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Antioxidant capacity and total phenols richness of *Cistanche violacea* hosting *Zygophyllum album*

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Abstract

Cistanche violacea is an Orobanchaceae holoparsite hosting chenopodiaceous, including Zygophyllum album. While *Cistanche violacea*, is known to have edible uses in the folk medicine, no data have been reported before. In the present study, chemical assays were undertaken in order to emphasize the *Cistanche violacea* antioxidant capacity and total phenols richness. Our data showed higher phenolic contents were measured in Cistanche flowers and Zygophyllum leaves, however Cistanche bulb was the most enriched in flavonoïds. Antioxidant activities were evaluated in methanolic extract of *Cistanche violacea* flowers and bulb among the host *Zygophyllum album* leaves. Cistanche flowers and Zygophyllum leaves exhibited the strongest scavenging 2,2-diphenyl-2-picrylhydrazyl radical (DPPH) activity. The metal chelating activity was the highest in Cistanche bulb and Zygophyllum leaves compared to Cistanche flowers. Conversely, the ferric reducing power was significantly higher in Cistanche flowers relative to its bulb and Zygophyllum leaves.

Keywords: *Cistanche violacea*, *Zygophyllum album*, ferric reducing power, metal chelating activity.

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Introduction

Many spontaneous aromatic and medicinal plants have very interesting biological properties, which find application in various fields, namely in medicine, pharmacy, cosmetics and agriculture. However, the assessment of properties as phytotherapeutic antioxidant remains limited, especially for less used or unknown plants such as holoparasites species (Orobanchaceae). Among these species is *Cistanche violacea*, a plant considered to be medicinal, but unfortunately not devoted by any accurate scientific consideration. Besides, this species became endangered in same country because of over exploitation for folk therapeutic and medicinal uses [1].

It was shown that Cistanche species contain higher amounts of bioactive compounds compared to other plants. Recently, Yamada et al. [2] described a first report on antiallergic activity of acteoside extracted from *Cistanche tubulosa* on basophilic cells. The influence of the *Cistanche tubulosa* extract on the proliferation of human fibroblasts have shown that it can prevent brain aging and may accelerate the transformation of fibroblasts neurons, suggesting that it can actually prevent brain aging and improve the functioning of the brain [3]. It was found that crude ethanol and water extract of *Cistanche deserticola* possessed sedative effect through prolonging the hexobarbital-induced sleeping time and reduce spontaneous motor activity in mice [4].

While, *Cistanche tubulosa* and *salsa* were well investigated, no available data on *C. violacea* could be found in the literature. The present work is a contribution to highlight the chemical composition and antioxidant properties of *Cistanche violacea* extracts and its host *Zygophyllum album* collected from Tunisian seashore.

Materials and Methods

Plant collection and essential oil extraction

Plants were collected in the South-East of Tunisia from seashore of Hassi Jerbi village located in the South-East of Tunisia (Latitude: 33°38'13.34"N, Longitude: 11° 0'22.49"E). The flowers and bulb of *Cistanche violacea* and leaves of *Zygophyllum album* were air-dried on the shadow, until weight stability (three weeks).

Methanolic extract preparation

Samples of 1 g of dried matter from each plant organ were finely ground using a homogenizer and extracted with 80% methanol at room temperature for 24 h. Each mixture was then filtered through Whatman No. 42 filter paper to remove the debris, and the extracts were then evaporated using a rotary evaporator. The crude extracts were suspended in 80% methanol.

Total Polyphenols and flavonoïds determination

Total phenolic content was determined by the Folin-Ciocalteu method modified by Heimler et al. [5] using gallic acid (GA) as phenolic standards, and expressing the results as gallic acid equivalents per g dry weight (mg GA/g DW). A slightly modified version of the spectrophotometric method [6] was used to determine the flavonoïds contents of samples. Flavonoïds contents were then calculated using quercetin (Q) as standard. Total flavonoïds content was expressed as mg quercetine/g dry weight (mg Q/g DW).

