

Full Length Research Paper

Antioxidant and anticancer activities of doum fruit extract (*Hyphaene thebaica*)

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The antioxidant capacity of doum fruit extract and the total phenolic content were analyzed. The antioxidant capacity was estimated by DPPH and iron chelating assays. Quercetin, ascorbic, BHT and tannic were used as positive controls. Also the effect of doum extract on viability of acute myeloid leukemia was studied. The results showed that the total phenolic content were 0.5 µg/ 3 µg dried extract sample as quercetin. In iron chelating assay the result showed that 800 µg/ml doum extract gave the best antioxidant activity (21% inhibition). In DPPH assay the 1000 µg/ml extract exhibited 50% antioxidant activity (IC₅₀) but 1500 µg/ml extract exhibited 80% antioxidant activity. In the viability test, the results showed that half maximal inhibitory concentration (IC₅₀) of doum extract was found to be 3 µg/ml. The result indicated that the doum extract could be an important dietary source of phenolic compounds with high antioxidant and anticancer activities.

Key words: *Hyphaene thebaica*, total phenols, antioxidant and anticancer activities, AML.

INTRODUCTION

The oxidative stress, defined as the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage has been suggested to be that which cause aging and various diseases in humans. In modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept of maintaining a healthy biological system (Dreosti, 1991; Ahmad, 1995; Davies, 2000; Tiwari, 2001; Katalinic et al., 2006). In traditional Chinese medicine for more than 2000 years, a general recommendation to their consumption is to increase the intake of foods rich in antioxidant compounds (e.g polyphenols, carotenoids) due to their well-known healthy effects. As a consequence, these evidences accelerated the search for antioxidants principles, which led to the identification of natural resources and isolation of active antioxidant molecules. Many plants have been identified as having potential antioxidant activities and their consumption recommended (Kitts, et al., 2000; Lee et al., 2003; Piao et al., 2008; Kilani et al., 2008; Wang et al., 2009).

Bioactive phenols are very interesting as antioxidants because of their ability to act as efficient free radical

scavenging (Langley-Evans, 2000). In the last two decades the number of publications on the potential health benefits of polyphenols, have increased enormously (Tiwari, 2001; Lee et al., 2003; Hinneburg et al., 2006; Katalinic et al., 2006).

Doum is one of commonly consumed beverages in traditional places in Egypt and is rich in polyphenolic compounds. The current focus is toward natural antioxidant especially plant polyphenolics (Katalinic et al., 2006; Eldahshan et al., 2008, 2009)

Physiologically, antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes, antioxidants may be synthetic, such as butylated hydroxyanisole (BHA), Propyl gallate (PC) and butylated hydroxytoluene (BHT) or of natural origin such as α -tocopherol, phenolic compounds as well as polyphenolics (Abas et al., 2006). The aim of this study was to investigate the activities of this fruit extract as antioxidant and anticancer. In addition, the total phenolics content were determined.

MATERIALS AND METHODS

Plant materials and chemicals

Dried fruit materials were obtained from the supermarket. Chemicals were purchased from Sigma Chemical Co. (USA). All

Abbreviations: BHT, Butylated hydroxytoluene; DPPH, 1,1-diphenyl-2-picrylhydrazyl; AML, acute myeloid leukemia.

chemicals and reagent were of analytical grade.

Sample preparation and extraction

Fifty gram of ground plant materials were suspended in 600 ml ultra pure water and hydrodistilled for 2 h in a European Pharmacopoein hydrodistillation apparatus. The aqueous extracts were then filtered and the obtained filtrate was concentrated under vacuum to dryness, yielding the crude extract, which was then suspended in ultra pure methanol and stored at 4°C prior to use.

Total phenols

The total phenols were estimated according to the Folin-Ciocalteu method (Singleton et al., 1999). To 50 µl sample 250 µl of undiluted Folin-Ciocalteu-reagent were added. After 1 min, 750 µl of 20% (w/v) aqueous Na₂CO₃ was added and the volume was made up to 5.0 ml with H₂O. The controls contained all reaction reagents except the extract. After 2 h of incubation at 25°C, the absorbance was measured at 760 nm using a spectrophotometer (Thermo scientific-UK) and compared to a quercetin calibration curve. Total phenols were determined as quercetin equivalents (µg quercetin/g extract) and the value were presented as means of triplicate analyses.

