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Antimicrobial Activity and Phytochemical Properties of Corn (*Zea mays* L.) Silk

Selim Morshed and S.M. Shahinul Islam*

Plant Genetic Engineering Lab., Institute of Biological Sciences, University of Rajshahi, Rajshahi - 6205 (Bangladesh)
*e-mail: *shahin_ibsc@ru.ac.bd*

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ABSTRACT

Corn silk (stigma of female flower) is used medicinally against some diseases caused by various pathogenic bacteria. The present study was aimed to identify some medicinal properties of corn silk and investigate its antimicrobial activities in different solvents *viz.*, ethanol, chloroform and methanol extracts (10 mg/ml each) in comparison to streptomycin (10 mg/ml). All the extracts showed variable degree of inhibitory zone by using disc diffusion method against tested bacteria. The highest inhibition zone (13.17 and 12.27 mm) was observed in 10.0 mg/ml of ethanol and methanol, respectively. Ethanol extract showed significant antimicrobial activity against both Gram positive bacteria (13.17 to 9.45 mg/ml) and Gram negative bacteria (12.36 to 8.15 mg/ml) bacteria. No extract was sensitive against *Escherichia coli* and yeast strain.

Key words: Antimicrobial activity, corn silk, extract, flavonoid glycosides, phytochemical

Maize (*Zea mays* L.), belonging to family *Poaceae*, is the 3rd important cereal crop world-wide as well as in Bangladesh. It is used as human food, animal feed and raw material for manufacturing a number of industrial products and is considered a potential valuable biofuel and forage crop. It has antimicrobial activities that prevent various diseases caused by viruses, bacteria and fungi. Drug resistance by human pathogens has necessitated the search for antimicrobial chemicals (Sandrasagaran *et al*., 2014). The plant originated antimicrobial agents show least side effects and have prospective therapeutic effect in healing many infectious diseases (Gulcin *et al*., 2004; Rahman *et al*., 2013). Despite remarkable advances in medical research, infectious diseases are still leading cause of premature deaths worldwide (Agrawal *et al*., 2014). There are a number of medicinally important plant-originated products which are used as mild stimulants, diuretic and demulcent, against acute and chronic cystitis and as remedy to bladder irritation of uric acid and phosphatic gravel. Some medicinal properties attributed to corn silk include antioxidant properties (El-Ghorab *et al*., 2007; Mohsen and Ammar, 2009), anti-diabetic effects (Rau *et al*., 2006; Guo *et al*., 2009), antibiotic activities towards corn earworm (Waiss *et al*., 1979), resistance to insects (Guevara *et al*., 2000) and antitumour activity (Habtemariam, 1998). The screening of various plants species for medicinal properties is important to overcome these emerging problems (Monica *et al*., 2013), since corn silk contains a number of flavonoids, chlorogenic acid, p-coumaric, ferulic acid, saponins, phytosterols, volatile oil, fixed oil, resin, sugars, allantoin, tannin and minerals (Snook *et al*., 1993; Sosa *et al*., 1997; Fazilatun *et al*., 2001; Ebrahimzadeh *et al*., 2008). Therefore, the present study was aimed to investigate the antimicrobial activities of corn silk extracts prepared in different solvents and assess its flavonoids, glycosides, steroids and sugars value.

MATERIALS AND METHODS

Plant materials

Mature seeds of corn cv. 'Mohar' were collected from Bangladesh Agriculture Research Institute, Gazipur, Bangladesh. Seeds were sown and grown in the research field of Institute of Biological Sciences, University of Rajshahi, Bangladesh during October, 2014 – March, 2015. The cobs were collected for corn silks. Analytical grade methanol, ethanol and chloroform (procured from Merck Ltd., Mumbai, India) were used as solvents.

