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RESEARCH ARTICLE

Investigation of bioactive compounds and determination of antioxidant potential of corn silk using spectrophotometric methods

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Abstract

Corn silks are yellowish-golden, long, hair-like extensions of the female inflorescence of the maize plant. Even though treated as an agricultural waste, corn silk has been used as medicine in certain parts of the world. It is widely acknowledged for its antioxidant benefits and consumed in the form of corn silk tea. Present study on corn silk revealed low amount of loss on drying, indicating a good shelf life, an essential parameter for valorisation of corn silk. Phytochemical analysis revealed high amounts of secondary metabolites, polyphenols and flavonoids, suggesting its potential as a rich source of antioxidants. Total polyphenols in YCS extract were measured to be 1.35 mg GAE/g, and WCS extract contained 4.51 mg GAE/g of powdered corn silk. Total flavonoids in YCS extract were found to be 0.19 mg QE/g whereas WCS extract had 1.67 mg QE/g. Amount of vitamin C in YCS extract was 44.2 µg AAE/g, while WCS showed 566 µg AAE/g. In DPPH assay, YCS extract showed an inhibition percentage of 50.96%, whereas the WCS extract exhibited a higher percentage of 90.99%. These findings highlight the promising antioxidant potential of corn silk, paving the way for synthesis of value-added products and their potential applications in the domain of natural antioxidants.

Keywords: Antioxidant, Corn silk, DPPH, Free radicals, Phytochemicals

Introduction

The use of natural resources for healing has been a fundamental aspect of human health practices throughout history. The pursuit of medicine has often led people to plants, as remedies for various ailments (Houghton 1995; Petrovska 2012). This traditional knowledge, passed across generations, has become an integral part of many communities (Prance 1991). Use of whole plant or plant parts as medicine became a common practice by native people. Corn silk (CS) has been utilized in indigenous American and traditional Chinese medicine as a potent diuretic and effective antioxidant (Hasanudin *et al*. 2012; Vijitha and Saranya 2017). Although corn silk is often treated as an agricultural waste after harvesting corn cobs, it is widely known for its therapeutic benefits (Kaur *et al.* 2023).

Antioxidants have potential to inhibit oxidation and scavenge free radicals. Free radicals, which are substances with unpaired electrons, include oxygen radicals such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$), and hydroperoxyl ($HO_2^{\bullet}$) radicals. Free radicals though associated with harmful effects, play significant roles in essential physiological processes, serving as secondary messengers in signal transduction pathways, apoptosis, and enzymatic reactions (Bayr 2005; Krumova and Cosa 2016). The cell has natural mechanisms to regulate free radicals. However, an excess of free radicals due to lifestyle or environmental factors can overwhelm these mechanisms, leading to an imbalance between their generation and scavenging. This imbalance ultimately causes oxidative stress (Yoshikawa and Naito 2002).

Corn silk, also known as *Stigma maydis*, is a combination of style and stigma, part of female inflorescence of *Zea mays* belonging to family Gramineae or Poaceae. These golden-yellow hairlike outgrowths on the corn cob serve

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the important function of capturing pollens as they fall from the tassels of the male flowers above. Each individual thread of corn silk is directly linked to a single kernel of corn. Once the silky hairs have received the pollen, they transport it to the ovules for fertilization (Hasanudin *et al.* 2012). Silk starts to emerge from the ovules at the base to the top of the ear. As it grows it emerges from the tip of the ear and become visible outside the husk. This stage is called silking stage. Silk stops growing after pollens are captured. Usually, elongation of silk seizes about 10 days after its emergence due to senescence of silk tissues even if it is not pollinated (Nielsen, 2016).

Phytochemical analysis of corn silk from different developmental stages showed that corn silk at silking stage has high amounts of polyphenols and flavonoids (Žilić *et al.* 2016). Polyphenols comprise second largest group of secondary metabolites in plants after terpenes. Most plant-derived polyphenols possess good antioxidant potential due to their ability to donate hydrogen or electron. Moreover, their metal chelating potential may also reduce iron and copper induced free-radical reactions (Rice-evans *et al.* 1995). This high content is positively correlated with high antioxidant activity of corn silk (Sarepoua *et al.* 2013; Singh *et al.* 2022). As the silk matures and is exposed to sunlight, it undergoes a darkening process. This high pigmentation is associated with elevated antioxidant activity (Tian *et al.* 2021). Despite its therapeutic properties, corn silk is still treated as an agricultural waste. Our research supports the documented data for corn silk usage as supplement functional food. Results of the study would help in facilitating production of corn silk-based products and to gain recognition.

