Antimicrobial and Gtase Inhibitory Activity of Crude Methanol Extracts of Plants from Java and Kalimantan

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Abstract

Some methanol extracts from Java and Kalimantan plants species were evaluated for in vitro anti-*Streptococcus sobrinus* and Glucosyltransferase (GTase) inhibitory activity. The extracts were screened for anti-*S. sobrinus* and its related enzyme activity by broth dilution method. IC_{50} and GTase were determined wherever activity was notice. The resutls revealed that the amount of phenolic and flavonoid compound varies from different plant species and the type of plant material (woody or herb). Out of 24 plant species, 9 species from methanol extracts showed good antibacterial activity in a range at 10 $\mu g\ ml^{-1} \leq IC_{50} \leq$ 100 $\mu g\ ml^{-1}$. The *Intsia palembanica* extract is the most antimicrobial. However, only 4 plants could exhibit GTase activity in a range at 18 $\mu g\ ml^{-1} \leq IC_{50} \leq$ 50 $\mu g\ ml^{-1}$. The most active extract is *Psidium guajava* (15.33 $\mu g\ ml^{-1}$). The result of the present study suggests that some Indonesian plant can prevent dental caries and periodontal diseases, since it demonstrated antimicrobial and GTase inhibitory activity against *S. sobrinus*.

Key words: antimicrobial, crude plant extracts, glucosyltransferase, *Streptococcus sobrinus*

Introduction

Throughout history and across the globe, the plant kingdom has provided a variety of medicines. Plant extracts have been used for centuries as a popular method for treating several health disorders. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases.

Plant species are sources of well known and medically useful secondary products. These compounds called secondary metabolites include: alkaloids, glycosides, essential oils and other organic constituents. These constituents are usually produced in different parts of the plants like the root, leaves, fruits and seeds and then translocated to other parts of plant for storage.

Phenolics are secondary metabolites in plants. They comprise a large group of biologically active ingredients (above 8000 compounds) from simple phenol molecules to polymeric structures with 30.000 Da. Phenolic possess a wide spectrum of biological activities such as antioxidant, antimutagenic, anticarcinoge nic, as well as ability to modify the gene

expression. Numerous epidemicological studies confirm significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk Arung *et al.* (2008).

Plants with antimicrobial activity are also known to be numerous. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants (Nita *et al.* 2002; Ates & Erdo. 2003; Bhattacharjee *et al.* 2006; Parekh & Chanda 2006, 2007). In the past two decades, antibacterial properties of various plants and plant parts like root, stem, leaves, seeds, and flowers have been well documented for some of the medicinal plants.

Different parts of the plant have shown various chemical components when analyzed by different methods. Several studies have indicated that contains substances that possess dental plaque-inhibiting and antimicrobial properties against oral microbes. *Streptococcus sobrinus* species cause important dental caries diseases. In countries where these diseases occur, medicinal plants have traditionally been used for centuries, and people found some relief without scientific data that could justify and support their use. Nowadays, investigations are carried out on extracts from medicinal plants to prove their efficacy.

Based on the evidence on the high biological activity of phenolic compound and not much data for their content in plants, this study aimed to focus on determination of total phenolic and total flavonoid content Thus the present study deals with the in vitro evaluation of the antimicrobial of plant species against *S. sobrinus*. The related enzyme glucosyltransferase of *S. sobrinus* inhibitory were also assessed.

Materials and Methods

Plant materials

Twenty eight plants were analyzed for the determination of total phenol and total flavonoid content. The study covered 40 plant materials including of woods, stems, barks, leaves, fruits, flowers and lichen.

Sample preparation

The dried samples were extracted with methanol. After evaporating the extracts to dryness, the extracts were dissolved with 40% ethanol for measuring the polyphenol content、antimicrobial of *S. sobrinus* and glucosyltransferase (GTase) inhibitory activity.

Total phenolic assay

Total polyphenol content was determined using the Folin-Cialteu method, 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-Ciealteu reagent was added and mixed. After exactly 1 min, 0.15 ml of sodium carbonate (0.5 g ml^{-1}) 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 (50-800 µg ml^{-1}).

Total flavonoid assay

The content was determined as described by other authors based on the total content of quercetin, adapted to a microscale. Briefly, 200 µl of aliquots were mixed with 200 µl 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 µl) and 5% $AlCl_3$ (200 µl). The percentages of flavanoids were calculated from standard curve of quercetin prepared in methanol, and expressed as mg/g quercetin equivalent.

