Evaluation of Antagonistic Action of Corn Silk Extract Towards Various Fungal and Bacterial Pathogens

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ABSTRACT: Corn silk (stigma of female flower) is widely exploited for medicinal purposes against various pathogenic microorganisms. The present investigation was carried out to evaluate the antimicrobial and antioxidant properties of corn silk. In this regard, antifungal and antibacterial assays of corn silk extract prepared using microwave assisted method were carried out. The results revealed that corn silk extract possessed better antibacterial potential than antifungal potential. The antifungal assays showed similar results in comparison to positive controls. The antibacterial assays revealed that the plant extract was able to retard the growth of gram positive bacteria better than gram negative bacteria. Highest inhibition zones were observed in case of gram positive bacteria. The free radical scavenging activity of corn silk extract was 50.58% which indicated low phenolics content. The qualitative analysis of the aqueous ethanolic corn silk extract showed positive results for the presence of flavonoids and phenolics.

Key words: Corn Silk, antioxidant, antibacterial, antifungal

INTRODUCTION

Plant extracts had been used as traditional treatment for various human diseases in many parts of the world for centuries. The rural population in developing countries used these medicinal plants as their primary source of medicine (Chitme et al., 2004). Around 80% of the people in developing nations rely on these traditional medicines for treatment of different diseases (Kim, 2005). Due to increased resistance of pathogens towards therapeutic agents (antibiotics and antiviral agents), novel anti-infective compounds have gained importance. Screening of plants against pathogenic microbes is the most important factor to identify the medicinal properties of plants. Different groups of phytochemicals from various plant extracts has been identified and their safe status has been established. Plant extracts exhibit significant antioxidant, antimicrobial, analgesic, anti-diarrheal and
wound healing properties. They are proven to be better alternatives having lesser side effects. According to WHO estimates, around 20,000 medically important plants have been discovered in 91 countries. Extraction, screening on pharmacological basis, isolation and characterization, toxicological and clinical evaluation are leading steps towards widespread use of bioactive compounds (Das et al., 2006).

Corn silk is considered a waste product and could be easily obtained in large quantities from food and feedstock industries. Corn silk is reported to display antioxidant activity because of presence of polyphenol compounds. It also contains various phytochemicals such as steroids, flavonoids, alkaloids and anthocyanins (Velazquez et al., 2005). Steroids in corn silk consists of sitosterol and stigmasterol (Ebrahimzadeh et al., 2008). Antimicrobial activity of the corn silk extract prepared through various extraction methods has been established against twelve pathogenic bacteria i.e. Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, Salmonella typhi, Salmonella paratyphi, Escherichia coli, Shigella sonnei, Shigella flexneri, Proteus vulgaris, Proteus mirabilis and one yeast Candida albicans (Nessa et al., 2012). The aim of this study was to investigate the antimicrobial and antioxidant potential of corn silk. Antimicrobial potential (due to presence of phytochemicals) of corn can be utilized to cope up with the problem of antibiotic resistance. Similarly, the antioxidative nature of corn silk may help to treat various diseases including cancer and cardiovascular disorders since antioxidants can demolish the injury caused by the formation of free radicals during oxidative stress.

MATERIALS AND METHODS

Sample Collection:

Fresh, healthy corn was taken from the local market of Lahore, Pakistan and was deposited to the laboratory of department of Biology, Lahore Garrison University, Lahore, Pakistan. Corn silk was excised from the corn fruit by removing husk. It was then rinsed with sterile distilled water, dried in sunlight and pulverized to form powder. The powdered corn silk was stored at room temperature until further use.

Preparation of Corn Silk Extract:

The active compounds from the corn silk were released using microwave assisted extraction (MAE) (Altemimi et al., 2017). About 1g of the powdered plant material was dissolved in 50ml of the chloroform and subjected to heating cycles with intermittent cooling to prevent the material from vigorous boiling which can cause the thermal degradation of heat labile phytochemicals present in plant material.

Screening for Antioxidant Activity:

The antioxidant activity was analyzed using the hydrogen peroxide scavenging assay proposed by Dua et al. (2012). For this purpose, 40 mM solution of hydrogen peroxide was prepared in phosphate buffer saline (pH 7.4). 10mg/ml of the ethanolic extract was added to 10ml of hydrogen peroxide solution. After 10 minutes, optical density was measured at 560nm against blank solution containing phosphate buffer with hydrogen peroxide. The percentage of hydrogen peroxide scavenging
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of the corn silk extract was calculated by formula:

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\text{Percentage scavenging} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100
\]

