J. Food and Dairy Sci., Mansoura Univ., Vol. 4 (6): 333 - 342, 2013

ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITIES OF TIGERNUT TUBERS PHENOLIC EXTRACT

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ABSTRACT

Phenolic compounds of tigernut tubers were extracted by methanol, colorimetrically determined, separated and identified using HPLC. Also, antioxidative assay of this extract was tested using DPPH radical scavenging activity method. Furthermore, these extracts were tested as antioxidants for corn oil compared to BHA. Also, these extracts were tested as antimicrobial agent against some microbial strains. The results showed that total phenolic compounds recorded about 197.20 mg/100g and the methanolic yield was 1.02%. ρ-hydroxy benzoic acid was the major phenolic compound (54.95 mg/100g) followed by salicylic acid (37.41 mg/100g); then cholchecien (35.25 mg/100g). Antioxidative activity of tiger nut phenolic extract was lower than that of BHA at all used concentrations. Phenolic extract at all used concentrations had lower antioxidative activity (indicated by peroxide, thiobarbituric acid and acid values) compared to BHA in corn oil. Antimicrobial test reflected that this extract had antimicrobial activity against gram (-) and (+) but lower than phenol 1%, as well as against *Saccharomyces spp.*, and only against *Aspergillus niger* at 50% concentration.

Keywords: Tigernut, phenolic extract, antioxidant, DPPH, corn oil, antimicrobial agent.

INTRODUCTION

Tigernut or chufa (*Cyperus esculentus* L.) a grass like plant of the family Cyperaceae is one of the finest nuts from the tropics to the temperature regions. Chukwuma *et al.* (2010) have investigated the presence and phytochemical composition of the raw and the roasted tigernut tuber. The phytochemical screening showed that a higher content of alkaloids, sterols, and resins than cyanogenic glycosides, saponins, and tannins were detected in raw tigernut tubers. Alkaloids, saponins, and tannins are known to have antimicrobial activity, as well as other physiological activities (Trease and Evans 2005). Some have been used as analgesic, antispasmodic, and antibacterial agents (Chukwuma *et al.,* 2010). Tannin compounds have antimicrobial activities and are responsible for preventing and treating urinary tract infections and other bacterial infections. The phytochemical compounds determined by Chukwuma *et al.* (2010) indicated that tigernut tubers have some biologically active compounds which could serve as potential sources of drugs. These phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals (Amadi *et al.,* 2006). Tigernut milk has been found to be good for preventing arteriosclerosis, since its consumption can help prevent heart problems and thrombosis and activate

blood circulation (Chukwuma *et al.,* 2010), mainly because its unsaturated fatty acid content is similar to that of olive oil (Linssen *et al.,* 1988). The total phenolic content in tigernut liquid co-product (TNLC) is 169.8 ± 10.5 mg gallic acid/L (Sánchez-Zapata *et al.,* 2012). Phenolic compounds may be present in TNLC because tigernut contains phenolic acids. Tigernut is a member of the Cyperaceae family that has been identified as having phenolic acids bound to cell walls (Parker *et al.,* 2000), and part of them could remain in the effluents. Parker *et al.* (2000) indentified some monomeric phenols in the tigernut cell wall, like ρ-hydroxybenzoic acid, vanillic acid, ρ-hydroxybenzaldehyde, vanillin, ρ-trans-coumaric acid, trans-ferulic acid, ρ-cis-coumaric acid, cis-ferulic acid, and other dimeric phenols. Also, some phenols in the drained-water may have come from food processing operations, like organic or inorganic cleaning agents or disinfectants (Oreopoulou and Russ 2006). Synthetic phenolic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are used in the food industry at low concentrations to prevent or retard the development of rancidity and extend the shelf-life of oils. However, these materials might cause undesirable effects for human body, since possess carcinogenic effects (Yen and Duh 1993; Serafini *et al.,* 1996). The aim of this study was to investigate the extracted phenolic compounds from tigernut tubers and identification of their components using HPLC. Also, assessment the antioxidative and antimicrobial activities of this extract.

