract Soxhlet; EU: Ethanolic extract Ultrasound; AU: Aqueous extract Ultrasound.

A score plot was used to assess the similarities and differences between the different extracts of *C. violacea* (Desf.) Beck. It was clear that the ethanolic extract using the maceration method was distinguished from the other extracts. Since the highest component with eigen values larger than 1.0 was chosen, two factors were retrieved. Our research revealed that factor 1 accounted for up to 83.53% of the total variation and factor 2 accounted for up to 8.90% of the total variance, totaling 92.43%. Therefore, the test samples were divided into factor 1 and factor 2 based on the PCA loading plot (Figure 5A), which showed differences in the test samples' TFC, TPC, and antioxidant activities as determined by the ABTS, DPPH, RP and Phen assays.

The PCA plot's first two principal components axes accounted for 92.43% of the total variance. PC1 described 83.53% of the total variability, while PC2 accounts only for 8.9%. According to the loading plot of PCA, two principal components PC1 and PC2 were applied to supply a convenient visual aid for recognizing the dissimilarity in the data and to show how strongly each characteristic (or variable) influences a principal

component. The results showed that the main contributors to the first component were TPC and TFC. The second component (PC2) was associated with DPPH, ABTS, RP and Phen antioxidant activities. The ethanolic extracts of *C. violacea* (Desf.) Beck were distinguished from the aqueous extracts based on their polyphenolic content and antioxidant activities by representing factor 1 *vs* factor 2 in the scatter plot (Figure 5B). Therefore, PC1 and PC2 explained the relation between the phenolic composition and the antioxidant activity of different extracts of *C. violacea* (Desf.) Beck. According to the results of HCA and PCA, polyphenolic composition and antioxidant activities can be used to distinguish between the different extracts of *C. violacea* (Desf.) Beck.

4. DISCUSSION

The phytochemical study, antioxidant capacity and antimicrobial activity of the ethanolic and aqueous extracts of *C. violacea* (Desf.) Beck obtained by maceration, Soxhlet and ultrasound extraction methods were investigated in this study.

4.1. Phytochemical Study

Many phenolic compounds found in plants have a positive effect on counteracting the oxidation process and function as reducing agents and antioxidants. To characterize *C. violacea* (Desf.) Beck, the TPC and TFC were determined for the different extracts obtained by two solvents (water and ethanol) and three different extraction methods (maceration, Soxhlet and ultrasound). The obtained results show that TPC in the ethanolic extracts were significantly higher ($p < 0.05$) than those in the aqueous extracts, ranging from 159.17 ± 3.3 to 188.95 ± 0.4 mg GAE/mg DE and from 71.48 ± 5.3 to 107.22 ± 5.7 mg QE/mg DE, respectively. Furthermore, ultrasound and maceration extractions were significantly higher for TPC than the obtained results for Soxhlet extraction when using ethanol as an extraction solvent. In contrast, Soxhlet extraction gave significant results compared to maceration and ultrasound extraction methods when using water as a solvent. For TFC, Highly significant results were obtained for maceration after using ethanol as an extraction solvent (40.26 ± 6.02 µg QE/mg DE), followed by ultrasound extraction (27.73 ± 0.8 µg QE/mg DE) and finally, Soxhlet (19.76 ± 3.7 µg QE/mg DE). No significant differences between the extraction methods for the aqueous extracts were observed despite the high TFC content of the ultrasound aqueous extract (18.35 ± 0.3 µg QE/mg DE).

