Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 6 Issue 12 December 2022

Research Article

Antioxidant Properties of Nine Commonly Used Medicinal Plants in Arabs Region

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DOI: 10.31080/ASNH.2022.06.1149

Received: October 25, 2022

Published: November 07, 2022

Abstract

The present study was conducted to investigate the total phenolics, total flavonoid, and Trolox equivalent antioxidant capacity of 9 commonly used medicinal plants and herbs among Arabs nation namely, *Trachyspermum ammi (Apiaceae*) seeds, *Ferula assa-foetida* (*Apiaceae*) dried latex, *Elettaria cardamomum* (*Zingiberaceae*) fruit 1, *Senna alexandrina (Caesalpinioideae*) *Leguminosae* leaves, *Adenium obesum* (*Apocynaceae*) leaves, *Elettaria cardamomum (Zingiberaceae*) fruit 2, *Trigonella foenum-graecum* (*Leguminosae*) seeds, *Lepidium sativum* (*Brassicaceae*) seeds, *Ziziphus spina-christi* (*Rhamnaceae*) fruit, and *Nigella sativa* (*Ranunculaceae*) seeds.

The methanol extract of each plant was obtained by dissolving 2g of plant powder in 20 ml methanol-water (4:1v/v) to overnight at room temperature. The supernatants were concentrated by using a rotary evaporator. Residues of each extract were dissolved in distilled water and the final volume was recorded. A serial dilution of 10, 100, 1000, and 5000 were made.

The mean values of the total phenolics content were 535.24 ± 70.68, 150.28 ± 36.89, 69.05 ± 14.18, 1358.7 ± 118.88, 605.83 ± 117.67, 228.28 ± 53.8, 2171.57 ± 296.2, 726.65 ± 130.73, 702.63 ± 32.93, and 132.4 ± 30.77 (mg GAE/100g) in *T. ammi, F. assa-foetida, E.cardamomum1, S. alexandrina, A. obesum, E.cardamomum2, T. foenum- graecum, L. sativum, Z. spina-christi and N. sativa* respectively, whereas the mean values of the total flavonoid content were 143.96 ± 6.44,0.0, 9.16 ± 3.14,761.66 ± 14.71, 210.09 ± 7.61,12.83 ± 0.88, 347 ± 60.15,94.84 ± 10.04, 94.07 ± 6.26, and 30.49 ± 0.33 (mg QE/100g) respectively. The determined scavenging activity against ABTS radical of the aqueous methanolic extracts of *T. ammi, F. assa- foetida, E. cardamomum1, S. alexandrina, A. obesum, E. cardamomum2, T. foenum- graecum, L. sativum, Z. spina-christi and N. sativa* were 1271.32 ± 273.35, 234.89 ± 42.2, 135 ± 21.45, 2551.83 ± 305.35, 2101.1 ± 298.97,794.9 ± 175.72, 794.73 ± 149.49, 2219.74 ± 382.12, 3093.11 ± 445.93, and 483.07 ± 98.17 (µmole TEAC/100g) respectively. Among studied plants *T. foenum-graecum* showed the highest phenolic content (2171.57 ± 296.2 mg GAE/100g) whereas *S. alexandrina* showed the highest total flavonoid content (761.66 ± 14.71 mg QE/100g). The highest IC_{50s} were noticed in *T. ammi, S. alexandrina*, *Z. spina-christi,* and *A. obesum* extracts (100.035 ± 19.38, 583.38 ± 45.11, 666.87 ± 79.62 and 720.51 ± 92.24µg respectively). The hydromethanolic extracts of *T. foenum-graecum, S. alexandrina*, *A. obesum, L. sativum* and *Z. spina-christi* showed a significant antioxidant property, further research is ongoing to identify their bioactive compounds, in particular phenolic acids. Our findings suggest the possibility of applying *T. foenum-graecum, S. alexandrina*, *A. obesum, L. sativum* and *Z. spina-christi* in food and/or pharmaceutical industries.

