Original Article

The potency of ethanolic extract from corn silk as natural antibiotics for acne-related bacteria: A preliminary study

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Abstract

Introduction: Corn silk or known as herb name *stigma maydis* is an important medicinal botanical in many traditional medicines worldwide, including jamu, an Indonesia traditional medicine. The exploration of corn silk to treat acne vulgaris is still lacking, therefore the current research was conducted to analyze the activity of ethanolic extract from corn silk (EECS) against 3 acne-related bacteria, *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. ***Materials and Methods***: The antibacterial activity of EECS at concentration range of 10 to 100% v/v was evaluated using the disk diffusion method. As comparation, distilled water was used as a solvent control, while 1% clindamycin was used as a positive control. ***Results***: Shinoda's test showed that flavonoid was detected in the EECS. The higher concentration of EECS exhibited higher diameter of inhibition zone indicating higher antibacterial activity on *P. acnes*, while the antibacterial activity of *S. epidermidis* was not increased at similar concentrations of EECS. The antibacterial activity of EECS against *S. aureus* decreased at the higher EECS concentration (>70%). ***Conclusion:*** Taken together, EECS is a potential as a bioactive source to inhibit the growth of acne-related bacteria *P. acne, S. epidermidis* and *S. aureus*. Further investigation is needed to explore the corn silk or *stigma maydis* as a medicinal botanical in jamu targeted to treat acne vulgaris.

Keywords: antibacterial; flavonoid; corn silk; *Propionibacterium acne*, *Staphylococcus aureus*; *Staphylococcus epidermidis*

Bangladesh Journal of Medical Science Vol. 21 No. 01 January'22 Page : 84-89
DOI: https://doi.org/10.3329/bjms.v21i1.56331

Introduction

Corn (*Zea mays* L.) or "jagung" is the second staple food after rice, which supports food security in Indonesia. In 2018 the corn production in Indonesia reached 30 million tons.[1] Corn grains are usually utilized as food and feed sources, whereas other parts of the corn plant like corn silk is a biological secondary product of corn cultivation. The utilization of corn silk as a botanical medicine can be an added value of corn to improve the income of farmer in Indonesia.

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Corn silk or known as herb name *stigma maydis* is a female inflorescence of corn in form of fine soft thread 10-20 cm long. Corn silk contained several compounds including flavonoids, tannins, terpenoids, steroids, alkaloids, saponins, carotenoids, anthocyanins.[2–4] Nowadays, corn silk is reported as an important medicinal botanical in many traditional medicines worldwide.[5,6] Bioactive constituents such as flavonoids and terpenoids showed potential antidiuretic[7], hypoglycemia[8], anti-cancer[9], hypocholesterolemia[10], hypopigmentation[11], hypothyroidism[12], antifungal, antiviral and antibacterial activity[3,13–15]. In Indonesian traditional medicines, s*tigma maydis* is used as one of ingredients in jamu claimed to treat urinary related problems, to relieve pain and inflammation of arthritis, to reduce uric acid, and to reduce body fat.[16] The exploration of corn silk to treat acne vulgaris is still lacking, although antibiotic resistance in acne patients raises a concern worldwide.[17]

Acne vulgaris is one the skin-related neglected tropical diseases which showed a highly prevalent inflammatory skin condition in up to 90% of teenager.[18] The disease has been associated with colonization of bacteria *Propionibacterium acnes* in sebaceous areas, *Staphylococcus epidermidis* and *Staphylococcus aureus* in moist human skin areas.[19–22] The aim of the current research was to analyze the antibacterial activity of ethanolic extract from corn silk and compare the activity with standard antibiotic clindamycin in the 3 acne-related bacteria. The results can be used to provide an evidence of the antibacterial activity of the corn silk extract as low-cost herbal antibiotic to treat acne vulgaris.

