Antioxidant Potential of Date Palm Leaves and *Acacia nilotica* Fruit in Comparison with Other Four Common Arabian Medicinal Plants

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Abstract: This study was conducted to investigate the antioxidant activities of date palm (Phoenix dactylifera) leaves (DPL) and Acacia nilotica Delile (Fabaceae) fruit (AN) and to compare their antioxidant potential with other four common Arabian medicinal plants namely, pumpkin (Cucurbita pepo L.) seeds (PS), flax (Linum usitatissimum) seeds (FS), cinnamon, Cinnamomum cassia (Lauraceae) bark (CIN), and ginger (Zingiber officinale) Roscoe (GNR). The mean values of the total phenol concentrations of investigated plants were 299, 34.63, 2.52, 6.51,2.21,and 2.4 (mg GAE/g) in AN, DPL, GNR, CIN, PS, and FS respectively, while the mean values of the total flavonoid concentrations were 2.88,11.55,1.55, 2.08, 0.3, and 1.98 (mgQAE /g) in AN, DPL, GNR, CIN, PS, and FS respectively. The mean values of Trolox equivalent antioxidant capacity (TEAC, µmol Trolox equivalents per g sample) were 113.31, 147.27, 8.33, 23.75, 6.17, and 21.49 in AN, DPL, GNR, CIN, PS, and FS respectively. These results showed that DPL and AN fruit are potential rich sources of total phenol content and TEAC radical-scavenging activity compared with the current studied plants as well as with other medicinal plants reported in the literature. It will be useful to further analyze DPL and AN fruit with sophisticated techniques such as chemiluminescence and high performance liquid chromatography in order to separate and identify their active principles.

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Key words: Date palm leaves, *Acacia nilotica* fruit, Total phenol, Total flavonoid, Trolox equivalent antioxidant activity

1. Introduction

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. The widespread use of traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties, the antioxidant properties of their constituents, and a wide range of their phenolic compounds. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and metal chelators (Scartezzini and Speroni, 2000; Ivanova et al., 2005; Kiselova et al.,2006). Numerous physiological and biochemical processes in the human body may produce oxygen-centered free radicals and other reactive oxygen species as byproducts. Overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases. Several reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans (Sabu and Kuttan,2002; Naik et al., 2003; Cai et al., 2004; Al-Mustafa and Al-Thunibat,2008). Ryan et al. (2007) reported that pumpkin seeds contain noticeable amounts of squalene and tocopherols. The phenolic phytochemical content of pumpkin reported to have the potential to contribute to the reduction of hyperglycemia-induced microvascular complications (Kwon et al., 2007).The health benefits of pumpkin seeds is attributed to the presence of molecules being able to scavenge radicals and inhibit lipid peroxidation catalyzed by lipoxygenase (Xanthopoulou et al.,2009). The antioxidant potentials and the health benefits of ginger have been reported (Eleazu and Eleazu, 2012; Skrovankova et al., 2012). Flaxseed reported to possess secoisolariciresinol diglucoside(SDG), an antioxidant phytochemical, which is capable to scavenge the hydroxyl radical and therefore a beneficial effect in some diseases as in breast cancer (Prasad, 1997; Chen et al.,2009). A study carried out by Yang et al. (2012) concluded that the ethanol extracts of cinnamon barks exhibited higher antioxidant activities. *Acacia nilotica* leaves and pods have been reported to have a high contents of phenolic and flavonoid as well as a potent antioxidant activity (Singh et al., 2009; Kalaivani and Mathew,2010). El Hadrami and Al-Khayri (2012) reported the presence of phenolic acids, hydroxycinnamates, and flavonoids including tannins in dates palm fruit and they

associated between the presence of these compounds and the beneficial effects on human health and chronic or degenerative illnesses such as cancer and cardiovascular diseases.