**Screening for Antimicrobial Activity:**

For antifungal assay, fungal cultures were maintained on potato dextrose agar (PDA) incubated at 28°C for 48 hours. Axicon were taken as a positive control while sterile distilled water was taken as a negative control. 1 ml of the prepared plant extract was plated onto petri plates containing potato dextrose agar. Fungal spores were then picked off and placed into the middle of the petri plates containing plant extract and incubated at 28°C for 48 hours. The same procedure was done for the control plates. The size of the colony diameter of experimental and control group was used as the index of antifungal activity. *Aspergillus nidulans, Aspergillus flavus* and *Aspergillus fumigatus* were used as test organisms. For estimation of antibacterial activity, bacteria were grown on the nutrient agar and incubated at 37°C. The antibacterial activity was measured using disc diffusion method. Test organisms used were gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus*) and gram-negative bacteria (*Escherichia coli* and *Acenobacter baumannii*). Piperacillin-tazobactam (TZP) was used as positive control while distilled water was used as negative control. All the experiments were carried out in triplicates. ANOVA test was used to determine the statistical significance of the results.

**Screening for Phytochemicals:**

Ethanolic extract of the corn silk was utilized for qualitative analysis of phytochemicals i.e., flavonoids and phenolics (Ngonda, 2013).

**RESULTS AND DISCUSSION**

**Hydrogen Peroxide Scavenging Assay:**

Corn silk extract was analyzed for antioxidant activity using hydrogen peroxide scavenging assay. Optical density was measured at 560 nm for experimental and control solution and came out to be 1.69 and 3.42 respectively. Percentage inhibition of hydrogen peroxide scavenging assay for corn silk was found out to be 50.58. This depicted that low quantities of free radicals were scavenged due to low phenolics content. Previous investigations confirmed that antioxidant activity of corn silk extract is dependent upon the extraction method and maturity stage (Morshed and Islam, 2015).

**Antimicrobial Activity of Corn Silk Extract:**

The antifungal potential of corn silk was investigated, and the results revealed that the corn silk extract was able to retard the growth of fungal pathogens (Fig. 1). However, when compared to the positive control (Axicon), the experimental group had the same effect. There was no significant size difference between the colonies obtained on corn silk extract and axicon supplemented media (P > 0.05).

Disc diffusion method was employed to test the antimicrobial action of corn silk extract against different types of bacteria. Corn silk extract antibacterial activity with varying degrees, as suggested by the diameter of inhibition zone, against Gram-positive \( \text{viz}, \)
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*Staphylococcus aureus* and *Enterococcus* and gram-negative bacteria viz., *Acenobacter baumannii* and *Escherichia coli*. Maximum zone of inhibition was obtained against gram-positive bacteria *i.e.*, *S. aureus* and *Enterococcus* (20 and 25 mm). The growth inhibition for gram-negative *i.e.*, *E. coli* and *A. baumannii* bacteria was estimated to be at 19 and 23 mm respectively.

The antimicrobial activities of corn silk extract can be attributed to the presence of various organic compounds such as tannins, phenols, alkaloids, flavonoids, terpenoids, glycosides, steroids, phytosterol (stigmasterol and β-sitosterol) and mixtures of fatty acids (dodecanoic acid, tetradecanoic acid, hexadecanoic acid and octadecanoic acid) (Fazilatun et al., 2001). Various studies have confirmed antimicrobial potential of corn silk extract prepared using different extraction methods (Alam, 2011; Saleh et al., 2017; Sharma et al., 2009). The antimicrobial potential is influenced by the extraction method used to release the phytochemicals of corn silk (Morshed and Islam, 2015). In the present study, the growth of gram-positive bacteria was impeded better than the gram-negative bacteria. The findings are in line with Grosvenor et al. (1995) who reported that the variable susceptibility might be attributed to the structural composition of cell wall of gram-positive and gram-negative bacteria (Grosvenor et al., 1995).

**Phytochemical Analysis of Corn Silk Extract:**

Flavonoids and phenolics are among the significant phytochemicals imparting the plant extract their antimicrobial activities (Cushnie and Lamb, 2005, Saavedra et al., 2010). Qualitative tests performed for the presence of flavonoids and phenolics in corn silk extract showed positive results. Presence of flavonoids was identified by the appearance of pink colour when hydrochloric acid, magnesium and ethanol was added to the plant extract. Presence of phenolics in the ethanolic extract was confirmed by the appearance of bluish green colour after addition of ferric chloride to the ethanolic extract of corn silk.
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Fig. 1: Antifungal action of corn silk extract against Aspergillus species

Fig. 2: Antibacterial action of Corn Silk extract against gram-positive bacteria (Staphylococcus aureus and Enterococcus) and gram-negative bacteria (Acenobacter baumannii and Escherichia coli)
CONCLUSION

This study is a preliminary work to establish the antimicrobial and antioxidant activity of corn silk. The positive results of antimicrobial and antioxidant assays revealed that corn silk extract harbors the potential to cope up with the problem of antibiotic resistance and the anti-oxidative nature of corn silk may help to treat various diseases including cancer and cardiovascular disorders.

REFERENCES


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