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Anticancer and Anti-oxidant Potentials of Ethanolic Extracts of *Phoenix dactylifera, Musa acuminata* and *Cucurbita maxima.*

Faten Abou-Elella[1]* and Rasha Mourad[2].

[1]Biochemistry Department, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.
[2] Food science Home economics Department, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.

ABSTRACT

The total phenols and flavonoids , anticancer and antioxidant activities ethanol extracts of three plants (*Phoenix dactylifera Musa acuminata* and *Cucurbita maxima*) was determined. The total phenolic contents was computed to be (342 µg/mL gallic acid equivalents in ethanol extract of banana fruit while the highest total flavonoids was in ethanol extract of molasses date (1424µM as rutin equivalent). *In vitro* anticancer activity was determined using EACC and HeLa cell lines . *In vitro* anticancer activity against EACC revealed that the maximum inhibition was observed in ethanol extract of pumpkin seeds (100% at 100µg/ml) while the maximum inhibition against HeLa cell line was observed in ethanol extract of date seeds (90% at 100µg/ml). The antioxidant activity was determined using three different methods (DPPH, ABTS scavenging activity and reducing power). DPPH scavenging activity was found to be 85 and 84 % in ethanol extracts of date seed and banana fruit, respectively. ABTS scavenging activity was found to be 98, 98, 95 and 95 % in ethanol extracts of seeds, molasses of date, fruit and peel of banana, respectively . The reducing power was 873, 833 and 871 µg/mL (GAE) in the ethanol extracts of molasses , seeds and fruit of date .Four different formulas were prepared from tested plants and the sensory evaluation of these formulas showed that prepared formulas were judged as highly accepted. The results showed that ethanol extracts of date parts , banana peel pumpkin seeds are promising new antioxidant and anticancer agents and prepared formulas could be used as a daily healthy supplement.

Keywords : Anti-cancer, antioxidant , *Phoenix dactylifera* , *Musa acuminata* and *Cucurbita maxima*

Corresponding author

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INTRODUCTION

Cancer is a multi-factorial diseases and economical burden worldwide. There are numerous chemopreventive agents used to cure various types of diseases including cancer. These drugs show an adverse side effect through alteration in gene normal action. The current treatments based on radiotherapy and chemotherapy which are effective but also show adverse consequences. Constituents of medicinal plants such as flavanoids and phenols play a significant role in cancer control through the regulation of genetic pathways without any side effect [1,2].

In the last few years, the metabolism of oxygen in humans has been investigated by biochemists all over the world. An excess of oxygen leads to formation of reactive oxygen species, which responsible for oxidative stress in the tissues–leading to cellular death and contributing to faster aging and causing diseases, like cancer, atherosclerosis, inflammatory injury, diabetes, hypertension, atherogenesis, and Alzheimers, [3,4,5]. Much attention has been focused on the activity of natural antioxidants which may reduce the level of oxidative stress [6] i.e. preventing free radicals from damaging proteins, DNA and lipids [7].

C maxima (pumpkin) belongs to the family *Cucurbitaceae*. It has received considerable attention in recent years because of the nutritional and health benefits of the bioactive compounds obtained from its seeds and fruits. Many studies demonstrated that pumpkin has extensive bioactivities, such as hepatoprotection [8], anti-diabetes [9], anti-cancer [10], and anti-obesity properties [11].

Phoenix dactylifera L are a main source of staple food in many countries. recent Studies have shown that date fruits are an excellent source of phenolics and therefore possess an extremely high antioxidant capacity. As of today, dates also have the unique distinction of being the only food to contain flavonoid sulfates, which have antioxidant properties [12,13,14]. Dates fruits has medicinal value are summarized in terms of therapeutic implications in the diseases control through anti-oxidant, anti-inflammatory, anti-tumour and anti-diabetic effect [15]

Musa species commonly known as banana, is one of the valuable plant species having a number of pharmacological activities [16]. Banana have been classified as one of the antioxidative foods [17]. The antioxidant compounds identified in *Musa acuminata* include ascorbic acid, tocopherol, beta carotene, phenolic groups, dopamine and gallocatechin [18, 19].The aim of this study is to evaluate the anticancer and antioxidant activities of ten ethanol extracts of different parts of three plans from Egypt and produce and evaluate highly accepted formulas from the ingredients that highly destroyed cancer cell .