Preparation of extracts

The collected corn silks were washed with sterile distilled water, after soaking the excess water was removed by using filter papers and then incubated at 40ºC for 5 days (Fig. 1A). The dried corn silk was powdered (Fig. 1B) with the help of a pulverizor. The material was dissolved in organic solvents namely methanol, ethanol and chloroform to applying ultrasound of the frequencies ranging from 20 to 2000 kHz by sonication method (Tiwari *et al*., 2011). The material was then distilled off on a water bath at atmospheric pressure and the last trace of solvent removed *in vacuo*. The crude extract was stored at 4ºC. The known amount of crude extract (10 mg/ml) was dissolved in DMSO (Fig. 1D). The stock solution was sterilized by passing through 0.2 µm pyrogesic filter and diluted with DMSO to make concentrations of 5.0, 2.5 and 1.25 mg/ml. For determination of antimicrobial activities, 5 mm dia. discs were prepared from Whatman filter paper No. 1 and sterilized by autoclaving (Bauer *et al*., 1966).

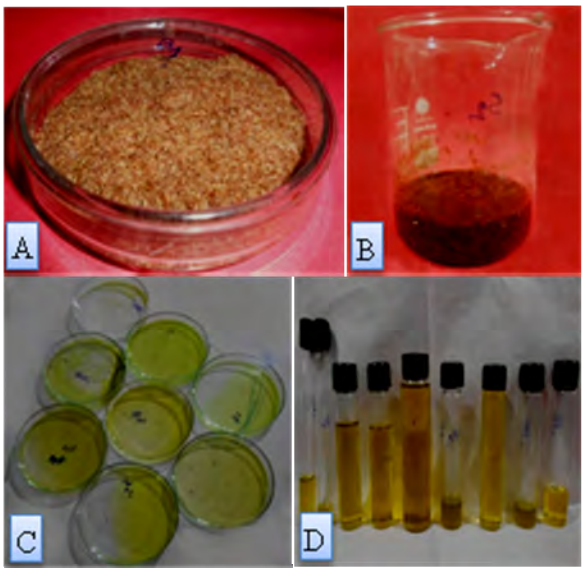

Fig.1: A) Dried corn silk of maize cv. Mohar, B) Corn silk dissolved in DMSO, C) Crude extract, and D) Stock solution

Microorganisms

Four Gram-positive bacteria *viz., Bacillus cereus, B. subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and eight Gram negative bacteria *viz., Shigella sonnei, S. flexneri, Proteus vulgaris, P. mirabilis, Enterobacter aerogenes, Salmonella typhi, S. paratyphi* and *Escherichia coli* were used along with a yeast strain of *Candida albicans* collected from Popular Diagnostic Centre (Pvt.) Ltd. Rajshahi, Bangladesh. Bacterial and fungal cultures grown nutrient agar and potato dextrose agar (PDA) media, respectively, were stored at 4°C till further use.

Screening for antimicrobial activity

For antibacterial activity, the bacterial strains were grown on nutrient broth and incubated at 37°C for 24 hours. Similarly for antifungal activity, yeast strain was grown on PDA and incubated for 24-48 hours at 28ºC. The bacterial and fungal cultures were grown in sterile petri-plates (90 mm dia.) containing nutrient agar/PDA medium. Preparation of agar disc and sterilization procedure was same as described previously. The 5 mm discs dipped in different concentrations (10.0, 5.0, 2.5 and 1.25 mg/ml) of corn extract (prepared in three solvent *viz.*, methanol, ethanol and

chloroform) were placed on the medium and incubated at 37°C for 24 hours. Control plates were also maintained wherein streptomycin (10 mg/ml) dipped discs were used. All the treatments were replicated thrice in completely randomized design. After incubation, the microbial growth was recorded.

