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Studies on antimicrobial activity of ethanolic extract of maize silk

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To study antimicrobial activity of ethanolic extract of maize silk. *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Bacillus coli* and *Candida albicans* were chosen as detected germs. Using methods of agar plated diffusions and tube continuous dilution to detect antimicrobial activity and MIC (minimum inhibitory concentration) of ethanolic extract of maize silk. Ethanolic extract of maize silk can inhibit microbial activity against bacteria such as *S. aureus*, *P. aeruginosa, B. cereus*, *B. coli* and *C. albicans* and the diameter of bacteriostatic circle is 19, 20, 28, 15 and 21 millimeter, respectively. Ethanolic extract of maize silk has the best antimicrobial activity against *Bacillus cereus*. The MIC to *B. coli* is 15.625 mg/ml, while the MIC to *S. aureus*, *P. aeruginosa*, *C. albicans* are all the 31.25 mg/ml. Ethanolic extract of maize silk has antimicrobial activity on *S. aureus*, *P. aeruginosa*, *B. cereus*, *B. coli* and *C. albicans*.

Key words: Maize silk, ethanolic extract, antimicrobial activity, minimum inhibitory concentration.

INTRODUCTION

Maize (*Zea mays L*) silk is the stylet or stigma of maize. Maize silk is line pattern and generally form a loose cluster. It has some colors such as lightgreen, kelly or brownish red. It is soft and bland and has no smell (BeiJing, 2010; Nanjing medicine college and Chinese Herbal Medicine Pharmacology, China, 1980). As the results of many researches show (Bobryshev, 1962; Hanping et al., 2009), maize silk contains lots of components such as fatty oils, volatile oils, gums, resins and so on. It also has large numbers of potassium nitrate and a-tecopherol quinone. Natural products are in great demand due to its extensive biological properties and providing source for the discovery of many types of effective bioactive compounds. Maize silk has been recognized for their many pharmacological activities such as reducing blood sugar (Wastl, 1947), inhibiting the growth of tumor and urinary stone (Habtemaram, 1998), inducing diuresis (Dzhamalieva, 1954) and accelerating the coagulation of blood. It also has some effects on circulatory system (Hahn, 1973). Therefore, maize silk has been used as traditional Chinese medicine for the treatment of hydragogue, cholagogue, hyperglycemia, antiphlogistic, etc (Nanjing medicine college and Chinese Herbal Medicine Pharmacology, China, 1980).

There is little research about antibacterial activity of ethanolic extract in recent years (Zhou et al., 2008; Lilian and Renxiang, 2001). Moreover, all the strains used in recent studies are bacteria. Whether ethanolic extract of maize silk can restrain the growth of funguses which are also related to the safety of food and the health of our bodies, more researches need to be done. Therefore, the aim of this study is to explore the antimicrobial activity of ethanolic extract of maize silk, and hopefully to get lots useful information for the exploit of maize silk.

MATERIALS AND METHODS

Plant material

Air-dried maize silk was collected in Yueyang, Hunan, China. Microorganisms *S. aureus* (ACCC 01331, *P. aeruginosa* (GIMT 1.074, *B. cereus* (CPCC 100005), Bacillus coli (CMCCB 44102) and *C. albicans* (CAU 0037) were selected as the

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Table 1. Results of the inhibition zone of ethanolic extract of maize silk.

Plant extracts	Zone of Inhibition (mm)				
	S. aureus	*B. coli*	*P. aeruginosa*	*B. cereus*	*C. albicans*
Ethanolic extract of maize silk (treatment group)	19	15	20	28	21
Sterile purified water (control)	—	—	—	—	—
Extract of andrographis paniculata (positive control)	29	23	25	30	31

Table 2. Results of the MIC of ethanolic extract of maize silk.

Strains	Concentration (mg/ml)					control
	250	125	62.5	31.25	15.625	
S. aureus	-	-	-	+	+++	+++
B. coli	-	-	+	++	+++	+++
P. aeruginosa	-	-	-	+	+++	+++
B. cereus	-	-	-	-	+++	+++
C. albicans	-	-	-	+	+++	+++

Note:"—"stands for no bacteria growth;"+"stands for very few bacteria growth;"++"stands for part bacteria growth;"+++"stands for vigorous bacteria.

tested microorganisms. Various microbial strains were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP).