MATERIALS AND METHODS

Materials:

Tigernut tubers (*Cyperus esculents* L.) yellow variety were purchased from local market at Tanta City, Egypt. Corn oil free of antioxidants was obtained from Tanta Company for Oils and Soaps, Tanta, Egypt. All microorganisms strains were kindly provided by the Plant Pathology Department, Faculty of Agriculture, Kafrelsheikh University. Such microorganisms were checked for purity and identity. The cultures were stored in refrigerator at 5°C until used. All chemicals used in this study were purchased from El-Gomhoria Co. for Chemicals and Drugs at Tanta City, Egypt. These chemicals were of analytical grade and were permitted for food processing applications.

Methods:

Sample preparation:

About 500g of tigernut tubers were crushed (Braun, Model 2001DL, Germany) then passed through a 60-mesh sieve. Oil content was extracted using the methods described by AOCS (1998). The defatted powder was kept in polyethylene bags and stored at -18 ^{0}C until used.

Determination of total phenolic compounds:

Extraction:

Total phenolic compounds were extracted using cold methanol 95% according to the methods described by Rodriguez de sotillo *et al.* (1994). Total phenolic compounds of this extract were colorimetrically determined (as

% of tannic acid) by the Folin- Ciocalteau method (Singleton *et al.,* 1999). The obtained extract was lyophilized. Each solution (100 and 200 mg powder/ml ethanol) was used as a sample solution for the following tests.

Identification of phenolic compounds:

Phenolic compounds were separated, identified and quantified by HPLC according to the methods of Goupy *et al.* (1999).

Determination of antioxidative activity by DPPH radical scavenging activity:

Dilutions of phenolic extract (100 and 200 µg/ml ethanol) were used to test the antioxidative activity of tigernut tubers phenolic extract. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was used to test the activity according to the method outlined by Yamaguchi *et al.* (1998)

Assessment of tigernut tubers phenolic extracts as antioxidant:

The antioxidative activity of phenolic compounds extracted from tigernut tubers were assayed by addition different phenolic extracts levels at 200, 250 and 300 ppm to corn oil (free of antioxidants) and put them in 100ml flasks. The oils were stored in air oven at 60◦C for 5 days and the degree of oxidation expressed as peroxide, thiobarbituric acid and acid values were determined each 12 hours through storage period. Acid value was determined according to the method of A. O. A. C. (2005). Peroxide value was tested using the method outlined by Leonard *et al.* (1987). Thiobarbituric (TBA) acid value was determined according to the method described by Tarladgis *et al.* (1960), which modified by Rhee (1978).

Assessment of antimicrobial activity of tigernut tubers phenolic extracts:

The disc-diffusion method was used for determination the antimicrobial activity of tigernut tubers phenolic extract against some micro-organisms. Some bacterial strains represent gram positive bacteria, namely *Bacillus subtilis* and gram negative bacteria, *Pseudomonas spp* were used. In addition, fungi isolate was also examined in this study, namely *Aspergillus niger*. Moreover, one strain of yeast namely *Saccharomyces spp* was also, tested.

Statistical analysis:

The obtained data were statistically analyzed using General Linear Models Procedure Adapted by Statistical Package for the Social Sciences (SPSS, 1997) for user's Guide Duncan Multiple Range Test was used to test the difference among means (Duncan, 1995).

RESULTS AND DISCUSSION

Phenolic compounds of tigernut tubers:

Total phenolic compounds in tigernut tubers are colorimetrically determined and the results recorded about 197.20 mg/100g and the methanolic yield was 1.02% (Table 1). Al-Saikhan *et al.* (1995) found that total phenolic contents of potato cultivars were ranged between 23.7 to 52.7 mg/100g. The obtained data agreed with the range of total phenolic acids of cabbage, carrot, aubergine, broccoli, pot-grown lettuce, spinach, radish and

red beet which ranged from 11 to 52 mg/100g as mentioned by Pirjo and Jarkko (2007).

Table (1): Total phenolic compounds (as % tannic acid) of tigernut tubers.

Properties	Tigernut tubers
Methanolic yield %	1.02%
Total phenolic (mg/100g)	197.20

Identification and quantification of phenolic compounds using HPLC:

Total phenolic compounds extracted from tigernut tubers were separated and identified by HPLC; the results are listed in Table (2). The sample contained 15 phenolic compounds. ρ-hydroxy benzoic acid was the major phenolic compound (54.95 mg/100g) followed by salicylic acid (37.41 mg/100g); then cholchecien (35.25 mg/100g) and ellagic acid (22.64 mg/100g). The results showed also that tigernut tubers methanolic extract contained small amounts of caffeic, ρ- coumaric and gallic acids.

