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The comparison of the antioxidant capacity of methanol extract in three species of Artemisia (A. sieberi Besser, A. aucheri, and A. deserti Krasch)

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Abstract

Artemisia deserti Krasch (*A. deserti*), *Artemisia aucheri* (*A. aucheri*) and *Artemisia sieberi* Besser (*A. sieberi*) are three members of Asteraceae (compositae) family, which grow widely in the even and high areas of Birjand, Iran. This study has attempted to compare the total antioxidant capacity of methanol extracts of these three plants using DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging assay in which spectrophotometry method was used at 517 nm. The results showed that the aerial parts (AP) of *A. sieberi* has the highest total antioxidant capacity (IC_{50}=11.054 mg/mL). The lowest amount of antioxidant capacity was found in the root (R) of A. *aucheri* (IC_{50}= 91.408 mg/mL).

Keywords: Antioxidant capacity; Artemisia sieberi Besser; Artemisia aucheri; Artemisia deserti Krasch; DPPH.

Introduction

Free radicals, which are generated in the human body as consequences of a number of endogenous metabolic processes involving bio-energetics electron transfer, redox enzymes and exposure to the plethora of exogenous chemicals, can cause many oxidative stress-mediated disease conditions such as cancer, atherosclerosis, diabetes, inflammation and aging [1]. Antioxidants may mediate their effect by directly reacting with free radicals, quenching them

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and chelating the catalytic metal ions [2]. By inhibiting the initiation or propagation of oxidizing chain reactions, antioxidants can delay or inhibit the oxidation of lipids or other molecules [3]. The antioxidative effect of plants is due to the phenolic compounds such as flavonoids, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [4 and 5]. Recent epidemiological studies have shown that fruits, vegetables and medicinal plants which are rich in phenolic compounds are able to reduce incidence of cardiovascular and chronic diseases like cancer [6]. Among two basic categories of antioxidant (synthetic and natural ones), the use of synthetic anti-oxidants because of their carcinogenicity is restricted [7 and 8]. Therefore, replacing the natural antioxidants instead of synthetic ones on the part of the preventive medicine are of interest. In many cases, methanol and ethanol have been extensively used to extract antioxidant compounds from various plants and plant-based foods (fruits, vegetables, etc.). Specially, plant parts extracted from methanol has considerable antioxidant property [9 and 10].

The genus *Artemisia*, small herbs and shrubs, is one of the largest and most widely distributed genera of the Asteraceae (or Compositae) family [11and12]. The members of this genus have botanical and pharmaceutical interest due to their characteristic scent or taste and use in the liqueur-making industry [11and13]. *A. deserti, A. aucheri* and *A. sieberi* are three members of this family (Asteraceae) which widely grow in the even and high areas of Birjand, Iran. In this study, we tried to compare the total antioxidant capacity of methanol extract of three species of *Artemisia* (*A. deserti, A. aucheri* and *A. sieberi*) by using DPPH radical scavenging assay in which spectrophotometry method was used.

Experimental

General

Methanol was purchased from Merck Company and DPPH was purchased from Sigma-Aldrich Company. The absorbance was read using UV-Win X-ma 2000 spectrophotometer.

Plant material

A. deserti, A. aucheri and *A. sieberi* were collected in July 2013 from South Khorasan province, Iran. The AP and R of these plants were separated, washed, shade dried in air and ground in a mixer [14].

Plant extract

The antioxidant activity of plant extracts (*A. deserti, A. aucheri* and *A. sieberi*) was

estimated in the terms of DPPH free radical scavenging activity [15]. One gram of each plant powder was added to 20 mL of 80% methanol and the mixture was sonicated for 45 min, incubated for 15 min in the dark at ambient temperature and centrifuged for 15 min at 14000 rpm to obtain clear extract.

Determination of antioxidant activity

The antioxidant activity of *A. deserti, A. aucheri* and *A. sieberi* was performed according to the method described previously with some modifications [16]. Briefly, 0.5 mL of each plant extract was added to 0.5 mL of 80% methanol to obtain sample with twofold concentration. Samples were serially diluted 1/4 (0.5 mL of 80% methanol was added to the twice diluted extract), 1/8, 1/16 and 1/32. Then, 200 µL of each plant extract was added to the methanol solution of DPPH (4 mL, 6×10^{-5} M) and incubated in a dark environment for 60 minutes.

Blank sample was prepared from the mixture of the same amount of methanol and DPPH solution and measured at 517 nm [17 and 18]. Radical scavenging activity was calculated by the following formula [19]:

Inhibition (%)= $[(A_B- A_A)/ A_B] \times100$

Note: A_B—the absorption of blank sample;

A_A—the absorption of extract solution.

Then, the results are reported as the percentage of inhibition or neutralization of the DPPH (IC_{50}-scale).