Materials and methods

Extraction preparation

Fresh corn silks were collected from the corn farmer in Grobogan, Purwodadi, Central Java, Indonesia. The silk was harvested at maturity stage (30 days after silking). The colors of silk were yellow-brown (Fig. 1a). A given amount (2 kg) of fresh corn silks was cleaned with tap water and was thereafter dried in an oven at 50 °C for 5 days to reach a constant weight. The oven-dried corn silk (Fig. 1b) was milled using an electric blender. The fine powder was extracted using 70% ethanol for 1 week at room temperature. The extract solution was filtered through a filter paper (Whatman No. 1) to remove insoluble material. The filtrate obtained was then dried using rotary evaporator and N_2 gas. The ethanolic extract were stored at 0–4 °C until analysis.

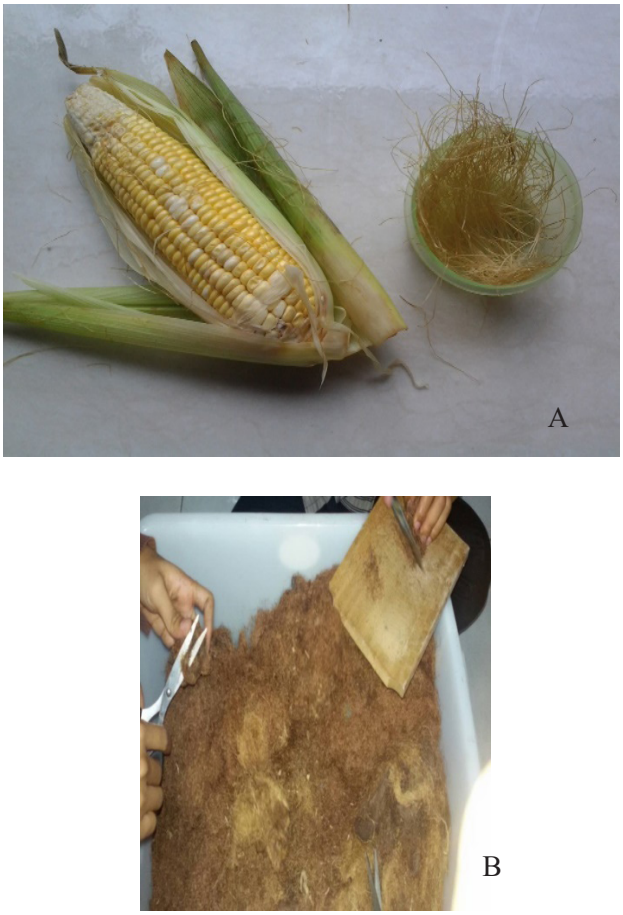

Figure 1. Characteristics of collected fresh corn silk (a) and oven-dried corn silk to be extracted (b)

Qualitative test of flavonoid content

The existence of flavonoid was qualitatively analyzed by Shinoda's test. The extract was reacted with few fragments of magnesium ribbons and concentrated hydrochloric acid gave drop wise. Appearance of magenta color indicates the presence of flavonoid.[2]

Assessment the antibacterial activity of ethanolic extract of corn silk (EECS)

Propionibacterium acnes ATCC 6919 was obtained from Laboratory of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta. *Staphylococcus aureus* and *Staphylococcus epidermidis* was obtained from Laboratory of Microbiology, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia. The bacteria were inoculated in Nutrient broth, incubated at 37°C and used as inoculums. The *Propionibacterium acnes* was incubated in anaerobic condition, while incubation of *Staphylococcus aureus* and *Staphylococcus epidermidis* were in aerobic condition. Inoculate density was adjusted to

a 0.5 McFarland turbidity standard [1.5×10^8 colony-forming units (CFU)/ml].

The antibacterial activity of ethanolic extract of corn silk (EECS) was evaluated on the 3 different strains of bacteria using the disk diffusion method.[23,24] A suspension of 200 ml of EECS was used as a 100% of EECS concentration, while EECS concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% was diluted from the 100% EECS concentration with distilled water. As comparation, distilled water was used as a solvent control (0% EECS), while 1% clindamycin (CDM) was used as a positive control. A volume the 20 µL of each test solution were applied into paper disk of 6.0 mm diameter, then the disk was placed on the agar surface. Upon incubation at 37 °C for 24 hours, diameter of inhibition zone was measured. The absence of a zone inhibition indicates the absence of antibacterial activity. Five replications of these tests were performed for each strain of bacteria evaluated.

