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Iceplant as Antioxidant/ Anticancer and Functional Food

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Abstract: In This study the first target is to study the phenolic content, antioxidant and anticancer activities of iceplant phenolics extract, where the second one is to incorporate of iceplant powder in cookies by substituting 2, 4 and 6% of wheat flour. Physical characteristics and sensory evaluation were also studied. HPLC analysis of iceplant powder methanolic extract resulted in the identification of 23 phenolic compounds, where catechin, pyrogallol and catechol were the highest three compounds among the phenolic compounds (41.15, 7.30 and 6.59%, respectively). Moreover, the activity of this extract against MCF7-1(carcenogenic cells of human breast) was higher than those of H1299 (carcenogenic cells of human lung) where their values were (IC50 values of 38.3 µg/ml and 22.1µg/ml, for the two previous types of cells respectively).Also, the results stated that with increasing adding iceplant powder from 0 (control) to 6% there was an increment in total phenols, flavonoids and antioxidant activity. Moreover, cookies integrated with iceplant powder have a lower spread factor, loss rate, leavening ration and L value (lightness) than control. Generally, adding iceplant powder to cookies up to 6% was acceptable.

Key words: Iceplant · Anticancer · Antioxidant activity · Cookies · *Mesembryanthemum crystallinum*

INTRODUCTION

Cancer is defined as a group of diseases identified via the abnormal cells spread due to uncontrolled growth as a result to external environmental agents (radiation, infectious microbes and chemicals) or internal agents (hormones, mutations and immunity variations) [1].

In 2011, About 15.8 million persons were patient with cancer around the world and cancer caused in mortal of 9.6 million persons in the same year [2]. The highest widespread types of cancers were Lung (12.7%) and breast (10.9%) [3].

Increasing evidence supports the hypothesis that many medicinal herbs and phytochemicals provide chemical protection against carcinogenic and mutagenic chemical substances. The previous studies revealed that there is a relation between anticancer activity and antioxidant capacity (scavenging activity and modulations of enzymatic antioxidants defense) [4].

Polyphenols which, considered the largest of secondary metabolites group in fruits and vegetables, show important antiallergic, anti-inflammatory and anticancer activities [5]. The epidemiological investigations stated that consuming diets rich with polyphenols caused a prevention of several pathological diseases, for instance, cancer and coronary diseases [6].

It is believed that dietary antioxidant help in enhancing cellular immunity and protect cellular components from oxidative damage [7]. Antioxidants biological activities are highly affected by the antioxidants interactions (antagonism, additive effects and synergism), there is a little number of investigations in connection with this topic in the whole food as a complicated system [8]. In addition, antioxidants biological characteristics may be dependent on its biological accessibility (release rate from the matrix of food during the process of digestion) and may qualitatively and quantitatively varied than those obtained within the chemical extractions applied in most investigates [9]. Thence, to study food constituents release, the structural changes and digestibility, a model for in vitro digestion is widely utilized.

Mesembryanthemum crystallinum belongs to the family of *Aizoaceae* which is the greatest spread planty families into Africa, is one of the most important wild halophytes located on the northern coast of Egypt [10]. There are trichomes named epiderm bladder cells covered

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the plant surface and these cells are reflected with its popular names: icicle plant or crystalline iceplant [11]. The iceplant grows in thick stems that spread horizontally on the ground and the stems and leaves have a succulent appearance [12]. It has a yellow or green color at the beginning of the growth and becomes orange color with age. Its flowers look like sea anemones with a colure ranged from yellow to purple and at the maturity stage it gave a fruits similar to figs with diameter of 3-4 cm [13].

Mesembryanthemum is used as a medicinal plant and functional food which contains a lot of biological and health promotion components. It has been known for its high content of bioactive components such as catechin, catechol, flavonoids (neohesperidin, rutin, hyperoside) and ferulic acid [14]. It is known for the presence of antioxidants enzymes such as catalase, superoxide dismutase and ascorbate peroxidase [15]. Additionally, iceplant has been contained a high quantity of D-pinitol, which has been shown a high antiinflammatory, anticancer, antidiabetic and antioxidant activities [16]. However, little studies were carried out on iceplant (*Mesembryanthemum crystallinum*) in Egypt.

