Evaluation of antioxidant and antibacterial activities of Egyptian *Maydis stigma (Zea mays* hairs) rich in some bioactive constituents

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Abstract: The main aim of this research work is to evaluate antioxidant and antibacterial activities of Egyptian *Maydis stigma (Zea mays* hairs" corn silk") rich in some bioactive constituents. Antioxidant activity of ethanolic extract of both upper parts of corn silk (dark brown parts, exposed to the air) and lower parts (light yellow parts, not exposed to the air) was determined spectrophotometrically using total antioxidant activity and DPPH scavenging activity methods. It was found that upper parts were found to have the highest total antioxidant capacity (2.735 mg/g GA equivalents). Regarding DPPH scavenging activity, it was found that upper parts were found to have the highest DPPH scavenging activity (IC_{50} = 0.704 mg/ml). Antibacterial activity of ethanolic extract of both upper and lower parts of corn silk was screened against six human pathogenic bacterial species (*Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli* and *Streptococcus pyogenes*) by disk diffusion assay. The pattern of inhibition, activity index and proportion index were studied. It was found that both upper and lower parts of corn silk have no effect on bacterial species under investigation. Total phenolics, total anthraquinones and total flavonoids were estimated, in these regard, upper parts contain more amounts of these phytochemicals (180 µg GAE_s/g F.W., 17.22 µg/g F.W. and 119.47 µg/g F.W. respectively) than lower parts of corn silk (151.33 µg GAE_s/g F.W., 8.61 µg/g F.W. and 101.66 µg/g F.W. respectively).
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Key words: Corn silk, Antioxidant activity, Antibacterial activity, Phenolics, Anthraquinones, Flavonoids.

Abbreviations:
GAE_s: Gallic Acid Equivalent.
DPPH: 1.1-Diphenyl-2- picryl hydrazyl.
IC_{50}: Concentration that gave 50% inhibition.
ppm, µg/g, mg/g and mm: part per million, microgram/gram, milligram/gram and millimeter respectively.
F.W.: Fresh Weight.

1- Introduction

Corn silk (*Maydis stigma "Zea mays* hairs") refers to the stigmas of the maize female flowers (Rosli *et al.,* 2008). Historically, it has been used as a therapeutic remedy for various ailments such as the inflammation of the urinary bladder and prostate as well as, treatment for irritation within the urinary system. To date, numerous commercially viable products prepared from corn silk are available (El-Ghorab *et al.,* 2007). Corn silk has long been reported in ancient literatures to be able to assist with prostate problems, bed-wetting, carpel tunnel syndrome, edema and obesity. It has also been used to lessen the effects of premenstrual syndrome, and said to promote relaxation. Corn silk was also reported to be useful to treat urinary infections and cystitis. It is helpful for frequent urination caused by irritation of the bladder and urethral walls as well as, for difficulty in passing urine, e.g. prostate disorders. It relaxes the lining of the urinary tubules and bladder, thus relieving irritation and improving urine excretion (Steenkamp, 2003).

Another biological activities of corn silk constituents well cited in literatures. These include: antibiotic activity toward corn earworm by a flavone glycoside maysin (Maksimovic and Kovacevic, 2003), isolated flavonoids from corn silk were found to act as anti- fatigue and antidiabetic agents (Hu *et al.,* 2010).Traditionally corn silk has been used also as antilithiasic, uricosuric, and antiseptic. It is used for the treatment of gout, kidney stones, nephritis, and prostatitis, phenolics and flavonoids found in corn silk were thought to give it its antioxidant properties (Ebrahimzadeh *et al.,* 2008). Corn silk also improves nutrient contents and physical characteristics of beef patties (Rosli *et al.,* 2011).

In the present work, we will study some chemical compositions and some biological activities of Egyptian corn silk (which is considered till now in our country as a waste regardless its medicinal and economic importance). Corn silk was divided

"according to morphological differences" to upper parts of corn silk (dark brown parts, exposed to the air) and lower parts (light yellow parts, not exposed to the air) in a trail to find differences also between these parts regarding their antioxidant activity, antibacterial activity against some human pathogenic bacteria, in addition to phytochemical screening, total phenolics, anthraquinones and flavonoids.

2- Materials and methods

Plant materials:

Upper parts of Egyptian corn silk (dark brown parts, exposed to the air) and lower parts (light yellow parts, not exposed to the air) were used as plant materials for biological and chemical investigations.

Antioxidant bioassay:

Total antioxidant activity was performed using phosphomolybdenum reagent solution method of Prieto *et al.,* (1999) and adopted by Kumar *et al.,* (2008). The antioxidant capacity was expressed as Gallic Acid Equivalent (GAE) by using the standard Gallic acid graph.

DPPH (1.1 diphenyl-2 picryl hydrazyl) scavenging activity was carried out by using the method of Gursoy *et al.,* (2009).

Tested microorganisms:

Antibacterial activity of ethanolic extracts of both upper and lower parts of corn silk was investigated against six human pathogenic bacterial isolates, obtained from Clinical Pathology Department, Faculty of Medicine (Kasr El- Eini) Cairo University, Egypt. These included three gram-negative bacteria including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603), three gram-positive bacteria including *Streptococcus pneumoniae* (ATCC 49619), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615). The purity and viability of cultures were checked by culturing on nutrient agar slants, incubated at 37ºC for 24 hours. Cultures were subcultured regularly (every week) and stored at 4ºC (Yaecob and Tolba, 2006 and Arya *et al*., 2010).