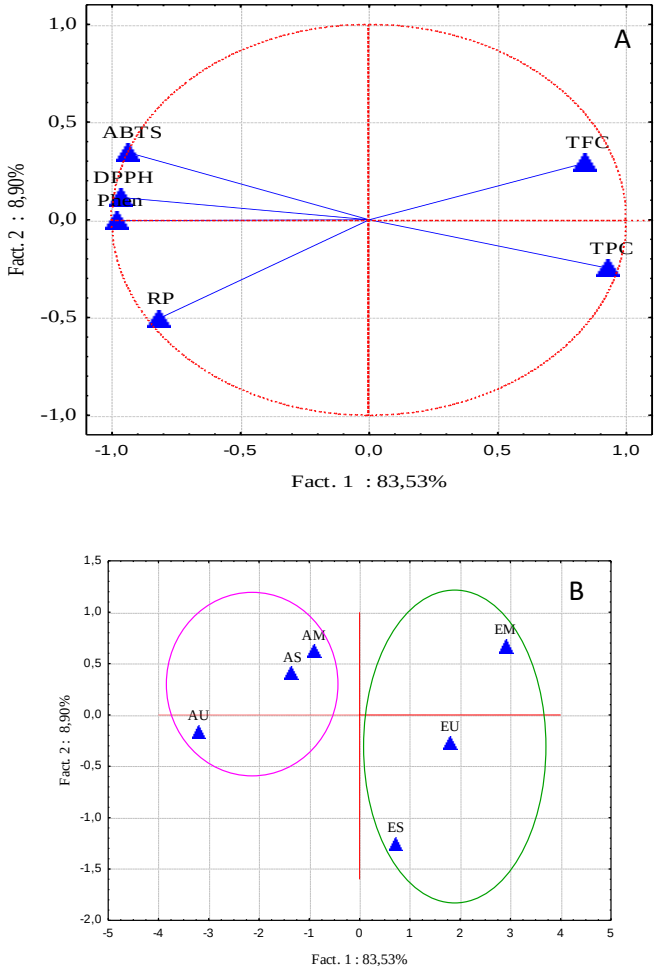


Figure 5: Principal component analysis loading plot (A) and score plot (B) for the different extracts of *Cistanche violacea* (Desf.) Beck based on TFC, TPC as well as ABTS, DPPH, RP and Phen antioxidant assays. EM: Ethanolic extract Maceration; AM: Aqueous extract Maceration; ES: Ethanolic extract Soxhlet; AS: Aqueous extract Soxhlet; EU: Ethanolic extract Ultrasound; AU: Aqueous extract Ultrasound.

A study conducted by Alia et al. (11) showed that the TPC and TFC of the methanolic extract of *C. violacea* collected from Oued Souf region (southeast of the Algerian Sahara) were 19.055 ± 0.197 mg GAE/mg DE and 5.769 ± 0.353 µg QE/mg DE, respectively. As was observed, our results were higher than those obtained by Alia et al. (11). This difference can be explained by the Folin-Ciocalteu reagent used in dosage, the biogenetic, geographical and environmental factors (25, 26). In addition, the composition of phenolic compounds is influenced by the solvent used and the extraction method. This fact was also reported by Ben Attia et al. (13), who showed that a cascade maceration using different solvents with increasing polarity (n-hexane, ethyl acetate, acetone, methanol, and water) gave different TFC and TPC contents for *C. violaceae* collected from Tataouine (southeastern Tunisia). According to Bouzayani et al. (14), *C. violaceae* (Desf) Hofmanns & Link, all extracts of the aerial parts and underground stems collected from Sfax, in the southeast of Tunisia, were rich in phenolic compounds, particularly the acetone–water extracts of the aerial parts (877 mg GAE/g DW) and the underground stems (814 mg GAE/g DW). Flowers were the most enriched in TPC compounds (394 mg GAE/g DW) compared to bulbs (116 mg GAE/g DW) in the study of Debouba et al. (12) on *C. violacea* collected from the seashore of Hassi Jerbi village located in the southeast of Tunisia. According to Piwowarczyk et al. (4), the quantity of polyphenolic compounds found in the stems and flowers of *Cistanche* was very diverse and depended on the host plant. *Cistanche armena* samples that were collected from southeastern Armenia gave a quantity of polyphenols of 568 mg/100 g (4).

In contrast to our TFC results, Bouzayani et al. (14) proved that *C. violaceae* extracts were rich in TFC. In their study, the highest TFC (342 mg QE/g DE) was derived from the underground stems acetone–water (8–2, v/v) extract, followed by 303 mg QE/g DE recorded in the underground stems methanol extract. In addition, Debouba et al. (12) showed that *C. violacea* flowers were the lowest enriched in TFC (95 mg QE/g DW) to bulbs (287 mg QE/g DW) but still higher than our TFC results. Our results are higher than those obtained by Ben Attia et al. (13), who reported that the highest TFC was observed in acetone and ethyl acetate for *C. violaceae* with 0.8 ± 0.08 and 0.79 ± 0.03 mmol CE/g, respectively.