Statistical analysis

Data of concentration–inhibition zone data were fitted with an interleaved bars graph as mean values ± SEM. Since the data were not normally distributed, comparisons between multiple groups were analyzed by Kruskal-Wallis test followed by a post hoc Dunn's Multiple Comparison Test. Statistically, $p < 0.05$ was considered significant. All analyses were done in GraphPad Prism software (version 5.00 for Windows, GraphPad software, San Diego, USA).

Results and Discussion

Flavonoid test

Shinoda's test showed that flavonoid was detected in the crude ethanolic extract from corn silk (EECS). EECS was extracted using 70% ethanol as reported by Limmatvapirat et al. (2020) that flavonoids content was higher in corn silk extracted using 40% v/v ethanol than those extracted by water.[2] Table 1 shows flavonoid content in corn silk revealed from the reported studies. Apart from flavonoid, ethanolic extract of corn silk contained flavonoids, tannins, terpenoids, steroids, and phenolic compounds like anthocyanins, *p*-coumaric acid, vanillic acid, quercetin, etc.[2,4,25] The phytochemical constituents of corn silk were influenced by corn hybrid, maturity stage of corn silk, cultivation region, and solvent polarity.[2,25] Therefore, a future research to quantitatively analysis the phytochemicals constituents of corn silk from Indonesia would be relevant to explore it as Indonesia's indigenous botanicals. The assessment will be of interest considering the high number of corn cultivation in Indonesia, and the fact that Indonesia government flags to develop Indonesian modern drug/ *Obat Modern Asli Indonesia* (OMAI).[26]

Table 1. Total flavonoid content (TFC) in corn silk based on hybrid, maturity stage, cultivation region, and solvent polarity

Cultivation region	Hybrid/Type of corn	Maturity stage	Solvent	Total flavonoid content (TFC)	References
Siam Ostrich Farm in Song Phi Nong District, Suphan Buri, Thailand	Pacific 271 hybrid	7 days (silking stage)	40% v/v ethanol	22.46 ± 0.48 mg RE/g extract	[2]
			distilled water	12.59 ± 0.35 mg RE/g extract	
	Zeba SG 17 hybrid		40% v/v ethanol	21.07 ± 0.52 mg RE/g extract	
			distilled water	11.25 ± 0.31 mg RE/g extract	
Vegetable Farm, Khon Kaen University, Khon Kaen, Thailand	five purple waxy corns, three white waxy corns and two super sweet corn	Silking stage	80% methanol	88.5 µg RE/g dried sample	[25]
		Milky stage		85.3 µg RE/g dried sample	
		Maturity stage (30 days after silking)		69.1 µg RE/g dried sample	
Changchun, China	-	fully developed, mature corn silk	95% ethanol	17.9 ± 1.7 µg RE/g dried sample	[27]
			n-butanol fraction	28.6 ± 2.3 µg RE/g dried sample	
Iran	-	-	ethanol-water (1:1) extraction	58.22 ± 1.34 mg/ g dried sample	[4]

Antibacterial activity of ethanolic extract (EECS) from corn silk

To assess the antibacterial activity of EECS, 3 acne-related bacteria *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* were exposed to increasing concentrations (10 to 100%) of EECS for 24 h. Figure 2 depicts the antibacterial activity of EECS against *P. acnes* increased in a concentration-dependent manner. The higher EECS exhibited higher diameter zone indicating higher antibacterial activity on *P. acnes*, while the antibacterial activity of *S. epidermidis* was not increased at similar concentrations of EECS. In the other hand, the antibacterial activity of EECS against *S. aureus* decreased at the higher EECS concentration (>70%).

