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SHORT COMMUNICATION article

Phytochemical characterization and radical-scavenging activity of solvent fractions from corn silk

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Abstract: This study aimed to quantify the total phenolic content (TPC) and total flavonoid content (TFC) in corn silk (*Zea mays* L.) extracts and evaluate their antioxidant potential using the DPPH radical-scavenging assay. The findings revealed that polar solvents were the most effective in extracting phenolic and flavonoid compounds, yielding the highest concentrations of these compounds. These results underscore the significance of solvent polarity in enhancing the recovery of antioxidant-rich phytochemicals from corn silk. The propanol extract showed the highest levels of both phenolic (274.82±151.11 µg/g) and flavonoid (193.0±61.0 µg/g) compounds, followed by the ethyl acetate extract. In contrast, the chloroform extract yielded the lowest concentrations of these bioactive constituents. The propanol extract showed the highest levels of both phenolic (274.82±151.11 µg/g) and flavonoid (193.0±61.0 µg/g) compounds, followed by the ethyl acetate extract. In contrast, the chloroform extract yielded the lowest concentrations of these bioactive constituents. dependent antioxidant activity. The propanol extract was superior to the others in its free radical scavenging activity, achieving the highest inhibition rate of 82.13% at a concentration of 46.69 mg/mL. The ethyl acetate extract showed 78.07% inhibition, and the chloroform extract showed 75.81%. These results are consistent with the calculated IC_{50} values, which indicate that the propanol extract had the highest antioxidant efficacy. The findings establish a strong correlation between the high content of phenolics and flavonoids in corn silk extracts and their elevated antioxidant activity. The propanol extract proved to be the most effective among the tested extracts, making it a promising candidate for use as a natural source of antioxidant compounds.

Introduction

Corn silk, the feathery strands from the female corn flower, is a valuable byproduct of corn cultivation [1]. It is harvested before pollination for its medicinal properties and can be used fresh or dried [2]. Corn (*Zea mays* L.) is a monoecious plant, bearing male and female reproductive structures on the same individual. The male flowers form the tassels at the top of the plant, while the female flowers develop into ears along the stalk. In addition, the female flowers develop corn silk, which functions as the stigma, capturing pollen for fertilization. As the fruit matures, the silk elongates beyond the cob, enveloping the edible portion of the plant [3]. It can aid in detoxification, treat urinary tract infections, and promote healthy skin and hair [4]. Corn silk has found application as a key ingredient in the development of various pharmaceutical products [5]. It is commonly

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used in Asia as a healthy and medicinal beverage, often consumed in the form of tea [6, 7]. It can stimulate the body's defense mechanisms, fight infections, and potentially reduce the risk of certain cancers need reference [5] It is safe for consumption, even in larger quantities [4]. Corn silk is a rich source of essential minerals and various bioactive compounds such as flavonoids, vitamins, phenolic compounds, alkaloids, steroids, carbohydrates, and proteins [8]. It is rich in nutritious bioactive compounds, including polyphenols, flavonoids, and ascorbate, and exhibits strong antioxidant activity [9]. Corn silk is a widely used traditional Chinese medicine employed in the treatment of various kidney-related ailments. It is recognized for its antimicrobial properties [10, 11]. It has demonstrated significant antioxidant activity, suggesting its potential as an antioxidant agent in both food and pharmaceutical applications [12].

