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Antioxidant Activity Of Two Different Extracts From Doum*(Hyphaenethebaica*) Fruits

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Abstract: *In the present study we have investigated antioxidant activity ofdoum(Hyphaenethebaica) fruit by using soxhlet apparatus for 9 hours to each of ethanol and ethyl acetate solvents. The antioxidant activity was done by using(DPPH) 2, 2 - diphenyl, 1- picrylhydrazyland (FRAP) ferric reducing antioxidant power assays.(BHT)butylatedhydroxytoluene was used as control. The results show that the scavenging effects of both extracts from our plant on DPPH radicals increased by increasing theconcentration. Ethyl acetate extract of the H. thebaica showed strong DPPH scavenging activity at concentrations (400, 600, 800 µg⁄ml)more than ethanolic extract and BHT which were 70.20, 88.70 and 95.80% respectively. Ethyl acetate extract showed highest FRAP scavenging activity at concentration 600 µg⁄mlwhich was 92.70 % more than BHT and ethanolic extract. The lower IC50 indicates a stronger free radical inhibition, However the IC50 of ethyl acetate extract was (229.383 and 205.507) in both DPPH and FRAP assays respectively. The results also revealed that doum fruits can be used as a natural antioxidants as well as the possibility of using this plant as food additives.*

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I. Introduction

Herbal medicine is still the most common source for Human health careof about 65-80% of the world's population, because of better cultural acceptability, better compatibility with the human body and fewer side effects. Roots, flowers, bark, leaves, fruits, seeds and stem can all be constituents of herbal remedies. The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. The most important of these components are alkaloids, tannins, flavonoid and phenolics compounds[1]. In present day the studies focus on natural antioxidants especially on plant phenolics[2,3], which act as an important free radicals removal and prevention disease, therefore the interest in plant products and extracts as a source of antioxidants is growing worldwide[4,5.]Doum palm (*Hyphaenethebaica*L.) is a desert palm belonging to the family of Arecaceae. It is widespread in the sub-Saharan Africa, west India and tends to grow in areas where groundwater is present and is found along the Nile River in Egypt and Sudan. It is registered as one of the beneficial plants of the world[6,7.] Various studies have revealed the fact that the doum fruit contain high levels of essential minerals such as potassium, sodium, calcium, magnesium, and phosphorus. As well as, Doum fruit contains B-complexvitamins, carbohydrates, and dietary fiber, which is essential for good nutrition[7,8.] Numerous studies have emphasized that the doum fruit extracts contain high levels of phenols and flavonoids, and possess significant antioxidantand antimicrobial activities[7,9.]Previous studies on doum had focused on the fruit because, besides its nutritional value, the fruit drink brewed from hot water infusion of the dried fruit pulp is widely consumed as a health tonic and has been valued in the Turkana region of Kenya, for its many anecdotal medicinal properties for centuries[10,11.]The water extract of doum fruits can reduce hyperlipidaemia in nephritic syndrome and leads to decrease the risk of glomerulosclerosis and atherosclerosis and consequently the natural, safe and nontoxic *H.thebaica* fruit could be of great merit for use as hypolipidaemic drug as found by[12.]. This extract also is used in the treatment of bilharziasis, haematuria, and bleeding especially after child birth and as haematinic agent [13,14.]According to the previous studies, few scientific evaluations were done concerning the characterization of alcoholic doum fruit (*H.thebaica*) extracts.

II. Material and Methods

Plant materials and chemicals

Doum fruits were purchased from the local markets in Saudi Arabia. Chemicals were obtained from Sigma Chemicals Co. (USA).

Samples preparation and extraction

The fruits were crushed and grinded. Twenty grams of fruits powder were extracted with two different solvents(Ethanol and Ethyl- acetate) by using soxhlet apparatus for 9 hours. The extract was evaporated by using rotary evaporator and then the extract was stored at 4C prior to use[15.]

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

2ml of each sample at different concentrations(200,400.600,800,1000 µg/ml) were separately added to 1ml solution of DPPH radical in methanol. The mixture was shaken and allowed to stand for 30 min of dark place. Then the absorbance of resulting solution (yellow color) was measured at 517 nm with spectrophotometer .

Inhibition of free radical DPPH as percentage I% was collected as follow :

$$I\ \% = 100 \times \frac{\text{A Blank} - \text{A sample}}{\text{A Blank}}$$

A blank =Absorbance of control (containing all reagents except the test compound) and A sample is absorbance of test compound. IC50 value µg/ml is the effective concentration of which DPPH radical are scavenged 50% ,it was calculated by using Excel programme depended on the logarithm(Log.) of each concentration. Butylatedhydroxytoluene (BHT) was used as control[16.]