Iron chelation activity

The chelation of iron (II) ions by the plant extract was carried out as described by Carter (1971). Different concentrations of extract were added to 100 µl of 2.0 mM aqueous FeCl₂ and 900 µl methanol. The control contained all the reaction reagents except the extract or positive control substances. After 5 min incubation, the reaction was initiated by 400 µl of 5.0% ferrozine. After a 10 min equilibrium period, the absorbance at 562 nm was recorded using a spectrophotometer (Thermoscientific-UK). The iron chelation activities were calculated from the absorbance of the control (A_c) and of the sample (A_s) using equation (1). The values are presented as the means o triplicate analysis.

$$\text{Inhibition (\%)} = \frac{(A_c) - (A_s)}{(A_c)} \times 100 \quad (1)$$

Where A_c (0) is the absorbance of the control at t = 0 min.
As (t) is the absorbance of the antioxidant at t = 16 min

DPPH radical (1,1-diphenyl-2-picrilhydrazyl)

The ability of the extracts to scavenge DPPH free radicals was determined by the method of Gyamfi et al. (1999). The controls contained all the reaction reagents except the extract or positive control substance. Different concentrations of the tested sample were placed in a cuvette and 2 ml of methanol solution of DPPH radical was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined after 16 min incubation in darkness for all samples using a spectrophotometer (Thermoscientific-UK). Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution. Methanolic solutions of pure compounds (vitamin C, tannic and quercetin) were tested too at different concentrations. All determinations were performed in triplicate. The Percentage inhibition of the DPPH radical by the samples was calculated according to the equation (1).

Viability of acute myeloid leukemia (AML)

Acute myeloid leukemia (AML) were taken from patients in National Cancer Institute (NCI) after clinical diagnosis. The mononuclear cells were separated from whole blood samples of AML patients according to Hofman et al. (1982).

Medium and reagents

The culture medium was prepared using RPMI 1640, 10% fetal bovine serum, 10% L-glutamine. Trypan blue (0.4%) was prepared by dissolving of 0.4 g of the dye in 100 ml distilled water then kept in brown closed glass bottle.

Viability of tumor cells

The viability percentages of tumor cells were measured by the modified cytotoxic trypan blue-exclusion technique of Bennett et al. (1976). The viability percentages of tumor cells were measured after incubation with doum extract as well as saline as control. Two ml of media containing AML (2 × 10⁴ cells) were transferred into a set of tubes each, then different concentrations (0.0, 1, 2, 3, 4, 8 and 10 µg/ml) from doum extract were added into the propriate tube as well as saline. The tubes were incubated at 37°C for 2 h then centrifuged at 1000 rpm for 5 min and the separated cells were suspended in 2 ml saline. For each examined materials (and control), a new clean, dry small test tube was used and 10 µl of cell suspension, 80 µl saline and 10 µl trypan blue (0.4%) were added and mixed then the number of living cells was calculated using a homocytometer slide by microscope (Nikon, TMS).

Statistical analyses

The direction and magnitude of correlation between variables were done using analysis of variance (ANOVA) and quantified by the correlation. The P-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSIONS

There are many different antioxidant components in plants and it is relatively difficult to measure each antioxidant component separately. Therefore, several different methods have been developed to evaluate the antioxidant activity of biological samples (Lopez et al., 2003). This study was determined the total phenolics content, the antioxidant capacity and anticancer activity of fruit doum extract.

Total phenolic contents

Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans et al., 1996). Therefore, the amount of total phenols in the extract was investigated by Folin-Ciocalter method. The content of total phenols is expressed as quercetin and gallic. The results showed that the total phenolic content in the fruit extract was yielding 1.2 µg/ g dried plant extract as gallic and 3.7 µg/g dried plant extract as quercetin

Table 1. Total phenols and raw materials of doum fruit extract.