Table (2): Identification and quantification of phenolic compounds using HPLC.

No.	Phenolic compounds	mg/100g sample
1	Gallic acid	1.15
2	ρ-OH Benzoic acid	54.95
3	Protocatechuic acid	1.72
4	Catechin	6.93
5	Chlorogenic acid	11.21
6	Catechol	4.04
7	Caffeic acid	0.97
8	Vanillic acid	2.29
9	Pyrogallol	6.21
10	Ferulic acid	4.01
11	ρ - Coumaric acid	1.01
12	Ellagic acid	22.64
13	Cholchecien	35.25
14	Salicylic acid	37.41
15	Caffeine	11.32

Determination of antioxidative activity:

Data in Table (3) showed that antioxidative activity of tigernut phenolic extract was lower than that of BHA at all used concentrations.

Table (3): Determination of DPPH radical scavenging activity of tigernut tubers methanol extract:

Sample	DPPH radical scavenging activity (%)	
	100 µg powder/ml ethanol	200 µg powder/ml ethanol
BHA	a83.76b	a94.55a
Phenolic extract	b43.42b	b60.33a

Means of values having the same case letter(s) within a column (right) are not significantly different (p > 0.05). Means of values having the same case letter(s) within a row (left) are not significantly different (p > 0.05).

Assessment of tigernut tubers phenolic extracts as antioxidant:

Changes in peroxide values (PV_s):

The primary products of lipid oxidation are hydroperoxides. Therefore, it seems responsible to determine the concentration of peroxides as a measure of the extent of oxidative deterioration occurred during storage of treated and untreated oil. The results in Fig. (1) reflected that PVs of treated and untreated oil samples increased as a function of increasing storage time. While, PVs of untreated samples were the highest. Apparent also from the same Figure that oil samples treated with 300 ppm of tigernut tubers phenolic extracts were found to be the best among all of the used concentrations and showed similar effect to BHA at 200 ppm for reducing primary products of oxidation. These results were in agreement in some measure with those reported by Salama (2000), who found that the antioxidants extracted from black and green tea (at 250 ppm) decreased the PVs of sunflower oil to 11.60 and 11.00 meq. O_2/kg; respectively.

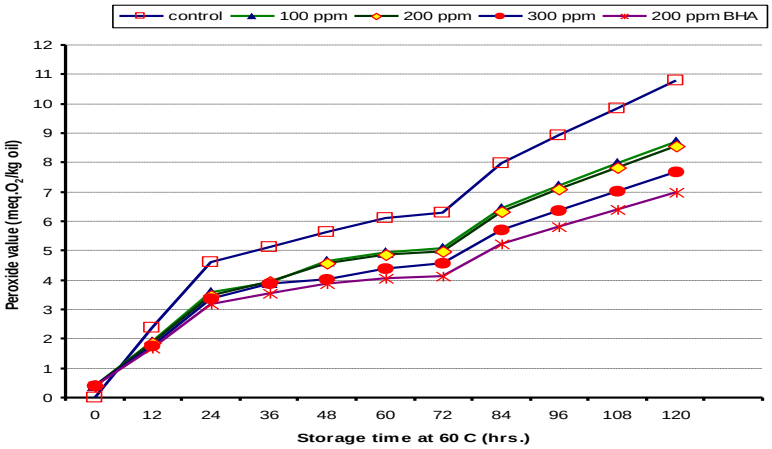

Figure (1): Peroxide values of corn oil treated with tigernut tubers phenolic extracts during storage at 60°C for 5 days.

Changes in thiobarbituric acid (TBA) values:

Thiobarbituric acid (TBA) measured by release of malondialdehyde that was the secondary oxidation products of polyunsaturated fatty acids (Pearson *et al.,* 1983). Data in Fig. (2) showed TBA values of corn oil treated

and untreated with tigernut tubers phenolic extract compared with synthetic antioxidants and stored at 60C° for 5 days. The data revealed that TBA values of untreated and treated oil samples increased as a function of increasing storage time. It may be due to the formation of secondary products of oxidation. Tigernut tubers phenolic extract possessed remarkable effects for reducing malondialhyde formation compared to control. The percentage of 300 ppm was similar to 200 ppm of BHA as antioxidant effect.

