

Antibacterial and glucosyltransferase enzyme inhibitory activity of *helmyntostachyszelanica*

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Abstract. *Helminthostachyszeylanica* is a terrestrial, herbaceous, fern-like plant of southeastern Asia and Australia, commonly known as tunjuk-langit. This kind of plant have a medicinal properties such as treatment of malaria, dysentery and can be eaten with betel in the treatment of whooping cough. To evaluate the scientific basis for the use of the plant, the antimicrobial activities of extracts of the stem and leaves were evaluated. The bacteria used in this study is *Streptococcus sobrinus*, a species of gram-positive, that may be associated with human dental caries. The dried powdered plant parts were extracted using methanol and 50% aqueous extract and screened for their antibacterial effects of *Streptococcus sobrinus* using the 96 well-plate microdilution broth method. The inhibitory activities of its related enzyme were also determined. The plant extracts showed variable antibacterial and Glucosyltransferase enzyme inhibitory activity while some extracts could not cause any inhibition. It was shown that 50% ethanolics of *Helminthostachyszeylanica* stem have a potency as anti dental caries agents.

1. Introduction

Plant species are sources of well known and medically useful secondary products. The most important secondary plant metabolites are phenols and flavonoids [1,2]. They have distinctive biological activity as natural antibacterials which exceed many other synthetic ones [3].

One of the most common infectious disease in the world is dental caries. It is well known that mutans streptococci are the major etiological agents in dental caries. *S. sobrinus* species cause important dental caries diseases because it produces acids, and extra cellular glucans and fructans from sucrose, which are critical factors in the expression of virulence by these microorganisms [4]. Therefore the use of antimicrobial agents to control the colonization and accumulation of these cariogenic bacteria on the tooth surface is a logical approach to prevent this common oral disease. *Helminthostachyszeylanica* Linn commonly called “tunjuklangit”. This is a pteridophyte and have high medicinal value and used in various parts of the world. The whole plant parts like roots and leaves use as medicine. Whole parts of this plant are used as various medicinal purposes not only Indonesia. In India, the rhizome of *Helminthostachyszeylanica* is chewed with areca to treat whooping cough or used for curing impotency.[5]. In Malaysia, the rhizome used as antidiarrheal agent [6].

Several studies reported that *Helminthostachyszeylanica* contained flavonoid such as ugonstilbene, ugonin and quercetin glucosides [7,8,9,10,11]. However, a detailed study on the antimicrobial, especially on the oral pathogen causing dental caries of this extract has not been conducted yet. Therefore, this research aims were screened for in vitro evaluation of the antimicrobial of plant species against *Streptococcus sobrinus*. The related enzyme glucosyltransferase inhibitory were also assessed.



2. Materials and Methods

2.1. Plant material

Fresh leaves of *C. sumatrana* were collected from East Kalimantan and were provided by the Department of Forest Product Technology, Mulawarman University. A voucher specimen is kept in our laboratory for future reference.

2.2. Preparation of Extracts

Plant material were extracted with methanol and 50% ethanol. After evaporating the extracts to dryness, the extracts were dissolved with 40% ethanol for measuring the polyphenol content, antimicrobial of *Streptococcus sobrinus* and glucosyltransferase (GTase) inhibitory activity.

2.3. Total Phenolic (TP) and Total Flavonoid (TF) Content

Total polyphenol content was determined using the Folin-Ciocalteu method[12], adapted to a microscale. In a 1.5-ml eppendroff tube, 0.79 ml distilled water, 0.01 ml sample extract appropriately diluted, and 0.05 ml Folin-Ciocalteu reagent was added and mixed. After exactly 1 min, 0.15 ml of sodium carbonate (20g/100ml) was added, and the mixture was mixed and allowed to stand at room temperature, for 120 min. The absorbance was read at 750 nm, and total polyphenol concentration was calculated from a calibration curve, using gallic acid as standard (50-800ug/ml), and expressed as mg/g gallic acid equivalent.

The content was determined as described by other authors [13]based on the total content of quercetin, adapted to a microscale. Briefly, 200 ul of aliquots were mixed with 200 ul of methanol containing 5% anhydrous aluminium chloride ($AlCl_3$) and completed to 1 ml with 40% ethanol. After 30 minutes, the absorbances were read at 420 nm against blank containing 40% ethanol (600 ul) and 5% $AlCl_3$ (200 ul). The percentages of flavanoids were calculated from standard curve of quercetin prepared in methanol, and expressed as mg/g quercetin equivalent

2.4. Antimicrobial assay

The microorganism that used in this study was *Streptococcus sobrinus* 6715, which are proven cariogenic pathogens. Since mutans streptococci are involved in caries formation, we choose *S. sobrinus* as the test microorganism in this study. *S. sobrinus* 6715 were cultured on Todd Hewitt Broth Agar containing 1% sucrose[14]. The medium was autoclaved at 121°C for 15 minutes and incubated at 37°C for 24 hours without shaking. The crude extracts were tested for bacterial inhibitory in sterile 96-well plates. Fifty micro liters of microbial inoculums were added into the wells containing sample and medium to achieve a final volume of 200µl. The concentration of test extract was prepared with the range of 0 - 450µg/ml using two-fold dilution method. Solvent and medium controls were included on each test plate. In order to dissolve the sample extracts 40% ethanol was used in this study, which showed no significantly inhibitory effect on *S. sobrinus* growth.

