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ANTIOXIDANT AND ANTIFUNGAL ACTIVITY OF CORN TASSELS EXTRACTS FRACTIONS

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ABSTRACT

Corn is economically considered the most important crop produced all-over the world. Tassels of corn plants (male spike collected after harvesting the crop) were chemically analyzed for their phenolic content and other compounds. The contents of phenolic fractions and other compounds especially those having value added were extracted by using different solvents i.e. water, ethanol, methanol, acetone, hexane, chloroform, butanol, petroleum ether and methylene chloride separately .Chemical compounds of the ethanolic extract were fractionated and identified by GC-MS. Antioxidant activities of corn tassels fractions were determined as oxygen radical absorbance capacity with 1,1-Diphenyl -2- Picrylhydrazyl (DPPH) in addition their efficiency at different concentrations on sunflower oil was evaluated .The antifungal activity of corn tassels extract fractions (extracted by using water and ethanol separately) at different concentrations (i.e. 2,5 and10%) against different strains of fungi was tested . The inhibitory action for this fungus sp. was determined by using media broth. The results indicated that ethanol was the best solvent followed by methanol and water for extraction of total phenols (i.e., 1575.0, 1125.0 and 737.5 mg/l, respectively) from ground corn tassels. Antioxidant capacities of corn tassels were ranged from 83.0 to 85.2, 69.9 to 83.7, 69.8 to 80.4, 22.2 to 49.1 and 14.8 to 19.3 for ethanol, methanol, acetone, butanol and water extracts, respectively. The induction period values proved that ethanolic extract of corn tassels possessed an antioxidant activity for lipid oxidation by two times compared to Tert.-Butyl Hydro-Quinone (TBHQ). Results also showed that water extract of corn tassels caused more inhibition to fungal growth than ethanol extract. The rate of fungal growth inhibition was clearly recorded for *Fusarium* sp., *Penicillium* sp., *A. parasiticus*, *A. flavus*, and *A. niger.* The inhibition reached to more than 75% which could be attributed to the presence of high content of imidazol in addition to polyphenols and flavonoids compounds in the corn tassel fractions. These results prove the utilization of these natural extracts as antioxidant agents as well as their ability to inhibit the growth of fungi producing toxins and consequently could be used in different purposes in food processing instead of synthetic compounds.

INTRODUCTION

Corn (*Zea mays L.*) is one of the most widely planted crops in the world (471 million tons Corn tassels are largely available in Egypt, where the production of corn increased from 5740 in 2003/2004 up to 5780 thousand tons in 2004/2005. Corn tassels as a waste part of the plant could be considered a great renewable raw material for producing a lot of added – value products. Some studies have been reported that the extracted volatile oil **(Buttery *et al* 1980**) flavonol glycosides of quercetin, isohamnetin and kaempferol (**Ceska and Styles 1984**) and lipids (**Blanchi *et al* 1990**) are considered as value – added products. **Mour *et al* (2001)** reported that isolation of antioxidants from food processing wastes increased the value added. (**Stoilova *et al* 2005**) showed the antimicrobial activity of poly phenol mangiferi against seven bacterial and five fungal species.

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Damintoti *et al* (2005) found that the inhibition of microorganisms by phenolic compounds could be due to iron deprivation or hydrogen bounding with vital proteins such as microbial enzymes. Phenolic compounds notabally proanthocyanidins are vulnerable to polymerization in air through oxidation reactions. **Balasundram *et al* (2006)** reported that Phenolic compounds (the natural source) exhibited a wide range of physiological properties, such as antiallergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, antithrombotic and cardioprotective effect. **Turkoglua *et al* (2006)** attributed the antioxidant and antimicrobial activity of *Morchella conica pers*. to its content of phenolic and flavonoids compounds. Different studies have showed the toxicity of BHA and BHT (**Burlow, 1990**) and also their promoting the development of cancerous cells in rats (**Ito *et al* 1986**).

Therefore the present work aimed to identify the different compounds of corn tassels extracts. In addition, total phenolic fractions and other compounds extracted by using different solvents and their effect as antioxidants and antifungal substances were evaluated.