The chemical variation between the different extracts of *C. violacea* (Desf.) Beck revealed that all the extracts are rich in phenolic compounds after HPLC analysis. Approximately twenty-nine compounds were revealed in each of the obtained extracts using ethanol as the solvent, while a lower number was obtained for the different aqueous extracts. Constituents including *para*-hydroxybenzaldehyde (phenolic aldehyde), cinnamic acid, dihydroxycinnamic, *n*-hydroxycinnamic, 3-hydroxy, 4-methoxycinnamic, 3, 4, 5-trimethoxy-trans-cinnamic, salicylic, and gallic acids (phenolic acids), and apigenin-7-glycoside, luteolin-7-glycoside, spiracoside and rutin (flavonoids) were detected. In addition, other compounds like resorcinol and hydroxyquinone were revealed. Rutin and gallic acid were also reported in the study of Alia et al. (11) on the crude extract of *C. violaceae*, whereas they also reported the presence of chlorogenic acid and naringin. On the other hand, Piwowarczyk et al. (4) identified polyphenolic compounds based on MS and MS/MS data of *C. armena*, revealing phenylethanoids and their glycosides in morphological parts (flowers and stem with tubers separately). In the phytochemical study carried out by Beladjila et al. (9) on *C. phelypaea n*-butanol extract, four new phenylethanoid glycosides (1-β-p-hydroxyphen-yl-ethyl-3,6-di-α-L-rhamnopyranosyl-4-p-coumaroyl-β-D-glu-copyranoside, 1-β-p-hydroxyphenyl-ethyl-3,6-O-di-α-L-rhamnopyranosyl-β-D-glucopyranoside, 1-β-p-hydroxy-phenyl-ethyl-2-O-acetyl-3,6-di-α-L-rhamnopyranosyl-4-p-coumaroyl-β-D-glucopyranoside and 1-β-p-hydroxy-phenyl-ethyl-2-O-acetyl-3,6-di-α-L-rhamnopyranosyl-β-D-glu-copyranoside) and the three known compounds brandioside, pinoresinol 4-O-β-D-glucopyranoside and apigenin 7-O-β-glucuronopyranoside were reported. This last compound was also revealed in our study.

Several studies have indicated that many of the identified compounds in our extracts have been isolated from various plants and have a wide range of biological effects, including anti-inflammatory, antioxidant, antimicrobial, antitumor, and others (27-35).

From the 32 identified compounds, ten were found in our six extracts, namely ascorbic acid, gallic acid, *para*-hydroxybenzoic acid, 1, 2 dihydroxybenzene, sinapic acid, vanillin, salicylic acid, naringenin-7-glucoside, coumarin, and m-anisic acid. These compounds were studied by many researchers and reported to possess many biological and pharmacological effects. Ascorbic acid, which was recorded in all analyzed extracts, is a known potent antioxidant and free-radical scavenger. It acts primarily as a donor of single hydrogen atoms, and the radical anion monodehydroascorbate reacts mainly with radicals (29). Gallic acid is a low molecular weight tri-phenolic molecule that has outstanding anti-inflammatory, antioxidant, antitumor, antidiabetes, antiobesity, antimicrobial, and anti-myocardial ischemia properties (31). *p*-hydroxybenzoic acid and its derivatives have been reported by Singab et al. (33) to have potential antimicrobial, antiviral action against SARS-CoV-2, antimutagenic, anti-inflammatory, antioxidant, hypoglycemic, antiestrogenic, and antiplatelet aggregating activities. This compound can be chemically synthesized or recovered from various plants (33). Sinapic acid is a prevalent component of the human diet since it is found in a wide variety of dietary and medicinal plants. The antioxidant, antibacterial, anti-inflammatory, anticancer, and antianxiety properties of sinapic acid are demonstrated by Nićiforović and Abramovič (27). This compound has been recommended for potential use in food processing, cosmetics, and the pharmaceutical industry, mostly due to its antioxidative action (27). Vanillin is a well-known natural aromatic flavoring compound that is widely