DPPH radical-scavenging assay

DPPH scavenging activity was measured by the slightly modified spectrophotometric method of Brand-Williams et al. [7]. An aliquot (3 ml) of freshly prepared DPPH solution was mixed with 100 ml of the samples at varying concentrations (25-500 µg/ml). The solutions in the test tubes were shaken well and incubated in the dark for 15 min at room temperature. The decrease in absorbance (A) was measured at 517 nm. The antiradical activity was expressed as IC_{50} (µg/mL), the antiradical dose required to induce a 50% inhibition. The percentage inhibition of the radicals due to the antioxidant property of the extracts was calculated using the formula: $\%inhibition= [(A_{control} - A_{sample})*100]/A_{control}$

Metal chelating activity

The chelating of ferrous ions by plant extracts was estimated as described by Zhao et al. [8]. Briefly, different concentrations of plant part extracts were added to a 0.05 mL $FeCl_2$, $4H_2O$ solution (2 mmol/L) and left for incubation at room temperature for 5 min. After the reaction was initiated by adding 0.1 mL of ferrozine (5 mmol/L), the mixture was adjusted to 3 mL with deionised water, shaken vigorously, and left standing at room temperature for 10 min. Absorbance (A) of the solution was then measured spectrophotometrically at 562 nm. The metal chelating activity was expressed as IC_{50} (µg/mL), the dose required to induce a 50% inhibition. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula: $\%inhibition= [(A_{control} - A_{sample})*100]/A_{control}$

Ferric-reducing activity

The reducing power of plant extracts was determined by the method of Yildirim et al. [9]. Sample solutions (0.5 ml) with different concentrations (25-500 µg/ml) were mixed with 1.25 ml of 0.2 M phosphate buffer (pH 6.6) and 1.25 ml of (10 g/l) potassium ferricyanide solution. The mixtures were incubated for 30 min at 50°C. After incubation, 1.25 ml of (100 g/l) trichloroacetic acid were added and the reaction mixtures were centrifuged for 10 min at 3000g. A 1.25 ml aliquot of the supernatant from each sample mixture was mixed with 1.25 ml of distilled water and 0.25 ml of (1.0 g/l) ferric chloride solution in a test tube. After a 10 min reaction time, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

Statistical analysis

Each experiment was conducted at least in twice. Data are means of three replicates ± SD followed at $P < 0.05$ according to Tukey test.

Results and Discussion

Total polyphenols and flavonoïds contents

Metabolites analysis showed that *C. violacea* flowers were the most enriched in total phenolic compounds compared to its bulb and *Zygophyllum album* leaves (Table 1). Lowest contents were measured in Cistanche bulb (115.83 mg GA/g DW), suggesting that bulb may constitute an overpass transporting phenols from Zygophyllum leaves to Cistanche flowers.

Conversely, flavonoïds contents were the lowest in Cistanche flowers relative to its bulb and Zygophyllum leaves (Table 1). We measured 3-fold and 2-fold higher contents of flavonoïds in Cistanche bulb and Zygophyllum leaves compared to Cistanche flowers, respectively. It seems that *C. violacea* bulb acts as sink of flavonoïds to be used during growth by aerial part of Cistanche.

Free radical scavenging activity

In the present work, DPPH was used to evaluate the free radical scavenging activity of different plant extracts. DPPH is a stable free radical, which accepts an electron or hydrogen radical to become a stable which does not initiate or propagate oxidation of the lipids [10]. Our results showed that all tested methanolic extracts behave an important free radical scavenging activity (Table 2). The highest activity was observed in Cistanche flowers and Zygophyllum leaves with IC_{50} ranging from 5.65 to 6.38 µg/mL (Table 1).

Higher DPPH scavenging activity in Cistanche flowers and Zygophyllum leaves relative to Cistanche bulb may be related to differential phenols richness in these organs. In fact, according to Table 1, phenols were about 3 to 4-fold higher in Cistanche flowers and Zygophyllum leaves compared to Cistanche bulb. Indeed, phenols are known to be responsible for the free radical scavenging and antioxidant activities of plants [11, 12]. Phenolic compounds can act as proton donating and show radical scavenging activity [13]. Sroka and Cisowski [14] found that the antioxidant and anti-radical activity of phenolic acids correlated positively with the number of hydroxyl groups bonded to the aromatic ring and that phenolic acids with three hydroxyl groups in an ortho position (such as gallic acid) have the strongest anti DPPH scavenging activity.

The observed free radical scavenging activity measured in Cistanche bulb extract was rather due to flavonoïds accumulation (Table 1). Antioxidant properties of flavonoïds have been attributed to their possible effects on intracellular redox status [15]. The precise mechanisms by which flavonoïds remain unclear while recent studies have speculated that their classical hydrogen-donating [16].

Metal chelating activity

Metal chelating activity is one of the most important antioxidant process occurring in plant cell, since iron at ferrous state (Fe^{2+}) is known to be the powerful prooxidant among the various species of metal ions [17]. In the present assay, ferrous ion chelating activity increased progressively with increasing extract concentration (Table 1).

Table 1: Total polyphenols and flavonoïds contents, DPPH scavenging activity and metal chelating activity of *Cistanche violacea* flowers, bulb and its host (*Zygophyllum album*) leaves. Each point is the average of three replications ± SD. Means sharing at least one same letter are not significantly different according to Tukey test at P<0.05.

