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Antioxidant Properties of Water Extracts for the Iraqi Plants *Phoenix Dactylifera, Loranthus Europeas, Zingiber Officinalis and Citrus Aurantifolia*

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Abstract

In the present study, the antioxidant activities (%AA) of the water extract for *Phoenix dactylifera, Loranthus europeas, Zingiber officinalis, Citrus aurantifolia* was measured by the TBARS method. Total phenol content, DPPH scavenger free radical activity and the iron chelation capacity of these extracts were also quantified. The results revealed that *Phoenix dactylifera, Citrus aurantifolia* had a significantly ($P < 0.05$) higher total phenol, and iron chelation ability, DPPH scavenging activity, and (%AA) than *Loranthus europeas* and *Zingiber officinalis*. Meanwhile the water extracts of *Phoenix dactylifera* and *Citrus aurantifolia* had the highest protective ability and this probably due to its higher antioxidant activity (AA%), total phenol content, iron chelation and DPPH scavenging activity.

Keywords: Antioxidant activity, Polyphenol, Pro-oxidant, $Fé^{+2}$, DPPH, Iron chelation

1. Introduction

The common free radicals are oxygen reactive species (ROS) namely superoxide radical, hydroxyl radical, and peroxyl radical which can be internally produced by cellular metabolism, inflammaiton by immune cells and externally by radiation, pharmaceuticals, hydrogen peroxide, toxic chemicals, smoke, alcohol, oxidized polyunsaturated fats and cooked food. Free radicals can cause damage to parts of cells such as proteins, DNA, and cell membranes by stealing their electrons through a process called oxidation. Free radicals may cause heart damage, cancer, and a weak immune system (Feinman 1988; Esterbauer *et al*. 2006; Maharaj et al., 2006; Puntel et al., 2007). Farther more, a strong relationship between atherosclerosis and acetaldehde formed from lipid peroxidation hás been reported (Glavind *et a*l.; 1992). Most living organisms possess enzymatic and nonenzymatic defence systems against excess production of reactive oxygen species. may be of great. However, different external factors such as smoke, diet, alcohol and some drugs and aging could decrease the capability of such protective systems resulting in disturbances of the redox eqilibrium that is established in healthy conditions. Therefore, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and / or the propagation of oxidative systems resulting in disturbances of redox equilibrium that is established in healthy conditions. There for, antioxidant that scavenge reactive oxygen species may be of great value in preventing the onest and or the propagation of oxidative disease (Whilet, 1994; Olalye and rocha, 2007). Antioxidants are also compounds that scavenge reactive oxygen species may be of great value in preventing the onset and or the propagation of oxidizing chain reaction (REF). Of late, more attention hás been paid to the role of natural antioxidants mainly phenolic compounds, which may have more antioxidant activity than vitamins C, E, β- carotene (Vinson *et al*., 1995; Haslam 2006). The antioxidative effects of natural phenolic compounds in pure formes or in thier extracts from different model systems of oxidation (Gazani *et al*., 1998; Heinonen, *et al*., 2003) . Therefore, antioxidants, which can neutrlize free radicals, may be of central importance in the prevention of carcinogenicity, cardiovascular and neurodegenerative changes associated with aging (Halliwell 1994; Yu 1994; Houghton 2003; _Felter 2008). Epidemiological studies show that the consumption of plants can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames *et al*., 1993; Chu *et al*., 2002; Materska and perucka 2005).

The aim of the present work is to evaluate in vitro the antioxidant activities of water extracts for *Phoenix dactylifera, Loranthus europeaus, Zingiber officinalis, Citrus aurantifolia* in relation to their Antioxidant activity measured by the TBARS method, total phenol content, Iron chelation,and DPPH

2. Material and methods

2.1 Materials

Thiobarbituric acid (TBA), malonaldehyde- bis-dimethyl acetal(MDA) 2,2-diphenyl-1-picrylhydrazyl (DPPH_), quercetin, rutin and phenanthroline were purchased from Sigma (St. Louis, MO,USA). Sodium nitroprusside (SNP) was obtained from Merck(Darmstadt, Germany) and iron (II) sulphate from Reagen (Rio de Janeiro, RJ, Brazil).

2.2 Preparation of plant extract

The plant was purchased from Iraq and authenticated by a botanist at University of baghdad/ Iraq. Dried plant material (25 g) was soaked in boiling water (250 ml) for 15 min, allowed to cool and filtered using Whatman filter paper.The obtained residues were further extracted, twice, and then concentrated using a rotary evaporator. Filtrates were dried to a powder in an oven at 40–50 _C.