Diets, which have a high content of polyphenols, can introduce some health advantages for the consumers. Bakery products, especially cookies, considered to be the most acceptable and viable supplementary carrier [17]. Wheat flour is the principal substance in cookies processing though is a rich resource of carbohydrates, however; it may suffer from the deficiency of minerals content and the bioactive components such antioxidants for meeting the increase in nutritional needs of the populations [18].Therefore, the first target of this work is to study the phenolic content, antioxidant and anticancer activities of iceplant phenolics extract, where the second one is to incorporate iceplant powder in cookies by substituting 2, 4 and 6% of wheat flour. Physical characteristics and sensory evaluation were also studied.

MATERIALS AND METHODS

Materials: Iceplant (*Mesembryanthemum crystallinum*) have been collected from both sides of the international coastal road near El-Boruls city, Kafr El-Sheikh Governorate, Egypt (latitude 32 35`N and longitude31 16`E) during March 2016. Drug cytotoxicity MCF7-1(Breast) and Drug cytotoxicity H1299-1(Lung) were obtained from National Institute of Oncology, Cairo, Egypt. All chemicals and solvents used in this study (HPLC grade) were obtained from Sigma Company of chemicals and drugs, St. Louis, MO, USA.

Methods

Sample Preparation: Iceplant powder were prepared according to Ibtissem *et al.* [19] as follow: iceplant samples were washed with distilled water, the excess water was removed by a white cloth, left in open air for 7 days in the dark and then oven dried for 2 h at 60°C.

Extraction of Iceplant Powder: Iceplant powder methanolic extract was prepared according to Gaur *et al.* [20] 5 gram of the fine iceplant powder was mixed with 100 ml of methanol (70%) for 24 hr at room temperature, filtered, evaporated under vacuum, then it was stored at 4°C until analysis.

Identification of Phenolic Acids: For HPLC analysis, one gram of the iceplant powder was mixed with acidified methanol (10 ml)for 4 min and centrifuged at 10000 rpm for10 min and the supernatant was filtered through a 0.2 um Millipore membrane. The filtrates were collected in a vial for injection into HPLC Hewllet packared (series 1050) equipped with auto-sampling injector, solvents (methanol and acetonitril) were degassed and ultraviolet (UV) detector was set at 280 nm and quarter HP pump. The column temperature was maintained at 35°C, where gradient separation was performed using the mobile phase (methanol and acetonitril) at flow rate of 1 ml/min following the method of Goupy *et al.* [21].

Anticancer Activity: Potential cytotoxicity of iceplant powder extract at (0,5,12.5,25 and 50 µg/ml) was tested using the Natural Red Uptake (NRU) assay[22].

Cookies Preparation: Cookies were made to contain the following ingredients flour(500 g), vegetable fat (250 g), sugar (250 g), baking powder (10 g) and eggs (180 g) according to A.A.C.C. [23]. Cookies contained different percentages of iceplant powder i.e. 0 (control), 2, 4 and 6% were prepared by substituting wheat flour with iceplant powder.

Total Phenolics, Flavonoids and Antioxidant Activity of Iceplant Powder and Cookies: Cookies methanolic extracts were prepared according to Ismail *et al.* [24], 5 gram of the fine iceplant or cookies powder was mixed with 100 ml of methanol (70%) for one hr at room temperature, filtered, evaporated under vacuum, Then it was stored at 4°C until analysis.

The total phenolics (mg gallic acid equivalent/100g extract) and flavonoids content were determined according to Andzi-Barhé *et al.* [25]. Antioxidant activity

(%) was estimated by using 1,1-diphenyl-2-picrylhydrazyl solution as mentioned by Alam *et al.* [26]

Physical Properties: Density, spread ratio and lose rate of cookies were determined by the method of Inglett *et al.* [27].Cookies color was estimated using a Hunter Lab Colorimeter (MiniScan XE Plus, Reston, VA) according to the method outlined by Žilić *et al.* [28].

Sensory Evaluation: Sensory assessment was done for measuring the response of consumer for overall acceptability of the 2%, 4% and 6% iceplant powder integrated cookies compared to the control. Twenty panelists evaluated texture, taste, color and overall acceptability according to the method described by Baumgartner *et al.* [29].