Analysis of phytochemicals

Ethanol, methanol and chloroform based corn silk extracts were subjected to qualitative chemical analysis for the identification of phytochemical constituents *viz.*, flavonoids, glycosides, terpenoids, phenols, saponins, tannins, steroids, amino acids, carbohydrates and sugar as per the standard methods (Harborne, 1998; Kalaiarasan *et al*., 2012; Chandrashekar *et al*., 2013). The data was analyzed using analysis of variance as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The various organic solvents and concentrations used for screening of antimicrobial activity and determination of phytochemical properties of corn silk extract showed sensitive responses in methanol and ethanol based extracts to 11 bacteria out of the 12 tested. Comparatively, chloroform extract showed less sensitivity except in case of *Bacillus cereus* (11.98 mm), *B. subtilis* (12.08 mm), *Staphylococcus aureus* (5.56 mm) amongst G+ve bacteria and *Shigella sonnei* (9.33 mm) and *Enterobacter aerogenes* (8.10 mm) amongst G-ve bacteria. However, *Pseudomonas aeruginosa, Shigella flexneri*, *Proteus vulgaris*, *P. mirabilis*, *Salmonella typhi* and *S. paratyphi* did not show any response to it. *Escherichia coli* and *Candida albicans* (yeast) showed no response in all the three solvents tested. Streptomycin (10 mg/ml) showed sensitivity to all tested bacteria, except yeast (Table 1; Fig 2 and 3). Ethanol extracts (10 mg/ml) showed highest sensitivity against *B. cereus* (13.17 mm) followed by *B. subtilis* (12.16 mm), *S. aureus* (11.45 mm), *S. sonnei* (11.25 mm),

Table 1: Antimicrobial activity observed by using different organic solvent extracts of corn silk (mean ± SE)

Strains	Microorganisms	Zone of inhibition (mm)			
		Streptomycin (10 mg/ml)	Ethanol extract (10 mg/ml)	Chloroform extract (10 mg/ml)	Methanol extract (10 mg/ml)
Gram positive	*Bacillus cereus*	8.00 ± 0.64	13.17 ± 0.67	11.98 ± 0.86	11.66 ± 0.81
	Bacillus subtilis	9.08 ± 0.51	12.16 ± 0.95*	12.08 ± 0.69*	12.27 ± 0.93*
	Staphylococcus aureus	8.00 ± 0.67*	11.45 ± 0.84	5.56 ± 0.59	9.68 ± 0.81*
	Pseudomonas aeruginosa	12.23 ± 0.80	9.45 ± 0.81	0	11.65 ± 0.80
Gram Negative	*Shigella sonnei*	10.68 ± 0.80*	11.25 ± 0.95*	9.33 + 0.65	11.25 ± 0.54*
	Shigella flexneri	7.01 ± 0.80	8.15 ± 0.75	0	8.03 ± 0.39
	Proteus vulgaris	9.00 ± 0.48*	11.78 ± 0.38	0	9.01 ± 0.48*
	Proteus mirabilis	9.05 ± 0.85	12.36 ± 0.79	0	7.19 ± 0.81
	Enterobacter aerogenes	11.00 ± 0.80	9.76 ± 0.93	8.10 ± 0.69	12.05 ± 0.91
	Salmonella typhi	12.95± 0.71*	10.25 ± 0.99	0	12.13 ± 0.88*
	Salmonella paratyphi	9.01 ± 0.48*	9.35 + 0.97*	0	6.54 + 0.67
	Escherichia coli	6.05 ± 0.84	0	0	0
Yeast	*Candida albicans*	0	0	0	0
$CD_{0.05}$		Microorganism - 6.31; Organic solvents - 11.15			

* Values within the row are non-significant

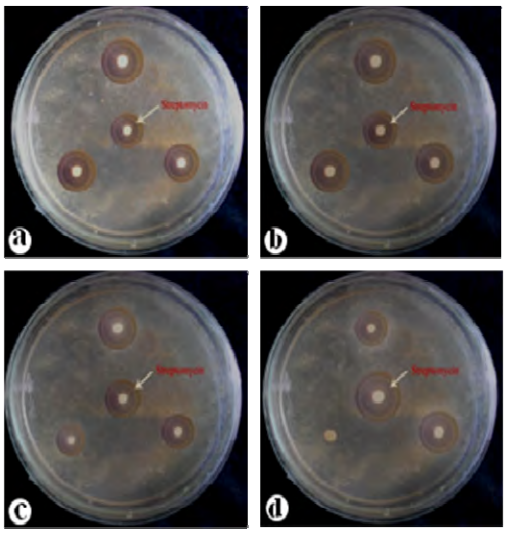

Fig. 2: Antimicrobial activities of Gram positive bacteria by using corn silk extract (a) *Bacillus cereus;* (b) *B. subtilis;* (c) *Staphylococcus aureus* & d) *Pseudomonas aeruginosa*