Phytochemicals, non-nutritive bioactive compounds found in plants, play essential roles in plant defense mechanisms against predators and harsh environmental conditions [13-16]. These compounds also hold significant promise in pharmaceutical and medicinal fields due to their diverse biological properties [17, 18]. Corn silk is a rich source of various bioactive phytochemical compounds and volatile oils [19]. These compounds have been isolated, characterized, and investigated for their potential therapeutic applications. Corn silk is a valuable natural source of bioactive compounds, including the flavonoid maysin. Maysin contains luteolin, a compound recognized for its potent antioxidant properties [14-22]. Maysin has also demonstrated efficacy in reducing fat deposition and body weight. Luteolin is another flavonoid found in corn silk [23, 24], inhibits cancer cell growth, and exerting antioxidant and anti-inflammatory effects [25-27]. Allantoin [28] and limonene [29, 30], isolated from corn silk, exhibit anti-cancer, antioxidant, and anti-inflammatory properties. Allantoin is used in skin cancer treatment and wound healing. It contains catechin [31] and exhibits antioxidant [32-35], antimutagenic [36-38], and other biological activities [39, 40]. Corn silk contains several phenolic acids, including syringic acid [41, 42], ferulic acid [43, 44], and caffeic acid, which exhibit antioxidant, anticarcinogenic, and antimutagenic properties [35-39]. Ferulic acid has been linked to various health benefits, including anti-inflammatory, antiviral, and anti-cancer effects [45, 46]. Stigmasterol, a compound isolated from corn silk [47], has been demonstrated to exhibit a broad spectrum of pharmacological activities [48]. These findings underscore the potential of corn silk as a valuable natural source of bioactive compounds with significant therapeutic implications. Chitosan and dextran found in water extracts of corn silk [49] possess antimicrobial and other beneficial properties [50-55]. These findings highlight the potential of corn silk as a valuable natural source of bioactive compounds with therapeutic applications. It contains various bioactive compounds with potential health benefits. Gallic acid [41, 42], a compound isolated from corn silk, exhibits a wide range of therapeutic activities, including neuroprotective, gastrointestinal, cardiovascular, and anti-inflammatory effects. Isoorientin-2-2"-O-α-L-rhamnoside and 3'-methoxymaysin [22, 38, 56, 57]. These findings highlight the potential of corn silk as a valuable natural source of bioactive compounds with therapeutic applications. Corn silk has emerged as a source of diverse bioactive compounds with potential health benefits. Studies have reported the isolation of 2"-O-α-L-rhamnosyl-6-C-quinovosylluteolin, 2"-O-α-L-rhamnosyl-6-C-fucosylluteolin, and 2"-O-α-L-rhamnosyl-6-C-fucosyl-3'-methoxyluteolin from corn silk, demonstrating their antioxidant properties [38]. The extraction of compounds 4,4,5,6-tetramethyl-tetrahydro-1,3-oxazin-thione, ethylhexadecanoate, and decyl decanoate revealed their potential as antibacterial agents, skin moisturizers, and antioxidants, respectively [58]. The compounds isolated and identified from corn silk can be broadly classified into six major categories: flavonoids, polyphenols, sterols, terpenoids, amino acids, and organic acids [59]. This study aimed to evaluate the antioxidant activity of corn silk extracts and to quantify their total phenolic content (TPC) and total flavonoid content (TFC) using spectrophotometric methods. By examining the correlation between these phytochemical concentrations and antioxidant activity, the study explores the potential of corn silk as a natural source of antioxidants for application in the food industry.

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Materials and methods

Plant collection: Stigma maydis (corn silk) was obtained from South Libya during September 2024. Corn cobs are cotton jerseys made from the green fibers squeezed from the cobs of the corn plant *Zea mays* L., which are originally the stigmas of the female flower.

Extraction with ethanol-water mixture: The sample, prepared by a predetermined quantity of dried plant material, was accurately weighed and placed in an Erlenmeyer flask. After that ethanolic solution was prepared by mixing 70.0 mL of ethanol with 30.0 mL of distilled water. Then, add the plant material was extracted with 100 mL of the prepared ethanolic solution. The mixture was vigorously shaken using a shaker for 24 hrs at room temperature and in the dark to maximize the extraction of target compounds. The mixture was filtered through Whatman filter paper to separate the extract from the plant debris. This extraction process was repeated three times using fresh solvent each time, and the filtrates were combined. Combined filtrates were concentrated using a rotary evaporator at a reduced pressure until a crude extract was obtained.