2.3 Phenolics content

The total phenol content was determined by adding 0.5 ml of the aqueous extract to 2.5 ml, 10% Folin–Ciocalteau's reagent (v/v) and 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated at 45 _C for 40 min, and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as a standard phenol (Singleton, Orthofer R, & Lamuela-Raventos, 1999). The mean of three readings was used and the total phenol content was expressed as milligrammes of gallic acid equivalents/ g extract.

2.4 Antioxidant activity toward lipid peroxidation in brain homogenate

Production of TBARS was determined using a modified method of Ohkawa, Ohishi, and Yagi (1979). The rats were killed by anaesthetizing them mildly in ether and the brain tissues were quickly removed and placed on ice. One gramme quantities of tissues were homogenised in cold 100 mM Tris-buffer Ph 7.4 (1:10 w/v) with ten up and down strokes at approximately1200 rev/min in a Teflon glass homogenizer. The homogenates were centrifuged for 10 min at 1400g to yield a pellet that was discarded and a low-speed supernatant (S1) used for the assay. The homogenates (100 µl) were incubated with or without 50 µl of the various freshly prepared oxidants (Fé SO_4) and different concentrations of the plant extracts, together with an appropriate volume of deionized water, to give a total volume of 300 µl at 37 _C for 1 h. The colour reaction was carried out by adding 300 µl of the 8.1% sodium dodecyl sulphate(SDS), acetic acid (pH 3.4) and 0.6% TBA, respectively. The absorbance was read after cooling the tubes at a wavelength of 532 nm in a spectrophotometer (TBARS 1). As the control, the homogenate was peroxidized by Fé SO_4 without the antioxidants (TBARS2). The reaction without Fé SO_4 were carried out for each of the test substance as the blank (TBARS3 is the blank for test and TBARS4 is the blank for control). The antioxidant potential of the sample was calculated by using the following equation: Antioxidant activity (%) = (1-(TBARS1 – TBARS3) / (TBARS2 – TBARS4) × 100. All tests were done in triplicate and the results averaged .

2.5 Iron chelation assay

The ability of the aqueous extract to chelate $Fé^{+2}$ was determined using a modified method of Puntel, Nogueira, and Rocha(2005). Briefly, 150 of freshly prepared 2 mM FeSO4 were added to a reaction mixture containing 168 of 0.1 M Tris–HCl (pH 7.4), 218 saline and the aqueous extract of the plant (3.5 -16.9 mg /ml). The reaction mixture was incubated for 5 min, before the addition of 13 of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the spectrophotometer

2.6 DPPH radical-scavenging

Scavenging of the stable radical, DPPH, was assayed in vitro(Hatano, Kagawa, Yasuhara, & Okuda, 1988). The extract (2.5 - 50 µg/ ml) was added to a 0.5 ml solution of DPPH (0.25 mM in 95% ethanol). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm in a spectrophotometer. Percent inhibition was calculated from the control. Vitamin C was used as a standard compound in the DPPH_ assay

2.7 Analysis of data

Quantitative data would be expressed as mean ± standard deviation. Statistical evaluation of the data would be performed by using one – way analysis of variance (ANOVA) followed by Duncan's multiple range test (Zar,1984).

3. Results and discussion

The yield and total phenolics content of the different plants extracts, *Phoenix dactylifera, Loranthus europeas, Zingiber officinalis, Citrus aurantifolia Showed in* table 1. The amount of extractable components expressed as percentage by weight of dried material . The results revealed that the yield of *Phoenix dactylifera (101.3* ± 1.0^{a}), *Citrus aurantifolia*

(94.2 ± 2.2 [d]) had a significantly (P<0.05) higher yield content than *Loranthus europeas* (86.8 ± 1.2[b]) *and Zingiber officinalis* (69.7 ± 2.3[c]). However, the total phenol content of *Phoenix dactylifera (498.9* ± 1.8[a]), *Citrus aurantifolia* (404.2 ± 2.8 [d]) had a significantly higher (P<0.05) total phenolic content than *Loranthus europeas* (335.5 ± 2.3 [b]) and *Zingiber officinalis* (266.3 ± 1.2 [c]). The results revealed that the yield and total phenol content of *Phoenix dactylifera* was higher than the yield and total phenol content reported by