Antimicrobial activity assay

Microorganism that used in this study was *S. 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 Hewwit Broth Agar containing 1% sucrose. The medium was autoclaved at 121°C for 15 minutes and incubated at 37°C for 24 h without shaking.

The crude extracts were tested for bacterial inhibitory in sterile 96-well plates. Fifty micro litters of microbial inoculums were added into the wells containing sample and medium to achieve a final volume of 200 µl and final sample concentration starting with 225 µg ml^{-1}. The concentration of test extract was prepared with the range of 3.5–225 µg ml^{-1} 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 to afford the concentration that inhibits 50% of growth (IC_{50}).

Minimum inhibitory concentration (MIC) values were measured with a modified micro dilution broth method described by Cai & Wu (1996). The MIC was defined as the minimum concentration of test sample that inhibits the visible growth of a microorganism after overnight incubation.

Preparation of GTase

S. sobrinus 6715 was grown for 16 hr at 37°C in 4L of Todd Hewwit (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 h 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.

Assay for GTase inhibitory activity

Insoluble glucan synthesized by GTase was measured tubidimetrically. GTase was incubated in 300 µl 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 h. 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.) IC_{50} means the sample concentration (µg ml^{-1}) giving 50% inhibition of GTase.

Results and Discussion

Total phenol and total flavonoid

The comparative evaluation of the polyphenolic composition of the plants

was based on three representative indices; the total phenol and total flavonoid contents. The plants studied gave methanol extracts with yields ranging from 2.5-33.1% (data not shown). The results for total phenolic and total flavonoid content of 40 plant materials are presented in Fig. 1. The increasing interest in powerful biological activity of plant phenolic and flavonoids outlined the necessity of determining their contents in some Indonesian woody and herb plants. The study comprised 24 plants. Total phenolic and total flavonoid content ranged from 20.23-90.21 mg g^{-1} GAE, and 5.21-38.57 mg g^{-1} QE extract, respectively.

The result showed the highest total phenolic content of *Guazuma ulmifolia* (90.21 mg g^{-1}) extracts followed by *Intsia palembanica* (79.28 mg g^{-1} GAE) *Swietenia macrophylla (fruit)* (74.63 mg g^{-1}), *Xylocarpus granatum* (72.99 mg g^{-1} GAE) and *Gynura divaricata leaves* (68.07 mg g^{-1} GAE).

The data clearly outline the richest flavonoid sources is *I. palembanica* (38.57 mg g^{-1} QE) *Helminthostachys zeylanica* stem (65.06 mg g^{-1} QE), followed by extracts of *H. zeylanica* flower (42.92 mg g^{-1} QE) *Gynura divaricata (leaves)* and *Hibiscus tilliaceus* (49.75 mg g^{-1} QE).

The amount of phenolic and flavonoid compound varies from different plant species. The composition of the extracts and the yield of phenol and flavonoid were found to depend on the plant species, the type of plant material, the type of plant material (woody or herb), and the polarity of the extracting solvent. In the case of the polar solvents, higher yields of phenol was obtained from the wood than from the herb plant material The presented data for total phenolic and total flavanoid content are a bassis assement of the woody and herb plants and will enrich the plants composition database. It will undoubtedly be used with increasing frequency in the future because of the chemicals extracted from plant parts i.e. (flavonoids) and (phenolic compounds) are found to be useful in the pharmaceutical and food industries.

Antimicrobial activity against *S. sobrinus*

The uses of plant extracts with medicinal properties represent a concrete alternative for the treatment of different pathological stages. The antimicrobial properties have been reported in a wide range of plant extracts and natural products attempting to contribute with the development of new drug, which can generate a significant improvement in managing several kinds of health disorders (Alviano *et al.* 2004).

It is well known that mutans streptococci are the major etiological agents in dental caries; *S. sobrinus* produces acids, and extra cellular glucans and fructans from sucrose, which are critical factors in the expression of virulence by these microorganisms (Hamada & Slade 1980).

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.

The *in vitro* antimicrobial and GTase inhibitory activities in different solvent extract are presented in Table 1. In this investigation most of the sample in this study showed inhibition of the growth of *S. sobrinus* and glucosyltransferase activity.