Separation of the crude extract: The crude extract was dissolved in 100 mL of hot distilled water and allowed to stand overnight to ensure complete dissolution of water-soluble compounds. The aqueous solution was subjected to liquid-liquid extraction using a separatory funnel. The following solvents were used sequentially: chloroform, ethyl acetate, and n-propanol. After that, the organic layers from each extraction were collected and evaporated under reduced pressure at 50°C, 45°C, and 40°C, respectively, to obtain the corresponding fractions. The extract is stored in the refrigerator for testing to be performed.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectra of corn silk powder, prepared as potassium bromide discs, were acquired using a Perkin-Elmer FT-IR S 300 spectrophotometer (with wave number maxima reported in cm^{-1} across the spectral range of 400 to 4000 cm^{-1}. The resulting spectra obtained from the various samples were subsequently analyzed following the established methodology outlined by Stuart [60].

Determination of total phenolic content (TPC): The TPC of corn silk powder was quantified using the Folin–Ciocalteu colorimetric method, with gallic acid employed as the calibration standard [61]. A standard curve was constructed using gallic acid concentrations ranging from 5.0 to 50 µg/mL (5.0, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/mL). The resulting calibration curve demonstrated excellent linearity, with a correlation coefficient of R^2=0.9997. This high degree of linearity confirms the reliability and sensitivity of the method for detecting phenolic compounds within the tested range. The use of gallic acid as a reference standard is a well-established approach, enabling TPC values to be expressed as gallic acid equivalents (GAE). This standardization facilitates meaningful comparisons across different studies and plant matrices, enhancing the reproducibility and interpretability of results.

Total flavonoid content (TFC): The TFC of corn silk extracts was determined using the aluminum chloride colorimetric method, with quercetin as the standard [54]. A 2.0 mL aliquot of the extract solution was mixed with 0.2 mL of 5.0% sodium nitrite solution. After five minutes, 0.2 mL of 10.0% aluminum chloride solution was added, and the mixture was incubated at room temperature. Following this, 2.0 mL of 0.1 M sodium hydroxide was added, and the solution was left to stand for six minutes. The reaction mixture was then diluted with 0.275 mL of distilled water, and the absorbance was measured at 510 nm using a UV-Vis Spectrophotometer. A standard calibration curve was prepared using quercetin, and the total flavonoid content was expressed as milligrams of quercetin equivalents per 100 g of dry weight (mg QE/100 g).

Inhibitory concentration (IC_{50}) value of antioxidant activity: The antioxidant activity in percentage inhibition was determined, and the absorbance was measured at 517 nm (UV/Vis Spectrophotometer), against the blank (mixture without extract). In the test, a 9.39 x 10^{-5} M alcoholic solution of 2, 2-diphenyl-1-picrylhydrazyl

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(DPPH) was utilized. 0.1 mL extract was mixed with 3.0 mL DPPH solution, which was then diluted with ethanol. The mixture was violently mixed before being placed in the dark for 30 min. The absorbance was measured against a blank (mixture without extract) at 517 nm (UV/Vis Spectrophotometer [62].

Results and discussion

The FTI spectroscopy analysis of corn silk powder provided valuable insights into its chemical composition by identifying key functional groups based on their characteristic vibrational modes. These observations help infer the presence of major classes of bioactive compounds, such as proteins, polysaccharides, and polyphenols, which contribute to its antioxidant potential. A prominent broad peak at 3398 cm^{-1} was observed, corresponding to N–H stretching vibrations. This is typically indicative of amino groups, suggesting the presence of proteins or amine-containing compounds. This region may also overlap with O–H stretching vibrations, which are common in hydroxyl-rich compounds such as phenolics and carbohydrates, thus reflecting the presence of multiple hydrogen-bonded functional groups. The absorption band at 2925 cm^{-1} is associated with C–H stretching vibrations of aliphatic chains. This peak reflects the symmetric and asymmetric stretching of $–CH_2$ and $–CH_3$ groups, which are commonly found in lipids, cellulose, and hemicellulose components. This band further suggests intra- and inter-molecular interactions within polysaccharide chains, confirming the presence of carbohydrate-rich structures in the corn silk matrix. A distinct peak at 1647 cm^{-1} corresponds to amide I (C=O and N–H) stretching vibrations, a hallmark of proteinaceous compounds. This band generally arises from C=O stretching in peptide bonds, indicating the presence of structural proteins or protein-bound phenolic compounds. The absorption at 1404 cm^{-1} is attributed to asymmetric carbonyl (C=O) stretching. This band is often associated with carboxylate ions, possibly originating from organic acids or esterified polyphenols, which are known to be abundant in plant-derived materials. This may reflect oxidized phenolic structures, which contribute to antioxidant activity. Also, a sharp peak at 1080 cm^{-1} was observed, corresponding to C–O–C and C–O stretching vibrations, which are indicative of sugar residues, particularly those in pyranose ring structures. This suggests the presence of simple and complex carbohydrates, including glucose, arabinose, and other saccharide units, commonly found in cell wall polysaccharides.