Keywords: Total Phenolics; Total Flavonoid; Scavenging Activity; *T. Foenum-Graecum; S. Alexandrina; Z. Spina-Christi*

Introduction

In developing countries, herbal remedies are used for treatment of human diseases such as atherosclerosis, cancer, diabetes, aging, various skin diseases and other degenerative diseases [1-5]. In recent years an increasing attention has been focused on the study of substances with antioxidant potential and protective effects against free radicals, and reactive oxygen and nitrogen species. Free radicals, and reactive oxygen and nitrogen species can cause oxidative stress resulting in damage to several biomolecules, and therefore leads to chronic disease and serious illness [6]. Plants, including vegetables, fruits, herbs, and cereals, have various bioactive compounds that have anti-diabetic, antibacterial, anti-cancer, anti-atherogenic, and anti-inflammatory effects [7]. The administration of *Senna alata* (*S. alata*) extract reported to improve the impaired lipid metabolism in HFD-induced obese mice by the reducing of serum lipid concentrations and hepatic triglycerides content [8]. Mercury chloride - induced nephrotoxicity rats when administered *Ziziphus spina-christi* leaf extract at a concentration of 300mg/Kg showed significant reduction in serum creatinine and urea, also kidney lipid peroxidation was significantly reduced as malondialdehyde levels diminished [9]. *A. obesum* ethanolic seeds extract reported to have a cytotoxic potential against MCF-7 breast cancer cells [10]. Serum levels of glucose, lipid profile, kidney, and liver enzyme were significantly reduced after treatment of streptozotocin-induced diabetic rats with oral administration of *L. sativum* extract at the dosage of 100 and 200 mg/kg [11]. Supplementation of *T. foenum-graecum* leaves in the diet to streptozotocin-induce diabetic rats daily for 45 days reduced serum and tissue concentration of total cholesterol, triglycerides, and free fatty acids [12]. *T. foenum-graecum* leave extract also showed a significant level of total phenolics and antioxidant properties and significantly reduced the lipid oxidation in chicken meat during refrigerated storage [13].

Here, we studied the total phenolics, total flavonoid, and ABTS radical scavenging activity of *T. ammi, F. assa- foetida, E. cardamomum1, S. alexandrina, A. obesum, E. cardamomum2, T. foenum- graecum, L. sativum, Z. spina-christi and N. sativa,* a commonly used medicinal plants in Arabs region. The possibility of applying extracts with potential antioxidant properties in food and/or pharmaceutical industries was also a focus.

Materials and Methods

Chemicals and reagents

Methanol and other chemicals were of analytical grade and were obtained from BDH (Poole, UK). Gallic acid, Folin-Ciocalteu reagent, quercetin, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS),and potassium persulfate were purchased from Sigma-Aldrich(St. Louis, MO,USA). Trolox (6-hydroxy-2, 5, 7, 8- tetramethylchroman-2- carboxylic acid) was obtained from Fluka Chemie AG (Buchs, Switzerland).

Plant materials and extraction

Trachyspermum ammi (*Apiaceae*) seeds, *Ferula assa-foetida* (*Apiaceae*) dried latex, *Elettaria cardamomum* (*Zingiberaceae*) Fruit 1, *Senna alexandrina* (*Caesalpinioideae*) *Leguminosae* leaves, *Adenium obesum* (*Apocynaceae*) leaves, *Elettaria cardamomum* (*Zingiberaceae*) Fruit 2, *Trigonella foenum-graecum* (*Leguminosae*) seeds, *Lepidium sativum* (*Brassicaceae*) seeds *Ziziphus spina-christi* (*Rhamnaceae*) Fruit, and *Nigella sativa* (*Ranunculaceae*) seeds. All plants were obtained from the local market, Jeddah, the western region of Kingdom of Saudi Arabia except *Adenium obesum* was obtained from Yemen.

Plants were identified by staff members of the Botany Department, College of Science, King Saud University, Saudi Arabia. Voucher specimens were deposited at the herbarium of the Botany Department. Powdered extracts were obtained by use of a mill and they were stored in amber bottles to prevent degradation. The methanol extract of each plant was obtained by dissolving 2g of each plant powder in 20 ml methanol-water (4:1v/v) to overnight at room temperature using an orbital shaker. Filtrates were collected and residues were extracted again with 20 ml solvent. Appropriate filtrates were pooled and centrifuged at 5000 rpm for 10 min, and the supernatants were concentrated under reduced pressure at 40ºC using a rotary evaporator. After solvent evaporation, residues of each extract were dissolved in distilled water and the final volume was recorded. A serial dilutions of 10, 100, 1000, and 5000 were made for each plant extract and stored at -20 ºC until analysis.