MATERIALS AND METHODS

Chemicals

All chemicals used were of the highest analytical grade.

Plant extraction and preparation

Date and its molasses , pumpkin, and banana were purchased from the local market . Four ethanolic extracts were prepared from fruit, stigma, molasses and roasted seeds of date , four ethanol extracts were prepared from fruit, peel, seeds and material surrounded the seeds of pumpkin and two extracts were prepared from banana (fruits and peel) the peels were cut into small pieces, while seeds were grinded . 40g of each material was extracted with 500 ml ethanol 70% . Each extract was evaporated using a rotary vacuum evaporator at 45 ˚C. The obtained extracts were kept in light-protected containers at −18°C until further use.

Measurement of total phenolic content

The amount of total phenolics of the different tested ethanol extracts was determined with the Folin-Ciocalteu reagent [20]. Gallic acid standards were prepared (0-500 µg).To 50 µl of each sample 2.5 ml diluted Folin-Ciocalteau's reagent (1/100) and 2 ml of Na_2CO_3 (7.5%, w/v) were added and incubated at 45C^o for 15 min. The absorbance of all samples was measured at 765 nm using a UV–Vis spectrophotometer (GAT UV-

9100). A standard curve was plotted using different concentrations of Gallic acid and the results were expressed as gallic acid equivalent.

Measurement of total flavonoids content

The amount of total flavonoids of the different tested ethanol extracts was determined [21]. A standard curve was plotted using different concentrations (10-100µM) of rutin and all results were expressed as rutin .

Anticancer activity using Trypan blue assay

A line of Ehrlich Ascities Carcinoma was obtained from National Cancer Institute (NCI) Cairo, Egypt . The tumor cell line was maintained in female Swiss albino mice by weekly intraperitoneal (ip) transplantation of 2.5×10^6 cells/ mouse. Cancer cells ($2x\ 10^4$ /ml) were incubated with various tested ethanol extracts as control for 2 h then the viability was determined by the modified cytotoxic trypan blue-exclusion technique [22]

Anticancer activity using MTT Assay

The culture medium was prepared using RPMI 1640 media with 1.2 g/l sodium carbonate and L-glutamine (Gibco, Grand island, USA), 10% inactivated fetal bovine serum (Gibco), and 100 units/ ml penicillin and 100 mg/ ml streptomycin were added. The anticancer effect of the different 5 ethanol extracts on HeLa cell line was determined by the MTT assay [23]. Five thousand cells per well (100 µl) were plated in 96-well plates in the presence of various concentrations of the extracts (25, 50 and 100µg /ml) for 24h at 37°C in 5% CO_2 incubator. The activity of mitochondrial succinic dehydrogenase was measured by incubation for 4 h in the presence of 0.5 mg/ml of MTT (3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide). Absorbance reflects the viable cell number and was measured at 570 nm . % Cell death was calculated using the following formulas % Cell death = (Control OD –Sample OD)/Control OD x 100 [24].

DPPH free-radical Scavenging Activity

DPPH radical scavenging activity of tested ethanol extracts were evaluated [25]. The reaction mixture contained 50 µl of test samples (or 80% MeOH as a blank) and 5 ml of a 0.04% (w/v) solution of DPPH in methanol. Different standard antioxidants butylated hydroxytoluene (BHT,) and Butylated hydroxylanisole (BHA) were used as a positive control. Discoloration was measured at 517 nm after incubation for 30. Measurements were performed in triplicate.

