

Antioxidant capacity and total phenol and flavonoid contents of *Teucrium polium* L. grown in Algeria

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Abstract.

BACKGROUND: *Teucrium polium* L. aerial parts are traditionally used in Algerian folk medicine due to its therapeutic properties.

OBJECTIVE: The present study was designed to evaluate the effect of different extracting solvents (methanol, ethanol, ethyl acetate, chloroform, hexane and water) on antioxidant capacity and total phenol and flavonoid contents of Algerian *Teucrium polium* aerial parts.

METHODS: The antioxidant capacity of *Teucrium polium* extracts was assessed by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) free radicals scavenging and reducing power assays. For each extract, the total phenol and flavonoid contents were investigated by the Folin-Ciocalteu and aluminium trichloride methods, respectively.

RESULTS: All the tested extracts showed an appreciable total phenolic and total flavonoid contents and strong antioxidant capacity. Among the different extracts, the methanolic extract was found to be containing the highest amount of total phenols (206.95 ± 1.82 mg of gallic acid equivalent per gram of extract) and flavonoids (42.16 ± 0.61 mg of quercetin equivalent per gram of extract). The same extract also exhibited a much better reducing power and scavenging capacity against DPPH ($IC_{50} = 6.77 \pm 0.15$ mg/l) and ABTS ($IC_{50} = 2.79 \pm 0.07$ mg/l).

CONCLUSION: These findings suggest that aerial parts of *Teucrium polium* methanolic extract could be used as natural source of antioxidants in food, pharmaceutical and cosmetic industry.

Keywords: *Teucrium polium* L., extracts, effect of solvent, antioxidant capacity, total phenol content, flavonoid

1. Introduction

Oxidative stress is defined in general as excess formation and/or incomplete removal of highly reactive molecules such as reactive oxygen species (ROS) [1]. Free radicals are produced in the human body from normal metabolism or induced by physical and/or chemical factors in the environment [2]. Oxidative radicals are reported to be a potential cause of mutations, damage to lipids, DNA and proteins which in turn lead to certain disorders including cancer [3]. The use of substances with antioxidant ability can be very important in the

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therapeutic prevention of diseases related to increased oxidative stress, such as cancer, heart disease and aging [4]. Antioxidants that have traditionally been used to inhibit oxidation in foods also quench dreaded free radicals and stop oxidation chains *in-vivo*, so they have become viewed by many as nature's answer to environmental and physiological stress and other disorders [5]. ROS can induce peroxidation of lipids generating secondary oxidants such as heptanol and hexanal, which contributes to oxidative rancidity, deteriorating the flavor of food [6]. The synthetic antioxidants used in the food industry namely, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), Tertiary-butyl hydroquinone (TBHQ) and propyl gallate (PG) are suspected to be toxic and carcinogenic. Recently, more attention has been given to medicinal plants of therapeutic potentials as antioxidants in reducing free radical induced tissue injury [7]. The antioxidant ability of many plants may be attributed to the presence of phenolic compounds includes phenolic acids (hydroxybenzoic and hydroxycinnamic acids), tannins (hydrolyzable and condensed), and flavonoids [8]. Polyphenols are natural substances capable to neutralise free radicals and reduce the oxidative stress damage on the human body [9]. Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents [10]. Therefore, there is an increasing interest in extracting these plant antioxidants and using them as natural antioxidants [11]. Extraction is the first step in analysis polyphenolic, which consists in isolation of phenolic compounds from plant materials [12]. One of the most important factors affecting the extraction efficiency of bioactive compounds from plant materials and their consequent health benefits is the extraction solvent [13].

The genus *Teucrium* (Lamiaceae) contains more than 340 species spread all over the world, twenty of which are that found abundantly in the flora of Algeria [14]. *Teucrium polium* L. (Feltly germander) called 'kheyata' is one of the species from this genus that grows wildy in Algeria. This species was traditionally used in Algerian folk medicine due to its hypoglycemic, hypolipidemic, anti-inflammatory, antibacterial and antioxidant properties [15–19]. Previous studies have described *Teucrium polium* extracts as a potential source of natural antioxidants and phenolic compounds [20–23]. There is no report in the literature on the impact of different solvents extraction including methanol, ethanol, ethyl acetate, chloroform, hexane and water on antioxidant capacity and amount of total phenol and flavonoid of Algerian *Teucrium polium* aerial parts.