Determination of total phenolics (TP)

Total phenolics content was determined by Folin-Ciocalteu's reagent method [14]. 0.5 ml of each diluted extract and 0.1 ml Folin-Ciocalteu's reagent were mixed and incubated at room temperature for 15 min. Then 2.5 ml of 7% sodium carbonate solution was added, the tubes were incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid standard curve was constructed (0 -50 µg). Total phenolics content of plant extracts were expressed as mg gallic acid equivalent (GAE)/100 g.

Determination of total flavonoid (TF)

Total flavonoid content was determined by aluminum chloride colorimetric method [15]. In brief, 0.5ml of each diluted extract

was mixed with 1.5 ml of 95% ethanol, 0.5 ml of 1.2% aluminum chloride, 0.5 ml of 120 mM potassium acetate were added in order. After incubation at room temperature for 30 min, the absorbance was measured at 415 nm against reagent blank. Quercetin standard curve was constructed (0- 50 µg). Total flavonoid content of plant extracts was expressed as milligram quercetin antioxidant equivalents (QE/100g).

ABTS radical cation scavenging activity

The ABTS free radical decolorization assay was performed [16]. ABTS radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation in dark for 16hr at room temperature. The ABTS solution was diluted with 80% ethanol to an absorbance of 0.700 ± 0.050 at 734 nm. 0.1 mL of each diluted extract and 3.9 ml ABTS solution were mixed thoroughly, the mixture was allowed to stand at room temperature for 6 min and the absorbance was immediately recorded at 734 nm. Trolox standard curve (0-15 µM) in 80% ethanol was prepared. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, µmol Trolox equivalents per 100g sample).

Data analysis

Results were presented as means ± standard deviation. All samples were analyzed in three replications.

Results and Discussion

The calibration curve of gallic acid for determining total phenolics content is shown in figure 1. The total phenolics content of the aqueous methanolic extracts of *T. ammi, F. assa- foetida, E. cardamomum1, S. alexandrina, A. obesum, E.cardamomum2, T. foenum- graecum, L. sativum, Z. spina-christi and N. sativa* were 535.24 ± 70.68, 150.28 ± 36.89, 69.05 ± 14.18, 1358.7 ± 118.88, 605.83 ± 117.67, 228.28 ± 53.8, 2171.57 ± 296.2, 726.65 ± 130.73, 702.63 ± 32.93, and 132.4 ± 30.77 (mg GAE/100g) respectively (Figure 2). *T. foenum- graecum* showed the highest phenolics content (2171.57 ± 296.2mg GAE/100g). A high phenolics content of the freeze-dried *Trigonella foenum-graecum* seeds was reported [17]. Another study also reported that fenugreek seeds have a high level of total polyphenol content (85.88 mg GAE/g) [18]. *S. alexandrina* also showed a considerable total phenolics content (1358.70 ± 118.88 mg GAE/100g). The association between the presence of phenolic compounds and the biological activity of the acetone extract of *Senna italica* has been reported [19]. *T. ammi* showed a total phenolics content of (535.24 ± 70.68 mg GAE/100 g), however,

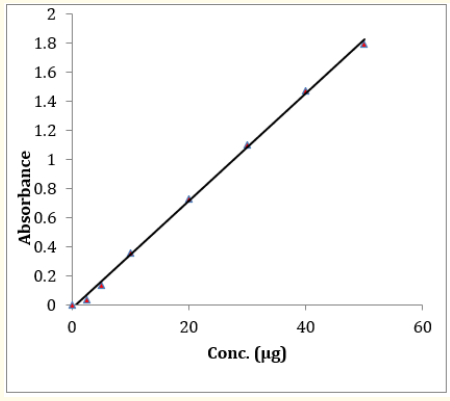

Figure 1: Standard curve of Gallic acid.

