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Determination of Total Polyphenols and Nutritional Composition of Two Different Types of *Ficus carica* Leaves Cultivated in Saudi Arabia

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Abstract: *Ficus carica* grows in tropical and subtropical regions of Saudi Arabia. The current study was conducted to evaluate the proximate analyses, minerals, alpha-tocopherol and vitamin C contents, and to investigate the antioxidant capacities of aqueous and methanolic extracts prepared from *Ficus carica* leaves. The antioxidant capacities in the forms of DPPH and FRAP were evaluated by spectrophotometric methods. Alpha-tocopherol and vitamin C contents were determined by using a high-performance liquid chromatography (HPLC)-UV method. Total phenolic content was determined by using the Folin-Ciocalteau method. The results indicated that TPC, DPPH and FRAP values were higher in methanolic extract of big *Ficus carica* leaf (412.37±57.9mg GAE/100 g, 63.29±2.51% and 131.39±13.96 mmol Fe^{2+}/100 g) in comparison to small leaf. Total phenolics were however higher in aqueous extract compared to methanolic. The results clearly demonstrate that these extracts have antioxidant capacity though there was no significant correlation between total phenolic content and antioxidant activity. Also, nutritional composition of *Ficus carica* leaves showed that they are good sources of minerals. *Ficus carica* leaf is therefore a rich source of polyphenols and has high antioxidant properties.

Key words: Antioxidant capacity, vitamins, minerals, proximate analysis, *Ficus carica* leaf

INTRODUCTION

Ficus carica (Teen) is one of only five plants mentioned in the Qur'an along with olives, grapes, pomegranates and dates. The medicinal properties of *Ficus carica* L. (Moraceae) has been known for centuries and people still use it as a source of traditional medicines. Many other plants are currently being used for medicinal purposes due to perceived lack of side effects and lower cost. Also, there is no doubting the fact that herbal medicines may have a similar degree of efficacy as conventional drug treatment without the associated side effects (Kozubek *et al*., 2001). Herbal plants contain many natural substances including natural antioxidants which are able to protect the body against viruses and the damaging effects of reactive oxide species (ROS) (Velkov *et al*., 2009). Figs (*Ficus carica*) are cultivated in the Kingdom of Saudi Arabia and the leaf has been reported to have health benefits including antidiabetic effects (Leoporatti and Ivancheva, 2003). The fruit and leaf of *Ficus carica* are traditionally used to cure throat diseases, and as stimulant, laxative, emollient, antitussive, resolvent, emmenagogue (Guarrera, 2003) and for constipation, hemorrhoid and high cholesterol (Cansaran and Kaya, 2010). In view of reports on its health benefits thus far, we hypothesized that figs may contain high amounts of antioxidants with significant antioxidant properties. Thus, we studied two types of fig leaves (large and small), grown in Saudi Arabia for their chemical and mineral compositions, vitamins C and E and phenolic contents, and antioxidant capacities in aqueous and methanolic extracts.

MATERIALS AND METHODS

Chemicals: The following were purchased from Sigma Aldrich (St. Louis, MO, USA): Folin-Ciocalteu; sodium acetate, gallic acid, 2,2-diphenyl-2-picrylhydrazyl (DPPH); ascorbic acid; 2,4,6-tris (2-pyridyl)-1,3,5- triazine (TPTZ), sodium acetate, glacial acetic acid, ferrous sulphate, chloride reagent and methanol. All chemical reagents used in this study were of analytical or HPLC reagent grade. Glassware was always rinsed with distilled water before use.

Sample preparation and extraction: Fig leaves collected from a farm in Taif, Saudi Arabia were washed with tap water, chopped into smaller pieces and freeze-dried. All samples were ground into a fine powder using a dry grinder and stored in a freezer (-20°C) before extraction, after which they were stored in an air-tight container until further analysis. The preparation of the aqueous and methanolic extracts followed the methods of Amin and Tan (2002) and Lee *et al*. (2007a) respectively, with slight modifications. One gram of samples was homogenized in 25 ml of aqueous or 80% methanol at room temperature. The mixture was shaken using an orbital shaker at 200 rpm for 2 hour at 50°C.

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The mixture was then centrifuged at 3000 rpm for 15 minutes at room temperature. The supernatant of methanolic extract was stored at -20°C and that of aqueous extract was stored at 4°C until further analysis.

Determination of total phenolic content: The total phenolic content was determined using the Folin–Ciocalteau reagent as described by Singleton and Rossi (1965). The sample extract (0.2 ml) was mixed with 1.5ml of Folin-Ciocalteu reagent (diluted ten-fold with distilled water) and allowed to stand at room temperature for 5 minutes. To this was added 1.5 ml of 6% sodium carbonate. The mixture was stirred and allowed to stand for 90 min; the absorbance was measured at 750 nm. The results were expressed as mg gallic acid equivalent (GAE) per 100g dry weight (DW).