Material and Methods

Collection and processing of plant material

Fresh material of yellow corn silk (YCS) of sweet corn was collected from local market in Thane city, Maharashtra. White corn silk (WCS) (Figure 1) was directly procured from corn fields of Kolhapur district, Maharashtra. To begin the preparation, fresh corn silk was air-dried and subsequently kept in an oven at 50°C. The dried silk was then finely powdered, carefully sieved, and kept in a clean, dry, air-tight container for future use.

Figure 1: White corn cob with silk (dark brown colour) from the corn fields of Kolhapur district, Maharashtra

Preparation of corn silk extract

To produce the corn silk (CS) extract, 10 grams of powdered corn silk, both YCS and WCS, were refluxed with 100 ml of specific solvent for 1 hour at temperature slightly below the boiling point of that solvent. Following this, the mixture was cooled and filtered using Whatman filter paper no. 1. The filtrate was used for further analysis.

Physicochemical analysis of corn silk

Physicochemical analysis was done to determine its properties including loss on drying, extractive values, and ash values. The differences were measured before and after treatment by using an empty container. Results of the experiment were reported as percentages of dry weight. To determine ash values, standard methods from the AOAC International were utilized. The methods outlined in Naik and Sellappan 2019 were employed to establish the loss on drying and extractive values.

Loss on drying

Finely powdered corn silk was placed into a pre-heated and precisely weighed crucible. Then, it was subjected to a controlled heating process at 110°C in an oven for a period of 3 hours. Subsequently, the crucible was cooled in a desiccator before being re-weighed. This process was repeated to get a consistent weight, ensuring accurate results.

Ethanol-soluble and water-soluble extractive values

To determine ethanol-soluble and water-soluble extractive values of CS extract, 5-gram powder was taken in flasks containing 100 ml ethanol and water separately. Mixtures were kept for 6 hours on a shaker, followed by an additional 18-hour period of standing. After filtration, 25 ml filtrate was kept for drying in a pre-weighed evaporating dish and the weight was recorded for further calculations.

Total ash content

To measure ash content, 2 grams of powder was carefully placed in a pre-ignited and weighed silica crucible. The powder was then subjected to incineration at 550°C for 3 hours until it was completely free of carbon. After cooling the sample in a desiccator, it was reweighed. Process of incineration at 550°C for 15 minutes, followed by cooling and reweighing was done until a constant weight was achieved.

Acid insoluble ash

To find out acid-insoluble ash content, total ash was acquired using the previously described procedure. Then, the ash should be heated for 5 minutes with 25 ml 4 N hydrochloric

acid. The insoluble matter carefully collected on ashless filter paper and thoroughly washed with hot water. Next, it was ignited in a pre-measured silica crucible at 550°C for 1 hour followed by cooling and reweighing it. This method was repeated until a fixed weight was established.

Water-soluble Ash

To evaluate the water-soluble ash content, previously obtained total ash was boiled for 5 minutes with 25 ml water. The insoluble material carefully collected by filtration on ashless filter paper was washed with hot water. The insoluble ash was transferred to a pre-measured crucible and ignited at 550°C for 1 hour, allowed to cool, and then weighed it. The procedure was repeated to obtain constant weight. Wight of the water-soluble ash content was obtained by removing the weight of the insoluble matter from the value of total ash.

Elemental Analysis

The elemental composition of CS was analysed using the highly sensitive ICP-MS (Inductively Coupled Plasma-Mass-Spectrometry) technique. To prepare the sample for analysis, the acid extract of CS was prepared using HCl-HNO_3 digestion method. The ICP-MS technique utilizes an argon (Ar) plasma to convert the sample into ions for detection using a mass spectrometer. This method effectively detects trace elements owing to its remarkably sensitive detection limit (Singh *et al.* 2022).

Phytochemical Analysis

Chemical tests for detecting both primary and secondary phytoconstituents in CS extract were conducted. Solvents selected for the study were water, 90% methanol, ethanol, methanol, chloroform, isopropanol and hexane based on their polarity. Qualitative tests performed are given in table 1.