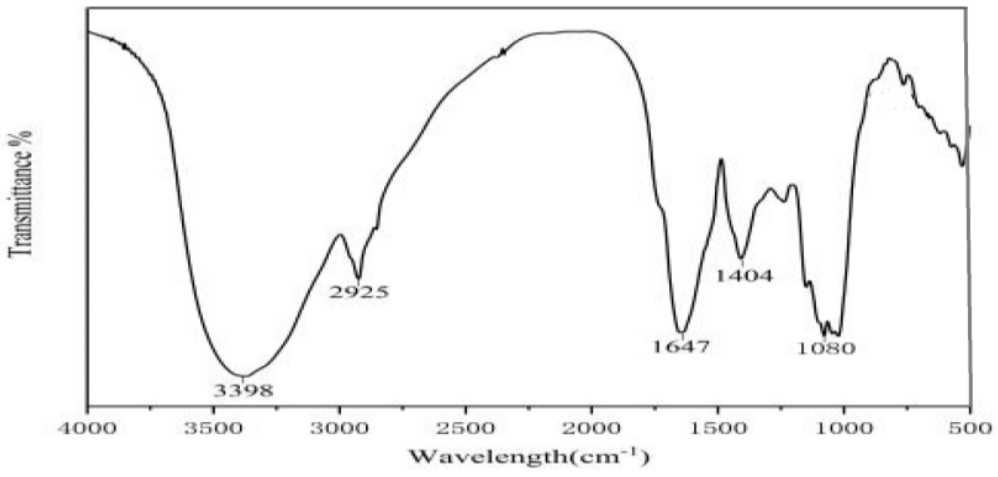

Figure 1: Infra-Red Spectra of corn silk powder

The TPC of corn silk propanol extract samples exhibited considerable variability, ranging from 50.38 µg/g extract to 499.35 µg/g extract, with a mean value of 274.8 ±151.11 µg/g extract. When expressed in milligrams per gram, the mean TPC was 0.274±0.151 mg/g extract. The relatively high standard deviation (about 55.0%) of the mean in both units suggests substantial variability among individual measurements or between extract batches. This variation may be attributed to differences in extraction efficiency, sample heterogeneity, or

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environmental factors affecting the phytochemical content of the corn silk, such as maturity stage, storage conditions, or plant genotype. Such variability highlights the need for standardized extraction protocols and possibly further fractionation or purification steps to ensure consistency and reproducibility in phenolic content, especially if the extract is intended for functional food or nutraceutical applications.