Determination of Reducing Power

The reducing power of all samples was determined [26]. 100 µL were added to 1mL of distilled water and mixed with phosphate buffer (2.5 mL, 36 0.2 mol/ L, pH 6.6) and potassium ferricyanide [K_3 $Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 C^o for 20 min. 2.5 mL of TCA, (10%) was added to the mixture, which was then centrifuged at 3000 rpm (MSE Mistral 2000, UK) for 10 min. The reaction was initiated by the addition 200µl $FeCl_3$ (0.1%) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. All results were expressed as gallic using a standard curve of gallic acid and a linear equation) was used to calculate the total phenols of extracts.

ABTS free radical scavenging assay

The antioxidant activity of the samples was also measured by ABTS assay [27]. $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS aqueous solution with 2.45 mM potassium persulfate in darkness for 12-16 hours at room temperature. Prior to assay, this solution was diluted in aqueous and equilibrated at 30^{o}C. Sample of 0.2 mL was mixed with 3.0 mL of diluted ABTS cation radical solution. The absorbance was measured at 734nm.

Preparation of different formulas and sensory evaluation

The stigma of date, pumpkin seed and the peel of banana (they showed highest anticancer effect in our experiment) were separately hand blended first then such materials were electrically blended till became very soft.

The base of the prepared formulas was a mixture of 20% honey and 80% date molasses. The composition of formulas was shown in Table 1. Taste, odor, texture, color, appearance and overall acceptability of the four formulas were assessed by 10 panelists [28]

Statistical Analysis

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

Determination of total phenolic content

The phenolic content of the ten tested ethanol extracts was evaluated. All results are expressed as gallic acid equivalents (GAE) using the following equation Y= 0.002 X + 0.004 at R^2 = 0.887 . Table 2 recorded that seeds , molasses of date , fruit, peel of banana ethanol extracts are rich in phenols which have 273, 292, 342 and 258 µg of gallic acid equivalents (GAE). The phenolic content of The fruit, stigma of date, fruit, peel, seeds, material around seeds of Pumpkin was found to be 126, 108, 126, 84, 133, 76µg (GAE), respectively (Table 2).

Estimation of total flavonoids contents

The flavonoids content of the tested 10 ethanol extracts was estimated. All results were expressed as rutin equivalents (µM) using the following equation Y=0.001x + 0.056 at R^2 = 0.992 . Table 1 illustrated that, molasses, fruit, seeds and stigma of date ethanol extracts are rich in flavonoids, which have 1424, 1279, 1027 and 1164 µM of rutin equivalents. The extracts of peel of banana and seeds of pumpkin came in the second category which have 493 and 347 µM of rutin equivalents. Table 2.

Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids [29]. Phenolic acids have repeatedly been implicated as natural antioxidants in fruits and vegetables. For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom [30]. Pumpkin exhibit high antioxidant activity through triterpenes, Tannins glycosides and vitamins E, A and C [31].

Dates have the highest concentration of polyphenols among the dried fruits [13,32] The antioxidant activity of phenolic compounds is a result of their redox properties, which can play an important role in absorbing and neutralizing free radicals [33]. Its seeds contain high levels of phenolics ,antioxidants and dietary fiber [34].

Anticancer activity against Ehrlich ascites carcinoma cells (EACC)

Table 3 summarized the anticancer activities of tested ethanol extracts against EACC line . The maximal inhibition (100% at 100µg/ml, IC_{50} =29.9 µg/ml) was observed in the ethanol extracts of seeds of pumpkin followed by banana peel (87% at 100µg/ml, IC_{50} =33.9 µg/ml) Extract of sitgma of date possessed anticancer activity of 76%, IC_{50} =45.6 µg/ml while extract from peel of *C. maxima* gave (81 % at 100µg/ml, IC_{50} =64.7 µg/ml) Table 3.