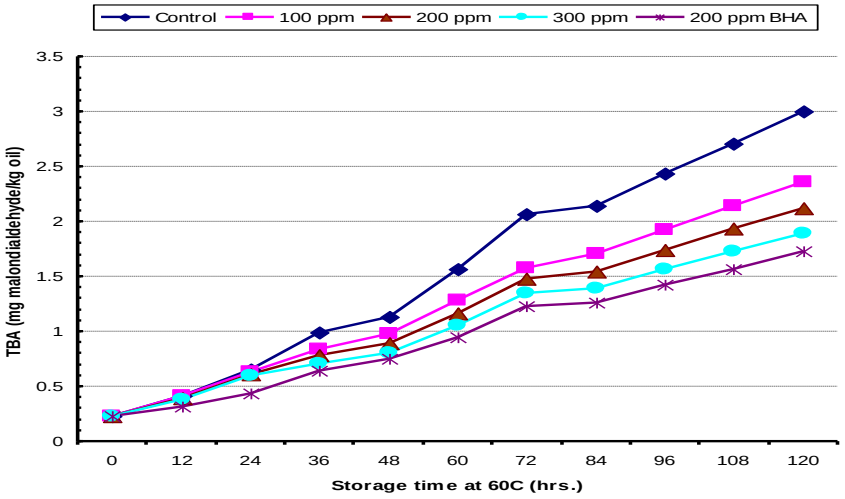

Fig. (2): Thiobarbituric acid (TBA) values of corn oil treated with tigernut tubers phenolic extracts during storage at 60 ◦C for 5 days.

Changes in acid values:

The results in Fig. (3) showed the effect of addition tigernut tubers phenolic extracts to corn oil on the acid values which refer to the third products of oxidation. It could be observed that acid values of treated and untreated oil samples increased gradually as a resulting of increasing storage time for 5 days at 60°C. But, acid values of untreated oil samples had the highest values. Tigernut tubers phenolic extracts had good effect for reducing acid values; 300 ppm was the best among all used concentrations and was similar to 200 ppm of BHA. These results were in agreement with these reported by Iskander *et al.* (2009), they found that acid value increased during storage period in cottonseed oil treated with BHA, BHT and PG, lower than that of untreated oil.

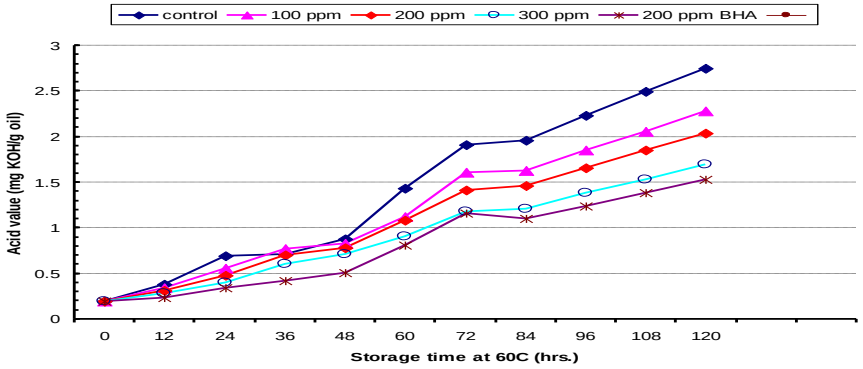

Figure (3): Acid values of corn oil treated with tigernut tubers phenolic extracts during storage at 60◦C for 5 days.

Assessment of tigernut tubers phenolic extracts as antimicrobial agent:

Three concentrations of phenolic extracts (25%, 50% and 100%) were examined and compared with 1% phenol and ethanol 70%. The phenolic extracts were diluted with 70% ethanol. The diameters of inhibition zones were taken as indicator of the antimicrobial effectiveness degree of the extracts. The obtained results are given in Table (4). Phenol 1% gave the most wide inhibition zones (11.50 mm). All used concentrations gave an inhibition zones against *Bacillus subtilis* and *Pseudomonas spp*. Inhibition zones increased as a function of increasing the used concentrations. These results are in agreement with these reported by Cueva *et al.* (2010) they found that phenolic acids at levels from 62.5 to 1000 µg/ml were effective against some microorganisms. Also, Osman, (2012) found that propolis phenolic extracts at 25, 50 and 100% levels had antimicrobial activity against *E. coli, Staphy. aureus, Aspergillus niger* and *Candida guilerimondii FTI 20037.* These results revealed also that, the harmful effects of tigernut tuber phenolic extract against *Aspergillus niger* were detected upon using only level 50%, which produced more size of inhibition zone 10.00 mm. The inhibitory effects of phenolic extracts against *Saccharomyces spp.* were noticed upon using levels 25 and 50%, but no inhibitory effect was detected in level of 100%. The effect of phenol 1% was the highest activity which had inhibition zone 7.30 mm. Level 50% gave 6.50 mm then level 25% gave 6.20 mm.