Furthermore, the bacterial inhibitory effects of isolated compounds were also measured. Triplicate samples were taken for each test concentration. After incubation for 12 hours at 37°C, *S. sobrinus* growth was estimated spectrophotometrically at 590 nm using micro plate reader. Percent growth was calculated with concentration tested [15].

2.5. Inhibition assay for glucosyltransferase enzyme activity

Preparation of GTase and assay for GTase-inhibitory activity followed the methods as previously described [16] :

2.5.1. Preparation of GTase. *Streptococcus sobrinus* 6715 was grown for 16 hr at 37 °C in 4L of Todd Hewitt (TH) broth. After centrifugation of the liquid medium at 5000 rpm for 10 min, the cell were collected and then extracted with 75 ml of 8M urea at 20 °C for 1 hr with stirring. The crude

enzyme solution containing urea was dialyzed against 10 mM potassium phosphate buffer (pH 6) until the urea was removed entirely. One ml of the crude enzyme solution was pipette into microtube and stored in a freezer at -80 °C.

2.5.2. Assay for GTase inhibitory activity. Insoluble glucan synthesized by GTase was measured turbidimetrically. GTase was incubated in 300µl of 0.1 M phosphate buffer (pH 6.0) containing 1% sucrose, 0.1% sodium azide, 0.5% dextran T-10, and in the presence or absence of sample at 37°C for 3 hr. The volume of the crude GTase solution used in the presence in the assay was determined by that giving an absorbance of 1.0 at 590 nm. Inhibition rate is expressed by the following equation: Inhibition rate (%) = 100 x (Ac – As)/Ac. (Ac and As represent absorbance obtained in the control and in the sample dose, respectively.).

3. Result and Discussion

The results for yield, total phenolic and total flavonoid content of *Helminthostachyszeylanica* are presented in Table 1.

Table 1.Yield, Total Phenolic, Total Flavonoids of *Helminthostachyszeylanica*

Part	Solvent	Yield (%)	Phenol (mg GAE/g)	Flavonoid (mg QE/g)
Stem	MeOH	26.62	45.11	31.05
	50% EtOH	21.00	63.44	39.00
Leaves	MeOH	28.21	55.7	36.77
	50% EtOH	20.17	30.34	0.77

Table1 show salist of the extraction yields, total phenolic concent (TPC) and total flavonoid content (TFC) obtained from the two solvent extractions of *Helminthostachyszeylanica* leaves. Themethanol extract of *Helminthostachyszeylanica* gave the greatest yield, whereas the 50% EtOH extract had the lower yield. According to some researchers, aqueous methanol and ethanol have been proven as effective solvents to extract phenolic compounds from different plants [17, 18]. Our results is similar to the report[19, 20]where methanol solvent was most effective in extracting phenolic components from oat bran and young ginger. The phenolics compounds often associated with other biomolecules (polysaccharides, proteins, terpenes, chlorophyll, inorganic compounds etc) and a solvent must be found that it is suitable for extracting them. However, ethanol/water or acetone /water were better solvents compared to ethanol or acetone [21, 22]. These authors also showed that the methanolic extract was better for flavonoid extraction such ascatechin, epicatechinand epigallocatechin. Based on the results of TPC and TFC ,the best extracting solvent for stem was 50% ethanol, while methanol was for leaves. According to the our results, it seems that the yield and efficiency of the phenolics extraction depends on the type and kind of the solvent as well on the flavonoids. For total phenolics and flavonoids extraction from stem parts, 50% ethanol was more efficient compared to methanol, while from leaves parts methanol was more efficient compare to 50% ethanol.

Table 2. Antimicrobial and Glucosyltransferase Inhibitory Activity of *Helminthostachyszeylanica*

Concentration ($\mu\text{g/ml}$)	Antimicrobial (%)					GTase inhibition (%)			
	0	56	112.5	225	450	0	112.5	225	450
Stem-MeOH	0	3.84	6.59	5.50	35.39	0	36.43	46.88	41.61
Stem-50% EtOH	0	3.23	6.81	11.62	11.77	0	28.39	41.25	47.88
Leaves-MeOH	0	0.00	0.00	0.00	5.38	0	37.19	36.46	44.21
Leaves-50% EtOH	0	8.60	11.19	12.36	12.35	0	69.35	75.42	83.01

Table 2 shows the antimicrobial of *Helminthostachyszeylanica*. The antimicrobial activity of *S. sobrinus* from the plants studied here showed that a direct extraction with methanol is effective to extract the antimicrobial component(s) from stem part of *Helminthostachyszeylanica*. Their effects on the growth of *S. sobrinus* were most likely due to the release of chemicals from the crude extracts into the medium when they were mixed. A number of studies have confirmed antibacterial mainly attributable to its content in polyphenol, even though the correlation was not perfectly matched in this study. The different reactions of *S. sobrinus* to the different extracts indicated that each solvent extracted different chemical components of plants. The differences in the antibacterial activity could be because of the presence of specific antibacterial compounds, in addition to the phenolic compounds [23]. Fifty percent ethanol extract of *Helminthostachyszeylanica* crude extracts showed the remarkable value in inhibiting the GTase enzyme at concentration 450 ppm. The mechanism of the toxicity of polyphenols against microbes may be related to inhibition of hydrolytic enzymes (proteases) or other interactions that inactivate microbial adhesins, cell envelope transport proteins and non-specific interactions with carbohydrates [24].

4. Conclusion

The result of the present study suggests that *Helminthostachyszeylanica* can prevent dental caries, since it demonstrated antimicrobial and GTase inhibitory activity against *S. sobrinus*. Therefore, the above active extracts deserve further studies in order to identify and characterize their active components.

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