Anticancer activity against HeLa cell line

Table 4 recorded The anticancer activity of different concentrations of five extracts molasses, fruit, and stigma of date, peel of banana and seed of pumpkin (have highest anticancer activity against EACC)

using MTT Assay and fytoside was used as positive control. The maximal inhibition was observed in the ethanol extracts of seeds of date , banana peel , seeds of pumpkin , positive control and e molasses of date which gave 90 , 87, 84, 81 % , and 75% at 100µg/ml respectively) . Data showed that extracts of , stigma of date possessed moderate anticancer activity (56 %) . The data also showed that the anticancer activity was dose dependent .These results are in agreement with [35] who showed that *C. maxima* has potent anticancer activity against EACC.

The potent anticancer activity of *C. maxima* may be due to some phytoconstituents including tocopherols (e.g., α- and ɤ-tocopherol), carotenoid (e.g., β-carotene, β-cryptoxanthin, lutein, and zeaxanthin), triterpenes, glycosides, tanins and β sitosterol , flavonoid, polyphenolics, saponin, which have has been reported for anti-inflammation [36], anti-oxidation [37], anti-carcinogenic activity [38]. Pumpkin seeds are considered an alternative treatment for stage I and II benign prostatic hyperplasia and for irritable bladder [39].

The banana extracts came in the second category .The ethanol extract of banana exhibits significant anti proliferating activity against Carcinoma of cervix cells [40]. The anticnacer effect of banana may be due to presence of anthocyanin which responsible for suppressed the proliferation of MCF-7 cell lines [41]. *musa sapientum* flower alkaloid extract was able to exert effects on cell-energy production required for the mitosis and interference with nucleic acid synthesis thereby inhibiting transcription of various genes [42]. Banana contains banlec, a jacalin-related lectin can binds to glycosylated viral envelopes and blocks viral entry, hence is a good microbicide; potent inhibitor of HIV-1 replication [43].

The antitumour and antioxidant activities of date may be due to presence of beta D-glucan [44]. The date has a great medicinal value due to its phytochemicals compounds such as flavonoids sterols carotenoids, anthocyanines, vitamins and minerals [45].

DPPH radical scavenging activity

Several methods have been developed to measure the efficiency of antioxidants. These methods focus on different mechanisms of the oxidant defense system that is scavenging active oxygen species and hydroxyle radicals, inhibiting of lipid peroxidation, or chelating of metal ions. [26] .DPPH assays often used to measure the ability of primary antioxidants in plants where these primary antioxidants react to scavenge the free radical from DPPH solution hence suppress the formation of initiation chain of free radical and destroy the propagation chain by donating hydrogen atom or electron so that the free radical can be changed to a more stable form of products . [46] Table 5 recorded the DPPH scavenging activity for tested ethanol extracts The data showed that maximum antioxidant inhibition was found to be in ethanol extracts of seeds, stigma, of date (85 and 84 %, respectively). Table 5. This study showed that these extracts have the proton-donating ability and could serve as free radical scavenger, acting possibly as primary antioxidant.

ABTS radical scavenging activity

Table 6 summarized ABTS scavenging activity of tested ethanol extracts. The data showed that scavenging activity of eight extracts was more than 80% . These ethanol extracts are seeds, , molasses, stigma of date, peel and fruit of banana and peel and seeds, fruit of pumpkin , stigma of date and material around seeds of pumpkin which gave (98, 98, 95, 84, 95, 96, 95 % and 83, respectively) Table 6.

Reducing power ($Y = 0.003x$ at $R^2 = 0.728$.)

Table 7 recorded the reducing power of different tested ethanol extracts. Amongst the tested ethanol extracts, the highest amount of reducing power was observed in ethanol extracts of fruit, seeds, molasses and stigma of date , which gave (817, 833, 873, and 750, µg/ml, respectively) followed by fruit of banana, fruit, peel, seeds and material around seeds of pumpkin which gave (470, 132, 143,156, and 151 µg/ml , respectively) Table (7).