Absorbance values from the corn silk ethyl acetate extract samples were then interpolated from this standard curve to determine their gallic acid equivalent concentrations. The calculated TPC values for the corn silk ethyl acetate extract ranged from 24.81 µg/g extract to 255.49 µg/g extract. The mean TPC was determined to be 146.26±77.62 µg/g extract. In milligrams per gram, the mean TPC was 0.146±0.077 mg/g extract. This considerable standard deviation (about 53.0% of the mean in both unit expressions) indicated significant variability among the individual measurements or samples of the corn silk ethyl acetate extract. The TPC of corn silk chloroform extract samples ranged from 12.31 µg/g extract to 119.58 µg/g extract, with a mean TPC of 65.97±38.07 µg/g extract. When expressed in milligrams per gram, the mean was 0.065±0.038 mg/g extract. The high standard deviation (58.0%) of the mean indicates considerable variability among individual measurements or extract samples, likely due to sample heterogeneity or inconsistencies in phenolic solubility within the chloroform phase. When comparing across solvents, the results clearly show that the propanol extract yielded the highest TPC, followed by the ethyl acetate extract, while the chloroform extract contained the lowest levels of total phenolic compounds. These differences are primarily attributed to the varying polarities of the solvents used during extraction. Phenolic compounds, being generally polar in nature, are more efficiently extracted by medium- to high-polarity solvents such as propanol and ethyl acetate, whereas low-polarity solvents like chloroform are less effective in solubilizing these compounds. Additionally, the chemical structure and solubility profile of specific phenolics present in corn silk may further influence their extraction efficiency with different solvents. This solvent-dependent variation in TPC underscores the importance of selecting appropriate solvents when aiming to maximize the recovery of phenolic antioxidants from plant materials.

The TFC of the corn silk propanol extract was determined to have a mean value of 193±61 µg/g extract, equivalent to 0.193±0.061 mg/g extract. The standard deviation of 61 µg/g corresponds to 31.6% of the mean, which reflects a moderate level of variability among the samples. This degree of dispersion is considered acceptable in phytochemical studies, suggesting that the individual data points are reasonably clustered around the mean, and the extraction process yielded relatively consistent flavonoid concentrations. Such consistency reinforces the effectiveness of propanol as a solvent for extracting flavonoid compounds from corn silk, likely due to its polarity and ability to dissolve a wide range of moderately polar phytochemicals. The relatively high TFC, combined with the manageable variability, further supports the suitability of propanol extracts for applications where flavonoid-rich natural antioxidants are desired.

The TFC of the corn silk ethyl acetate extract was determined to have a mean value of 90.0±25.0 µg/g of the extract. This is equivalent to 0.090±0.025 mg/g. The standard deviation (25) represents about 27.8% of the mean (90). This ratio is also logical and realistic, suggesting that the samples were relatively homogeneous. The TFC in the chloroform extract of corn silk is expected, as chloroform is a predominantly non-polar solvent, while flavonoids are generally polar compounds due to the presence of hydroxyl groups in their structure. The low polarity of chloroform limits its ability to effectively solubilize and extract these polar phytochemicals. As a result, the extraction efficiency of flavonoids using chloroform is significantly reduced, leading to lower total flavonoid content in the chloroform extract compared to more polar solvents such as propanol or ethyl acetate. The standard deviation (5) represents 33.3% of the mean, which is scientifically acceptable and confirms the consistency of the measurements despite the low concentration. These results conclusively demonstrate that the polarity of the solvent plays a critical role in the efficiency of flavonoid extraction from corn silk. Extracts obtained using moderately polar solvents, such as propanol and ethyl

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acetate, were significantly more effective in extracting flavonoid compounds than the non-polar solvent, chloroform. These findings are in complete agreement with the chemical principle of "Like Dissolves Like" and confirm that selecting the appropriate solvent is a fundamental step in ensuring the highest possible concentration of biologically active compounds in a plant extract. In this study, the percentage inhibition of free radicals was determined for various extracts obtained from corn silk. A linear trend is observed between the extract concentration and the percentage inhibition, implying a directly proportional relationship.