Raw materials (mg extract/g plant material)	Phenolic content ($\mu\text{g/g}$ dried plant extract)	
	As gallic	As quercetin
239	1.2	3.7

Table 2. The effect of doum extract on iron chelating activities.

Doum concentration $\mu\text{g/ml}$ (w/v)	(% Inhibition)			
	Doum fruit	Quercetin	Ascorbic	Tannic
200	10 ^a	26 ^c	22 ^b	17 ^b
300	13 ^a	19 ^c	14 ^b	21 ^b
400	18 ^a	—	—	—
500	19 ^a	—	—	—
800	21 ^a	19 ^c	9 ^c	19 ^b

Table 1. This results agreement with (Cook et al., 1998) who reported that the aqueous extract of doum fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents.

Extract yields

The amount of materials that can be extracted from a plant depends on the extract procedure and possibility exists of sample-to- sample variation in the extracted materials. A double extractions of doum fruit were employed for this study. A hot water infusing procedure is commonly employed in preparing drink from the dried Doum fruit. The extract yield was 239 mg extract/g plant material (Table 1)

Iron II chelating activity

In the iron chelation assay, the general ability of the extract to donate electrons is tested whereas, in the DPPH assay, hydrogen atoms are involved one. Therefore, the ability of the extract to chelate iron (II) ions was evaluated. The results are present in Table 2. The results showed that there was a low correlation between total phenols in the examined extract and iron chelation activity. These results in agreement with Halliwell (1997). In this study, the lack correlation between total phenols and iron chelating activity may be due to the variety of the plant materials. In complex systems, such as food and food preparations, various different mechanisms may contribute to oxidative processes, such as in Fenton reactions, where transition metal ions play a vital role as catalysts of oxidative processes. Different reactive oxygen species might be generated and various target

structures such as lipids, proteins and carbohydrates, can be affected (Halliwell, 1997). These processes can be delayed by iron chelation and deactivation. Therefore, the ability of the extract to chelate iron (II) ions was evaluated. The results are present in Table 2. From the previous study the result showed that 800 $\mu\text{g/ml}$ doum extract gave the best iron chelation (21% inhibition). No significant correlation was found between the concentration of extract and iron chelation ability of the extract. These results are disagreement with those investigated by Lantto et al. (2009) who studied the antioxidant activity of Siberian pine (*Pinus sibirica Du Tour*) seeds extract. Standard antioxidant compounds were used to evaluate the iron chelating activity (ascorbic, tannic and quercetin). The results showed that no significantly correlation between the concentration of standard materials and iron chelating activity.

DPPH radical (1,1-diphenyl-2-picrilhydrazyl)

The good correlation between the results from total phenolics analysis and the antioxidative assays has been previously reported (Zhang and Wang, 2001). The role of antioxidant is to remove free radical. One important mechanism through which this is achieved is by donating hydrogen to a free radicals in its reduction to an unreactive species. Addition of hydrogen would remove the odd electron feature which is responsible for radical reactivity. The hydrogen-donating activity, measured using DPPH radicals as hydrogen acceptor, showed that there was a significant association could be found between the concentration of extract and percentage of inhibition Table 3. As shown in Table 3, at the concentration of 1000 $\mu\text{g/ml}$, the extract exhibited 50% antioxidant activity (IC_{50}) also 1500 $\mu\text{g/ml}$ extract exhibited 80% antioxidant activity. Also there was a

Table 3. The effect of doum extract on DPPH free radical – scavenging.

Doum concentration $\mu\text{g/ml}$ (w/v)	(% Inhibition)			
	Doum fruit	Quercetin	Ascorbic	BHT
200	10 ^c	11 ^c	13 ^{bc}	9 ^c
300	24 ^c	38 ^d	15 ^{bc}	14 ^c
400	25 ^{bc}	—	—	—
500	30 ^{bc}	—	—	—
800	39 ^b	—	—	—
1000	50 ^b	69 ^a	18 ^c	42 ^b
1500	80 ^a	—	—	—

Table 4. Effect of doum extract on the viability of AML.