Table (4): Diameters of inhibition zones (mm) resulted from antimicrobial effects of phenolic compounds extracted from tigernut tubers toward some microorganisms.

Extract	Diameters of inhibition zones (mm) of microorganisms			
	***Bacillus subtilis* (G+)**	***Pseudomonas sp.* (G-)**	***Aspergillus niger***	***Saccharomyces spp.***
Ethanol 70%	0.00	0.00	0.00	0.00
Phenolic extract (25%)	5.80	7.50	0.00	6.20
Phenolic extract (50%)	7.50	8.30	10.00	6.50
Phenolic extract(100%)	8.60	9.00	0.00	0.00
Phenol 1%	10.00	9.20	11.50	7.30

CONCULSION

Finally, from the obtained data, it can be concluded that tigernut phenolic extract can be used as natural antioxidants against oxidative rancidity in corn oil. Although, it had antioxidative activity but lower than that of BHA. Also, this extract possessed remarkable activity as antimicrobial agents against some microorganisms.

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النشاط المضاد للاكسدة والمضاد للميكروبات للمستخلص الفينولى لدرنات حب العزيز

***مصطفى احمد عون ، *محمد فوزى عثمان ، *محمد عبدالباسط عبدالعزيز و **عواطف ابراهيم اسماعيل**
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أجريت هذه الدراسة على درنات حب العزيز بهدف الاستفادة من المستخلص الفينولى لها كمضادات اكسدة طبيعية وكذلك مضادات للميكروبات حيث تم استخلاص المركبات الفينولية بالميثانول وكذلك تقديرة كميا بالطرق اللونية ثم فصل والتعرف على تلك المركبات الفينولية على جهاز كروماتوجرافى السائل عالى الكفاءة (HPLC). وايضا اختبار النشاط المضاد للاكسدة لهذا المستخلص بطريقة DPPH وايضا اختبار ذلك على زيت الذرة مقارنه بمضاد الاكسدة الصناعى BHA . ايضا تم اختبار النشاط المضاد للميكروبات لهذا المستخلص الفينولى بطريقة Disc-diffusion method ضد سلالة بكتيرية موجبة وسالبة لصبغة جرام وكذلك نوع من الفطر وآخر من الخميرة.

حيث اظهرت النتائج ما يلى:

١- المركبات الفينولية الكلية سجلت ١٩٧,٢ مجم/١٠٠جم عينة. والمستخلص الميثانولى حوالى ١,٠٢%.
٢- كما اظهرت النتائج ان باراهيدروكسى حامض البنزويك كان اكبر المركبات الفينولية المفصولة بطرق التحليل الكروماتوجرافى حيث سجل ٥٤,٩٥ مجم/١٠٠جم يلية حمض السلسليك حيث سجل ٣٧,٤١ مجم/١٠٠جم ثم الكوليشسين ٣٥,٢٥ مجم/١٠٠جم.
٣- كما اظهرت النتائج ان النشاط المضاد للاكسدة لهذا المستخلص الفينولى كان اقل منه فى مضاد الاكسدة الصناعى BHA .
٤- عند استخدام هذا المستخلص الفينولى كمضاد اكسدة طبيعى لزيت الذرة مقارنه ب BHA حيث تم قياس هذا النشاط بتقدير رقم البيروكسيد ورقم حامض الثيوباربتيوريك وكذلك رقم الحموضة حيث وجد أن هذا المستخلص اقل نشاطا من BHA مع كل التركيزات المستخدمة.
٥- أيضا" اوضحت النتائج أنه عند استخدام هذا المستخلص الفينولى كمضاد للميكروبات وجد أنه ذو نشاط مضاد للبكتريا الموجبة والسالبة لصبغة جرام ولكنه أقل من الفينول ١%. كما كان له نشاط مضاد لخميرة *Saccharomyces spp.* لكنه اظهر نشاط مضاد لفطر *Aspergillus niger* عند تركيز ٥٠% فقط.

قام بتحكيم البحث

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