The literature search has indicated a total absence of information about the antioxidant potential of date palm leaves and *Acacia nilotica* fruit. In the current study an attempt was made to investigate the antioxidant activities of date palm (*Phoenix dactylifera*) leaves and *Acacia nilotica Delile (Fabaceae)* fruit and to compare them with other four common Arabian medicinal plants namely, pumpkin (*Cucurbita pepo L.*) seeds, flax (*Linum usitatissimum*) seeds, cinnamon,*Cinnamomum cassia* (*Lauraceae*) bark, and ginger (*Zingiber officinale*) *Roscoe.*

2. 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 material and extraction

Pumpkin (*Cucurbita pepo L.*) seeds, flax (*Linum usitatissimum*) seeds, cinnamon,*Cinnamomum cassia* (*Lauraceae*) bark, and ginger (*Zingiber officinale*) *Roscoe* were obtained from local market in Riyadh, Kingdom of Saudi Arabia. Date palm (*Phoenix dactylifera*) leaves were collected from a private farm in Riyadh. *Acacia nilotica* fruit was collected from the western province of Sudan. 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. Powder extracts were obtained by use of a mill and they were stored in amber bottles to prevent degradation. The methanol extract of each plant powder was obtained by dissolving 5g of each plant powder in 50 ml methanol-water(4:1v/v) at room temperature overnight using an orbital shaker. Filtrates were collected and residues were extracted again with 50 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 either in distilled water or in 80% ethanol and the final volume was recorded. A serial dilutions of 10, 100, 500, 1000, and 5000 were made for each plant extract.

Determination of total phenol

The concentration of total phenol was measured by the method originally described by Singleton and Rossi (1965) and modified by Kim et al.(2003). Briefly, an aliquot of 1ml diluted extracts or standard solutions of gallic acid (10, 20, 40, 60, 80 and 100 mg/L) was added to a 25 ml volumetric flask containing 9 ml of distilled water. A reagent blank using distilled water was prepared. 1ml of Folin Ciocalteu's reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% sodium carbonate solution was added with mixing. The solution was then diluted to 25ml with distilled water and mixed thoroughly. After incubation for 90 min at room temperature, the absorbance was read at 750 nm against blank.The standard curve of gallic acid was constructed. Total phenol contents of plant extracts were expressed as mg gallic acid equivalents (GAE)/100 g sample.

Determination of total flavonoid

The total flavonoid content was determined by aluminum chloride colorimetric method described by Chang et al.(2002). Briefly, 0.5ml of samples was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of deionized water were added in order. After incubation at room temperature for 40 min, the absorbance was measured at 415 nm against a deionized water blank. Quercetin standard curve was constructed (0- 50 mg /L). The total flavonoid content in samples was expressed as milligram quercetin antioxidant equivalents (QAE)/100g sample.

Radical cation ABTS scavenging activity

The ABTS free radical decolorization assay was performed as described by

Re et al. (1999). ABTS radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature in dark for 16 hr. The ABTS solution was diluted with 80% ethanol to an absorbance of 0.700 ± 0.050 at 734 nm. 0.1 mL of sample, 3.9 ml ABTS solution and 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 g sample).

Data analysis

The data are presented as means ± standard deviation. All samples were analyzed in three replications.

3. Results and Discussion

The standard curve of total phenol using gallic acid as standard is presented in Fig.1. The mean of the

total phenol concentrations of investigated plants were 299, 34.63, 2.52, 6.51,2.21, and 2.4(mgGAE/g) in AN, DPL, GNR, CIN, PS, and FS respectively(Fig.2).