The main objective of this study was to determine the effect of different extracting solvents on total phenolic and flavonoid contents and antioxidant potency of *Teucrium polium* aerial parts grown in Algeria. The total phenol and flavonoid contents of the investigated extracts were screened using the Folin-Ciocalteu and aluminium trichloride methods, respectively. The antioxidant capacity of the all extracts was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) free radicals scavenging capacity and reducing power.

2. Materials and methods

2.1. Plant material

The aerial parts of *Teucrium polium* were collected at flowering stage from Oum El Bouaghi (east of Algeria) during March 2015. The taxonomic identification of the plant was performed by the botanists of the High National School of Agronomy of Algiers, Algeria. The plant material was air-dried in the shade at room temperature.

2.2. Preparation of extracts

Dried and powdered aerial parts (30 g) from *Teucrium polium* were extracted with 400 ml of six different solvents (methanol, ethanol, ethyl acetate, chloroform, hexane and water) in a Soxhlet apparatus for 6 h. The solvent was evaporated using a rotary evaporator. Each extract was weighed and kept at 4 °C in the dark before analysis.

2.3. Determination of total phenol content

The total phenol content in the examined plant extracts was determined using Folin-Ciocalteu method described by Singleton et al. [24]. 0.25 ml of extract dissolved in methanol was mixed with 1.25 ml of Folin-Ciocalteu. After 3 min of reaction, 1 ml of solution of sodium carbonate (Na_2CO_3) at a concentration of 75 g/l was added. The samples were incubated for 30 min in the dark at room temperature. The absorbance was determined using spectrophotometer at 765 nm. Gallic acid was used as standard for the calibration curve. Total phenol content was expressed as milligrams Gallic Acid Equivalent (GAE) per gram of extract.

2.4. Determination of total flavonoid content

The flavonoid content in each extract was determined by aluminium trichloride (AlCl_3) method as described by Lamaison et al. [25] using quercetine as a reference compound (standard). The sample contained 1 ml of extract dissolved in methanol and 1 ml of solution of aluminium trichloride (2% w/v). The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at 420 nm. The flavonoid content was expressed as mg of Quercetin Equivalent (QE) per gram of extract.

2.5. Antioxidant capacity

2.5.1. Determination of DPPH radical scavenging capacity

The radical scavenging capacity of the examined extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. All the extracts were tested for the concentration of 1, 3, 5, 10, 20, 50 and 100 mg/l. 25 μl of various concentrations of the sample in methanol were added to 975 μl of methanolic solution of DPPH (60 μM). After 30 min incubation time in darkness at room temperature, the absorbance measurements were recorded at 517 nm. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Scavenging capacity of DPPH radical was calculated as follows:

$$\% \text{ of scavenging of DPPH radical} = [(\text{Abs blank} - \text{Abs sample}) / \text{Abs blank}] \times 100 \quad (1)$$

Where Abs blank is the absorption of the blank sample ($t=0$ min) and Abs sample is the absorption of the tested sample ($t=30$ min). Absorption of a blank sample containing the same amount of methanol and DPPH solution was acted as the negative control and BHT as the positive control. The IC_{50} value (concentration of sample providing 50% inhibition) was calculated using the graph plotted the percentage inhibition against concentration of sample.

2.5.2. Determination of ABTS radical scavenging capacity

The radical scavenging capacity of the tested extracts against ABTS (2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) radical cation was measured for the evaluation of the antioxidant activity. $\text{ABTS}^{\bullet+}$ radical cation was produced by reacting ABTS stock solution with potassium persulfate and allowing the mixture to stand for 12–16 h at 4 °C in the dark before use. The $\text{ABTS}^{\bullet+}$ radical cation solution was diluted with methanol to an absorbance of 1 ± 0.02 at 734 nm and equilibrated at 30 °C. Each concentration of the sample in methanol (25 μl) was mixed with 1 ml of diluted $\text{ABTS}^{\bullet+}$ radical cation solution. After reaction at 30 °C for 7 min, the absorbance at 734 nm was measured. Scavenging capacity of ABTS radical was calculated according to the following formula:

$$\% \text{ of scavenging of ABTS radical} = [(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{blank}}] \times 100 \quad (2)$$

Where Abs_{blank} is the absorption of the blank ($t=0$ min) and Abs_{sample} is the absorption of the tested sample (extracts and standard) ($t=7$ min). The ABTS was used as the negative control and trolox as the positive control. The IC_{50} value was calculated by plotting the percentage inhibition against concentration of sample.