Total Polyphenols from Corn Silk

Polyphenols of CS were estimated using a modified Folin-ciocalteu method and Gallic acid as standard (Waterhouse 2002; Sengul *et al*. 2009). The Folin-ciocalteu reagent (Singleton *et al.* 1999), diluted with distilled water in a 1:1 ratio, was combined with 0.5 ml of CS extract and thoroughly mixed. Following a 5-minute incubation period, 2 ml of 2% Na_2CO_3 was added, and allowed to stand for 2 hours with intermittent shaking. Utilizing a V-630 UV-visible spectrophotometer (Jasco, Japan), absorbance measurements were taken at 750 nm. A standard curve was plotted for standard Gallic acid (ranging from 0 µg-10

Table 1: Qualitative tests for phytochemical analysis

Secondary metabolite	*Chemical test*	*Observation*
Carbohydrates	Benedict's test- Extract + Benedict's reagent, boil	Formation of reddish brown ppt
	Fehling's test – extract + Fehling's solution A + Fehling's solution B	Formation of red ppt
Proteins and amino acids	Biuret test – extract + Biuret reagent	Formation of purple colour
	Ninhydrin test – extract + ninhydrin solution	Formation of purple colour
Fats and oils	Saponification test - Extract + alcoholic KOH, boil	Appearance of foam on top
Terpenes/ Terpenoids/ Isoprenoids	Salkowski test - Extract + chloroform + conc.H_2SO_4	Appearance of reddish-brown colour of the interface
Phenolics/ Polyphenols	Folin-Ciocalteu test – Extract + Folin-ciocalteau reagent + Na_2CO_3, incubate	Formation of blue colour
Flavonoids	Alkaline reagent test – Extract + dilute NaOH + Dilute acid	Appearance of bright yellow colour Solution becomes colourless
	Ammonium hydroxide test – Extract + NH4OH	Yellow effervescence
	Lead acetate test – Extract + lead acetate	Formation of white or yellow ppt
Tannins	Ferric chloride test – extract + FeCl3	Formation of blue/purple colour
Alkaloids	Wagner's test - Extract + Wagner's reagent	Formation of reddish brown ppt
	Dragendorff's test – Extract + Dragendorff's reagent	Formation of orange red ppt
Glycosides	Keller-kiliani test – Extract + glacial acetic acid + $FeCl_3$ + conc. H_2SO_4	A reddish-brown ring forms at the interface, the upper acetic acid layer soon turns bluish green
	Kedde's test – Extract + equal volume of ethanolic dinitrobenzene and NaOH	reaction mixture immediately turns purple-violet and colour disappears after a few min.
Steroids	Extract + equal volume of chloroform and conc.H_2SO_4	The upper layer in the test tube turns red and sulphuric acid layer showed yellow with green fluorescence.

µg). Total polyphenols were ultimately represented as mg of Gallic acid equivalents per unit of dry mass of corn silk.

Total Flavonoids from Corn Silk

To quantify total flavonoids, present in corn silk, a spectrophotometric assay was employed to create an aluminium complex, with quercetin serving as standard reference (Pękal and Pyrzynska 2014; Deshmukh and Theng 2018). The procedure started by combining 0.5 ml of CS extract with 0.3 ml of 5% $NaNO_2$. Following a 5-minute interval, 0.5 ml of 2% $AlCl_3$ was introduced into the mixture. Subsequently, at the 6-minute mark, 0.5 ml of 1M NaOH was added. The spectrophotometric readings were taken at 510 nm, utilizing a V-630 UV-visible spectrophotometer (Jasco, Japan). The quantification of flavonoids was performed via the utilization of the standard curve for quercetin, encompassing a range of 0 to 50 µg in methanol. Ultimately, the measured flavonoid content was extrapolated and expressed as mg of quercetin equivalents per unit of dry mass of corn silk.

Vitamin C Content from Corn Silk

The vitamin C content of CS extract was estimated by with the help of DNPH (2,4-Dinitro- phenylhydrazine) method, with ascorbic acid as standard (Rahman *et al.* 2007; Sadasivam 1996). Bromine water was used to convert ascorbic acid to dehydro-ascorbate. Then, 0.5 ml CS extract was combined with 1 ml DNPH and 100 µl of thiourea. Resulting mixture was vortexed and left to incubate at 37°C for 3 hours, allowing osazone crystals to form. Subsequently, 2.5 ml of 80% H_2SO_4 was added, and the absorbance was measured at 520 nm. Standard curve for ascorbic acid, ranging from 100 ppm to 1000 ppm was plotted using the same procedure. Finally, total vitamin C was quantified as milligrams of ascorbic acid equivalents (AAE) per unit of dry mass of corn silk.