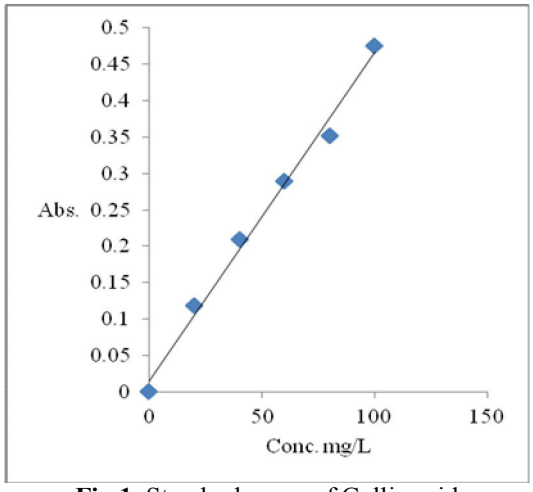

Fig.1. Standard curve of Gallic acid

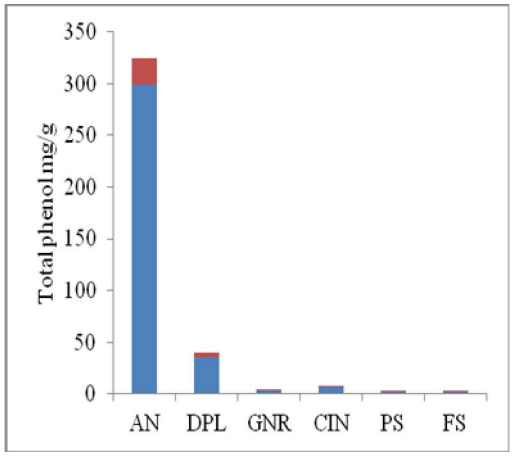

Fig. 2. Total phenol content as gallic acid (mg/g) in Acaia nilotica (AN), Date palm leaves (DPL), Ginger (GNR), Cinnamon (CIN), Pumpkin seeds (PS), and Flax seeds (FS)

Data are expressed as mean ±SD. All samples were analyzed in three replications

A. nilotica (AN) showed the highest phenolic content (299 mg / g), when compared with the highest reported total phenol data from literature, AN by virtue possesses the highest total phenol content as compared with the seventeen Ecuadorian fruits reported by Vasco et al. (2008). Also AN concentration of total phenol is about 13 folds of Chrysanthemum, a traditional chinese medicinal herb which reported to have a total phenol concentration of 13505 µ mole gallic acid / 100 g dry weight (Lee et al.,2008). AN concentration of total phenol is about 5 times higher than Dioscorea bulbifera L., another chinese medicinal plant which reported to have a total phenol concentration of 59.43 mg / g (Song et al., 2010). A recent report conducted by Abuelgassim (2013) showed that *A.nilotica* fruit extract has a strong hypolipidemic effect in alloxan-induced diabetic rats and this hypolipidemic effect was attributed to its high concentration in total phenol.

Our findings that AN fruit is a rich source of polyphenol is coincided with the report of Batanouny et al. (1999) stated that AN fruit contain high percentage of m-gallic acid, gallic acid, as well as methyl and ethyl gallic acid. The high content of gallic acid and and other polyphenols in AN were also observed by Kaur et al. (2005) who reported that the acetone extract of AN exhibits antimutagenic and cytotoxic activities.

Date palm leaf (DPL) also showed a high concentration of total phenol (34.63 mg/g) compared with the concentration of total phenol reported by Biglari et al. (2008) in various date palm fruits from Iran that ranged from 2.89 to141.35 mg gallic acid equivalent / 100g dry weight.This result reveal that total phenol concentration in DPL is about 25 times higher than the concentration of the same parameter in date palm fruit (DPF) (Biglari et al., 2008). Also this noticed result is in a full agreement with the fact that polyphenols are found in higher concentrations in leaves and their biosynthesis is accelerated by light exposure and serves as a filtration mechanism against Ultraviolet B- radiation of DPL extract have a hypocholesterolaemic effect and significantly reduce the serum (Harborne and Williams,2000). An earlier report concluded that the antioxidants concentrations of TC and LDL-C in alloxan-induced diabetic rats (Abuelgassim, 2010). GNR, PS, and FS have a low concentration of total phenol 2.52, 2.21,and 2.4(mg/g) respectively.Our finding that GNR has a low concentration of total phenol is in a full agreement with Hinneburg et al. (2006) who reported that ginger does not have any polyphenols and possess gingerol and zingerone that has antioxidant capacity. CIN total phenol concentration obtained in our investigation (6.51 mg / g) is comparable to that of 9.71 mg / g reported by Song et al., (2010).

The standard curve of total flavonoid using quercetin as standard is presented in Fig.3. The mean of the total flavonoid concentrations of investigated plants were 2.88,11.55,1.55, 2.08, 0.3, and 1.98 (mgQAE/g) in AN, DPL, GNR, CIN, PS, and FS respectively(Fig.4).