DPPH radical scavenging assay: The free radical scavenging activities of *Ficus carica* leaves extracts and ascorbic acid standard were determined according to the method described by Lee *et al.* (2007b). Briefly, 0.2 ml of sample extract (1, 2, 4 and 8 mg/ml in 80 % (v/v) methanol) or ascorbic acid (standard) (0.2-3.2 mg/ml) were mixed with 1 ml of 0.05 mM DPPH in absolute methanol, and the mixture shaken vigorously and allowed to stand in the dark for 30 minutes at room temperature. The absorbance was read at 517 nm. The scavenging activity was determined by comparing the absorbance with control containing equal volumes of DPPH solution and methanol. The radical scavenging activity was obtained by the following equation:

Scavenging effect (%) = [1 / (absorbance of sample / absorbance of control)] x 100

Ferric reducing / antioxidant power assay: Determination of antioxidant activity using ferric reducing/antioxidant power (FRAP) reagent method was done according to the method by Benzie and Strain (1996) with modifications. The FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyls- triazine (TPTZ) solution, and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio prior to use and heated to 37°C in a water bath. FRAP reagent (1.8 ml) was placed in a test tube and the absorbance read (Initial, $A_{initial}$ = 0 minute) at 593 nm using a UV-V spectrophotometer. Sample solution or standard (0.1 ml) was mixed with 0.1ml of distilled water and incubated at 37°C for 4 minutes. After that, the second absorbance was read (Final, A_{final} = 4 minutes). FRAP value was determined using the formula below and compared with the standard curve of ferrum sulphate (concentrations ranging of 0.05-0.5 ml).

$$\text{FRAP value} = A_{final} - A_{initial}$$

A_{final} = Final absorbance at 593 nm (4 min)
$A_{initial}$ = Initial absorbance at 593 nm (0 min)
The FRAP value was expressed as mmol Fe (II) / 100g DW.

Proximate analysis, mineral and vitamin contents: The total ash, protein, fat and carbohydrate contents were determined through the method of association of official analytical chemists (AOAC, 2000). All the proximate analyses were reported per 100g of leaves. The minerals including calcium (Ca), potassium (K), Iron (Fe), magnesium (Mg) and manganese (Mn) were analyzed by Atomic Absorption Spectrophotometry (AAS). The ascorbic acid and vitamin E were extracted according to the modified method of Abdulnabi *et al.*, (1997). Vitamin C was evaluated by a reverse-phase HPLC technique. A Hewlett Packard HPLC Series 1100 (USA) equipped with degasser, quaternary pump, auto sampler and diode array detector was used. An Ultrasphere octadecylsilyl (ODS) Hypersil C18, 5 mm particle size, in a 250 mm length x 4.0 mm I.D stainless steel column (Hewlett Packard) was used to evaluate vitamin C. The mobile phase used was a mixture of acetonitrile-water (50:50) at a flow rate of 1 ml/min, and monitoring at 225 nm. Vitamin E was determined by a reverse-phase HPLC technique. An Ultrasphere octadecylsilyl (ODS) Hypersil C18, 46 mm particle size, in a 250 mm length x 4.0 mm I. D stainless steel column (Hewlett Packard) was used to evaluate vitamin E. The mobile phase used was 100 % methanol at a flow rate of 1 ml/min, and monitored at 294 nm.

Statistical analysis: Data was presented as mean ± standard deviation. Data was statistically analyzed using SPSS (Version19.0 software, Chicago, IL, USA). One - way ANOVA and Pearson correlation coefficient were used to determine whether there were correlations between total phenolic content and antioxidant activity in *F. carica* leaves. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Proximate composition of dry figs leaf and mineral: Table 1 shows the proximate analyses of *Ficus carica* leaves. Total carbohydrate of the small leaf was 16.8 ± 0.64g while that of the big leaf was 17.3 ± 0.74g. Protein content of big leaf was 5.1 ± 0.46g, higher than the small leaf which contained 4.6 ± 0.69g. The small leaf contained 0.9 ± 0.14g of fat, whereas big leaf had 1.3 ± 0.35g of fat. Total ash contents of both leaves were quite similar at 4.2 ± 0.69g and 4.4 ± 0.41g respectively. The contents of *Ficus carica* leaf from this study are similar to previous report by El-shobaki *et al.* (2010), who reported fat and carbohydrate contents of 0.81g and 17.59g respectively. However, ash and protein contents (5.30g and 5.90g respectively) were higher than, and not in agreement with, the current study.