Date palms play a significant role in neutralization of free radical and finally suppress the various types of diseases development and progression due to suppress free radicals [47,48]. Another finding in the support of dates as antioxidant reported that dates are a good source of antioxidants due to the carotenoids

and phenolics and antioxidants constituents [49]. The highest reducing power of date may be due to presence of phenolics compounds such as protocaechuic, p-hydroxy benzoic, vanillic, syringic, caffeic, coumaric, ferulic, , mainly cinnamic acids [50].There is better correlation between total phenols in banana and antioxidant [40,51]. The highest antioxidant activity and reducing power, suggesting that there may be relationship between antioxidant activity, reducing power and phenolic compounds [52,53,54].

These results also indicated that compounds with strongest reducing power were concentrated in ethanol extracts of date parts and peel of banana [40]. The bioactivity of phenolic compounds in pumpkin could be related to their antioxidant capacity, which is attributed to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals [55].

Sensory analysis

Table 8 recorded that there was no significant difference at $P \leq 0.05$ between control and prepared formulas concerning taste, odor, texture, color, appearance and overall acceptability , this may reflect the high acceptability of studied formula. Moreover formulas (2, 3 and 4) were preferred in taste than control and had good acceptance level Table 8. It is worth to mention that high acceptance found in this study ,these formulas can be used as a daily functional food supplement.

Table 1: The composition of different four formulas

Materials %	Formula (1)	Formula (2)	Formula (3)	Formula (4)
Base formula	100	50	50	50
Stigma of date	-	12.5	8.75	5
Peel of banana	-	12.5	8.75	5
Seed of pumpkin	-	25	32.5	40

Table 2: Total phenolic and flavonoids contents

Plant extracts	Total phenolic content as Gallic	Total Flavonoids content as Rutin (µM)
Phoenix dactylifera L (date) Fruit	126^{c}	1279^{b}
Seeds	273^{b}	1027^{d}
Molasses	292^{b}	1424^{a}
Stigma	108^{c}	1164^{c}
Musa acuminata (banana) Fruit	342^{a}	534^{e}
Peel	258^{b}	493^{e}
Cucurbita maxima (pumpkin) Fruit	126^{c}	11^{g}
Peel	84^{c}	113^{g}
Seeds	133^{c}	347^{f}
Material around seeds	76^{c}	56^{g}
LSD	42.36	95.0

Each value is presented as mean of triplet treatments, means within each row with different letters (a-f) differ significantly at $P \leq 0.05$ according to Duncan's multiple range test

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Table 3: *in vitro* anticancer activity of tested ethanloic extracts against EACC cell Each value is presented as mean of triplet treatments, means within each row with different letters (a-d) differ significantly at P ≤0.05 according to Duncan's multiple range test

	%Dead cells			
Plant extract	25µg/ml	50µg/ml	100µg/ml	IC_{50}50µg/ml
Control	0.0^{c}	0.0^{d}	0.0^{d}	-
Phoenix dactylifera L Fruit	0.0^{c}	0.0^{d}	3^{d}	-
Seeds	0.0^{c}	0.0^{d}	0.0^{d}	-
Molasses	0.0^{c}	0.0^{d}	48^{c}	-
Stigma	28^{b}	36^{c}	76^{b}	45.6
Musa acuminata Fruit	0.0^{c}	0.0^{d}	0.0^{d}	-
Peel	0.0^{c}	58^{b}	87^{ab}	58.06
Cucurbita maxima Fruit	0.0^{c}	0.0^{d}	0.0^{d}	-
Peel	0.0^{c}	45^{bc}	81^{b}	64.7
Seeds	54^{a}	83a	100^{a}	33.9
Material around seeds	0.0^{c}	0.0^{d}	0.0^{d}	-
LSD	6.9	14.40	14.31	

Table 4: *in vitro* anticancer activity of some tested ethanloic extracts against Hela cell line

	%Dead cell			
Plant extracts	25µg/ml	50µg/ml	100µg/ml	IC50 µg/ml
Control	0.0^{d}	0.0^{e}	0.0^{g}	-
Phoenix dactylifera L				
Seeds	44^{c}	45^{d}	90^{a}	39.6
Molasses	44^{c}	62^{b}	75^{e}	50.65
Stigma	50^{b}	53^{c}	56^{f}	65.8
Peel of *Musa acuminata*	47^{bc}	48^{cd}	87^{b}	49.5
Cucurbita maxima seeds	74^{a}	81^{a}	84^{c}	29.9
Fytoside (Positive control)	78^{a}	76^{a}	81^{d}	30.4
LSD	3.7	5.4	2.5	

Each value is presented as mean of triplicate treatments. LSD significantly at p≤0.05 according to Duncan's multiple range test.