MATERIALS AND METHODS

1. Corn tassels (obtained from the Faculty of Agriculture farm, Cairo University, Egypt) after harvesting corn crop were collected, air dried, ground to a size of 1 mm and stored until use. Sunflower oil (obtained from Cairo Company for oils and soap, Cairo, Egypt.) and 1,1-Diphenyl -2- Picrylhydrazyl (DPPH) (bought from Fluka Chemika, Buchs, Switzerland), were used.

2. Strains of fungi

Fungal species (*A. parasiticus*, *A. flavus*, *A. niger*, *Penicillium sp.* and *Fusarium sp*.) were obtained from (National Research Center), Cairo, Egypt.

3. Chemical composition

Chemical composition of corn tassels was carried out according to **AOAC (2000).** Sunflower oil used was analyzed for Color (bleachability) by using the Lovibond Tentometer (The Colour Laboratory, Salisburg, England). Refractive index (by using Abbe Refractometer), free fatty acids of sunflower oil and peroxide value were determined according to **AOCS (1998)**. All analyses were carried out in triplicate.

4. Extraction of corn tassels phenolic compounds

Ground corn tassels compounds were extracted by using nine solvents separately (acetone, methanol, ethanol, water, hexan, chloroform, butanol, petroleum ether and methylene chloride) at solvent: corn tassels ratio of 10:1. Extraction was carried out using shaking incubator at room temperature for 24 hrs., then filtered through Whattman no.1 filter paper. The residue was re-extracted in the same manner and the two filtrates were combined. The ethanol extract was evaporated on a rotary evaporator (BUCHI- Rotavapor R-205 Switzerland) at 55ºC to near dryness. The total phenolic compounds were determined according to the Folin-Ciocalteu Method (**Vernon *et al* 1999**). Gallic acid was used as standard.

5. Chromatographic analysis

Chemical compounds of the ethanolic corn tassels extract were identified by GC-MS (HP 6890/5973) according to the method reported by (**Fiamegos *et al* 2004**). The compounds were identified by comparison with Library Spectra supplied by NIST v. 3.0 databases.

6. Determination of antioxidant activity

The DPPH assay was done according to the method of **Lee *et al* (2003)** with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at – 20 ºC until used. The working solution was obtained by mixing 10 ml stock solution with 45 ml methanol to obtain absorbance 1.1±0.02 units at 515 nm using the spectrophotometer (Jenway 6300). Corn tassels extracts (0.15 ml) produced by using the tested solvents were allowed to react with 3 ml of the DPPH solution for the desired time, then absorbance was read at 515 nm. The antioxidant activity of Tert-Butyl Hydro-Quinone (TBHQ) at 50 and 200 ppm was also determined. Blank by using no extracts was carried out. The results are expressed as Radical Scavenging Activity (%RSA):

% RSA= A control – A sample ×100 / A control. A= absorbance at 515nm

7. Antioxidant activity of ethanolic corn tassels extract on Sunflower oil

Antioxidant activity of ethanolic extract of corn tassels was determined on a sunflower oil model

system **(Table 5)**. The corn tassels extract was dissolved in the oil at the following concentrations (0, 100, 500 and 1000 ppm). A sample with TBHQ at 200 ppm was also prepared. Induction periods of the prepared extract samples (the induction period: the time needed for the peroxide value to become 20.) were determined by plotting the peroxide value of samples vs. time as described by **Banias *et al* (1992).**

8. Preparation of corn tassels extract tablets

Three grams of sodium carboxy methyl cellulose (CMC-sod.) were well mixed with 3 ml of the concentrated ethanolic corn tassels extract to get a homogenous paste, and then pressed in hydraulic pressure former to produce tablets.

9. Antifungal activity of corn tassels fractions

1. Preparation of Fungal spores suspension

A spore suspension of each fungal species (*A. parasiticus*, *A. flavus*, *A. niger*, *Penicillium.* sp and *Fusarium*. sp) were prepared by maintaining the fungal species on Potato Dextrose Agar (PDA) (Difco) slants for 7 days at 28ºC. The spore suspension was prepared using saline solution containing 0.05% Tween 80 and gently dislodging the spore with a flamed wire loop. The spore suspension was then obtained by passing through sterile cheesecloth to remove mycelium debris. Known amounts of spore suspension were used for determination of antifungal activity. (**Bullerman *et al* 1990**)

2. Determination of antifungal activity

The antifungal activity of the extracted corn tassels by either water or ethanol was determined by using malt extract media broth (Difco) against the previous mentioned fungus sp. Different concentrations i.e. 2, 5 and 10% of corn tassel extracts separately were used. Flasks containing 100 ml malt extract broth were inoculated by known amount of spore suspension from each fungal strain. All flasks were incubated at 25ºC for 7 days. Fungal growth especially the extent of sporulation was evaluated by comparing the color and halo size of the colonies with those in the control flasks.