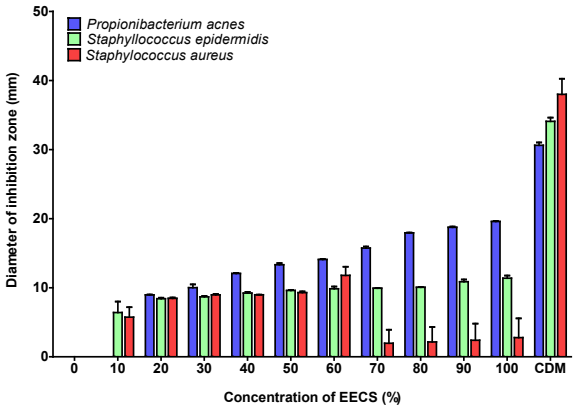

Figure 2. The antibacterial activity of EECS against 3 bacterial species, *Propionibacterium acne, Staphylococcus epidermidis* and *Staphylococcus aureus*

The diameter of inhibition zone at EECS 100% reached 19.6 ±0.09 mm, 11.4 ±0.4 mm and 2.8±2.8 for *P. acnes*, *S. epidermidis* and *S. aureus*, respectively. Standard antibiotics clindamycin was effective against *P. acnes*, *S. epidermidis* and *S. aureus* with inhibition zone of 30.6±0.4 mm, 38.0±2.2 mm, 34.1±0.5 mm respectively (Figure 2). Jannah et al. (2017) reported that minimum inhibitory concentration (MIC) of ethanolic extract from sweet corn silk extract against *S. aureus* was 125 mg/mL, while Minimum Bactericidal Concentration (MBC) was 250 mg/mL.[28] EECS exhibited significantly lower antibacterial activity to the standard antibiotic clindamycin maybe due to the slower diffusion rate of EECS than the antibiotic through the agar. The use of water as EECS solvent can be the one factors which influenced the diffusion rate of EECS in agar as detected previously when using DMSO to dissolve the ethanolic extracts of *Landolphia owerrience* root.[29] Water extracts showed no antibacterial activity, while acetone extracts of *Tragia involucrata* L. displayed the highest MIC against *Escherichia coli*.[30]

The preliminary chemical examination of EECS has shown the presence of flavonoids, which may be responsible of inhibitory of bacterial growth. Maysin and maysin-3′-methyl ether, 2 flavonoid compounds detected in petroleum ether extracts of corn silk showed antibacterial activity on 11 bacteria including *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella sonnei*, *Shigella flexneri*, *Proteus vulgaris*, and *Proteus mirabilis*.[31] The promising antibacterial effect of flavonoid in EECS can be attributed to apoptosis induction via the ROS-mediated mitochondrial pathway in the anticancer activity of corn silk extract against human breast cancer (MCF-7) cells.[9]

Moreover, it has been recognized that flavonoids are very effective antioxidants[2–4,27] which may contributed to the antibacterial activity. Further research, probably involving co-cultured of the 3 bacteria assays, would be needed to analyze the antagonism effect of EECS against *P. acne, S. epidermidis* and *S. aureus* and to determine at which acne-related bacteria the EECS are effective in treatment of acne vulgaris. The mechanism of antagonism *S. epidermidis* to *P. acne* is reported by excretion of succinic acid[32] or polymorphic toxins[33].

Conclusions

Taken together, EECS is a potential as a bioactive source to inhibit the growth of acne-related bacteria *Propionibacterium acne, Staphylococcus epidermidis* and *Staphylococcus aureus*. Further investigation is needed to explore the corn silk or *stigma maydis* as a medicinal botanical in jamu targeted to treat acne vulgaris.

Acknowledgment

This research was funded by *Hibah Program Kreativitas Mahasiswa bidang Penelitian* (PKM-P) from Ministry of Research, Technology and Higher Education Indonesia with contract Number No. 428/F/SA/V/2015 at 6th April 2015.

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Conflicts of interest

No potential conflict of interest was reported by the authors.