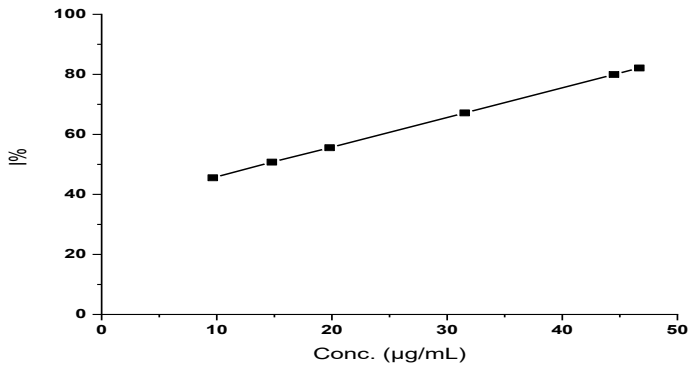

Figure 2: DPPH radical scavenging activity of propanol corn silk extract

Figure 2 illustrates a clear positive correlation between the concentration of the propanol extract of corn silk and its free radical scavenging activity. The data reveal a concomitant increase in the percentage of free radical inhibition with increasing extract concentration, indicating a direct dose-dependent relationship. The maximum observed free radical inhibition, 82.13%, occurred at an extract concentration of 46.69 mg/mL (equivalent to 46,690 µg/mL). Conversely, the minimum inhibition, 45.52%, was recorded at a concentration of 9.66 mg/mL (equivalent to 9,660 µg/mL). These findings demonstrate the concentration-dependent antioxidant efficacy of the propanol extract derived from corn silk. Additionally, similar trends were observed for ascorbic acid, as depicted in **Figure 3**.

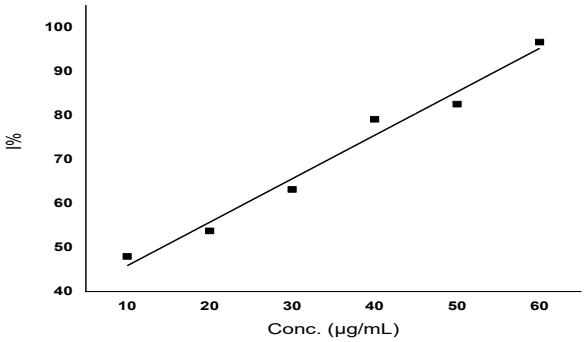

Figure 3: A correlation exists between ascorbic acid concentration and the observed inhibition

Figure 4 illustrates a clear positive correlation between the concentration of the ethyl acetate extract of corn silk and its free radical scavenging activity. As the concentration of the extract increases, there is a concomitant increase in the percentage of free radical inhibition, demonstrating a dose-dependent relationship. The highest inhibition of free radicals, 78.07%, was observed at an extract concentration of 42.58 mg/ml (equivalent to 42580 µg/ml). Conversely, the lowest inhibition, 38.05%, was recorded at a concentration of 2.10 mg/ml (equivalent to 2100 µg/ml).

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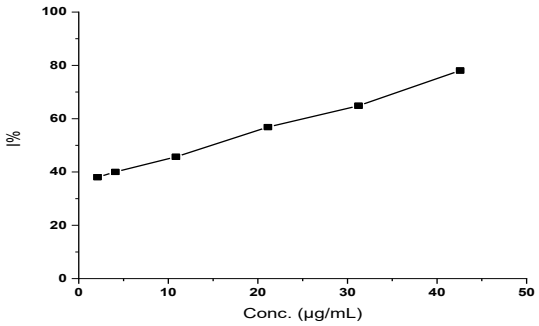

Figure 4: DPPH Radical scavenging activity of ethyl acetate corn silk extract

Figure 5 depicts a concentration-dependent increase in the free radical scavenging activity of the corn silk chloroform extract. The highest inhibition, reaching 75.81%, was observed at an extract concentration of 40.29 mg/ml (equivalent to 40290 µg/ml). The lowest inhibition, 38.54%, was recorded at a concentration of 2.60 mg/ml (equivalent to 2600 µg/ml).

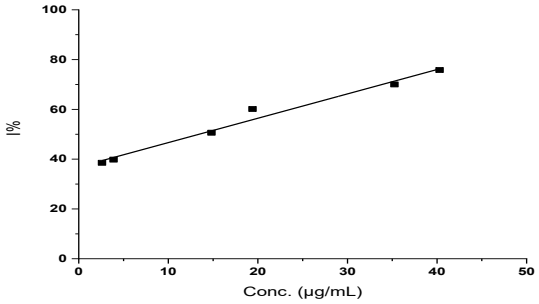

Figure 5: DPPH radical scavenging activity of chloroform corn silk extract

The IC_{50} value, defined as the concentration of antioxidant extract required to inhibit 50.0% of DPPH radicals. The studies were determined through linear regression analysis of the inhibition percentage against three different extracts in **Figure 6**.