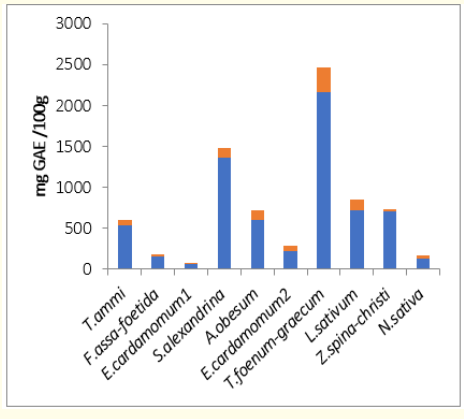

Figure 2: Total phenolics content as mg gallic acid (GAE) /100g of *T. ammi, F. assa-foetida, E. cardamomum1, S. alexandrina, A. obesum, E. cardamomum2, T. foenum-graecum, L. sativum, Z. spina-christi* and *N. sativa.* Data are expressed as means ± standard deviation and calculated from three determinations.

an early study suggested that phenolic compounds are important components of *Trachyspermum ammi*, and the pharmacological effects of the plant could be attributed to the presence of these polyphenols [20]. Our finding regarding the total phenolics content of *A. obesum* (605.83 ± 117.67 mg GAE/100g) is coincided with the report concluded that *A. obesum* extract collected from Yemen is a rich source of anthocyanins and possesses a significant antioxidant activity [21]. The total phenolics content of *L. sativum* seeds and *Z. spina-christi* flower was 726.65 ± 130.73 and 702.63 ± 32.93 mg GAE/100g respectively.

The total phenolics content of *L. sativum* is in a full agreement with the reported concentration range between 6.33 - 7.40 mg GAE/g [22]. A total phenolics content of 1644.00 ± 3.20 mg GAE/100g in *Z. spina-christi* flower has been reported [23], however, the discrepancy between the current investigation and that report, concerning the total phenolics content in *Z. spina-christi* flower could be attributed to the source of the plant and the extraction protocol. The observed total phenolics content in *E. cardamomum1* and *E. cardamomum2* were 69.05 ± 14.18 and 228.28 ± 53.8 mg GAE/100g respectively. The total phenolics components of the *E. cardamomum* at different methanol concentrations has been studied and found to range from 0.317 to 1.66 g GAE/100g [24]. Our finding regarding the total phenolics content in *F. assa- foetida* is in contrast to an earlier reported data of the plant, this contrast could be attributed to extraction protocol [25]. Our obtained result regarding the total phenolics content of *N. sativa*, (132.40 ± 30.77 mg GAE/100g) is consistent with the report stated that the phenol content of *N. sativa* is 122.67 ± 3.03 mg GAE/100g [26].

The calibration curve of quercetin for determining total flavonoid content is shown in figure 3. The total flavonoid content of the aqueous methanolic extracts of *T. ammi, F. assa- foetida, E. cardamomum1, S. alexandrina, A. obesum, E. cardamomum2, T. foenum- graecum, L. sativum, Z. spina-christi and N. sativa* were 143.96 ± 6.44,0.0, 9.16 ± 3.14,761.66 ± 14.71, 210.09 ± 7.61,12.83 ± 0.88, 347 ± 60.15,94.84 ± 10.04, 94.07 ± 6.26, and 30.49 ± 0.33 (mg QE/100g) respectively (Figure 4). The highest content of total flavonoid content was observed in *S. alexandrina* leaves (761.66 ± 14.71 mg QE/100g). The total flavonoid content in the bark of *Senna tora* which belongs to the family *Caesalpiniaceae*, another species of *Senna*, was reported to be 21.58 mg QE/100g [27]. Another study showed that the hydroalcoholic extract of *Cassia acutifolia*, another *Senna* species, has a total flavonoid content of 20.8 ± 0.40 mg QE/g [28].