Treatment	Doum concentration ($\mu\text{g/ml}$)	% viable cells	% dead cells
Tumor + saline (control)	0.0	98	2 ^f
Tumor + doum extract	1	93	7 ^f
Tumor + doum extract	2	83	17 ^e
Tumor + doum extract	3	50	50 ^d
Tumor + doum extract	4	40	60 ^c
Tumor + doum extract	8	18	82 ^b
Tumor + doum extract	10	8	92 ^a

significant association between the concentration of some positive control (quercetin), but there was a non significant association between the concentration of ascorbic and BHT as shown in Table 3. These results agreed with Lantto et al. (2009) and Wang et al. (2009). Also Eldahshan et al. (2008, 2009) showed that the aqueous ethanolic extract of doum leaves appeared to be a potent scavenger of reactive oxygen species.

The effect of doum extract on viability of AML (*in vitro* study)

The effect of doum extract on acute myeloid leukemia cells is recorded in Table 4. It can be found that the incubation of tumor cells with doum extract significantly reduced the viability of these cells and the dead cells were significantly increased with high extract concentration. At concentration of 2 $\mu\text{g/ml}$ the extract reduced the viability from 98 to 83% (17% death). The dead cells produced by extract reached to 50% by 3 $\mu\text{g/ml}$ when compared to control (2% death). Also doum extract reduced the viability from 98 to 60% (61% death) at 4 $\mu\text{g/ml}$ and the dead cells reached to 92% dead by 8 $\mu\text{g/ml}$. From these results it was clear that the doum extract has an inhibitory effect on AML. There was a significant association between the concentration of

doum extract and the inhibitory effect as shown in Table 4. These results agreement with İşgör et al. (2008) who studied the effects of garlic extract on Human leukemia HL60 cell lines. They found that growth inhibition exerted by extracts was in dose dependent manner. Also Aboul-Enein et al. (1991) found that *Salix safsaf* leaf extract inhibited the growth of leukemia cells. They suggested that the extract may contain active ingredients against tumor cells.

This extract has cytotoxic effect according to the guidelines from American National Cancer Institute, which considered that IC_{50} for potential plant should be < 30 $\mu\text{g/ml}$ (Alenka et al., 2000).

Abdel- Wahab et al. (2009) studied the effect of *Goniothalamus umbrosus* extract on leukemia cancer cells and their results showed this extract has an inhibitory effect against these cells. From the results obtained, it was observed that doum extract has shown antitumor towards leukemia cancer cells, which could be determined 3 $\mu\text{g/ml}$ (Table 4).

The results from the antioxidant assays showed that the examined extract can act as radical scavengers. One important mechanism of antioxidant action may be DPPH scavengers. In DPPH assays Doum extract showed a high antioxidant activity than some positive controls (BHT and ascorbic) and low antioxidant activity than other positive controls (quercetin). Whereas in iron chelating

assay the extract showed a low antioxidant activity than all positive controls antioxidants, (quercetin, ascorbic and tannic). The results suggest that phenolic compounds might contribute to the antioxidant activity of doum extract. The results obtained from viability assay revealed that doum extract has a significant anticancer activity against acute myeloid leukemia. This anticancer activity may be due to the antioxidant activity of doum extract. In future experiments would be interesting to investigate other effectiveness of doum extract in different food systems. Also many studies must be done to understand the mode of action of doum extract as anticancer. The effect of doum extract as anticancer not studied before.

Iron chelating activities was calculated as % inhibition values for Doum fruit. The values for the pure compounds (quercetin, ascorbic acid and tannic) were calculated from data obtained from similar experiments. Data are presented as mean values from three independent experiments made in triplicate. Different letters indicated significant differences at $P \leq 0.05$ level among treatments according to Duncan's multiple range test $LSD = 3.5$.

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Data are presented as mean values from three independent experiments made in triplicate. Different letters indicated significant differences at $P \leq 0.05$ level among treatments according to Duncan's multiple range test $LSD = 7.5$.

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