Results and discussion

Biological and chemical research in Life Science evidenced that free radical and reactive oxygen species can be involved in a high number of diseases [20]. Numerous physiological and biochemical processes in the human body may produce oxygen centered free radical and other reactive oxygen species and byproducts. Overproduction of such free radical causes oxidative damage to biomolecules leading to many chronic diseases [21]. The DPPH assay is technically simple, rapid and needs only a UV-Vis spectrophotometer that might explain its widespread use in antioxidant screening [22]. The DPPH free radical with odd electron has a strong absorption maximum at 517 nm and is purple in color [23]. Decrasing in the molar absorptivity of DPPH free radical from 9660 to 1640 changes the color of DPPH free radical from purple to yellow at 517 nm (Figure 1). This is due to the acceptance of an electron or hydrogen radical from an antioxidant compound forming a stable diamagnetic spin paired molecule [24]. Thus, reducing the optical density proportional to the ability to neutralize free radicals and DPPH shows the antioxidant power of the sample.

Figure 1. Color change of methanol extracts

The scavenging activity of the DPPH radical was found to be strongly dependent on the concentration of extract [25]. Figure 2 shows the linear correlation between the percentage of DPPH radical scavenging and the concentration of different extracts. As shown in Figure 2, the percentage of DPPH inhibition depends directly on the concentration of extracts.

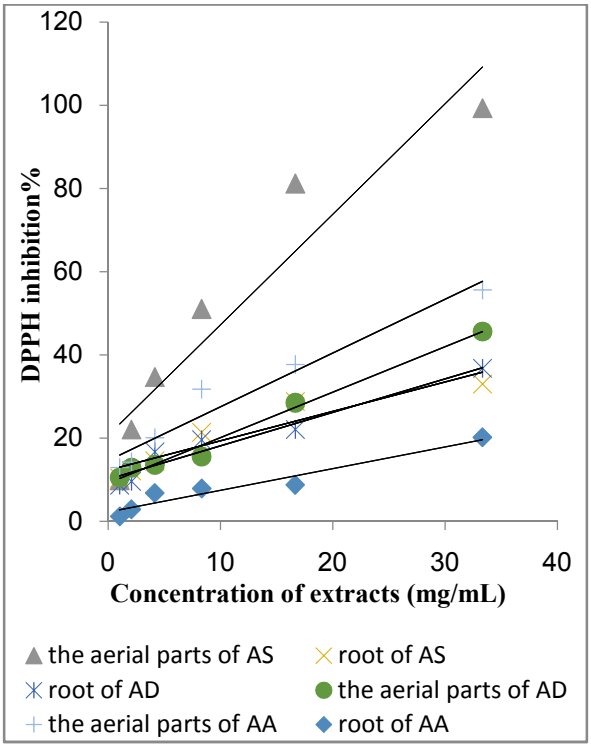

Figure 2. Linear correlation between the percentage of DPPH radical inhibition and the concentration of different extracts

Table 1 shows the antioxidant capacity of antioxidant activity of *A. deserti, A. aucheri* and *A. sieberi* (IC50-scale). The scale indicates the concentration of the extract that scavenging 50% of DPPH radical activity. As the results show in Table 1, the antioxidant activity of AP of all three species is higher than Rs. The variation of free radical scavenging activity may be due to the differences in their secondary constituents [26].

Moreover, the AP of *A. sieberi* has the highest total antioxidant capacity (IC_{50}=11.054 mg/mL). The lowest amount of

antioxidant capacity is in the R of *A. aucheri* (IC_{50}= 91.408 mg/mL).

Table 1. Antioxidant activity of methanol extracts

Plant	Organ	IC_{50} (mg/mL)
A. sieberi	AP	11.054
	R	53.336
A. deserti	AP	37.359
	R	49.579
*A. aucher*i	AP	27.369
	R	91.408

We also compared the antioxidant activity of these plant extracts with the literature. As shown in Table 2, the antioxidant activity of some plants such as *A. absinthium* and *Sambucus niagr* is the highest (Table 2, Entries 1 and 2), but the antioxidant activity of *A. sieberi* is higher than the other reports (Table 2, Entry 10).

Table 2. Antioxidant activity of various extracts of plant materials

Entry	Plant	IC_{50}(mg/mL)	Ref.
1	*A. absinthium*	5.87	27
2	*Sambucus niagr*	10	28
3	*Arnica montana*	100	28
4	*Sidastrum micranthum*	111.123	29
5	*Chenopodium quinoa*	22.4	30
6	*Amaranthus hypochondriacus*	13.6	30

7	*Amaranthus cruentus*	15.9	30
8	*A. deserti*	37.359	This work
9	*A. aucheri*	27.369	"
10	*A. sieberi*	11.054	"

Conclusion

The results which are shown in Table 1 indicated that the aerial parts of all three species have antioxidant activity higher than roots. Moreover, the aerial parts of *A. sieberi* (IC_{50}=11.054 mg/mL) has the highest antioxidant activity and the root of *A. aucheri* (IC_{50}=91.408 mg/mL) has the lowest antioxidant activity.

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