S. flexneri (8.15 mm), *P. vulgaris* (11.78 mm), *P. mirabilis* (12.36 mm) and *S. paratyphi* (9.35 mm) than streptomycin (10 mg/ml), except *P. aeruginosa* (9.45 mm), *E. aerogenes* (9.76 mm), *S. typhi* (10.25 mm) and *S. flexneri* (8.15 mm). On the other hand, *S. paratyphi* and *S. sonnei* showed non-significant results in comparison to control. Methanol extract comparatively performed better against *B. cereus* (11.66 mm), *B. subtilis* (12.27 mm), *S. aureus* (9.68 mm), *S. sonnei* (11.25 mm), *S. flexneri* (8.03 mm), *P. vulgaris* (9.01 mm) and *E. aerogenes* (12.05 mm) as compared to streptomycin against eleven bacteria.

Chloroform extract showed comparatively poor sensitivity than ethanol, methanol extracts and control (streptomycin) against both G-ve and G+ve bacteria. Three bacteria each from G+ve (*B. cereus*, *B. subtilis* and *P. aeruginosa*) and G-ve (*S. flexneri*, *P. mirabilis* and *E. aerogenes*) when compared with control (streptomycin) showed significant results

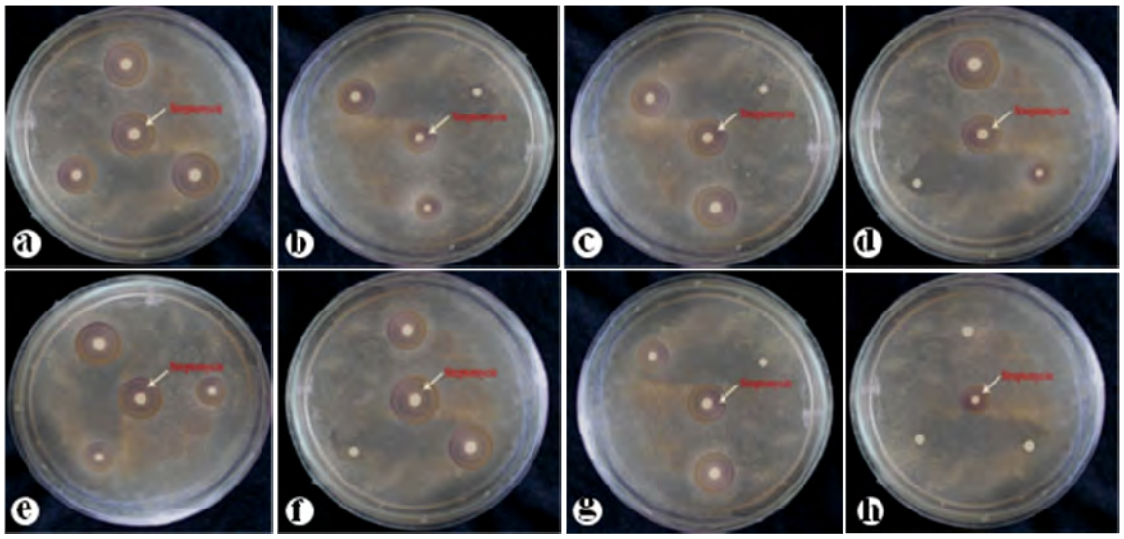

Fig. 3: Antimicrobial activities of Gram negative bacteria by using corn silk extract a) *Shigella sonnei;* b) *S. flexneri;* c) *Proteus vulgaris;* d) *P. mirabilis;* e) *Enterobacter aerogenes;* f) *Salmonella typhi;* g) *S. paratyphi* and h) *Escherichia coli*

in ethanol, chloroform and methanol extracts at $p < 0.05$ level. Overall ratio of multiplication, G+ve bacteria was better than G-ve bacteria by creating clear inhibition zone (Fig. 4). Phytochemical study revealed that the corn silk extracts had some bioactive compounds. Study showed that flavonoides and glycosides were presents in all tested organic solvent (ethanol, methanol and chloroform) extracts. Steroids and sugars appeared only in ethanol and methanol extracts, but terpenoids, phenols saponins, tannins, amino acids and carbohydrates were not detected (Table 2). For antimicrobial and phytochemical study, the extraction capacity was determined by different organic chemical extracts that were expressed at $p < 0.05$ significant level.