The FRAP (ferric reducing antioxidant power) method.

Various concentrations of the extracts (µg/ml) in distilled water were mixed with phosphate buffer (2.5 mL , 0.2 M , pH 6.6) and 1% of potassium ferricyanide water solution (2.5 mL , K3[Fe(CN)6]). The mixture was incubated at 50 C for 20 min. Aliquots of trichloro acetic acid (2.5 mL, 10%) were added to the mixture which was then centrifuged at 3000 rpm for 10 min . the supernatant (2.5mL) was mixed with distilled water (2.5 mL) and a freshly prepared FeCl3 solution (0.5 mL , 0.1%). The absorbance was measured at 700 nm, the reducing power of the tested samples increased with the absorbance values . BHT was used as a positive control.The reducing power of the (doum fruits extracts) were determined according to the method of [17].

III. Results and Discussion

The results in Table no (1) show that the antioxidant activity increased by increasing the concentrations. The activity of doum ethyl acetate extract was higher than ethanolic extract in the two different assays.At the concentration (400,600,800µg/ml),the highest DPPH activity was observed with theethyl acetate extract as compared with control.

Table no 1: DPPH and FRAP scavenging activities of two different extracts from *Hypheanthebiaca*fruits

Concentrations µg/ml	BHT		DoumEthanolic extract		DoumEthyl acetate extract	
	I % - DPPH	I % - FRAP	I % - DPPH	I % - FRAP	I % - DPPH	I % - FRAP
200	46.40	48.70	20.30	42.90	43.40	48.50
400	66.30	81.20	40.70	71.20	70.20	80.40
600	81.40	89.70	60.20	78.90	88.70	92.70
800	93.00	95.20	71.20	88.40	95.80	95.20
1000	99.20	100.00	95.30	90.20	97.40	99.50

The lower IC50(the half maximal inhibitory concentration) indicates a stronger free radical inhibition (strong free radical inhibitors are active at low concentrations)[18.]The IC50 of doum extracts for the DPPH and FRAP assaysare presentedin table no 2, for the ethanolic extract the IC50 was (462.190 and 211.072 µg/ml) respectively as compared with BHT(223.582 and 205.623 µg/ml) .The results also revealed that the IC50 of ethyl acetate extract was (229.383 and205.507) in both DPPH and FRAP assays respectively .

Table no 2: The IC50 of doum fruit extracts and BHT in DPPH and FRAP assays

Assay	IC50 /BHT	IC50 /DoumEthanolic extract	IC50 /Doum Ethyl acetate extract
DPPH	223.582	462.190	229.383
FRAP	205.623	211.072	205.507

Table no(3) shows that in DPPH and FRAP assays there was a significant difference at $p < 0.05$ exist between the (200 and 400 µg/ml) concentrations ofethanolic extract with each of control and ethyl acetate extract.However, there was no significance difference at $p > 0.05$ appeared between the ethyl acetate extract and control in FRAP assay in these two concentrations in addition to the 200 µg/ml concentration in DPPH assay. On the other hands ,ethyl acetate extract of doum fruit appeared to be higher than control and ethanolic extract in the concentration 400 µg/ml in DPPPH test. Figure(1)

Table no 3: DPPH and FRAP scavenging activities of(200 and 400µg/ml) in two different extracts from *Hypheanthebiaca*fruits

Concentration / Treatments	DPPH Test		FRAP Test	
	200 µg/ml	**400 µg/ml**	**200 µg/ml**	**400 µg/ml**
BHT	46.400 a ± 1.039	66.300 b ± 0.924	48.700 a ± 1.212	81.200 a ± 1.039
Ethanolic	20.300 b ± 0.808	40.700 c ± 0.924	42.900 b ± 1.097	71.200 b ± 0.866
Ethyl acetate	43.400 a ± 1.097	70.200 a ± 1.039	48.500 a ± 0.693	80.400 a ± 0.981
LSD P ≤ 0.05	**3.424**	**3.335**	**3.548**	**3.339**

Small letter s indicate to comparison in column , similar letters are non-significantly differences betweenmeans at (p≤ 0.05) using LSD test