Alfarisi, Morris and Baron (2007), while that of *Loranthus europeas* was within the same range with the value reported by Chopra, Nayar and Chopra (1997). The yields and phenol content of *Citrus aurantifolia* and *Zingiber officinalis ,* was higher than phenol content of some tropical leafy vegetable (Oboh , 2005). This study showed that the antioxidant activity (% AA) of *Phoenix dactylifera* (46.7 – 92.2%), *Citrus aurantifolia* (42.6 – 88.3%) had a significantly (P< 0.05) highest anti oxidant activity (% AA) than *Loranthus europeas* (25 – 73.7 %) and *Zingiber officinalis* (32.9 – 64.8) at the concentration (3.5 – 16.9 mg/ ml) (table2). The increasing of antioxidant acivity for each plant related with increasing plant extract concentration could be attributed to the presence of antioxidants, especially phenols (Chu *et al*., 2002; Matsufuji et al., 1998). Numerous studies have conclusively shown that the majorty of the anti oxidant activity May be from compounds such as flavanoids, catechin and isocatechin (Marin et al., 2004; Materska and Perucka, 2005). The $Fé^{+2}$ - chelating ability of the water extractable phytochemicals in the plants (*Phoenix dactylifera, Citrus aurantifolia , Loranthus europeas and Zingiber officinalis*) were determined and the results showed in table 3. The water extract of *Phoenix dactylifera* (81.5 – 96.8 %), *Citrus aurantifolia (*61.7 – 83.9 %) had a higher $Fé^{+2}$ - chelating ability than *Loranthus Europeas* (62.3 –75.8 %) and *Zingiber officinalis* (54.7- 64.1 %) at the concentration tested (3.5-16.9 mg/ml). However, the water extract of the *Phoenix dactylifera, Citrus aurantifolia* had a significantly higher (P<0.05) chelating ability than the water extract of *Loranthus europeas and Zingiber officinalis* . The use of iron chelation is a popular therapy for the management of $Fé^{+2}$ -associated oxidative stress in brain. The iron chelating ability of the plants under study was an indicator of the neuroprotective property of the plant because iron is involved in the pathogenesis of Alzeimer's and others diseases by multiple mechanisms(Elise & James, 2002). There was an agreement between table 1, 2, 3 extracts with the highest total phenol content had a highest $Fé^{+2}$ chelating ability and a higher antioxidant activity (AA%). Some authors (Katsube *et al.,* 2004; Djeridane et al., 2006; Katalin *et al.,* 2006) have demonstrated a linear correlation between the content of total phenolic compounds and thier antioxidant capacity. The results obtained in our study showed a good correlation between (% AA) and phenolic content. However, the DPPH radical scavenging activity of plant extract under study as shown in table 4. *Phoenix dactylifera* (21.7- 81.3 %), *Citrus aurantifolia* (15.4 -73.8 %), *Loranthus europeas* (10- 61.8%) *Zingiber officinalis* (6.4 – 54.8) at the concentration (2.5-50 µg/ ml). The water extract of *Phoenix dactylifera and Citrus aurantifolia* significantly (P<0.05) have the highest scavenging activity than *Loranthus europeas and Zingiber officinalis* at the highest concentration (50 µg/ ml). A high correlation between free radical scavenging and the phenolic contents hás been reported for creals (Peterson, 2001) fruits (Gao, 2000) and culinary herbs (Zheng and Wang, 2000). The results of DPPH radical scavenging assay revealed that the extracts by hydrogen and / or electron donation, might prevent reactive radical species from reaching biomolecules such as lipoproteins , poly unsaturated fatty acids (PUFA) , DNA,amino acids, proteins and food systems (Halliwell et al., 1995). Therapies developed along the principles of modern medicine are often limited in their efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. Therefore, treating diseases with plant-derived compounds, such as *Phoenix dactylifera, Loranthus europeas, Zingiber officinalis, Citrus aurantifolia*, which are easily available and do not require laborious pharmaceutical synthesis seems highly attractive. Phytochemical analysis of the plant showed the presence of high contents of phenolics content which may be responsible for the activity of the plant, beside other phytochemicals. Herbals and herbal extracts, which contain different classes of polyphenols, are very attractive, not only in modern phytotherapy, but also for the food industry, due to their use as preservatives. It has been reported (Calliste, Trouillas, Allais, Simon,& Duroux, 2001) that phenolic acids and their glycosides,aglycones, and monoglycosyl or diglycosyl flavonoids are distributed in the different solvents as a function of polarity and water extracts contain the most polar compounds. These facts might explain the strong scavenging and antioxidant activity of water extracts of *Phoenix dactylifera, Citrus aurantifolia*. In conclusion, the results of this study demonstrated the high efficacy of the crude aqueous extracts of *Phoenix dactylifera and Citrus aurantifolia* in free radical scavenging, inhibition of reactive oxygen species and lipid peroxidation, which may be associated with its high medicinal use as a functional food and effectiveness in treatment of different diseases, among brain and liver disease is the most important.