T. foenum- graecum seeds content of total flavonoid was 347 ± 60.15mg QE/100g (Figure 4). However, the aqueous methanolic extract of the plant seed reported to have a total flavonoid content (as Catechin) of 20.8 mg CE/100g [29]. The total flavonoid content in *A. obesum* and *T. ammi* were 210.09 ± 7.61 and 143.96 ± 6.44 mg QE/100g respectively. Flavonoids have been reported to have antibacterial, antineoplastic activity, anti-inflammatory, anti-allergic and antiviral activity. Our applied extraction protocol as well as the protocol for total flavonoid content determination failed to detect flavonoid in the dried latex of *F. assa- foetida*, however, it has been

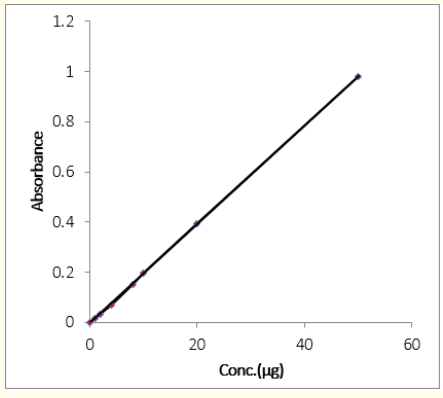

Figure 3: Standard curve of Quercetin.

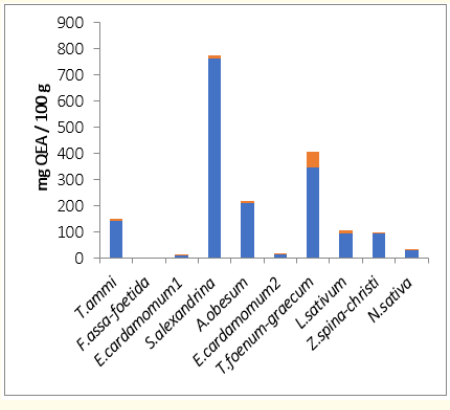

Figure 4: Total flavonoid content as mg Quercetin (QEA)/100g of *T. ammi, F. assa-foetida E.cardamomum1, S. alexandrina, A. obesum, E.cardamomum2, T. foenum-graecum, L. sativum, Z. spina-christi* and *N. sativa*. Data are expressed as means ± standard deviation and calculated from three determinations.

reported that the hydroalcoholic extract of *F. assa-foetida* leaves and *F. assa-foetida* leaves essential oil have a total flavonoid content of 12.53 ± 3.20, 0.015 ± 0.002 mg QE/100g respectively [30]. *L. sativum* and *Z. spina-christi* have the same total flavonoid content (94 mg QE/100g) (Figure 4). Our finding regarding the total flavonoid content of *Z. spina-christi* is comparable with the report stated that the fruit of *Z. spina-christi* grown in Oman has a total flavonoid content of 47 mg CE/100 g [23].

The calibration curve of Trolox scavenging activity against ABTS radical is shown in figure 5. The recorded scavenging activity of the aqueous methanolic extracts of *T. ammi, F. assa- foetida, E.cardamomum1, S. alexandrina, A. obesum, E.cardamomum2, T.*

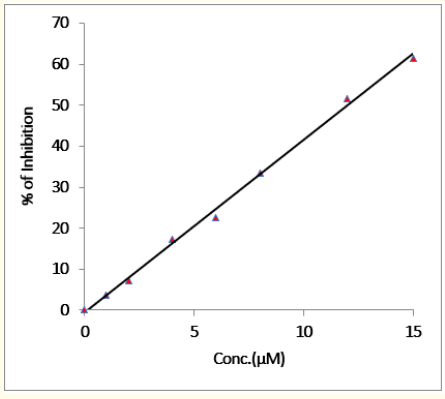

Figure 5: Standard curve of Trolox.

foenum- graecum, L. sativum, Z. spina-christi and N. sativa were 1271.32 ± 273.35, 234.89 ± 42.2, 135 ± 21.45, 2551.83 ± 305.35, 2101.1 ± 298.97,794.9 ± 175.72, 794.73 ± 149.49, 2219.74 ± 382.12, 3093.11 ± 445.93, and 483.07 ± 98.17 (µmole TEAC/100g) respectively (Figure 6).