2.5.3. Determination of reducing power

The method of Oyaizu [26] was used to determine the capacity of the tested extract and BHT to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The samples (0.125 ml) were mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and $\text{K}_3\text{Fe}(\text{CN})_6$ (2.5 ml, 1% w/v) and the mixture was incubated for 20 min at 50 °C. 2.5 ml of trichloroacetic acid solution (10%) was added to the mixture, which was then centrifuged at 1500 g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl_3 (0.5 ml, 0.1% w/v). Absorbance of all solutions was measured at 700 nm.

2.6. Statistical analysis

In this study, all determinations were conducted in triplicate and the data are expressed as the average of three measurements \pm standard deviation. Statistical analysis was performed using IBM SPSS Statistics 20 software. Statistical comparisons were carried out with oneway analysis of variance (ANOVA) and the probability p value less than 0.05 was considered significant.

3. Results and discussion

3.1. Extraction yield and total phenol and flavonoid contents

The percentage yield obtained from the aerial parts of *Teucrium polium* using different solvents extraction is presented in Table 1. The extraction yield of *Teucrium polium* based on the dry weight of the plant varied from 8.47 ± 0.79 to $23.33 \pm 0.61\%$ (g/g) and decreased as follows: methanol > ethanol > water > chloroform > hexane > ethyl acetate. The methanol showed the greatest extraction yield ($23.33 \pm 0.61\%$ (g/g)) compared to other solvents. These findings are in agreement with those obtained by Zazouli et al. [27], who shown that the methanol was the most appropriate solvent for recovering of the extractable constituents from plants. Variations in recovery percentage of extractable compounds have been probably related to polarity of the extracting solvent. According to Butsat and Siriamornpun [28], the difference in polarities of the extraction solvents might influence the solubility of the chemical constituents in a sample and its extraction yield.

Being plant secondary metabolites, the phenolic compounds (simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives and flavonoids) are very important judging from the virtue of their antioxidant capacities by chelating redox-active metal ions, inactivating lipid free radical chains, and avoiding the hydroperoxide conversions into reactive oxyradicals [29, 30]. The total phenol and flavonoid contents of dif-

Table 1
Extraction yield, total phenol and flavonoid contents of *Teucrium polium* extracts

Extracts	Yield (% (g/g))	Total phenols (mg GAE/g)	Total Flavonoids (mg QE/g)
Methanol	23.33 ± 0.61^a	206.95 ± 1.82^a	42.16 ± 0.61^a
Ethanol	21.97 ± 0.28^b	157.42 ± 4.12^c	34.46 ± 2.24^c
Ethyl acetate	8.47 ± 0.79^e	113.23 ± 3.54^d	5.74 ± 0.19^f
Chloroform	10.62 ± 1.15^d	102.36 ± 2.83^e	12.83 ± 1.92^e
Hexane	9.80 ± 0.43^{de}	83.7 ± 0.15^f	23.65 ± 0.60^d
Water	20.15 ± 0.85^c	184.84 ± 2.25^b	38.95 ± 1.13^b

Data were expressed as mean of three measurements \pm standard deviations. Values with different letters in the same experiment and same column differ significantly at $p < 0.05$.