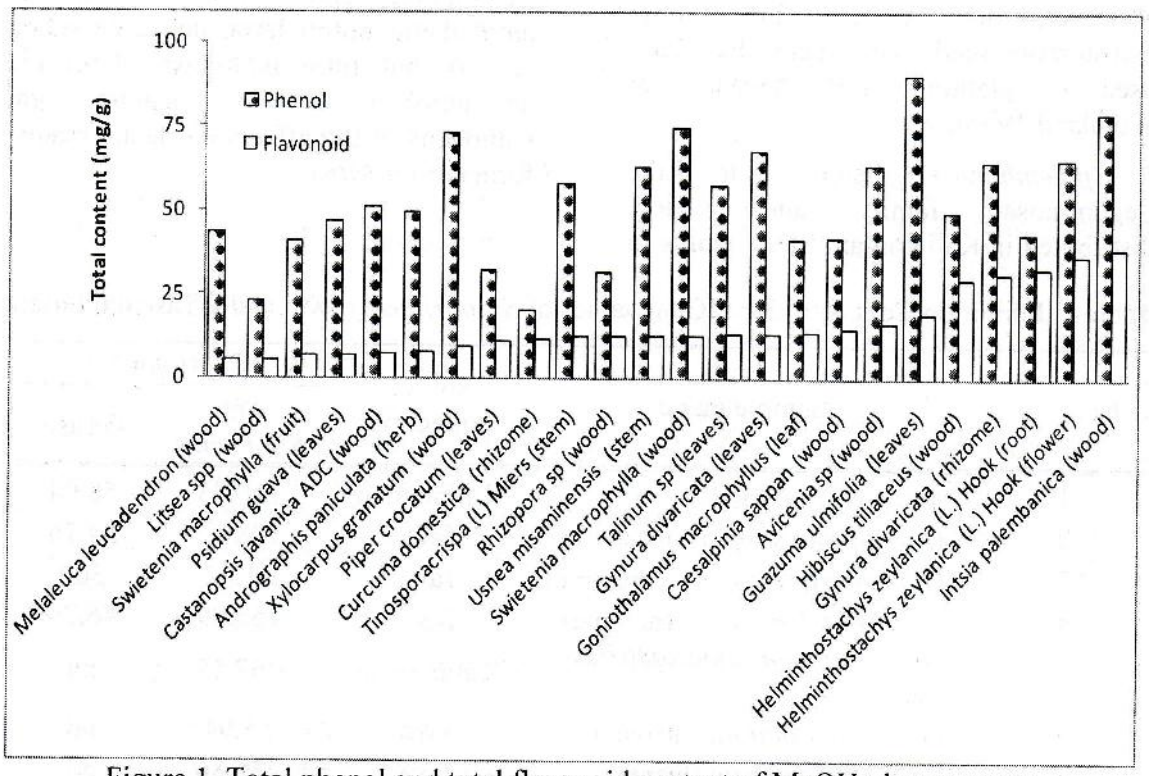

Figure 1 Total phenol and total flavonoid content of MeOH plant extracts

According to the findings of this study, the crude extracts inhibited, reduced or enhanced the growth of the test microorganisms. Most of the extracts exerted their MIC value only at the highest concentrations used. Regarding inhibition, the most potent extract was the woody plants extract. 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. The different reactions of *S. sobrinus* to the different extracts indicated that each solvent extracted different chemical components of plants.

Table 1 demonstrates the inhibitory concentration of plant extracts against *S. sobrinus* growth. Out of 24 plant species, 9 species from methanol extracts showed good activity in a range at 10 $\mu g\ ml^{-1} \leq IC_{50} \leq 100\ \mu g\ ml^{-1}$. They were included *I. palembanica, S. macrophylla* (wood) and *G. ulmifolia.*

I. palembanica shows promising as antimicrobial agent against *S. sobrinus. I. palembanica* wood is also called merbau in Kalimantan. Merbau is used locally for heavy constructional work, sleepers, axe and tool handles and furniture. The wood has a moderate to high resistance to wear, and is therefore suitable for flooring. It is also used for joinery, furniture and paneling. If used for exterior joinery it will need to be sealed to avoid any stain leaching out. The texture is course but even, and the grain is interlocked and often wavy. Sulphur-yellow and dark coloured deposits are characteristic. These can be extracted for dye. Merbau has good strength properties. *I. palembanica* is a slow-growing species; growth data from sample plots recorded an annual diameter increment of 0.6 cm only.

However, due to its fine timber and storable seed, the species has been used in planting trials (Appanah & Weinland 1993).

I. palembanica belongs to the Leguminosae family and widely distributed in Kalimantan. Many plants in Leguminosae show bioactivities such as antidiabetic, antimalaria, and antioxidant activity but little is known about the antimicrobial activity against oral pathogens or the effects on dental plaque formation *in vitro*.