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Table 5 : DPPH radical-scavenging activities of tested ethanloic extracts

Plant extracts	% antioxidant
Phoenix dactylifera L Fruit	62b
Seeds	85a
Molasses	73a
Stigma	84a
Musa acuminata Fruit	33c
Peel	79a
Cucurbita maxima Fruit	18c
Peel	71a
Seeds	20c
Material around seeds	20c
BHT	92a
BHA	85a
LSD	20.6

Each value is presented as mean of triplet treatments, means within each row with different letters (a-c) differ significantly at P ≤0.05 according to Duncan's multiple range test

Table 6: ABTS radical-scavenging activities of tested ethanloic extracts

Plant extracts	% antioxidant
Phoenix dactylifera L Fruit	74[c]
Seeds	98[a]
Molasses	98[a]
Stigma	83[b]
Musa acuminata *Fruit*	95[a]
Peel	95[a]
Cucurbita maxima Fruit	84[b]
Peel	96[a]
Seeds	95[a]
Material around seeds	57[d]
LSD	4.4

Each value is presented as mean of triplet treatments, means within each row with different letters (a-d) differ significantly at P ≤0.05 according to Duncan's multiple range test

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Table 7: Reducing power of tested ethanolic extracts

Plant extracts	GAE (µg/g)
Phoenix dactylifera L Fruit	817[b]
Seeds	833[b]
Molasses	873[a]
Stigma	750[c]
Musa acuminata Fruit	470[e]
Peel	679[d]
Cucurbita maxima Fruit	132[f]
Peel	143[f]
Seeds	156[f]
Material around seeds	151[f]
LSD	37.85

Each value is presented as mean of triplet treatments, means within each row with different letters (a-f) differ significantly at P ≤0.05 according to Duncan's multiple range test

Table 8: Sensory evaluation of prepared formulas

Items	Control(1)	Formula (2)	Formula (3)	Formula(4)
Taste	7.3 ±0.54a	7.9±0.38 a	8.4 ±0.4 a	8.4±0.48 a
Odor	8.3 ±0.47 a	8.6 ±0.43 a	7.7 ±0.88 a	8.8 ±0.25 a
Texture	8.7 ±0.5 a	7.4 ±0.45a	7.4 ±0.43 a	7.7 ±0.52a
Color	8.2 ±0.44 a	8.2 ±0.36 a	7.5±0.9 a	8.5 ±0.37 a
Appearance	8.5 ±0.58 a	7.1 ±0.48 a	7.5 ±0.45 a	7.9 ±0.4 a
Total acceptability	8.7 ±0.5 a	8.1 ±0.57 a	8.4 ±0.43 a	8.0 ±0.47 a

Each value is presented as mean of ten treatments. LSD significantly at p≤ 0.05

CONCLUSION

Based on the results of this study, it was revealed that pumpkin, date seeds and banana peel ethanol extracts have potential *in vitro* antioxidant activity against various antioxidant systems and anticancer due to the presence of various phytoconstituents which counteract the free radicals responsible for various health complications. The current study showed an increase in dose-dependent treatment of extracts when exposed to HeLa and EACC cell lines. Further works are needed to be carried out to isolate, identify and characterize the potential antioxidant or anti- proliferative compound (s) in the tested extracts for potential clinical use. Our results suggest that inclusion of antioxidant and anticancer -rich extract of date constitutes pumkin seeds banana peel as a dietary supplementary has beneficial effects for human health.

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