ferent *Teucrium polium* extracts were estimated through the Folin-Ciocalteu and aluminium trichloride (AlCl_3) methods, respectively. The amounts of total phenolic and flavonoid present in various *Teucrium polium* extracts (methanol, ethanol, ethyl acetate, chloroform, hexane and water) are given in Table 1. The Total phenol content was expressed as mg Gallic Acid Equivalent (GAE) per gram of extract (the equation of calibration curve: $y = 0.0107x + 0.0065$, $R^2 = 0.9993$). Total phenol content in different tested extracts was ranged between 83.7 ± 0.15 and 206.95 ± 1.82 mg of GAE/g and decreased in the order of methanol > water > ethanol > ethyl acetate > chloroform > hexane. The total flavonoid content in extracts was expressed as mg of Quercetin Equivalent (QE) per gram of extract ($y = 0.0343x + 0.0058$, $R^2 = 0.9999$). The flavonoid content of examined extracts was came in a range from 5.74 ± 0.19 to 42.16 ± 0.61 mg of QE/g and decrease in the following order: methanol > water > ethanol > hexane > chloroform > ethyl acetate. The results of the present study indicated a wide variation of the total phenol and flavonoid contents in the different *Teucrium polium* extracts (Table 1). This could have been due to solubility of phenolic compounds in the extracting solvent. Our data showed that methanolic extract from *Teucrium polium* aerial parts exhibited the highest amount of total phenols and flavonoids with values of 206.95 ± 1.82 mg of GAE/g and 42.16 ± 0.61 mg of QE/g, respectively. These findings were in good agreement with previous reports, which also found that methanol was the most effective solvent in extracting phenolic components from plants [20, 31]. The recovery of phenols from plant materials is influenced by solubility of the phenolic compounds in the solvent used for the extraction process [27]. Addai et al. [31] have been reported that with increase in solvent polarity, total phenol and total flavonoid content increased in extract. Phenolic compounds are often extracted in higher amounts in more polar solvents [32]. According to Khorasani Esmaeili et al. [33], a possible justification would be due to the formation of complexes by a part of phenolic compounds with carbohydrates and proteins, which are more extractable in methanol than in other solvents that have been emphasized in this study.

3.2. Antioxidant capacity

In the present study, the *in vitro* antioxidant capacity of the all *Teucrium polium* extracts was assessed by DPPH, ABTS and reducing power assays. The BHT and trolox were used as reference standards.

The DPPH assay was the most commonly method used for determination of scavenging activity of antioxidants [34]. The DPPH free radical scavenging capacities of the six tested extracts are shown in Fig. 1. All the examined extracts were able to reduce the stable free radical of DPPH to the yellow-colored diphenylpicrylhydrazine radical due to donation of a hydrogen atom. All the extracts showed a dose-dependent radical scavenging capacity. The IC_{50} values (concentration required for 50% inhibition) of each extract are presented in Table 2. Lower IC_{50} value indicates higher scavenging capacity of the extracts. All the *Teucrium polium* extracts revealed various DPPH scavenging capacities, with an IC_{50} values ranged from 6.77 ± 0.15 to 9.90 ± 0.04 mg/l. According to Adaramola and Onigbinde [35], different extractability of antioxidant constituents by extraction solvents due to variations in the polarity of the solvents may be responsible for the differences in the antioxidant capacity of extracts in different solvents. The DPPH scavenging capacity of the tested extracts was found to be decrease in the following order: methanol > ethanol > ethyl acetate > water > chloroform > hexane. As shown in Table 2, all the examined extracts exhibited higher scavenging capacity than the synthetic standard BHT ($\text{IC}_{50} = 27.99$ mg/l). The methanol extract showed the greatest DPPH radical scavenging capacity with an IC_{50} value of 2.79 ± 0.07 mg/l.

The ABTS free radical scavenging assay was used to test *in vitro* the antioxidant capacity of different *Teucrium polium* extracts. The antioxidants of tested extracts react with the stable ABTS^+ free radical cation (deep blue/green colour) and convert it to its colorless neutral form, by their hydrogen donating ability to ABTS. The IC_{50} values in the ABTS radical scavenging capacity assay of the different extracts are given in Table 2. All the examined extracts exhibited a concentration-dependent inhibition of the ABTS radicals as shown in Fig. 2. The investigated extracts denoted differential radical scavenging capacity against ABTS radical cation, with an IC_{50} values ranked between 2.79 ± 0.07 and 6.90 ± 0.05 mg/l. All the examined extracts showed lower scavenging capacity compared to the synthetic antioxidant trolox ($\text{IC}_{50} = 0.82$ mg/l). However, at a concentration