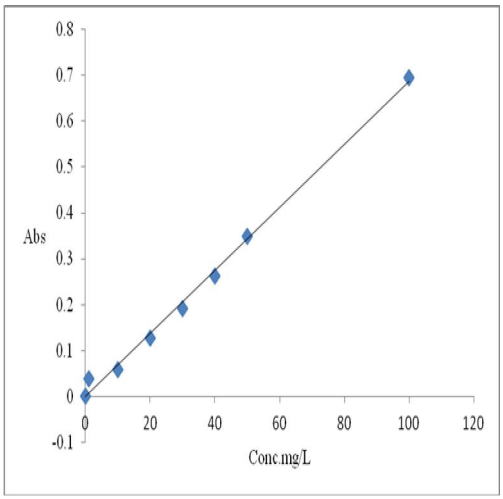

Fig.3. standard curve of Quercetin

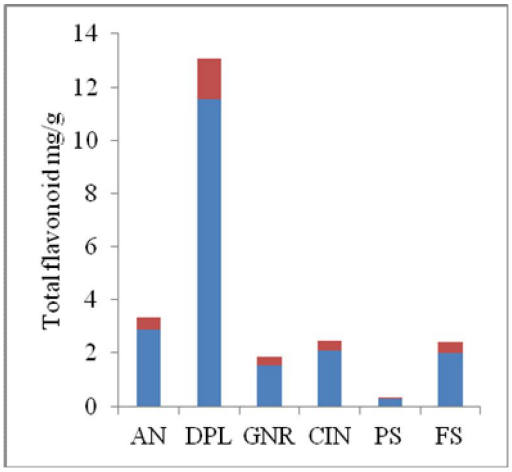

Fig. 4. Total flavonoid content as quercetin (mg/g) in Acaia nilotica (AN), Date palm leaves (DPL), Ginger (GNR), Cinnamon (CIN), Pumpkin seeds (PS), and Flax seeds (FS)

Data are expressed as mean ±SD. All samples were analyzed in three replications.

The DPL showed the highest concentration in total flavonoid 11.55 mg / g, the DPL content of total flavonoid is 14 times higher than the concentration of total flavonoid in the date palm fruit (DPF) which is reported to be 81.79 mg / 100 g (as catechin equivalents) (Biglari et al., 2008) while in our report the concentrations of total flavonoid were expressed as quercetin equivalents.

The standard curve of Trolox for determining the radical cation ABTS scavenging activity is presented in Fig.5. The mean of Trolox equivalent antioxidant capacity (TEAC, µmol Trolox equivalents per g sample) of investigated plants were 113.31, 147.27,8.33,23.75, 6.17, and 21.49 in AN, DPL, GNR, CIN, PS, and FS respectively(Fig.6).

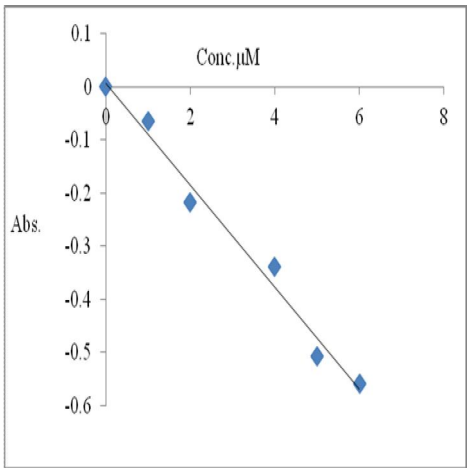

Fig.5. Standard curve of Trolox

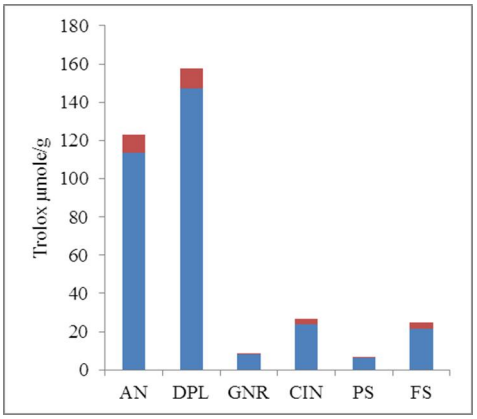

Fig. 6. Trolox equivalent antioxidant capacity (TEAC) as µmol/g in Acaia nilotica (AN), Date palm leaves (DPL), Ginger (GNR), Cinnamon (CIN), Pumpkin seeds (PS), and Flax seeds (FS)

Data are expressed as mean ±SD. All samples were analyzed in three replications

TEAC of AN and DPL are comparable to Chinese commonly used medicinal plants that are also common herbs in Arabia namely licorice root and hibiscus which are reported to have TEAC of 13322 ± 705 and 11559 ± 897 µmol Trolox equivalent per 100 g dry weight respectively(Lee et al.,2008).