RESULTS AND DISCUSSION

1. Proximate composition of corn tassels

The results in **Table (1)** show the proximate analysis of corn tassels. It could be noticed that corn tassels contained total carbohydrates (70.26%), ash 11.06, fat 10.10 and protein 6.26 and total phenols.052 %. This indicates that these unused parts of corn plant (corn tassels) could be utilized as a source of carbohydrate, fat,and ash in addition to their contents of phenolic compounds and consequently with value added.

Table 1. Chemical composition of corn tassels (dry wt. basis)

Constituents	%
Protein	6.26
Fat	10.10
Ash	11.06
Total carbohydrate	70.26
Total phenol	0.52

2. Total phenolic compounds of corn tassels as affected by solvent extraction

Table (2) shows the total extracted phenolic compounds from corn tassels using nine extracting solvents separately (i.e., Ethanol, Methanol, Acetone, Petroleum Ether, Butanol, Chloroform, Methylene Chloride, Hexane and Water.). Results generally, revealed that Ethanol was the best solvent in extracting phenolic compounds, followed by Methanol then Water (1575.00, 1125.00 and 737.50 mg/l, respectively). On the other hand, fewer amounts of phenolic compounds were extracted by either acetone, Petroleum Ether, Butanol, Chloroform, Methylene Chloride or hexane compared to the above mentioned three solvents.

Table 2. Total phenolic compounds extracted from the corn tassels using different solvents

Solvent type	Total phenolic compounds (mg/l)
Ethanol	1575.00
Methanol	1125.00
Acetone	200.00
Petroleum Ether	77.50
Butanol	50.00
Chloroform	33.75
Methylene chloride	16.25
Hexane	13.75

3. Identified compounds in ethanolic corn tassels extract

The ethanolic corn tassels extract was fractionated and identified by GC-MS as presented in **Table (3)** Results showed that the ethanolic extract of corn tassels contained different compounds i.e., N – (3-phenylbezo(b) selenophene, 1- Naphthalenepentanoic acid, 1H-Imidazole, 1- (trimethylsilyl), Perphenazine, Franchetine / Calcimycin, 10-Bromo-6-(3-bromophenyl),benzimid,7-chloro (methyoxycarbonylmet), 2,3-diazabicyclo-oct-7-en, Silane, (5.beta.-cholestan-3.beta.), Demethylchelerythrine, Pimozide / 2H-Benzimidazol-2-one, Methyl 5,7-dibromo-8-hydroxy, 1,1,8,8-tetrakis (t- butyl)-1,8-disi, 2-Benzoyl-6-methyl-4,5-diphenyl, 6,8-dihydro-4,10-di isopropyl, 14-Acetyltalatisamine, 9,10,11,12-tetraphenyl-3-oxabicycl, Cyclopentadienyl, 1,3,7-trimethyl-8 hydroxy-alloxazin, Coumarin (3- benzoyl- 4- phenyl, Cholestane-3,5,6,7,-tetrol, 1-cyclopentene-1-propanoic acid, 1-naphthacenecarboxylic acid, 4,8-diphenyl-1-(methylphenyl), 2-thiophenepropiolaldehyde, 2-(4-bromo-phenyl)-3-chlorophenyl and Chromene (oxo-2H-1-benzopyran-2-yl)-5-metho.Among these compounds ethanolic corn tassels extract contained 1575 mg/l total phenolic compounds in addition to other compounds **(Table 3)** i.e., polyphenols (i.e., tetrakis), flavonoids (i.e., chromene), alkaloide (i.e., acetyltalatisamine), steroide (i.e., cholestan) and others. This indicates that such extract contained different compounds most of them have antioxidant and antifungal activity (**Domintoti *et al* 2005**).