used in foods, drinks, pharmaceuticals, perfumes, and cosmetics. Many studies have documented the pharmacological effects of vanillin, including its diuretic, anticancer, antidiabetic, antioxidant, antisickling, antibacterial and anti-inflammatory properties (34). Salicylic acid is a phenolic derivative that is widely present in plants. It is known to be a regulator of several physiological and biochemical processes, including thermogenesis, plant signalling or plant defense, and response to biotic and abiotic stresses. Salicylic acid plays an important role in protection against pathogens and has anti-inflammatory and antioxidant effects (28). Coumarins are naturally and widely occurring in plants and have been reported to possess varied biological actions like antitumor, anti-HIV, antimicrobial, anti-inflammatory and anticoagulant properties, as well as triglycerides lowering and stimulant effects on the central nervous system (30). *p*-hydroxybenzaldehyde found in the maceration of the aqueous extract and the ultrasound for both aqueous and ethanolic extracts has various pharmacological effects, such as antioxidant, anti-inflammatory, vasodilation effects, and a protective effect on transient focal cerebral ischemia in normal rats (35). Moreover, it has been demonstrated that other polyphenols reported in different plants, including quercetin, apigenin, syringic acid, caffeic acid, rutin, and ferulic acid, boost the plant's antioxidant capacity by altering antioxidant processes and preventing lipid peroxidation (32). Some of the identified compounds were not found in the obtained extracts from our study. This can be explained by the fact that the production of secondary metabolites by plants is a very complex process that depends on many factors, including internal developmental genetic circuits (regulated genes and enzymes), as well as aspects related to external environmental factors (light, temperature, water, salinity, etc.) (36).

On the other hand, some of the peaks were not identified in our study. This is due to the lack of standards; thus, it could be interesting to search for phenylethanoid glycosides as Deyama et al. (37) revealed their isolation from many *Cistanche* species, including *C. deserticola*, *C. salsa*, *C. tubulosa*, *C. sinensis* and *C. phelypaea*.

4.2. Biological Activities Study

It is known that plants develop defense mechanisms to survive in their ecosystems, and hence, they represent a rich source of antimicrobial agents and other compounds of pharmaceutical interest. As the biological activities of *C. violaceae* are largely unknown and not all its active substances are identified, we assessed the antioxidant (DPPH, ABTS, reducing power and phenanthroline) and antimicrobial capacities of six different extracts.

Many antioxidant tests are employed for the assessment of the antioxidant properties of plant extracts. This is caused by the complicated composition of bioactive molecules, which react differently to different assay techniques (38). Four *in vitro* antioxidant assays were employed in our study to assess the scavenging and reducing the potential of the aqueous and ethanolic extracts from *C. violaceae* for this purpose. The obtained results showed mild antioxidant capacities of the analyzed extracts. The ethanolic extracts exhibited important antioxidant activities compared to the aqueous extracts, with the highest activity recorded for the ABTS test (IC_{50} values of < 12.5 µg/mL). These differences in the antioxidant activity between the extracts could result from the difference in the composition (39).

Generally, the antioxidant activity of our extracts was of average values, which was comparable to the obtained results of Alia et al. (11). Ben Attia et al. (13) evaluated the antioxidant activity by DPPH, ABTS and Ferric Reducing Antioxidant Power (FRAP) assays of hexane, ethyl acetate, acetone, methanol, and water extracts obtained by sequential maceration. Their results revealed that the highest antioxidant activity, based on all the tests combined, was obtained with *C. violacea*. Bouzayani et al. (14) used five complementary methods to assess the antioxidant activity of *C. violacea*, namely DPPH, ABTS, Nitric Oxide (NO), Total Antioxidant Capacity (TAC), and FRAP. These authors showed that most tested extracts displayed notable antioxidant capacities for aerial and underground parts. Our results are not comparable with those obtained by Debouba et al. (12), who reported that *Cistanche* flowers had the highest free radical scavenging activity and the strongest reducing power while *Cistanche* bulb was the most efficient in chelating ferrous ions.