Linear positive correlations were observed between the total phenolic content and TEAC radical-scavenging activity, as well as total flavonoid content, of the six selected plants. Both TEAC radical-scavenging activity and total flavonoid content showed high correlation coefficients of 0.91 and 0.84 respectively, an explanation of this finding could be that the phenolic compounds are significantly attributed to the total antioxidant activity of the plants(Fig.7a,b).The positive correlation between the total phenol content and the TEAC radical-scavenging activity is coincided with earlier reports that studied

different medicinal plants (Lee et al.,2008; Song et al., 2010). On the other hand, the positive relationship obtained between the total flavonoid content and the TEAC radical-scavenging activity of the six selected plants(r = 0.92) (Fig.7c) is in agreement with the findings of Silva et al. (2007) stated that total flavonoid correlate to TEAC radical-scavenging activity.

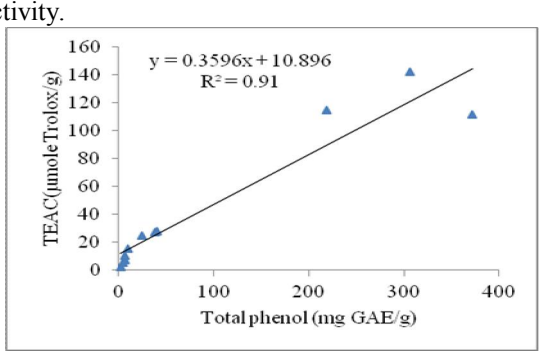

Fig. 7a. Correlation between the total phenolic content as gallic acid equivalents (GAE) and the Trolox equivalent antioxidant capacity (TEAC)of the six selected plants

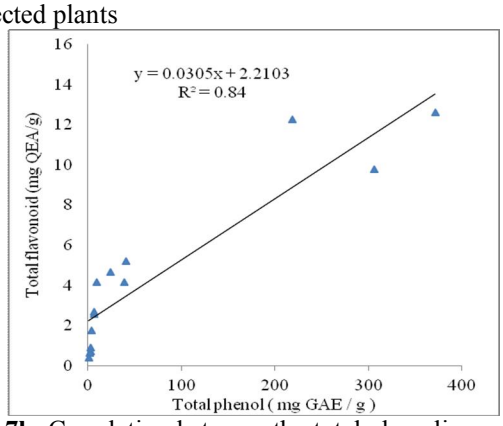

Fig.7b. Correlation between the total phenolic content as gallic acid equivalents (GAE) and the total flavonoid content as quercetin equivalents (QEA) of the six selected plants

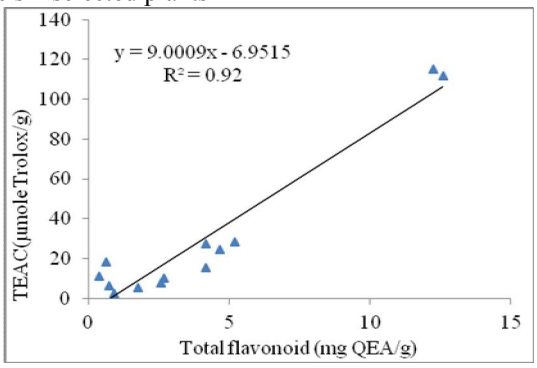

Fig. 7c. Correlation between the total flavonoid content as quercetin equivalents (QEA) the Trolox equivalent antioxidant capacity (TEAC)of the six selected plants

Conclusions

In this study, we determined the total phenolic content, total flavonoid content, and TEAC radical-scavenging activity of six Arabian plants among them, date palm leaves and *Acacia nilotica*, have not been studied before. The results showed that date palm leaves and *Acacia nilotica* fruit are potential rich sources of total phenol content and TEAC radical-scavenging activity compared with the current studied plants as well as with other medicinal plants reported in the literature. The study also showed that date palm leaves possess high total flavonoid content. Our future work will focus on the use of sophisticated techniques such as chemiluminescence and high performance liquid chromatography for the separation of the active principles of date palm leaves and *Acacia nilotica* fruit extracts and identification of their specific activities and roles in animal models induced with diabetes or atherosclerosis.

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