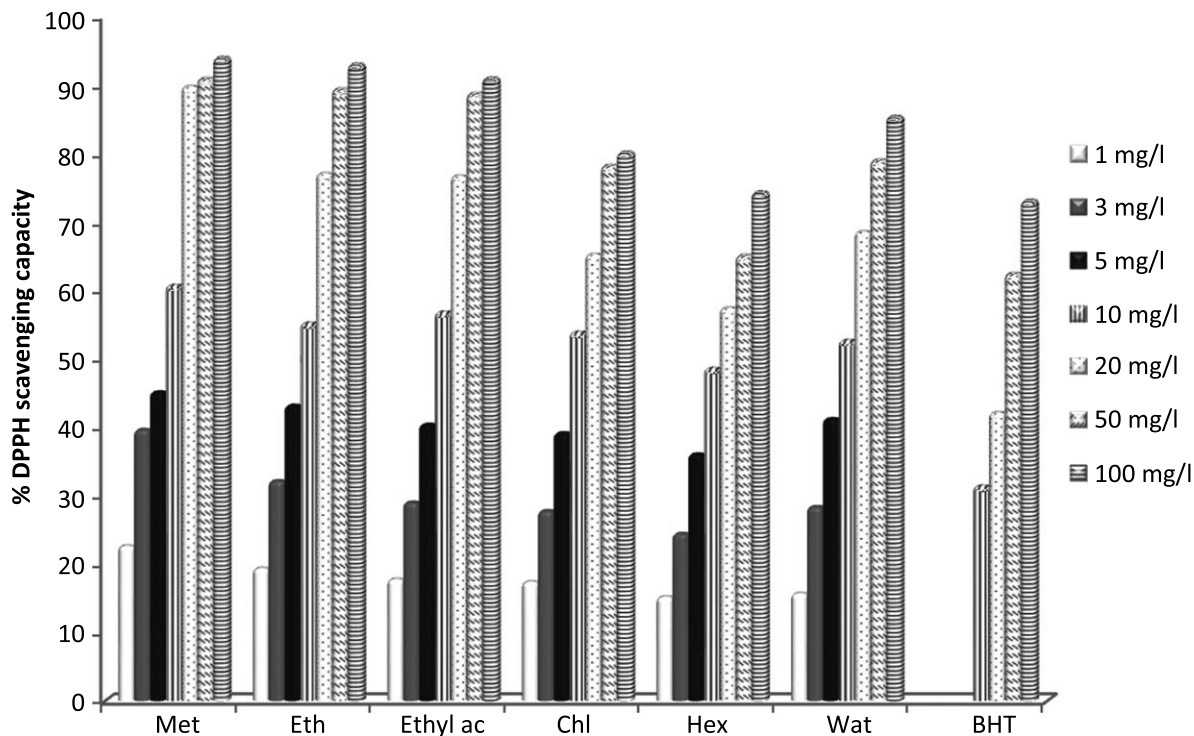


Fig. 1. DPPH radical scavenging capacity of *Teucrium polium* extracts. Met: Methanol, Eth: Ethanol, Ethyl ac: Ethyl acetate, Chl: Chloroform, Hex: Hexane and Wat: water. Data were expressed as mean of three measurements. Values differ significantly at $p < 0.05$.

Table 2
IC₅₀ values in DPPH and ABTS radicals scavenging capacity

Extracts	DPPH IC ₅₀ (mg/l)	ABTS IC ₅₀ (mg/l)
Methanol	6.77 ± 0.15 ^f	2.79 ± 0.075 ^b
Ethanol	8.01 ± 0.069 ^e	3.49 ± 0.1 ^c
Ethyl acetate	8.02 ± 0.07 ^e	3.82 ± 0.02 ^d
Chloroform	8.61 ± 0.32 ^c	4.60 ± 0.08 ^e
Hexane	9.90 ± 0.04 ^b	6.90 ± 0.05 ^f
Water	8.37 ± 0.28 ^d	3.68 ± 0.25 ^{cd}
BHT	27.99 ± 0.0 ^a	–
Trolox	–	0.82 ± 0.0 ^a

Data were expressed as mean of three measurements ± standard deviations. Values with different letters in the same experiment and same column differ significantly at $p < 0.05$.

of 5 mg/l, the methanolic extract exhibited a comparable ABTS free radical scavenging ability with standard trolox. The ABTS scavenging capacity of investigated *Teucrium polium* extracts was found effective in this order methanol > ethanol > water > ethyl acetate > chloroform > hexane. Among the six tested extracts, the methanol extract exhibited the greatest radical scavenging capacity (IC₅₀ = 2.79 ± 0.07 mg/l) when it reacted with the ABTS free radicals.