References

1. Deptan-RI. Produksi dan Kualitas Jagung Indonesia Tidak Kalah Saing dengan Impor. Published 2019. Accessed December 17, 2020. https://www.pertanian.go.id/home/?show=news&act=view&id=3934
2. Limmatvapirat C, Nateesathittarn C, Dechasathian K, Moohummad T, Chinajitphan P, Limmatvapirat S. Phytochemical analysis of baby corn silk extracts. *J Ayurveda Integr Med*. 2020;**11**(3):344-351. doi:https://doi.org/10.1016/j.jaim.2019.10.005
3. Maksimović Z, Malenčić Đ, Kovačević N. Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. *Bioresour Technol*. 2005;**96**(8):873-877. doi:https://doi.org/10.1016/j.biortech.2004.09.006
4. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant activities of iranian corn silk. *Turkish J Biol*. 2008;**32**(1):43-49. https://www.scopus.com/inward/record.uri?eid=2-s2.0-39849106157&partnerID=40&md5=016178a9335f7b43ce4ca8565d0b5e84
5. Hasanudin K, Hashim P, Mustafa S. Corn silk (*Stigma Maydis*) in healthcare: A phytochemical and pharmacological review. *Molecules*. 2012;**17**:9697-9715. doi:10.3390/molecules17089697
6. Mendoza-López ML, Alvarado-Díaz CS, Pérez-Vega SB, Leal-Ramos MY, Gutiérrez-Méndez N. Compositional and free radical scavenging properties of *Zea mays* female inflorescences (maize silks) from Mexican maize landraces. *CyTA - J Food*. 2018;**16**(1):96-104. doi:10.1080/19476337.2017.1343866
7. Du Dat D, Ham NN, Khac DH, et al. Studies on the individual and combined diuretic effects of four Vietnamese traditional herbal remedies (*Zea mays*, *Imperata cylindrica*, *Plantago major* and *Orthosiphon stamineus*). *J Ethnopharmacol*. 1992;**36**(3):225-231. doi:https://doi.org/10.1016/0378-8741(92)90048-V
8. Zhang X-G, Wei G-X, Wang W-N, Ma G-D, Tang P, Chen X-Q. Effects of Fe-YM1504 on iron deficiency anemia in rats. *Food Funct*. 2016;**7**(7):3184-3192. doi:10.1039/C6FO00423G
9. Al-Oqail MM, Al-Sheddi ES, Farshori NN, et al. Corn Silk (*Zea mays* L.) Induced Apoptosis in Human Breast Cancer (MCF-7) Cells via the ROS-Mediated Mitochondrial Pathway. *Oxid Med Cell Longev*. 2019;2019:9789241. doi:10.1155/2019/9789241
10. Cha JH, Kim SR, Kang HJ, Kim MH, Ha AW, Kim WK. Corn silk extract improves cholesterol metabolism in C57BL/6J mouse fed high-fat diets. *Nutr Res Pr*. 2016;**10**(5):501-506. https://doi.org/10.4162/nrp.2016.10.5.501

11. Choi SY, Lee Y, Kim SS, et al. Inhibitory effect of corn silk on skin pigmentation. *Molecules*. 2014;**19**(3):2808-2818. doi:10.3390/molecules19032808

12. Bhaigyabati T, Ramya J, Usha K. Effect of methanolic extract of sweet corn silk on experimentally induced hyperthyroidism in Swiss albino rats. *Int Res J Pharm*. 2012**;3**(3):241-245.

13. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents*. 2005;**26**(5):343-356. doi:https://doi.org/10.1016/j.ijantimicag.2005.09.002

14. Marmouzi I, Ali K, Harhar H, et al. Functional composition, antibacterial and antioxidative properties of oil and phenolics from Moroccan *Pennisetum glaucum* seeds. *J Saudi Soc Agric Sci*. 2018;**17**(3):229-234. doi:https://doi.org/10.1016/j.jssas.2016.04.007

15. Ismail AM, Mohamed EA, Marghany MR, Abdel-Motaal FF, Abdel-Farid IB, El-Sayed MA. Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of *Faidherbia albida* legume extracts. *J Saudi Soc Agric Sci*. 2016;**15**(2):112-117. doi:https://doi.org/10.1016/j.jssas.2014.06.002

16. Afendi FM, Okada T, Yamazaki M, et al. KNApSAcK Family Databases: Integrated Metabolite–Plant Species Databases for Multifaceted Plant Research. *Plant Cell Physiol*. 2012;**53**(2):e1-e1. doi:10.1093/pcp/pcr165