Different extracts made from other *Cistanche* species presented relevant antioxidant properties. For example, Rahim et al. (38) showed that the aqueous extract of *C. phelypaea* roots presented an interesting antioxidant potential related to the synergistic effects of several antioxidant compounds. Similarly, Aboul-Enein et al. (40) tested the DPPH scavenging abilities of the aqueous and ethanolic extracts from the whole *C. phelypaea* plants from Egypt and found that the aqueous extract produced better results. Moreover, the water extracts of roots and flowers exhibited the highest antioxidant capacity for *C. phelypaea*, which was collected from Ludo, Ria Formosa (Portugal) (41). According to the study of Wang et al. (42), all tested extracts had good dose-dependent inhibitory activity against the DPPH radical test. However, all of them were less potent than the reference antioxidants Trolox and ascorbic acid for *C. deserticola* from China.

For the antimicrobial activity, only one extract showed mild antibacterial activity against *S. aureus*. This last, when compared to the positive control that was tested under identical conditions, was less active in absolute terms with a high significant difference.

The activity against *S. aureus* can be explained by the presence of phenylethanoid glycosides, as suggested by Deyama et al. (37), who isolated them from many *Cistanche* species, including *C. deserticola*, *C. salsa*, *C. tubulosa*, *C. sinensis* and *C. phelypaea* and reported their anti-*Staphylococcus* activity.

Our results corroborate those obtained by Hamed and El-Kamali (43) for the methanolic extract of *C. phelypaea* roots that did not show any activity

against the tested bacteria. On the other hand, Keymanesh et al. (44) revealed mild antimicrobial activity of hydro-alcoholic extract *of C. tubulosa* aerial parts collected in Iran. The only antibacterial activity of *C. violacea* methanol and ethanol extracts was recorded against *S. epidermidis* ATCC® 12228™ in the study of Abdallah (15), while for the other microorganisms, the results showed weak activity or not at all. In contrast to our results, the study of Ullah et al. (45) revealed that the crude methanolic extract and aqueous, chloroform, *n*-hexane, *n*-butanol and ethyl acetate fractions of *C. tubulosa* from Pakistan were active against six tested bacterial strains. Also, the butanolic extract of *C. tubulosa* at concentrations of 2 and 4 mg/mL showed mild inhibition against *E. coli* (46). Bouzayani et al. (14) revealed that the aerial parts and underground stems of *C. violacea* had good efficiency against the tested bacterial strains. These authors showed that the methanolic extract at the concentration of 10 mg/mL was active against *S. aureus* with an inhibition zone of 15 mm.

4.3. Correlation

The DPPH and ABTS assays use a similar mechanism of action, which is a scavenging activity that may be the reason for the correlation between them. Similar studies have reported a strong relationship between the antioxidant capacity using these tests, including Bouzayani et al. (14), Fidrianny et al. (47) and Ali et al. (48). Also, Wang et al. (49) showed that the different antioxidant tests like DPPH scavenging capacity, Trolox Equivalent Antioxidant Capacity (TEAC), and RP correlated well with each other. Therefore, the antioxidant effect of the extracts may be assigned to phenolic compounds and flavonoids. Furthermore, positive correlations were observed in the study of Bouzayani et al. (14) between the TPC, TFC and CT and antioxidant activity of *C. violacea*. Other studies reported that the mechanism of action of flavonoids is through scavenging or chelating processes (50). In addition, Kalia et al. (51) and Amri et al. (52) reported that the potential antioxidant activity of an extract depends on its content in polyphenols and flavonoids.

5. CONCLUSIONS

This study was conducted to valorize a parasitic plant from the south of Algeria, *C. violaceae*. This research revealed that the plant had phenolics and flavonoids contents, antioxidant and antimicrobial activities. Furthermore, statistical methods using HCA, PCA, and Pearson correlation were employed based on the results to highlight the existing correlation between the TPC, TFC and antioxidant activity. Furthermore, the composition of the parasitic plant, identified using HPLC analysis, testifies to the plant's potential properties as for example, the presence of active ingredients, including ascorbic acid, that possesses a strong antioxidant activity. Thus, our findings support the role and value of the parasitic plant *C. violaceae* as a source of bioactive compounds from the harsh climate conditions of Ghardaia Province (Algeria). Further studies on the isolation and purification of bioactive components from this plant are highly recommended to verify their practical use.

6. CONFLICT OF INTEREST

There is no actual or potential conflict of interest in relation to this article.

7. ACKNOWLEDGMENTS

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