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 comparison, 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 acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*

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 *sigma 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. Nowadays, corn silk is reported as an important medicinal botanical in many traditional medicines worldwide. Bioactive constituents such as flavonoids and terpenoids showed potential antidiuretic, hypoglycemia, anti-cancer, hypocholesterolemia, hypopigmentation, hypothyroidism, antifungal, antiviral and antibacterial activity. In Indonesian traditional medicines, *sigma 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. The exploration of corn silk to treat acne vulgaris is still lacking, although antibiotic resistance in acne patients raises a concern worldwide.

Acne vulgaris is one the skin-related neglected tropical diseases which showed a highly prevalent inflammatory skin condition in up to 90% of teenager. The disease has been associated with colonization of bacteria *Propionibacterium acnes* in sebaceous areas, *Staphylococcus epidermidis* and *Staphylococcus aureus* in moist human skin areas. 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 N₂ gas. The ethanolic extract were stored at 0–4 °C until analysis.

**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.

**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. 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 comparison, 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. 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. The phytochemical constituents of corn silk were influenced by corn hybrid, maturity stage of corn silk, cultivation region, and solvent polarity. 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).

**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 (siling stage) | 40% v/v ethanol distilled water | 22.46 ± 0.48 mg RE/g extract | 2          |
|                            | Zeba SG 17 hybrid  |                | 40% v/v ethanol distilled water | 12.59 ± 0.35 mg RE/g extract | 2          |
| 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 | 21.07 ± 0.52 mg RE/g extract | 2          |
|                            |                    | Milky stage    | 80% methanol | 11.25 ± 0.31 mg RE/g extract | 2          |
|                            |                    | Maturity stage (30 days after silking) | 95% ethanol n-butanol fraction | 88.5 µg RE/g dried sample | 25         |
|                            |                    |                |                  | 69.1 µg RE/g dried sample | 2          |
|                            |                    |                |                  | 17.9 ± 1.7 µg RE/g dried sample | 27         |
| Changchun, China            |                    |                |                  | 28.6 ± 2.3 µg RE/g dried sample | 4          |
| Iran                       |                    |                |                  | 58.22 ± 1.34 mg/ g dried sample | 4          |

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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%).

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. acnes, 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. acnes is reported by excretion of succinic acid\(^3\)\(^2\) or polymorphic toxins\(^3\)\(^3\). 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.\(^2\)\(^9\) Water extracts showed no antibacterial activity, while acetone extracts of Tragia involucrata L. displayed the highest MIC against Escherichia coli.\(^3\)\(^0\)

Conclusions

Taken together, EECS is a potential as a bioactive source to inhibit the growth of acne-related bacteria Propionibacterium acnes, 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.
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Conflicts of interest

No potential conflict of interest was reported by the authors.

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