17. Xu H, Li H. Acne, the Skin Microbiome, and Antibiotic Treatment. *Am J Clin Dermatol*. 2019;**20**(3):335-344. doi:10.1007/s40257-018-00417-3

18. Lee YB, Byun EJ, Kim HS. Potential Role of the Microbiome in Acne: A Comprehensive Review. *J Clin Med*. 2019;**8**(7):987. doi:10.3390/jcm8070987

19. Bek-Thomsen M, Lomholt HB, Kilian M. Acne is Not Associated with Yet-Uncultured Bacteria. *J Clin Microbiol*. 2008;**46**(10):3355 LP - 3360. doi:10.1128/JCM.00799-08

20. Khorvash F, Abdi F, Kashani HH, Naeini FF, Narimani T. *Staphylococcus aureus* in Acne Pathogenesis: A Case-Control Study. *N Am J Med Sci*. 2012;**4**(11):573-576. doi:10.4103/1947-2714.103317

21. Dreno B, Martin R, Moyal D, Henley JB, Khammari A, Seité S. Skin microbiome and acne vulgaris: Staphylococcus, a new actor in acne. *Exp Dermatol*. 2017;**26**(9):798-803. doi:https://doi.org/10.1111/exd.13296

22. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol*. 2011**;9**(4):244-253. doi:10.1038/nrmicro2537

23. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am J Clin Pathol*. 1966;**45**(4_ts):493-496. doi:10.1093/ajcp/45.4_ts.493

24. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*. 2016;**6**(2):71-79. doi:https://doi.org/10.1016/j.jpha.2015.11.005

25. Sarepoua E, Tangwongchai R, Suriharn B, Lertrat K. Influence of variety and harvest maturity on phytochemical content in corn silk. *Food Chem*. 2015;**169**:424-429. doi:https://doi.org/10.1016/j.foodchem.2014.07.136

26. BPOM-RI. Informatorium Obat Modern Asli Indonesia (OMAI) di Masa Pandemi Covid-19. Published 2020. Accessed November 17, 2020. https://online.flipbuilder.com/aeqr/texx/mobile/index.html

27. Liu J, Wang C, Wang Z, Zhang C, Lu S, Liu J. The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (*Zea mays* L.) and related flavone glycosides. *Food Chem*. 2011;**126**(1):261-269. doi:https://doi.org/10.1016/j.foodchem.2010.11.014

28. Jannah A, Rachmawaty DU, Maunatin A. Uji aktifitas antibakteri ekstrak etanol, etil asetat dan petroleum eter rambut jagung manis (*Zea mays ssaccarata* Struct) terhadap bakteri Staphylococcus Aureus dan Escherichia coli. *ALCHEMY; Vol 5, No 4 ALCHEMY*. 2017;**5**(4). doi:10.18860/al.v5i4.4182

29. Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. *J Ethnopharmacol*. 2001;**78**(2):119-127. doi:https://doi.org/10.1016/S0378-8741(01)00307-5

30. Gobalakrishnan R, Kulandaivelu M, Bhuvaneswari R, Kandavel D, Kannan L. Screening of wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrata* L. *J Pharm Anal*. 2013;**3**(6):460-465. doi:https://doi.org/10.1016/j.jpha.2013.07.001

31. Nessa, Fazilatun; Ismailb, Zhar; Mohamedb N. Antimicrobial Activities of Extracts and Flavonoid Glycosides of Corn Silk (*Zea mays* L). *Int J Biotechnol Wellness Ind*. 2012;**1(**2):115-121. doi:10.6000/1927-3037/2012.01.02.02

32. Wang Y, Dai A, Huang S, et al. Propionic acid and its esterified derivative suppress the growth of methicillin-resistant *Staphylococcus aureus* USA300. *Benef Microbes*. 2014;**5**(2):161-168. doi:10.3920/BM2013.0031

33. Christensen GJM, Scholz CFP, Enghild J, et al. Antagonism between *Staphylococcus epidermidis* and *Propionibacterium acnes* and its genomic basis. *BMC Genomics*. 2016**;17**:152. doi:10.1186/s12864-016-2489-5