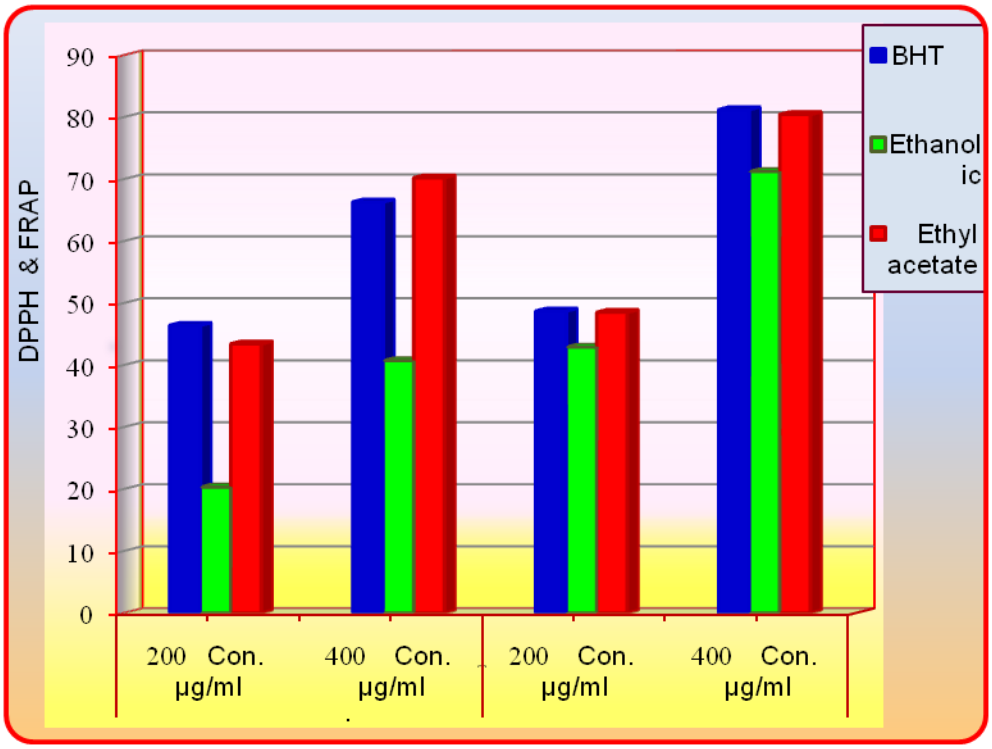

Figure 1: Antioxidant activity of(200 and 400 µg/ml) in two different extracts from *Hypheanthebiaca*fruits

IV. Discussion

The proton radical scavenging action is known as an important mechanism of antioxidants. DPPH is usually used as a substrate to evaluate the antioxidative activity of natural antioxidants because it is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[19].The effect of antioxidants on DPPHradical scavenging was thought to result from their hydrogen donating ability[20]. The decrease in absorbance of the DPPH radical caused by reacting between antioxidant molecules and the radical, progresses, which results in the scavenging of the radical by hydrogen donation. The scavenging effects of both extracts (Ethanol and ethylacetate) from our plant (*H. thebaica*) on DPPH radicals increased with concentration (Fig.1).The scavenging effects of doum fruit extracts and standard on the DPPH radical decreased in the order of BHT, ethyl acetate extract ,Ethanolic extractwhich were 46.40, 43.40 and 20.30% (Table 1)at the concentration of 200 µg/ml, respectively. Ethyl acetate extract of the *H. thebaica* shown strong DPPH scavenging activity at concentrations 400, 600, 800 µg/mlmore than ethanolic extract and BHT.These results indicated that ethyl acetate extract of doum fruithas a noticeable effect on scavengingfree radicals.The FRAP assaydepend on the reduction of a ferric tripyridyltriazinecomplex to ferrous –(2,4,6-tripyridyl-s-triazine)$_2$ i.e.: ferric (III) colorless will change to ferrous (II) blue color. The absorption readings are related to the reducing

power are related to electron-donating antioxidants present in the test compound.The scavenging effects of both extracts (Ethanol and ethyl acetate) on FRAP increased with concentration (Fig. 1). Ethyl acetate extract of the *H. thebaica* shown strong FRAP scavenging activity at concentrations 400 µg/mlmore than ethanolic extract. These results indicated that the ethyl acetate extract of *H. thebaica*has a noticeable effect on scavengingfree radicals. Phenolic compounds of the *H. thebaica*extracts are probably involved in their antiradical activity[21.] Although the activity of ethyl acetate extract of doum fruit in some concentration is relatively more than of BHT, the extract may be viable source of bioactive compounds with better activities after fractionation.

V. Conclusion

Since the presence of free radicals, especially their increased production,appears to be a feature of most, if not all human diseases.The modern research is directed towards "Natural antioxidants" from the herbal plants due to safe therapeutic. The findings of this study support the view that some medicinal plants like doum fruits are promising sources of natural antioxidants as well as tothe possibility of using this plant as food additives**.**

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