Plant tissues	*Cistanche violacea*		*Zygophyllum album*
Parameters	Flower	Bulb	Leaves
Phenolic content (mg GA/g DW)	394.00a	115.83b	296.83c
Flavonoïds contents (mg Q/g DW)	94.69a	287.19b	216.41c
DPPH· scavenging activity (IC_{50} µg/ml)	6.38a	17.15b	5.65c
Metal chelating activity (IC_{50} µg/ml)	638.57a	354.53b	220c

We found that leaves extract of the host plant exhibited the highest metal chelating activity, except that at 500 µg/mL. Cistanche bulb showed the strongest activity and lowest one was measured in Cistanche flowers (Fig. 1B). Again, we could not correlate metal activity pattern to phenolic contents (Table 1). Wong et al. [18] mentioned a poor correlation of cupric ion chelating activity with total phenol of all tested plants and this might indicate that phenolic compounds could not be the main chelators of copper ions.
Rather, we suggest that metal activity could be related to flavonoïds contents (Table 1) since they showed similar pattern within tested organs. Free hydroxyl groups in the flavonoïds ligands chelating the central metal ion may scavenge free radicals [18]. As antioxidants, flavonoïds have been reported to be able to quench free radicals, chelate transition metals and rendering them redox inactive in the Fenton reaction [19].

Ferric reducing power

The reducing power of different tested plant extracts is based on their ability to transform Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). This transformation is followed by an increasing optic density at 700 nm. Our results showed that the ferric reducing power of all extracts increased concentration dependently (Fig. 1). Significantly higher reducing power could be observed in Cistanche flowers extracts starting form 100µg/ml relative to its bulb and Zygophyllum leaves (Fig. 1). Extracts of Cistanche bulb and Zygophyllum leaves showed comparable reducing power at higher concentration (Fig. 1). It seems that Cistanche flowers are the main source of reductones among the other investigated organs. Meir et al. [20] showed that these reductones exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom.

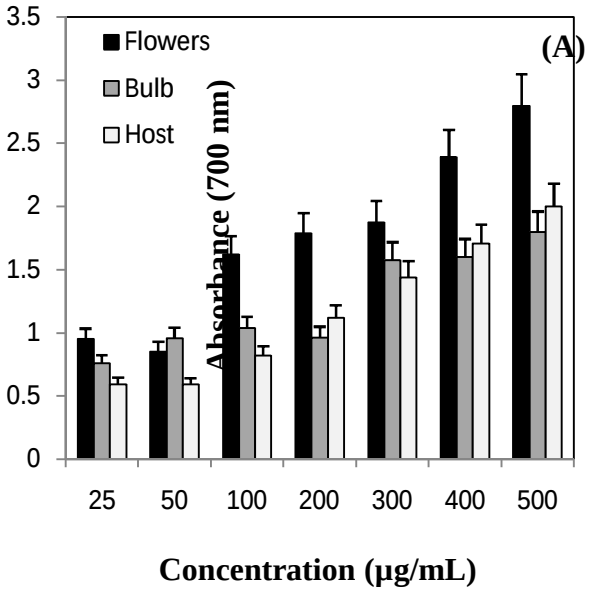

Figure 1: Pattern of Ferric reducing power of *Cistanche violacea* extracts (flowers and bulb) and its host (*Zygophyllum album*) leaves. Data are means of at least three replicates ± SD followed at *P* <0.05.

It could be stated that while Cistanche flowers and Zygophyllum leaves have similar phenolic contents and free radical scavenging activity (Table 1), Cistanche flowers exhibited higher reducing power than Zygophyllum leaves (Fig. 1). The lower reducing power in Zygophyllum leaves could be due the presence of phenolic compounds that are more efficient in reducing DPPH than Fe^{3+}/ferricyanide complex. In fact, the efficiency of phenolic compounds as anti-radicals or reductones depends on the number of hydroxyl groups bonded to the aromatic ring, the site of bonding and mutual position of hydroxyls in the aromatic ring [21, 14].

Conclusion

We are persuaded that obtained results will contribute to give further clarification into antioxidant proprieties and mechanism of *C. violacea* and its host *Zygophyllum album*. Comparing *C. violacea* extracts among host one reveled that (i) Cistanche flowers and host leaves have the highest free radical scavenging activity, (ii) Cistanche flowers exhibited the strongest reducing power, (iii) Cistanche bulb and host leaves were the most efficient in chelating ferrous ions. Particularly, the high reducing power of Cistanche flowers may underline specific reductones that would be synthesized in parasite cells and not sucked from the host plant.

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