Total Antioxidant Activity of Corn Silk by DPPH Method

The total antioxidant activity of CS extract using DPPH (2,2-diphenyl-1-picrylhydrazyl) method was performed as described by Vijitha and Saranya in 2017 and Senphan in 2019. Firstly, 0.1 ml of CS extract was introduced in 3 ml of methanolic DPPH solution (0.5 mM), with methanolic DPPH serving as control. Following this, test tubes were placed at 37°C in incubator for 30 minutes. After incubating the sample, readings were noted at 517 nm. To quantify the radical scavenging activity, the percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = 1 - (A_S / A_B) \times 100$$

Where, A_B is absorbance of the blank and A_S is absorbance of the sample after 30 minutes of incubation. Additionally, a calibration curve of ascorbic acid for the concentration of 50-500 µg was plotted.

Statistical Analysis

All experiments were performed in multiple sets for ensuring reliability and reproducibility. Independent student t-test for samples was performed comparing the means at $p < 0.05$. All readings in the data were represented as mean ± standard deviation (SD).

Results and Discussion

Physicochemical parameters such as loss on drying, extractive values, and ash values of powdered corn silk were quantified in the study (Table 2). Loss on drying was assessed for presence of mainly water and other volatile substances in sample. Extractive values provided information about capacity of the solvent to solubilize particular compounds. Water as well as ethanol soluble extractive values were higher for WCS than YCS. The analysis revealed total ash values and water-soluble ash values more in WCS than YCS except for acid-insoluble ash content which was higher in YCS. ICP-MS analysis of CS detected the presence of essential elements such as copper (0.34 ppm), zinc (0.8 ppm), manganese (0.43 ppm), and selenium (0.009 ppm). These elements are important in the superoxide dismutase family of enzymes, with manganese being an essential component of Mn-SOD, copper contributing to the catalysis of Cu-Zn superoxide dismutase, and zinc serving both structural and functional roles (Tainer *et al.* 1983; Liu *et al.* 2022).

Both YCS and WCS extracts were screened for presence of phytochemicals (Table 3). The analysis revealed higher amounts of polyphenols, flavonoids while alkaloids, glycosides and steroids were found comparatively in low amounts. As polyphenols and flavonoids are well known for antioxidant activity, their quantitative assays are performed.

The amounts of total polyphenols were calculated using a linear standard curve of gallic acid ($y = 0.0666x + 0.0246$; $R^2 = 0.9928$). Total polyphenols of YCS extract were measured to be 1.35 mg GAE/g, and WCS extract contained 4.51 mg GAE/g of powdered corn silk. Notably, polyphenols are

Table 2: Physicochemical analysis of corn silk

Sr. No.	*Parameter*	*% dry weight basis Mean ± SD*	
		Yellow corn silk	*White corn silk*
1.	Loss on drying	6.7835 ± 0.15	7.7144 ± 0.18
2.	Ethanol soluble extractives	1.516 ± 0.02	6.592 ± 0.16
3.	Water soluble extractives	9.668 ± 0.04	21.332 ± 0.1
4.	Total ash content	4.5016 ± 0.19	5.7303 ± 0.05
5.	Acid insoluble ash content	0.4180 ± 0.02	0.3825 ± 0.02
6.	Water soluble ash content	2.8212 ± 0.02	4.4297 ± 0.02

Table 3: Phytochemical analysis of corn silk

Secondary metabolite	*Chemical test*	*Result*		*References*
		YCS	*WCS*	
Carbohydrates	Benedict's test	Present in alcoholic extracts and absent in all other solvents including isopropanol	Present in alcoholic extracts and absent in all other solvents	Banu and Cathrine 2015
	Fehling's test	Present in water, alcoholic extracts, chloroform and absent in all other solvents including isopropanol	Present in all solvents except hexane	Banu and Cathrine 2015
Proteins and amino acids	Biuret test	Not detected in any of the solvents	Not detected in any of the solvents	Banu and Cathrine 2015
	Ninhydrin test	Present in ethanol and absent in all other solvents including isopropanol		Yadav and Agarwala 2011
Fats and oils	Saponification test	Not detected in any of the solvents	Not detected in any of the solvents	Banu and Cathrine 2015
Terpenes/ Terpenoids/ Isoprenoids	Salkowski test	Not detected in any of the solvents	Not detected in any of the solvents	Yadav and Agarwala 2011
Phenolics/ Polyphenols	Folin-Ciocalteu test	Present in water, alcoholic extracts and absent in all other solvents	Present in water, alcoholic extracts and absent in all other solvents including isopropanol	Banu and Cathrine 2015
Flavonoids	Alkaline reagent test	Present in water, alcoholic extracts and absent in all other solvents	Present in water, ethanol, isopropanol and absent in all other solvents	Yadav and Agarwala 2011
	Ammonium hydroxide test		Present in alcoholic extracts and absent in all other solvents	Edeoga *et al.* 2005
	Lead acetate test		Present in water, alcoholic extracts and absent in all other solvents including isopropanol	Vimalkumar *et al.* 2014
Tannins	Ferric chloride test	Present in water, alcoholic extracts and absent in all other solvents	Present in water, alcoholic extracts and absent in all other solvents including isopropanol	Yadav and Agarwala 2011
Alkaloids	Wagner's test	Present in alcoholic extracts and absent in all other solvents	Present in 90% methanol and absent in all other solvents	Banu and Cathrine 2015
	Dragendorff's test		Not detected in any of the solvents	Banu and Cathrine 2015
Glycosides	Keller-kiliani test	Not detected in any of the solvents	Present in 90% methanol, chloroform, isopropanol and absent in all other solvents	Pooja and Vidyasagar 2016
	Kedde's test	Present in chloroform and hexane and absent in all other solvents	Present in chloroform, isopropanol, hexane and absent in all other solvents	Borokini and Ayodele 2012
Steroids	Con. H_2SO_4 test	Not detected in any of the solvents	Present in ethanol, methanol, chloroform, hexane absent in all other solvents	Pooja and Vidyasagar 2016