Preparation of crude ethanolic extract of maize silk

For ethanolic extraction, 50 g of air-dried powder was placed in ethanol and boiled for 6 h. At intervals of 3 h, the solvent was removed in vacuum. The supernatant was then collected. After 6 h, the supernatant was concentrated to make the final volume to 100 ml. Finally, the 500 mg/ml concentration of ethanolic extract of maize silk was obtained. It was then autoclaved at 121℃ and 15 lbs pressure and stored at 4℃.

Agar diffusion method

The qualitative antimicrobial assay of the ethanolic extracts of maize silk was performed by the disc diffusion method (Cauwelier et al., 2004; Felten et al., 2002; Huys et al., 2002; Wilkins and Thiel, 1973). It was performed by using culture growth at 37℃ for 18 h and adjusted to approximate ly 3×10^8 colony-forming unit per milliliter (CFU/ml). The culture medium used for the bacteria was Mueller Hinton Agar (MHA), whereas Potate Dextrose Agar (PDA) was used for growing the fungi. 500 microliters of the inoculum was spread over plates containing MHA or PDA and a Whatman paper disc (5 mm) impregnated with 1 ml of the extract that was placed on the surface of the media. The plates were left 30 min at room temperature to allow the diffusion of the oil. They were incubated 24 h at 37℃ for the bacteria and 48 h at 37℃ for the fungi. After incubation period, the inhibition zone obtained around the disc was measured. Two controls were included in the test. The first involved the presence of microorganisms without test material and the second was one standard antibiotic: that is, the extract of Andrographis paniculata was used as control. The experiments were run in triplicate, and the developing inhibition zones were compared with those of reference discs (Table 1) (Bauer et al., 1966; Marmonier, 1987).

MIC determination

The minimal inhibitory concentration (MIC) of ethanolic extracts was determined by using the Mueller Hinton Broth (MHB) dilution method (Eloff, 1998). Bacterial strains were cultured overnight in MHB at 37℃. Tubes of MHB containing v arious concentrations of extracts (Table 2) were inoculated with 10 µl of 3×10^8 CFU/ml of standardized microorganism suspen-sions. They were incubated in a shaker (120 rpm) at 37℃ for 24 h. Control tubes without tested samples were essayed simultaneously. All samples were tested in triplicate. The MIC was defined as the lowest concentration preventing visible growth (Vanden and Vlietinck, 1991; Burt, 2004; May et al., 2000; Delaquis et el., 2002).

Statistical analysis

One-way analysis of variance (ANOVA) was performed on the data of all tables and figures, and significant differences among the means were determined by multiple range tests.

RESULTS AND DISCUSSION

Antimicrobial activity

The results of the antimicrobial activity of ethanolic extracts of maize silk determined by diameters of

inhibition zones are presented in Table 1. It indicated that the diameters of inhibition zones varied from 17 to 28 mm and 23 to 32 mm for the extracts of maize silk and andrographis paniculata, respectively. Extract of Andrographis paniculata was used as a standard antibiotic at the concentration of 500 mg/disc and it exhibited higher diameters of inhibition than ethanolic extracts of maize silk. Ethanolic extracts of maize silk revealed interesting antimicrobial activities on three of the five enterobacteria tested. Maximum antimicrobial activities were shown against *Bacillus cereus*, followed by *Candida albicans* and *Staphylococcus aureus*. Ethanolic extracts of maize silk was not able to inhibit *Bacillus coli* and *Pseudomonas aeruginosa*. By comparing the diameters of inhibition zone, ethanolic extracts of maize silk showed less activity than standard antibiotic.