Table 1: Nutritional composition (per 100g), mineral, and vitamins (mg/100g) of dried *F. carica* leaves

	Dried leaves	
Component	Small	Big
Protein	4.6 ± 0.69	5.1 ± 0.46
Fat	0.9 ± 0.14	1.3 ± 0.35
Carbohydrates	16.8 ± 0.64*	17.3 ± 0.74*
Ash	4.2 ± 0.69	4.4 ± 0.41
Calcium	1398.14 ± 0.62*	2551.31 ± 0*
Iron	75.7 ± 0.23*	66.3 ± 0.05*
Potassium	117.67 ± 0*	496.71 ± 0
Magnesium	396.36 ± 0*	307.88 ± 0*
Manganese	21.9 ± 0.12*	23.12 ± 0.1*

Values expressed as means ± standard deviations.
*Signifies statistically, significant difference at 0.05 level (2-tailed) on each row.

Table 1 shows that indigenous *Ficus carica* leaf samples contain high amounts of potassium in both small and big leaves (117.67±0 and 496.71±0 mg/100g respectively), and also high manganese (21.9±0.12 and 23.12±0.13 mg/100g respectively). The abundance of potassium may suggest its usefulness for those suffering from hypertension. Calcium in these leaves were found to be 1398.14±0.62 and 2551.31±0 mg/100g respectively, while magnesium was 396.36±0 and 307.88±0 mg/100g respectively, and iron was 75.7±0.23and 66.3±0.05 mg/100g respectively.

Vitamins content: Ascorbic acid content in *Ficus carica* extracts ranged from 21.78 ± 1.78mg to 22.42 ± 0.01mg ascorbic acid/100g DW. Big and small leaf extracts possessed high amounts of vitamin E (1.9±0.57 and 1.8±1.09mg vitamin E/100g DW respectively). The contents of *Ficus carica* leaf were different from previous report by Konyalioglu *et al.* (2005) who showed *Ficus carica* leaf from Turkey as having 3.279mg/100g alpha-tocopherol using a HPLC-UV method. *Ficus carica* leaf is known to contain 10 times more alpha-tocopherol (vitamin E) compared to industrially grown soya bean, with only 0.0051-0.0111% alpha-tocopherol content (Slover *et al.*, 1983; Perez *et al.*, 2003). In addition, other vitamins like vitamin C and minerals are reportedly abundant in figs (Doymaz, 2005; Ishurd *et al.*, 2004), making it a potentially rich source of beneficial vitamins and minerals for an optimal metabolism in humans.

TPC compound of fig leaf, DPPH and FRAP: Total phenolic contents of extracts of the leaves were determined using Folin-Ciocalteu method. The highest total phenolic contents were found in big and small leaves in aqueous extracts (907.02 ± 33.24mg GAE/100g and 629.78±7.88mg GAE/100g respectively). On the other hand, their methanolic extracts yielded 412.37±57.9mg GAE/100g and 275.35±10.36mg GAE/100g respectively. When the different solvents are compared, the yield of aqueous extract had higher total

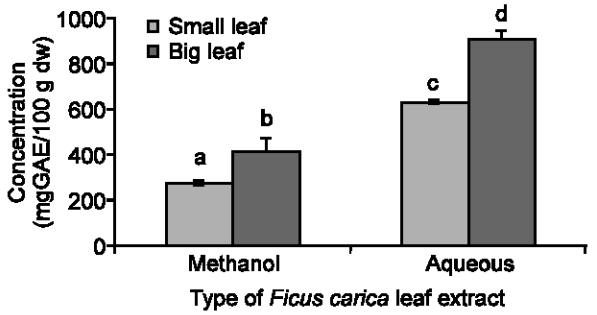

Fig. 1: Total reducing contents of *Ficus carica* leaves aqueous and methanolic extracts produced by Folin-Ciocalteu method.
Note that bars represent means while error bars represent standard deviation (SD) and different letters on error bars indicate significant difference at the level of $p < 0.05$.