Table 1 Inhibition Concentration (IC_{50}) values of *S. sobrinus* growth and GTase inhibition

No.	Sample name	Source collection	IC_{50} ($\mu g\ ml^{-1}$)	
			Anti *S.sobrinus*	GTase
1	*Avicenia sp* (wood)	Kalimantan	110.73	58.04
2	*Caesalpinia sappan* (wood)	Java	108.02	54.76
3	*Castanopsis javanica* (rhizome)	Java	71.42	300
4	*Curcuma domestica* (rhizome)	Java	155.09	46.76
5	*Goniothalamus macrophyllus* (leaves)	Kalimantan	267.55	nd
6	*Guazuma ulmifolia* (leaves)	Java	55.94	nd
7	*Gynura divaricata* (leaves)	Java	235.86	nd
8	*Gynura divaricata* (rhizome)	Java	346.77	nd
9	*Helminthostachys zeylanica* (flower)	Java	257.82	204.43
10	*Helminthostachys zeylanica* (root)	Java	Nd	102.45
11	*Hibiscus tiliaceus* (wood)	Kalimantan	141.42	nd
12	*Intsia palembanica* (wood)	Kalimantan	28.12	27.7
13	*Litsea spp* (wood)	Kalimantan	333.26	nd
14	*Melaleuca leucadendron* (wood)	Kalimantan	245.26	56.25
15	*Piper crocatum* (leaves)	Java	65.04	nd
16	*Psidium guajava* (leaves)	Java	50.25	18.75
17	*Rhizopora sp* (wood)	Kalimantan	150.67	56.25
18	*Swietenia macrophylla* (fruit)	Java	28.125	56.25
19	*Swietenia macrophylla* (wood)	Kalimantan	Nd	18.75
20	*Talinum sp* (leaves)	Java	45.45	nd
21	*Tinosporacrispa (L) Miers* (stem)	Java	56.25	nd
22	*Usnea misaminensis* (lichene)	Java	56.25	nd
23	*Xylocarpus granatum* (wood)	Kalimantan	159.99	184.23

In our previous study, isolated compound taxifolin and flavanonol rhamnoside as antimicrobial were found from 50% ethanol extracts of *Koompassia malaccensis* and have good activity for antimicrobial against *S. sobrinus* (Kuspradini *et al.* 2009). *K. malaccensis* is also a plant species in the same group of Leguminosae family.

GTase inhibitory activity

In each assay, the IC_{50} value for each sample was derived by the concentration-response curve. IC_{50} of Gtase activity were ranged at 18.75–204.43 $\mu g\ ml^{-1}$, respectively. The result of GTase activity in Table 1. reveal that only 4 plants could exhibit GTase activity in the range.of 18 $\mu g\ ml^{-1} \leq IC_{50} \leq 50\ \mu g\ ml^{-1}$). They were *Curcuma domestica, I. palembanica, S. macrophylla* (fruit) and *Psidium guajava.* The most active extracts is *P. guajava* (15.33 $\mu g\ ml^{-1}$).

P. guajava and *C. domestica* studied here shown a promising activity as GTase inhibitor. In our previous study, it was found that the compounds of the *C. domestica* in hexane fractions indicated as sesquiterpene (Germacrone) (Kuspradini *et al.* 2008). It has been reported that sesquiterpene compound has activity against GTase (Koo *et al.* 2002).

P. guajava herb extract as a most potent inhibitor on hydroxyapatite-adsorbed glucosyltransferase and *S. mutans* have been studied in Thailand (Benjavongkulchai *et al.* 2006). The glucosyltransferase inhibitory activity of *S. sobrinus* from the plants studied here showed that a direct extraction with methanol solvent is effective to extract the GTase inhibitor compound(s).

Conclusion

The amount of phenolic and flavonoid compound varies from different plant species and the type of plant material (woody or herb).

Methanol extracts proved to exert in vitro antibacterial activity against *S. sobrinus* and GTase inhibitory activity. The most active extract for antimicrobial was that from extract of *I. palembanica.*

Again from *I. palembanica* extracts could exhibit the IC_{50} of GTase at low concentration. *P. guajava* is most potent for inhibing activity of GTase followed by *C. domestica.* However, it is possible that extracts active between 5 and 100 $\mu g\ ml^{-1}$ could contain small amounts of very active components, which could be isolated and/or concentrated using simple procedures, or even obtained from other plants known to produce it in larger amounts.

The result of the present study suggests that some Indonesian plant can prevent dental caries and periodontal diseases, since it demonstrated antimicrobial and GTase inhibitory activity against oral bacteria (*S. sobrinus).*

Finally, the variation in the chemical constituents in plants will make some more medicinal than others. It also improves the identification of plant species. And note that the function of medicinal plant is depends on its active ingredients. Therefore, the above active extracts deserve further studies in order to identify and characterize their active components.

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