4. Antioxidant activity of corn tassels extracts

Data in **Tables (4&5)** show the antioxidant activity (% RSA) of different extracts of corn tassels using different organic solvents, and the characteristics of the used sunflower oil. It was clear from **(Table 4)** that all extracts exhibited antioxidant activity. The antioxidant power of the examined extracts could be arranged in descending order Comparing to Tert-Butyl Hydro-Quinone (TBHQ) as control as follow: TBHQ-200 > TBHQ-50 > Ethanol > Methanol > Acetone > Butanol > Water. It is also worth to note that the extracts with relatively higher antioxidant activity (Ethanol and Methanol) contained higher amount of total phenolic compounds **(Table 2)**. Results also revealed that,the prepared tablets of the ethanolic corn tassels extract possessed good antioxidant power. Therefore, this natural antioxidant could be applied successfully in food processing instead of the synthetic one.

5. Effect of ethanolic corn tassels extract on induction period of sunflower oil

Results in **Table (6)** showed that the induction period of sunflower oil was increased by increasing the concentration of the ethanolic corn tassels extract (i.e., from 1000 to 5000 ppm). Increasing the ethanolic corn tassels extract up to 4000 ppm increased the induction period to 40. This indicates that addition of the ethanolic corn tassels extract caused an increase in the induction period of sunflower oil by two times than those caused by TBHQ (at 200 ppm). This means that such extract had high antioxidant activity compared to TBHQ. It could also be concluded that such extract of corn tassels has a beneficial effect as antioxidant agent and could retard oxidative degradation of lipids and thereby improve the quality characteristics and nutritional value of food. In addition, the utilization of food crops wastes in the preparation of useful compounds especially antioxidant agents for the maintenance of health and protection from coronary heat diseases and cancer is also raising interest among scientists and food processors.

6. Effect of corn tassel compounds on fungal growth

The extracted fractions of corn tassels by either water or ethanol were evaluated for their effect on the rate of growth of some fungal sp. (*A. parasiticus*, *A. flavus*, *A. niger*, *Penicillium.* sp and *Fusarium*. sp) as presented in **Tables (7 and 8)**. Results indicated that corn tassel compounds caused an inhibition to the growth of the studied fungal species. The rate of growth inhibition was clearly recorded for water extract and also increased by increasing the corn tassel fractions concentrations. The rate of fungal growth inhibition for the studied strains was varied in which *A. parasiticus*, *A. flavus*, *A. niger*, and *Penicillium sp*. showed a very clear and large halo (maximum inhibition rate) while it was small halo (minimum inhibition rate) for *A. flavus and Fusarium sp. when treated* by water extract of corn tassels. When these strains were treated by ethanolic corn tassels extract, the rate of inhibition was reduced to small halo in case of *A. parasiticus*, *Penicillium sp.* and *Fusarium sp.* while *A. flavus*, and *A. niger* showed no inhibition growth.

Table 3. Identified main compounds in ethanolic corn tassels extract, retention time and relative percent

Identified compounds	Retention time	% (area)
N – (3-phenylbezo(b)selenophene	3.58	0.02
1- Naphthalenepentanoic acid	4.40	0.02
1H-Imidazole, 1- (trimethylsilyl)	5.40	97.94
Perphenazine	6.60	0.06
Franchetine / Calcimycin	16.39	0.05
10- Bromo-6-(3-bromophenyl) benzimid	17.47	0.05
7- chloro-1-(2-(methyoxycarbonylmet)	17.93	0.03
2,3-diazabicyclo-oct-7-en	18.03	0.06
Steroide: Silane, (5.beta.-cholestan-3.beta.)	18.39	0.05
Demethylchelerythrine	19.28	0.06
Pimozide / 2H-Benzimidazol-2-one	19.58	0.06
Methyl 5,7-dibromo-8-hydroxy	20.35	0.03
Polyphenol: 1,1,8,8-tetrakis (t- butyl)-1,8-disi	20.67	0.06
2-Benzoyl-6-methyl-4,5-diphenyl	21.76	0.05
6,8-dihydro-4,10-di isopropyl	22.56	0.07
Alkaloide: 14-Acetyltalatisamine	22.59	0.06
9,10,11,12-tetraphenyl-3-oxabicycl	22.62	0.04
Cyclopentadienyl	23.99	0.07
1,3,7-trimethyl-8 hydroxy-alloxazin	24.87	0.04
Coumarin (3- benzoyl- 4- phenyl	27.15	0.03
Cholestane-3,5,6,7,-tetrol	28.33	0.06
1-cyclopentene-1-propanoic acid	29.46	0.07
1-naphthacenecarboxylic acid	29.90	0.03
4,8-diphenyl-1-(methylphenyl)	30.25	0.05
2-thiophenepropiolaldehyde	30.34	0.04
2-(4-bromo-phenyl)-3-chlorophenyl	30.58	0.05
Flavonoids: Chromene (oxo-2H-1-benzopyran-2-yl)-5-metho	30.73	0.03