prominent plant secondary metabolites acclaimed for their robust antioxidant activity (Kaur *et al.* 2023).

The quantification of total flavonoids of the YCS extract was achieved through the utilization of a linear standard curve of quercetin ($y = 0.0179x$; $R^2 = 0.9968$), yielding a concentration of 0.19 mg QE/g. Similarly, the WCS extract displayed a significant content of 1.67 mg QE/g of powdered corn silk. Flavonoids, a subset of polyphenols, are pivotal contributors to multiple biological functions, including antioxidant activity, and protection against UV radiation (Hasanudin *et al.* 2012; Hano and Tungmunnithum 2020). Furthermore, mechanism of action of flavonoids involves inhibition of specific free radical-producing enzymes within the cell. This inhibition occurs due to highly reactive hydroxyl group (OH^-) of flavonoids, enabling them to interact with reactive species, thereby neutralizing them and safeguarding cellular components from oxidative damage (Panche *et al.* 2016).

The YCS extract was found to contain 44.2 µg AAE/g of powdered corn silk, while the WCS extract contained 566 µg AAE/g of vitamin C. Vitamin C, referred to as L-ascorbic acid, a water-soluble vitamin, with powerful antioxidant properties. It serves as an effective reducing agent by donating electrons faster than any other cell compounds. When vitamin C donates electrons, it forms dehydroascorbic acid, which has a relatively stable and less reactive (Padayatty *et al*. 2003). It also plays a crucial role as a cofactor in reactions catalysed by metal-dependent oxygenases (Linster and Van Schaftingen 2007).

Total antioxidant activity was assessed in terms of the percentage inhibition of oxidation of DPPH by a reducing agent. The YCS extract showed an inhibition percentage of 50.96%, whereas the WCS extract exhibited a higher percentage of 90.99%. In conclusion, WCS extract possesses a greater total antioxidant activity compared to the YCS extract.

Antioxidant activity is attributed to polyphenols especially to flavonoids. In DPPH assay WCS showed much more antioxidant activity than YCS which can be correlated to higher water-soluble extractives found in WCS than YCS. As flavonoids are water- soluble compounds these findings align with expected outcomes.

In Maharashtra, white corn is cultivated only during specific seasons in particular regions, which restricts its availability and use to those areas. Conversely, yellow sweet corn is both seasonally grown as well as imported, ensuring its availability for most of the year. Despite the fact that white corn silk has greater antioxidant benefits compared to yellow sweet corn silk, the latter is more accessible as a raw material for synthesis of value-added products. Given its higher efficacy, a smaller quantity of white corn silk is required.

Conclusion

In the present study, corn silk was evaluated for its physical and chemical properties employing a range of physicochemical parameters. The phytochemical analysis of both white corn silk (WCS) and yellow corn silk (YCS) revealed a rich presence of polyphenols and flavonoids. These results align with traditional ethnobotanical claims highlighting valuable antioxidant properties attributed to corn silk, largely due to the well-established antioxidative capabilities of flavonoids.

Even though corn silk is an overlooked and deemed as mere agricultural waste, our findings open exciting avenues for the development of high-value products derived from corn silk. It shows the potential to transform under-utilized material into beneficial applications emphasizing sustainability.

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