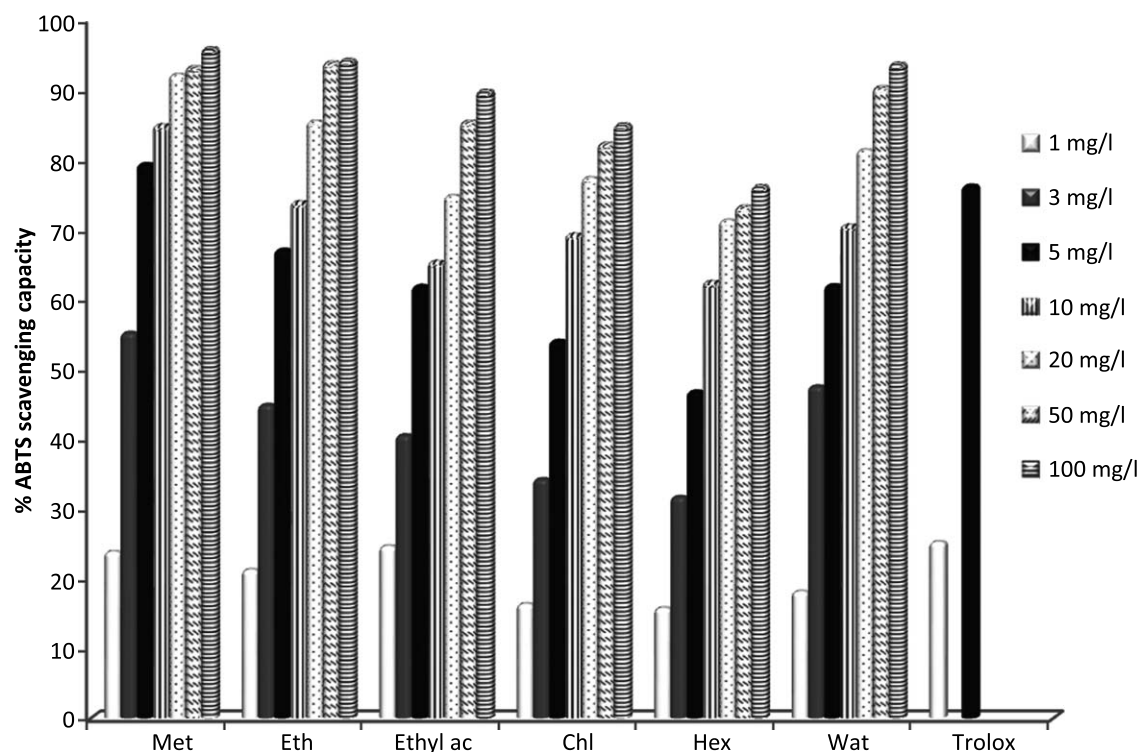


Fig. 2. ABTS radical scavenging capacity of *Teucrium polium* extracts. Met: Methanol, Eth: Ethanol, Ethyl ac: Ethyl acetate, Chl: Chloroform, Hex: Hexane and Wat: water. Data were expressed as mean of three measurements. Values differ significantly at $p < 0.05$.

The results of DPPH and ABTS free radicals scavenging assay revealed that the methanolic extract present the best antioxidant potential. This could be attributed to the presence of high amount of total phenols in the methanolic extract (Table 1). There are some studies in the literature that report a high linear correlation between antioxidant capacity and total phenolic content [36–38]. Viuda-Martos et al. [39] have been reported that phenolic content can be used as an important indicator of antioxidant capacity of plants and can be used as a preliminary screen for any product when intended to be used as a natural source of antioxidants in functional foods. Phenolics possess one or more aromatic rings, extended conjugated aromatic system to delocalize an unpaired electron, and one or more hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical; therefore, they have ideal structure for free radical scavenging capacities [40].

The different *Teucrium polium* extracts were also subjected to screening for their possible *in vitro* antioxidant capacity by reducing power assay. Reducing power is widely used to evaluate the antioxidant capacity of plant extracts [41]. In the reducing power assay, the presence of antioxidants in plant extracts causes the reducing of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by giving away an electron. The reductive abilities of different *Teucrium polium* extracts are shown in Fig. 3. All the examined extracts exhibited good reducing power capability. Ferric reducing power increases with the increase in concentration of the extract. The antioxidant capacity of the examined extracts by reducing power assay can be ranked in the following order: methanol > chloroform > ethanol > ethyl acetate > hexane > water. The methanolic extract showed the strongest reducing power ability in comparison to other extracts and standard BHT (Fig. 3). These results were in agreement with those found by Pavithra and Vadivukkarasi [42] on leaves from *Kedrostis foetidissima*, which reported that methanolic extract exhibited highest reducing power ability when compared to other extracts.