Table 4. The antioxidant activity (as scavenging capacity) of the different corn tassels extracts at room temperature at 0, 5, 10, 20 min

Time (min.) / Extracts	Zero	5	10	15	20
Ethanol B	84.3	83.0	83.2	84.6	85.2
Ethanol A	76.0	74.8	75.3	76.1	77.0
Methanol	69.9	82.1	82.0	83.3	83.7
Acetone	69.8	73.6	76.6	79.0	80.4
Butanol	22.2	26.3	31.1	37.9	49.1
Tablets	77.7	77.6	76.9	77.8	77.0
Water	14.8	19.3	19.2	17.3	15.3
TBHQ 50	85.4	85.5	85.1	85.0	85.0
TBHQ 200	89.4	88.1	88.5	88.9	88.3

B= before concentration A= after concentration

Table 5. Characteristics of the used sunflower oil

Criteria	
Color (Bleachability)*	4
Free fatty acids (acid value as Oleic)	0.792
Peroxide value	1.0 meq O_2 /kg oil
Refractive index	1.4730

* Lovibond units, 5 ¼ in. cell, yellow 35 units.

Table 6. Induction period of sunflower oil as affected by ethanolic corn tassels extract

Ethanolic corn tassels extract (ppm)	Induction period (hr)*	R.I.P.#
None	12.0	1.00
1000	12.5	1.04
2000	15.0	1.25
3000	25.0	2.08
4000	40.0	3.33
5000	40.0	3.33
TBHQ (200)	20.0	1.66

* Induction period: the time needed for peroxide value to become 20.0 at 75± 1.
# R.I.P.: Relative induction period, induction period for control=1.

Table 7. Fungal growth Inhibition at different concentrations of water corn tassels extracts

Fungi	Control	2%	5%	10%
A. parasiticus	++++	+++	++	+
A. flavus	++++	++++	+++	++
A. niger	++++	+++	++	+
Fusariums sp.	++++	++++	++++	++
Penicillium sp.	++++	+++	+++	+

Table 8. Fungal growth Inhibition at different concentrations of ethanolic corn tassels extracts

Fungi	Control	2%	5%	10%
A. parasiticus	++++	+++	+++	++
A. flavus	++++	++++	+++	+++
A. niger	++++	+++	+++	+++
Fusarium sp.	++++	++++	++++	++
Penicillium sp.	++++	+++	+++	++

+: A very clear and large halo ++: A small halo
+++: No fungal inhibition ++++: Similar to control

The results also revealed that the fungal inhibition was more than 75% at 10% concentration of water extract corn tassels. Such inhibitory action could be attributed to the effect of high imidazol content in addition to polyphenols and flavonoids of corn tassel extract **(Table 3)**. **Elena *et al* (2006)** showed the antifungal and antimicrobial activity of imidazol and triazol derivatives. Moreover the obtained results agreed with those reported by (**Turkoglua 2006**) concerning the effect of phenolic and flavonoids compounds as antimicrobial substances.

Therefore the inhibition of fungi would greatly reduce its ability to produce toxins. These results need more studies especially the reduction of fungal growth and its relation to toxin production by using corn tassels extract fractions. Moreovert the application of corn tassels extract in food processing as natural source for antioxidants and antifungal would be of great interest.

The above results proved that corn tassels could be utilized for the production of value added products containing natural antioxidants and antifungal agents. In addition such process could be eliminate the effect of corn tassels wastes as environmental contaminants.

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