The results of the antimicrobial activity of ethanolic extracts of maize silk determined by MICs are represented in Table 2. The MIC tests of ethanolic extracts of maize silk against 5 microbial species were carried out using the broth dilution technique. The MIC values of ethanolic extracts ranged between 62.5 and 500 mg/ml. These results revealed that ethanolic extracts of maize silk showed maximum activity with MIC 62.5 mg/ml for *B. cereus*. The MICs of the ethanolic extract against *S. aureus* and *C. albicans* were 500 and 250 mg/ml. Ethanolic extracts had very low or no activity, even in the highest concentration (500 mg/ml) MIC against *B. coli* and *P. aeruginosa*.

In this study, ethanolic extracts of maize silk was active against most of the investigated microbial strains. The plant extracts were more active against the Gram-positive microorganisms than against the Gram-negative microorganisms. This is in agreement with previous reports which showed that plant extracts are more active against Gram-positive bacteria than against Gram-negative bacteria (Vlietinck et al., 1995; Rabe, and Van Staden, 1997; Marjorie, 1999). This higher resistance among Gram-negative bacteria may be due to the differences in the cell membrane of these bacterial groups. Indeed, the external membrane of Gram-negative bacteria renders their surfaces highly hydrophilic (Smith-Palmer et al., 1998), whereas the lipophilic ends of the lipoteichoic acids of the cell membrane of Gram-positive bacteria may facilitate penetration by hydrophobic compounds (Ultee et al., 1999; Cox et al., 2000).

B. cereus was the most susceptible bacteria amongst all the bacterial strains investigated in the present work. An interesting antifungal activity against fungi (*C. albicans*) was noted with ethanolic extracts of maize silk. Such result proved that the ethanolic extracts may possess antifungal properties.

The correlation between two different screening methods examined in this study was in accordance, larger inhibition zone related to lower minimum inhibitory concentration. Additionally, with the concentration of ethanolic extract lowering, the growth of microorganism was better. Therefore, it showed that ethanolic extract's antimicrobial activity was associated with the concentration. The effect is more significant with the higher concentration of the extract.

It was found that the ethanolic extract of maize silk contains fatty acids and flavone, with the latter as main component. This is in accordance with what have been reported (Bobryshev, 1962; Hanping et al., 2009; Nozzolillo and Reid, 1992). The flavone is said to exhibit antibacterial activity (Huang, 2008; Wang et al., 2005; Augustin, 1994). Fatty acids also have antibacterial and antifungal properties (Hayes and Berkovitz, 1979), in addition to being selective against Gram-positive organisms by targeting the structure and function of bacterial cell walls and membranes (Kabara et al., 1972). These components may be the contributing factors to the effect demonstrated by the crude extract of maize silk.

Conclusion

Ethanolic extracts of maize silk showed the broad spectrum of antibacterial activity on the tested microorganisms. It exhibited high inhibitory potency against *S. aureus*, *B. subtilis* and *C. albicans*. These derivatives could be potential alternatives to the traditional control of bacteria and fungi at the storage of food. Furthermore, our approach will be focused on isolating and purifying some unknown compounds from the ethanolic extracts of maize silk. Moreover, further studies also shoud be carried out to ascertain the mechanism of these compounds.

REFERENCES

Augustin E (1994). Prenylated is of lavanone from the roots of erythrina sigmoidea, Phyochem., 35: 1074-1075.

Bauer AW, Kirby WMM, Sherries JC, Tuck M (1966). Antibiotic Susceptibility Testing by A Standardized Disc method, Am. J. Clin. Pathol., 45: 493–496.

Bobryshev NE (1962). Corn a medicinal plant, Kukuruza, 9: 59-60.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods: A review, Int. J. Food Microbiol., 94: 223–253.

Cauwelier B, Gordts B, Descheemaecker P, Van Landuyt H (2004). Evaluation of a disk diffusion method with cefoxitin (30 microg) for detection of methicillin-resistant Staphylococcus aureus European J. Clin. Microbiol. Infect. Dis., 23: 389-392.

Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE (2000). The mode of antimicrobial action of the essential oil from *Malaleuca alternifolia* (tea tree oil), J. Appl. Bacteriol., 88: 170–175.

Delaquis PJ, Stanich K, Girard B, Mazza G (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils, Int. J. Food Microbiol., 74: 101–109.

Dzhamalieva IBD (1954). Pharmacological action of an extract of corn silk, Ser. Fizioli Med., 3: 80-83.