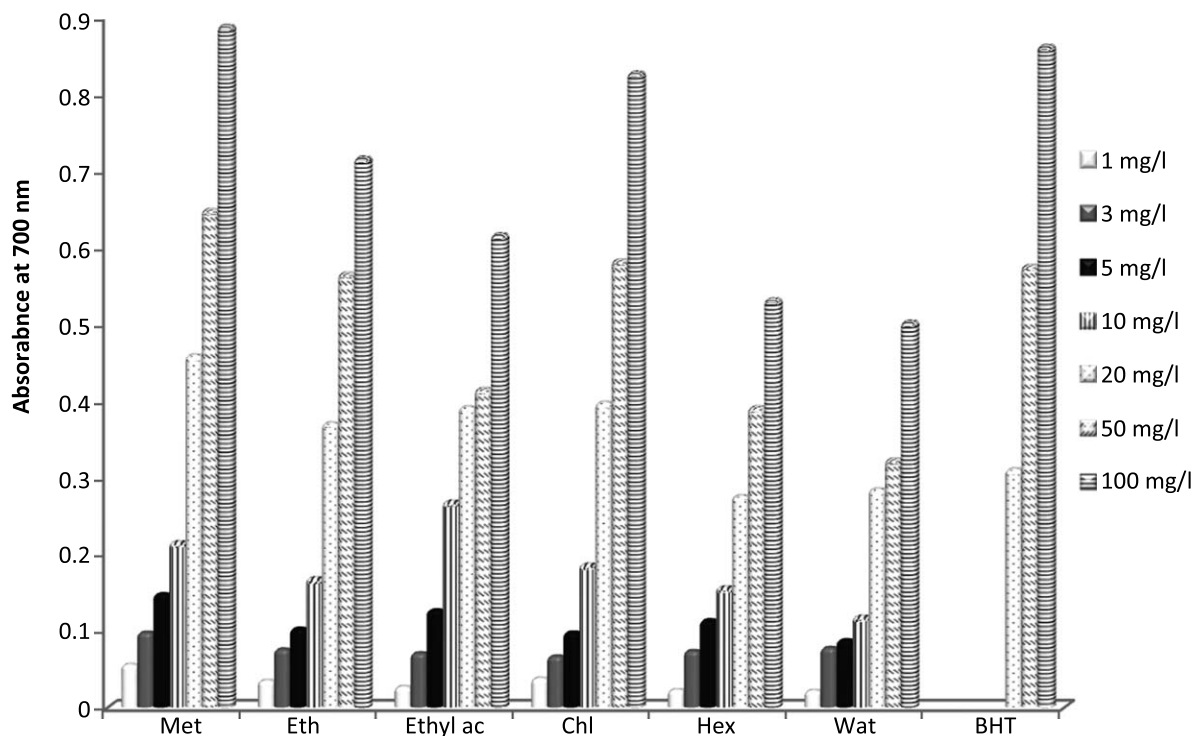


Fig. 3. Reducing power ability of *Teucrium polium* extracts. Met: Methanol, Eth: Ethanol, Ethyl ac: Ethyl acetate, Chl: Chloroform, Hex: Hexane and Wat: water. Data were expressed as mean of three measurements. Values differ significantly at $p < 0.05$.

4. Conclusion

In conclusion, results of this study demonstrated that the type of solvents used significantly affected the extraction efficiency of bioactive compounds and antioxidant potency of *Teucrium polium* aerial parts grown in Algeria. Variations in phenolic compounds content and antioxidant capacity between extracts were probably related to polarity of the extracting solvent. All the examined extracts contained appreciable contents of total phenolic and flavonoid and exhibited good antioxidant potential. Among the six tested extracts, methanolic extract was found to have the strongest antioxidant capacity when was assessed by DPPH, ABTS and reducing power assays. The obtained data also showed that methanol was the most efficient solvent for extraction of the highest amount of total phenolic and flavonoid from *Teucrium polium* aerial parts. Thereby, it can be said that antioxidant capacity of the methanolic extract was probably due to the presence of high content of phenolic compounds which possess ideal structural chemistry for free radical scavenging capacity. Our results revealed that *Teucrium polium* methanolic extract could be considered as good source of natural antioxidants capable to reduce oxidative stress damage.

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