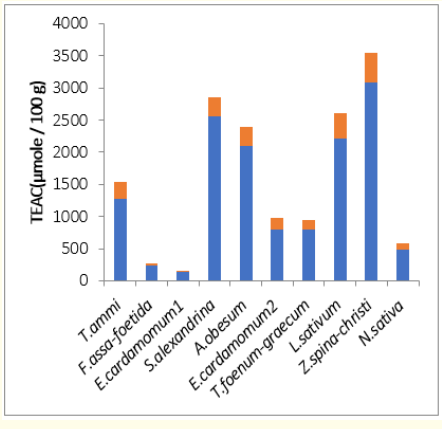

Figure 6: Trolox equivalent antioxidant capacity (TEAC) as µmole/100g of *T. ammi, F. assa-foetida, E. cardamomum1, S. alexandrina, A. obesum, E. cardamomum2, T. foenum- graecum, L. sativum, Z. spina-christi* and *N. sativa.*

Data are expressed as means ± standard deviation and calculated from three determinations.

The obtained IC_{50} of Trolox standard against ABTS radical was determined to be 11.63 µM (Figure 5), whereas the calculated IC_{50s} of *T. ammi, F. assa-foetida, E.cardamomum1, S. alexandrina, A. obesum, E.cardamomum2, T. foenum- graecum, L. sativum, Z. spina-christi and N. sativa* were 100.035 ± 19.38, 3471.5 ± 213.76, 1015.52 ± 166.04, 583.38 ± 45.11, 720.51 ± 92.24, 1569.4 ± 217.94, 1558.96 ± 206.24, 817.01 ± 81.21,666.87 ± 79.62, and 2673.52 ± 241.59 µg respectively (Figure 7). The highest IC_{50s} were noticed in *T. ammi, S. alexandrina, Z. spina-christi,* and *A. obesum* extracts (100.035 ± 19.38, 583.38 ± 45.11, 666.87 ± 79.62 and 720.51 ± 92.24µg respectively). A recent report showed that the aerial part of *S. alexandrina* that undergo ultrasound-assisted extraction exhibited a powerful antioxidant activity against DPPH and ABTS when ascorbic acid is used as standard [31]. *Alternaria alternata* that had been isolated from *Ziziphus spina-christi* reported to have as potential antioxidant activity against DPPH as the IC_{50} reached 409µg/ml [32]. Our finding that *T. ammi* seed extract has a strong ABTS scavenging activity as the IC_{50} reached 100.035 ± 19.38µg is in full agreement with the report that studied 24 commonly used medicinal plants from Nepal and Japan and stated that *T. ammi* seed showed a strong activity against DPPH as the recorded % of inhibition was 90.67 ± 0.58 [33].

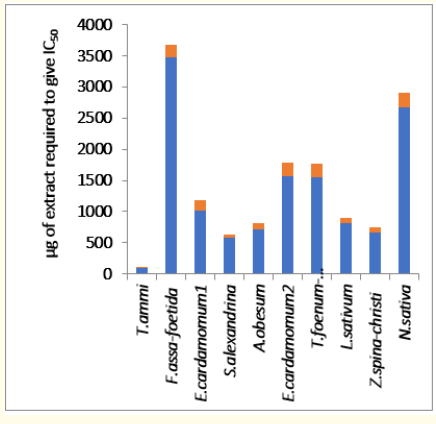

Figure 7: Micrograms required of *T. ammi, F. assa-foetida, E. cardamomum1, S. alexandrina, A. obesum, E. cardamomum2, T. foenum- graecum, L. sativum, Z. spina-christi* and *N. sativa* extracts to give IC50s against ABTS radical.

Data are expressed as means ± standard deviation and calculated from three determinations.

Conclusion

The hydromethanolic extracts of *T. foenum-graecum, S. alexandrina*, *A. obesum, L. sativum* and *Z. spina-christi* showed a significant antioxidant property, further research is ongoing to identify their bioactive compounds, in particular phenolic acids. Our findings suggest the possibility of applying *T. foenum-graecum, S. alexandrina*, *A. obesum, L. sativum*, and *Z. spina-christi* in food and/or pharmaceutical industries.

Acknowledgements

The author would like to thank the research center, college of science for supporting this project. The author also thanks the Deanship of Scientific Research and RSSU at King Saud University for their technical support.

Conflict of Interest

The author declares no conflict of interest.

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