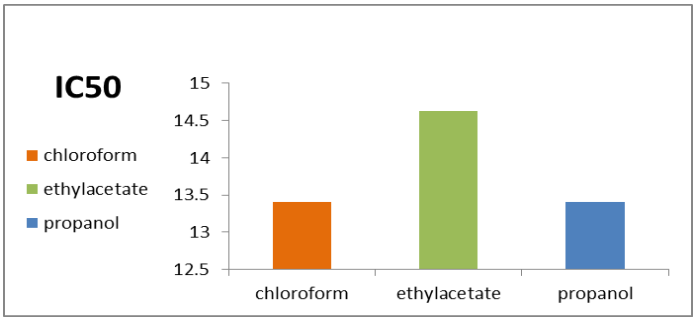

Figure 6: Comparison of IC_{50} values for different extracts

The IC_{50} value, which represents the concentration of extract required to inhibit 50.0% of DPPH radicals, is inversely proportional to antioxidant capacity-that is, lower IC_{50} values indicate stronger free radical scavenging activity. As shown in **Figure 6**, the propanol extract exhibited the lowest IC_{50} value, indicating the highest antioxidant activity among the tested extracts. In comparison, the ethyl acetate and chloroform extracts showed higher IC_{50} values, reflecting comparatively weaker DPPH radical scavenging capabilities. These results further support the effectiveness of propanol in extracting antioxidant-rich compounds from corn silk.

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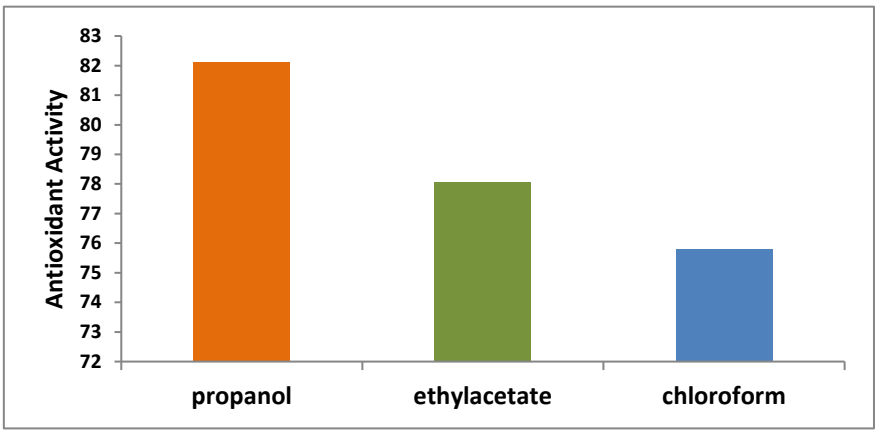

Figure 7: Antioxidant activity in various organic extracts of corn silk

Analysis of the various corn silk extracts revealed that the propanol extract exhibited the strongest antioxidant capacity, achieving 82.13% DPPH radical inhibition at a concentration of 46.69 mg/mL. In contrast, the chloroform extract demonstrated the weakest antioxidant activity, with 75.81% inhibition at a concentration of 40.90 mg/mL. The enhanced antioxidant activity observed in the propanol extract is likely attributable to its higher content of phenolic compounds and flavonoids, which are known for their free radical scavenging properties. These support the correlation between phytochemical composition, particularly polar antioxidants-and the effectiveness of corn silk extracts in neutralizing oxidative stress.

Conclusion: The current findings establish a strong correlation between the high content of phenolics and flavonoids in corn silk extracts and their elevated antioxidant activity. The propanol extract proved to be the most effective among the tested extracts, making it a promising candidate for use as a natural source of antioxidant compounds.

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