phenolic content than 80% methanolic extract, with that of big leaf has higher than the small leaf. The total phenolic contents of *Ficus carica* leaves in this study are comparable to those of previous studies from different cultivars. Konyalioglu *et al.* (2005) reported *Ficus carica* leaf cultivars grown in Izmir, Turkey as having high total phenolic content based on analysis using Folin-Ciocalteau reagent. The total phenolic content in *Ficus carica* leaf, from their study, was highest in aqueous extract (6.909±0.108 mg/g dry weight) compared to methanolic extract (4.727±0.095 mg/g dry weight). On the other hand, using the same Folin-Ciocalteu assay on ethanol extract of *Ficus carica* leaf, it was shown that fresh frozen samples had the highest total phenolic content of 345.81mg GAE/g, followed by oven-dried samples with 151.36mg GAE/g. When 95% ethanol was used, fresh frozen leaf samples yielded higher phenolic content 66.55±1.58mg GAE/g over oven-dried leaf samples (52.41±1.64mg GAE/g) (Jahangiri *et al.*, 2011). Scavenging activity in aqueous extracts was found to be highest in big leaf (74.58±1.60%), followed by small leaf (21.44±4.88%). On the other hand, higher DPPH values were given by methanolic extracts of big leaf (63.29±2.51%) and small leaf (59.42±1.53%). El-Sayed and Abdel-Hamed (2009) also reported that methanolic extracts of leaves of all eleven Ficus species including *Ficus carica* grown in Egypt had phenolic compounds as major components with significant radical scavenging activity. Therefore, not only *Ficus carica* but all species may have significant relevance in the prevention of diseases. The current study is in agreement with these studies in terms of the antioxidant capacity of figs suggesting that the evidence presented demonstrates that the leaves have a potential to reduce and/or prevent free radicals. This potential may translate into prevention of chronic diseases associated with oxidative stress for humans who consume figs.

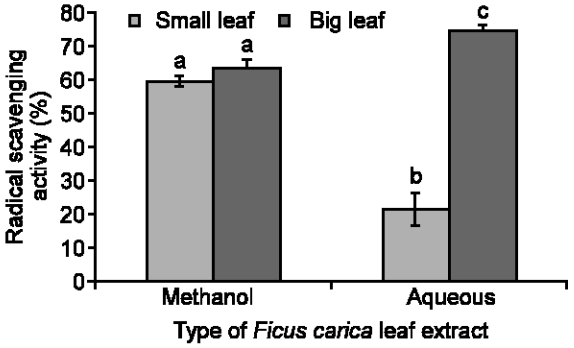

Fig. 2: Mean radical scavenging effects (%) at highest concentration mg/ml of the sample assayed by DPPH radical scavenging method using aqueous and methanolic extracts
Note that bars represent mean while error bars represent standard deviation, and bars with different letters are significantly different at the level of $p < 0.05$

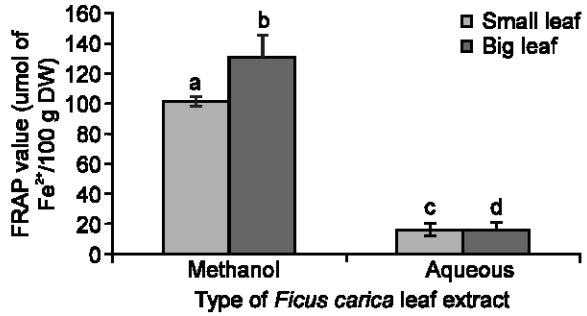

Fig. 3: Antioxidant capacity of *Ficus carica* leaves methanolic and aqueous extracts as determined by FRAP assay
Note that bars represent mean while error bars represent standard deviation, and bars with different letters are significantly different at the level of $p < 0.05$

FRAP assay was determined for aqueous and methanolic extracts of *Ficus carica* leaves. In relation to the solvent used, highest FRAP was found in methanolic extracts of big leaf (131.39±13.96 mmol Fe^{2+}/100 g), followed by small leaf (101.46±3.01 mmol Fe^{2+}/100 g). With regards aqueous extract, big leaf (16.66±4.40 mmol Fe^{2+}/100 g) had higher than small leaf (16.25±4.09 mmol Fe^{2+}/100 g). In this study, results showed that methanol extracts of leaves have higher antioxidant activity compared to aqueous extracts. The potential significance of figs is therefore as source of antioxidants that could help in reducing the level of oxidative stress and by extension prevents development of chronic diseases.

Total phenolics content in *Ficus carica* showed weak negative correlation with DPPH ($R = - 0.142$; $p < 0.05$) and FRAP ($R = - 0.512$; $p < 0.05$). This result was in agreement with Azlim-Almey *et al.* (2010) who had reported a weak negative correlation between total phenolic content and antioxidant activity. The possible explanation for negative correlation between total phenolic content and antioxidant activity could be that total phenolic content may not account for all the antioxidants present in an extract, as there could also be other non-phenolic antioxidants (Tawaha *et al.*, 2007). However, strong correlation was found between the amount of total phenolics and the antioxidant capacity similar to what was reported previously (Makris *et al.*, 2007; Pinelo *et al.*, 2005; Turkmen *et al.*, 2006; Solomon *et al.*, 2006; Veberic *et al.*, 2008).

Conclusion: The results obtained in the present study show that aqueous extract of *Ficus carica* leaf has the highest amounts of phenolics when compared to other extracts studied. It also had higher radical scavenging activity. This reveals that the leaves contain considerable amounts of antioxidants with good antioxidant activity. It is interesting to conduct more research in depth on the leaves in order that consumers benefit from them as food additive or in nutraceutical and biopharmaceutical industries.

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