Amongst the corn silk extracts, ethanol extract showed best performance by giving highest zone of inhibition than methanol and chloroform extracts. Such effects were described by Rahman *et al.* (2015) in olive for several microorganisms i.e. *S. aureus*, *S. epidermidis*, *E. coli* and *S. typhimurium*. On the other hand, chloroform extract of *V. tessellata* leaves exhibited higher degree of inhibition (11.00 mm) against *S. aureus* and *E. coli* and 2.6 to 4.4 mm for *Vanda coerulea* leaves extract (Shanmuga *et al.*, 2011). The chloroform extract of orchid plant (*Vanda tessellata)* produced inhibition zone of 15.40 - 5.20 mm (Bhattacharjee *et al.*, 2015). In our study the inhibition zones for microorganisms revealed that ethanol based extract of corn silk had higher antimicrobial activity potential. Reports reveal that corn silk extracts depict highest performance on petroleum ether (12.17 - 8.50 mm) than chloroform, methanol and antibiotic gentamycin (Nessa *et al.*, 2012). It appears that ethanol based corn silk extract performed equally (13.17 - 8.15 mm) which might be to different genotypes along with differences in organic solvents.

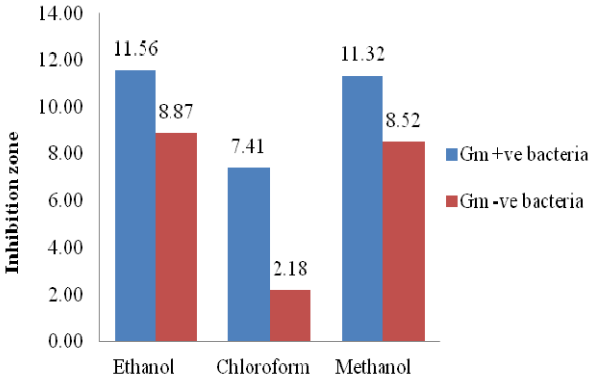

Fig. 4: Inhibition zone between Gram positive and Gram negative bacteria using different extracts of corn silk

Table 2: Presences of phyotochemical properties in corn silk extracts

Phytoconstituents	Solvent extracts		
	Ethanol	Chloroform	Methanol
Amino acids	-	-	-
Carbohydrates	-	-	-
Flavonoids	+	+	+
Glycosides	+	+	+
Phenols	-	-	-
Saponins	-	-	-
Steroids	+	-	+
Sugars	+	-	+
Tannins	-	-	-
Terpenoids	-	-	-

+ = present, - = absent

In present study, G+ve bacteria produced higher inhibition zone than G–ve bacteria with tested extracts. The findings are supported by Grosvenor *et al*. (1995) who argued that the variable susceptibility might be due to the differences in cell wall composition of G+ve and G-ve bacteria.

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) in different corn silk varieties were studied by Fazilatun *et al*. (2001) and it was reported that methanol extract contained a number of flavonoids such as maysin, quercetin and maysin-3′- methyl ether (Snook *et al*., 1993; Sosa *et al*., 1997; Ebrahimzadeh *et al*., 2008). In our findings, flavonoids and glycosides were observed in ethanol, methanol and chloroform based extracts; and steroids and sugar were in ethanol and methanol based extracts only. However, tannins, saponins, phenolic compounds, steroids, glycosides, alkaloids, anthro-quinones and flavonoids were reported in methanol based extracts in case of *Catharanthus roseus* leaves (Maithili and Mekala, 2015).

Conclusion: Of the three solvent based extracts of corn silk ethanol based extract proved best in exhibiting maximum antimicrobial activity against G+ve and G-ve bacteria as compared to other extracts and control. Flavinoids and glycosides were present in all the tested extracts which showed

some phytochemical properties but steroids and sugars were present in ethanol and methanol based extracts only. For overall multiplication, Gram +ve performed better than Gram –ve bacteria.

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