Statistical Analysis: All data (except phenolics content) were done in triplicate independent analyses and analyzed using one-way ANOVA according to Steel *et al.* [30] procedure. The significance level was set at $p \leq 0.01$.

RESULTS AND DISCUSSION

Identification of Phenolic Acids Content of Methanolic Iceplant Powder Extract: HPLC procedure was used to fractionate and identificate the polyphenolic components extracted from the tested sample. The results were tabulated in Table (1). The data obtained revealed that, iceplant methanolic extract have 23 phenolic components. Catechin was the highest compound of polyphenolic compounds found in methanolic iceplant extract (41.15%) followed by pyrogallol (7.30%) then catechol (6.59%), where Cinnamic (0.14%) and 3,4,5 Methoxycinnamic (0.33%) were the lowest ones. These results are in the same line with others obtained by Hamed *et al.* [14].

Anticancer Activity of Methanolic Iceplant Powder Extract: Methanolic extract of iceplant powder was evaluated for anticancer activity *in vitro* disease orient antitumer screening using neutral red up take (NRU)assay including two human tumor cell lines representing two different cancer types, where MCF7-1(Human breast carcinoma)and H1299(Human lung carcinoma)cancer cell lines.

As shown in Table (2) the methanolic extract of iceplant powder revealed different cytotoxic activities toward the two investigated human cancer cell lines. In general, a dose dependent decrement in the survival of the two tumor cell lines was observed. However, the dead cells were increased by increasing the concentration of methanolic extract of iceplant powder.

The highest MCF7-1 dead cell percentage was recorded by using (50µg/ml) with (61.8%) meanwhile, the lowest dead cell percentage was recorded by (5.0 µg/ml) with (9.8%). On the other hand, the highest H1299 dead cell percentage was recorded by using (50µg/ml) with (79.2%) meanwhile, the lowest dead cell percentage was recorded by applying (5.0 µg/ml) with (21.4%). These results are in the same line with those of Lee *et al.* [16].

In addition, the data in the same Table revealed that, methanolic extract of iceplant powder exhibited excellent cytotoxic activity against MCF7-1(Human breast carcinoma) (IC_{50} = 38.3 µg/ml) and H1299 (Human lung carcinoma) (IC_{50} = 22.1 µg/ml). Generally, MCF7-1 cells were less sensitive than H1299cell lines toward the investigated sample.

Gerhäuser *et al.* [31] found that phenolic compounds play an active part as antitumor substance. Oxidative stress could be very important for causing damage to DNA, hence, inducing mutations that may contribute to progressive tumor growth. Moreover, Fang *et al*. [32]

Table 1: Phenolic compounds in methanolic extract of iceplant powder

Compounds	% of the Total phenolics	Compounds	% of the Total phenolics
Gallic acid	4.15	Ferulic	0.62
4-Amino benzoic acid	0.56	Isoferulic	1.76
Protocatchuic	3.04	Ellagic	4.21
Catechin	41.15	Alpha-Coumaric	2.06
Chlorogenic	3.86	e-Vanillic	3.97
Catechol	6.59	Benzoic	6.41
Epicatechin	6.02	3,4,5 Methoxycinnamic	0.33
Caffeine	0.74	Coumarin	0.45
Caffeic	1.39	Salycilic	1.82
Vanillic	2.13	Cinnamic	0.14
â-coumaric acid	0.64	P-OH-benzoic	0.66
Pyrogallol	7.30	Total	100%

Table 2: Anticancer activity of methanolic iceplant powder extract

	Dead cell percentage	
Concentration µg/ml	MCF7-1	H1299
0.0	-----	----
5.0	9.8[d]	21.4[d]
12.5	15.3[c]	34.5[c]
25.0	37.2[b]	53.1[b]
50.0	61.8[a]	79.2[a]
IC_{50}	38.3µg/ml	22.1µg/ml

Mean followed by different letters in the same column differs significantly (P≤0.05).