Eloff J (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica, 64: 711-713.

Felten A, Grandry B, Lagrange PH, Casin I (2002). Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J. Clin. Microbiol., 40: 2766-2767.

Habtemaram S (1998). Extract of silk (stigma of aea nays) inhibits the

tumour necrosis factor and bacterial lipopolysaccharide induced cell adhesion adhesion and expression, Planta, Medica, 64: 314-318.
Hahn SJ (1973). K' at ollik taehak vihakpu, Nonmunjip. 25: 127-129.
Hanping, Li H, Hou C (2009). Chemical composition of maize silk and the progress in the research of its application, Mod. Agric. Sci. Technol., 18: 17-18.
Hayes ML, Berkovitz BK (1979). The reduction of fissure caries in Wistar rats by a soluble salt of nonanoinic acid, Arch. Oral. Biol., 24: 663-666.
Huang X (2008). Study on the Extraction and Antibacterial Activity in Vitro of Flavonoids from *Syzygium buxifolium* Leaves, J. Anhui Agri. Sci., 36: 5257-5258.
Huys G, D'haene K, Swings J (2002). Influence of the culture medium on antibiotic susceptibility testing of food-associated lactic acid bacteria with the agar overlay disc diffusion method. Lett. Appl. Microbiol., 34: 402-406.
Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP (1972). Fatty acids and derivatives as antimicrobial agents, Antimicrobial. Agents Chemother., 2: 23-28.
Lilian JI, Renxiang TAN (2001). Antimicrobial Activities of Maize Silk Extracts against Food Spoilage and Food-borne Pathogens, Life Sci. Res., 5: 68-72.
Marjorie CM (1999). Plant products as antimicrobial agents, Clin. Microbiol. Rev., 12: 564-582.
Marmonier A (1987). Bacte ´riologie me ´dicale, Techniques usuelles, in: Antibiotiques, Technique de diffusion en ge ´lose me ´thode des disques, SIMEP SA-PARIS, France, pp. 238–244.
May J, Chan CH, King A, Williams L, French GL (2000). Time-kill studies of tea tree oils on clinical isolates, J. Antimicrob. Chemother., 45: 639–643.
Nanjing Medicine College, Chinese Herbal Medicine Pharmacology1 (1980). China, p. 234
Nozzolillo C, Reid L (1992). Anthocyanin pigmentation of maize silk, Bull Liaison Groupe Poly. Phenol., 16: 187-188.
Rabe T, Van Staden J (1997). Antibacterial activity of South African plants used for medicinal purposes, J. Ethnopharmacol. 56: 81-87.
Smith-Palmer A, Stewart J, Fyfe L (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens, Lett. Appl. Microbiol., 26: 118–122.
Ultee A, Kets EPW, Smid EJ (1999). Mechanisms of actions of carvacrol on the food-borne pathogen Bacillus cereus, Appl. Environ. Microbiol., 65: 4606–4610.
Vanden Berghe DA, Vlietinck AJ (1991). Screening methods for antibacterial and antiviral agents from higher plants, in: Dey PM, Harbone JB, Hostettman K (Eds.), Methods in plant biochemistry. Assays for Bioactivity, Academic Press, London, pp. 47–69.
Vlietinck AJ, van Hoof L, Totte J (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties, J. Etyhnopharmacol., 46: 31-47.
Wang Y, Zhang Y, Fang M (2005). Anti-inflammatory and antibacterial effects of total flavones of oldenlandia diffusa willd, Chinese Pharmacol. Bull., 21: 348-350.
Wastl H (1947). Influence of corn sild extract on blood pressure in hypertensive rats, Arch Intern Pharmacodynarmie, 74: 1-2.
Wilkins TD, Thiel T (1973). Modified broth-disk method for testing the antibiotic susceptibility of anaerobic bacteria. Antimicrob. Agents Chemother., 3: 350-351.
Zhou Y, Chen Y, Mao X (2008). Preliminary Study on Antimicrobial Activities of Maize Silk, J. Gannan Med. Uni., 28: 477-488.