Inoculum preparation:

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37ºC for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 MC farland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1-2X10^8 colony-forming units per milliliter (cfu/ml), the suspension was then used for further testing (Arya *et al*., 2010).

Antibacterial bioassay:

Antibacterial bioassay was carried out following Disc Diffusion Method according to Arya *et al*., (2010). The concentration of each extract per disc 12.5, 25 and 50 mg/disc in case of both upper and lower parts of corn silk and positive controls (synthetic drugs; Cefotaxime, Cephradine and "Amoxycilin, Flucloxacilin"). Negative controls were ethanol, water and empty discs. The diameter of inhibition zone (measured in mm) is indicated by clear area in the Petri dish which was devoid of bacterial cells growth was measured. Each Petri dish contains four centered disks, r value of each disk = 5 mm, one layer, Whattman number 1 filter paper.

Determination of activity and proportion indexes:

Calculations were carried out following the methods of Singh *et al*., (2002) and Borgio *et al.,* 2008.

Assay for total phenolics:

Total phenolics were estimated following the method of Gursoy *et al.,* (2009) involving Folin–Ciocalteu reagent and Gallic acid as standard. 1 ml of each extract of upper and lower parts of corn silk contains 100 mg F.W. . Concentrations of phenolic compounds were calculated according to the following equation that was obtained form the standard Gallic acid graph.

Absorbance = 0.0167 Gallic acid (µg) + 0.017 (R^2: 0.99)

Assay for total anthraquinones:

Total anthraquinones were estimated using the method of Sakul Panich and Gristsanapan, (2008).
1 ml of each extract of upper and lower parts of corn silk contains 100 mg F.W., using Emodin as standard.

Assay for total flavonoids:

Total flavonoids were determined using the method of Gursoy *et al.,* (2009). 1ml of each extract of upper and lower parts of corn silk contains 100 mg F.W.. Concentrations of flavonoid contents were calculated according to the following equation that was obtained from the standard Quercetin graph:

Absorbance = 0.0228 Quercetin (µg) – 0.0045 (R^2: 0.9979)

Statistical analysis:

Statistical analysis of all results was done using Fisher analysis of variance methodology.

A least significant difference test was applied at 5% and 1% probability level to determine differences

among treatment means (Steel and Torrie, 1984). The MSTAT computerized package program was subjected to the regular statistical analysis of variance (Nissen *et al*., 1985). Each reading = mean of three replicates ± SD.

Results and Discussion:

Results of antioxidant activity and DPPH scavenging activity (Table: 1) of upper and lower parts of corn silk revealed that, there were non significant variations between upper and lower parts

of corn silk. It was found that upper parts were found to have the highest total antioxidant capacity and DPPH scavenging activity (2.735 ±1.180 mg/g GAE_s, IC_{50} = 0.704±0.067 respectively).

Table (1): Antioxidant activity of upper and lower parts of corn silk (Total antioxidant activity and DPPH scavenging activity methods).

Corn silk	Total antioxidant activity (mg/g GAE_s)	DPPH scavenging activity (IC_{50} in mg/ ml)
Upper parts	2.735±1.180	0.704±0.067
Lower part	2.150±1.180	1.001±0.067
		Quercetin (positive control) = 0.801±0.260
L.S.D.(0.05)	3.947	0.263
L.S.D.(0.01)	5.839	0.436

Antibacterial activity studies of upper and lower parts of corn silk revealed that, using 12.5, 25 and 50 mg ethanolic extract/disc, both of them have no antibacterial activities against bacterial species under investigation "*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603), *Streptococcus pneumoniae* (ATCC 49619), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615).

Concerning total phenolics, total anthraquinones and total flavonoids (Figure : 1), it was found that, upper parts of corn silk contain more amounts of these phytochemicals (180 µg GAE_s/g F.W., 17.22 µg/g F.W. and 119.47 µg/g F.W. respectively) than lower parts (151.33 µg GAE_s/g F.W., 8.61 µg/g F.W. and 101.66 µg/g F.W. respectively).

These results match morphological differences "in color" between upper darker parts and lower lighter parts of corn silk, according to Stapleton and Walbot, (2009) flavonoids and other phenolics can protect maize DNA from the induction of ultraviolet radiation damage, since upper parts of corn silk were exposed to sun more than lower parts, so accumulation of phenolics, anthraquinones and flavonoids in upper parts of corn silk more than their correspondings in lower parts can be considered as a defensive mechanism in the plant.

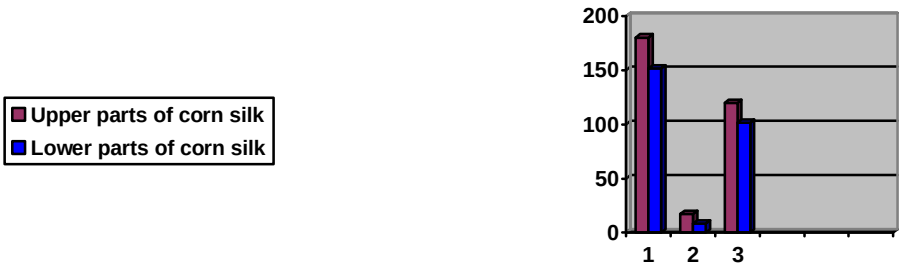

Figure:1. Total phenolics, anthraquinones and flavonoids in upper and lower parts of corn silk.
1- Total phenolics 2- Total anthraquinones 3- Total flavonoids

These results agreed with Ebrahimzadeh *et al.*, 2008 who found that, phenolics and flavonoids found in corn silk were thought to give maize silk its antioxidant properties.

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