Table 3: Total phenolics, flavonoids and antioxidant activity of iceplant powder and cookies

	Component		
Samples	Total Phenolic Contents (mg GAE/100g)	Total Flavonoids (mgquercetin/100g)	Antioxidant activity (%)
Iceplant powder (IP)	580.70[a]	212.59[a]	75.86[a]
Cookies control	99.25[e]	128.41[cd]	32.06[e]
Cookies with 2% IP	109.41[d]	133.58[c]	45.92[d]
Cookies with 4% IP	118,96[c]	135.07[c]	56.14[c]
Cookies with 6% IP	129.88[b]	136.63[b]	71.80[b]

Mean followed by different letters in the same column differs significantly (P≤0.05).

Table 4: Some physical properties of cookies incorporated with iceplant powder

	Percentage of iceplant powder			
Properties	Control	2%	4%	6%
Spread factor	8.10[a]	7.93[b]	7.72[b]	6.46[c]
Loss rate	13.41[a]	13.20[a]	12.90[b]	12.73[b]
Leavening ratio	99.00[a]	93.55[b]	81.76[c]	73.15[d]
Color				
L*	74.55[a]	70.32[b]	65.13[c]	61.54[d]
a*	4.58[a]	4.30[a]	3.97[b]	3.18[b]
b*	33.76[a]	32.88[a]	31.09[b]	30.77[b]

Mean followed by different letters in the same row differs significantly (P≤0.05).

reported that Gallic acid had a remarkable effect on lung cancer, it induced apoptotic enzyme and reduce the rate of tumor growth.

Total Phenolics, Flavonoids and Antioxidant Activity of Iceplant Powder and Cookies: Iceplant powder appeared the highest percentages of total phenols (580.70 mg GAE/100 g), flavonoids (212.59 mg quercetin/100g) and correlating DPPH antioxidant activity (75.86%) (Table3).

Also, data in the same Table demonstrated that there was a significant increase in cookies contents from total phenol and flavonoids from 99.25 to 129.88 mg/100 g and from 128.41to 136.63mg/100 g, respectively as a linear function for increasing the adding percentage of iceplant powder. In addition, the same trend was found in case of scavenging activity of cookies enriched with iceplant powder. These results were in the same trend obtained by Seelam *et al.* [33].

Cookies Physical Properties: Table (4) shows the effect of adding plant powder when preparing cookies on some of its physical properties. As appeared in Table 4, enriching cookies with iceplant powder caused a modest decrement in spread factor and loss rate values of made cookies when the enrichment percentage rose from 0 up to 6%. Also, the leavening ratio was a highly decreased with increasing the substitution rates. The decrement in spread factor, loss rate and leavening ratio may be due to increasing moisture absorbance percentage when adding iceplant powder.

All cookies made by replacing of wheat flour by iceplant powder had higher a* and lower b* values and L* values than the control (L* = 74.55, a* = 4.58, b* = 33.76) due to the darker color of iceplant. Addition of iceplant powder 2%, 4% and 6% significantly (P < 0.05) lowered the lightness (L) compared to the control (Table 4). These results were in the same line with Ismail *et al.* [24].

Table 5: Sensory evaluation cookies incorporated with different levels of iceplant powder

	Percentage of iceplant powder			
Properties	Control	2%	4%	6%
Taste	7.26[a]	6.48[b]	6.12[b]	6.03[b]
Color	7.64[a]	6.87[b]	6.66[b]	6.53[b]
Crispiness	7.43[a]	7.15[a]	6.76[b]	6.61[b]
Texture	7.20[a]	6.81[b]	6.52[b]	6.00[b]
Overall acceptability	7.38[a]	6.83[b]	6.52[b]	6.29[b]

Mean followed by different letters in the same row differs significantly ($P \leq 0.05$).

Sensory Evaluation: The results presented in Table 6 indicate that the control sample was higher in its sensory characteristics than all other investigated samples. There were no significant differences between all cookies samples containing iceplant powder. In general, all samples were sensory acceptable.

CONCLUSIONS

The results of this study demonstrated that iceplant methanolic extract contains 23 phenolic components. These compounds helped to improve iceplant powder extract as antioxidant and anticancer agents that can be used to prepare cookies without any negative effect on its physical properties which provide human with the required nutrients and offer protection against certain dieses.

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