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Table 1. Characterization of the plants material and extraction yield for water extracts.

PLANTS	Botnical Family name	Yield of extraction (mg/g)	Phenolic compounds (mg GA/100G)
Phoenix dactyliefera	Arecaceae	101.34±1.02[a]	498.97± 1.89[a]
Loranthus europeas	Loranthaceae	86.82±1.22 [b]	335.5± 2.37 [b]
Zingiber officinalis	Zingiberaceae	69.7±2.23 [c]	266.3±1.20 [c]
Citrus aurantifolia	Rutaceae	94.2±2.24 [d]	404.27±2.89 [d]

Values represent means of triplicate.
Values with the different alphabet along the same column are significantly different (P>0.05).

Table 2. Antioxidant activity (AA %) of water extract for *Phoenix dactylifera, Loranthus europeas, Zingiber officinalis, Citrus aurantifolia.*

Conc. mg/ml	*Phoenix dactylifera*	*Citrus aurantifolia*	*Loranthus Europeas*	*Zingiber officinalis*
3.5	46.7±6.3 [a]	42.6±5.4 [a]	25±4.9 [a]	32.9±7.1 [a]
6.9	51.1±4.6 [b]	55.4±1.4 [b]	29±3.2 [b]	45.1±4.6 [b]
10	67.3±3.4 [c]	73.8±5.5 [c]	40.8±2.4 [c]	49.3±6.6 [c]
13.5	78.7±6.8 [d]	79.2±3.7 [d]	60.3±3.5 [d]	56.7±4.1 [d]
16.9	92.2±4.8 [e]	87.8±5.4 [e]	73.7±4.7 [e]	64.8±5.1 [e]

Values represent means of triplicate.
Values with the different alphabet along the same column are significantly different (P>0.05).

Table 3. $Fé^{+2}$ chelating ability of aqueous extracts of *Phoenix dactylifera, Loranthus europeas, Zingiber officinalis, Citrus aurantifolia*

Conc. mg/ml	*Phoenix dactylifera* %	*Citrus aurantifolia* %	*Loranthus europeas* %	*Zingiber officinalis* %
3.5	81.5 ± 2.4 [a]	61.7 ± 4.1 [a]	62.3 ± 5.3 [a]	54.7 ± 1.4 [a]
6.9	92.2 ± 3.7 [b]	80.3 ± 3.4 [b]	71.7 ± 3.3 [b]	61.3 ± 3.2 [b]
10	93.7 ± 1.4 [c]	82.5 ± 1.3 [c]	73.8 ± 1.3 [c]	62.2 ± 4.2 [c]
13.5	94.1 ± 1.9 [d]	83.1 ± 3.4 [d]	74.1 ± 4.6 [d]	63.8 ± 3.8 [d]
16.9	96.8 ± 4.3 [e]	83.9 ± 2.2 [e]	75.8 ± 3.1 [e]	64.1 ± 1.6 [e]

Values represent means of triplicate.

Values with the different alphabet along the same column are significantly different (P>0.05).

Table 4. DPPH radical scavenging activity of water extract of *Phoenix dactylifera, Loranthus europeas, Zingiber officinalis, Citrus aurantifolia.*

Conc.(µg/ml)	*Phoenix dactylifera* %	*Citrus aurantifolia* %	*Loranthus europeas* %	*Zingiber officinalis* %
2.5	21.7±2.4 [a]	15.4±3.1 [a]	10±6.0 [a]	6.4±3.3 [a]
5.0	37.4±4.5 [b]	21.6±5.4 [b]	17.3±2.6 [b]	13.8±4.8 [b]
12.5	51.8±3.8 [c]	43.5±3.2 [c]	27.8±1.4 [c]	25.4±5.2 [c]
25.0	67.2±4.1 [d]	52.3±4.7 [d]	49.5±3.7 [d]	37.2±4.7 [d]
50	81.3±2.8 [e]	73.8±22.4 [e]	61.8±3.4 [e]	54.8±1.8 [e]

Values represent means of triplicate.
Values with the different alphabet along the same column are significantly different (P>0.05).