Inhibitory Effect of Temulawak (*Curcuma xanthorrhiza*) and Kunyit (*Curcuma domestica*) on Glucosyl Transferase Activity

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ABSTRACT

The present study evaluated the chemical composition and glucosyltransferasse activity of the extracts and fractions of *Curcuma xanthorrhiza* (Temulawak) and *Curcuma domestica*. (Kunyit). Methanol, 50% ethanolic extract, hexane, ethyl acetate and water fractions of *Curcuma xanthorrhiza* and *Curcuma domestica* were chemically identified by chromatographic methods and tested on glucosyltransferase of *Streptococcus sobrinus* activity. Glucosyltransferase activity was tested by incubating a crude enzyme preparation with sucrose and determining the amount of water-insoluble glucan formed. Some compounds were identified in both *Curcuma* extracts, including -curcumene, cynammyl tiglate, bicycle [3.3.1] non-ene-9-ol and germacrone (*Curcuma domestica*) and camphor, zingiberene, -curcumene, -farnese, -cedrene, -elemenone, and xanthorrhizol (*Curcuma xanthorrhizol*). Both Temulawak and Kunyit extracts could inhibited the formation of water-insoluble glucan in all fractions, except Temulawak water fraction. The 50% inhibitory doses of Temulawak extracts against the glucosyltransferase of *S. sobrinus* were ranged at 37.27 >250 g/ml. The concentration of IC_{50} of Kunyit extracts were ranged at 13.78 > 250 g/ml. Our results suggest that Temulawak and Kunyit extract may prove effective for the inhibition of glucosyltransferase. Thus, these extracts may be of great interest for future studies about treatment of oral diseases,considering their potent inhibitory activity on glucosyltransferase of *S.sobrinus*.

Keyword : Glucosyltransfrase, *Curcuma xanthorrhiza*, *Curcuma domestica*

INTRODUCTION

Dental plaque when allowed to accumulate may lead to caries formation and discomfort due to the inflammation of the gingival area. Both conditions are direct consequences of poor oral hygiene measures of an individual. Glucosyltransferase (GTase; sucrose 6-glucosyltransferase, EC 2.4.1.5) is produced mainly by *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*), which are major causative agents of dental caries. Mutans strepcococci grow inside the oral cavity and transfer sucrose to insoluble adhesive glucans. Insoluble adhesive glucans, in turn, attach to the surface of the teeth while oral bacteria produce organic acids that break down the enamel of the tooth surface. This process is recognized as the primary stage of cavity development. Therefore, *mutans streptococci and GTase should be the* primary targets in the pathogenesis of dental caries[1].

There are several approaches to preventing the formation of tooth cavities, such as inhibiting the *growth of mutans streptococci, dismantling and dissolution of insoluble glucans, and* suppression of glucan formation by GTase inhibition [2,3,4].

Using GTase inhibitors is considered to be a useful means of preventing glucan formation without disturbing the balance of helpful oral bacteria. Recent studies have demonstrated the GTase inhibitory activity of natural sources such as propolis and oolong tea polyphenols [5,6]. The initiation or progress of these common plaque-related diseases is very much affected by the level of oral hygiene of the individual. Effective plaque removal procedures are expected to prevent the development of these diseases

Curcuma xanthorrhiza known as temu lawak and *Curcuma domestica* or known as turmeric, has been traditionally used in Indonesia for food and medicinal purposes. As little attention has been focused on the role of these species of Curcuma in glucosyltransferase activity. In the present study, we demonstrate the inhibitory activities of extracts from *Curcuma xanthorrhiza (Temulawak) and Curcuma domestica* (Kunyit) on glucosyltransferase of *S. sobrinus* activity.

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MATERIALS AND METHODS

Extraction and fractionation of samples

The air-dried powdered of Temulawak and Kunyit was extracted with methanol and 50% ethanol. The hexane fractions were prepared using the methanol extracts of Temulawak and Kunyit using n- hexane. The fractions were concentrated using rotary evaporator obtaining the hexane fractions: H-*Cx*) and H-*Cd*. The residues of the extractions described above were used to prepare the ethyl acetate fraction. The fractions of ethyl acetate: EA-*Cx* and EA-*Cd*. The residues of the ethyl acetate were used to get the water fraction : W-*Cx* and W-*Cd* .The methanol and hydroethanolic extracts (ME-*Cx* and ME-*Cd*, and EE-*Cx* and EE-*Cd*), hexane fractions (H-*Ml* and H-*Mg*) , ethyl acetate fractions (EA-*Cx* and EA-*Cd*) and the water fractions (W-*Cx* and W-*Cd*) were analyzed for inhibitory activity on glucosyltransferase. All extracts and fractions were analyzed by high pressure liquid chromatography.

HPLC method

Obtaining extracts and fractions were analyzed by high performance liquid chromatography (HPLC) with reversed phase column monitored at 280 nm. The solvent system used was as follows: a gradient program for 60 min from 5 to 100% solvent B (100% methanol) in solvent A (0.01% trifluoroacetic acid in H_2O) at a flow rate 10 mL/min.

GC for identification of component

The hexane fraction of *Curcuma domestica* was analyzed by gas chromatography coupled with a mass detector. GC-MS system (Shimadzu GCMS-QP 5050A) equipped with a J&W scientific 0.25 mm x 30 (L) m i.d. DB-5 MS column was used.

The electron impact technique (70eV) was used. The carrier gas was helium (42ml/min). The injector temperature was 250°C, and that of detector was 250°C. The GCMS peaks were identified by computer searches in commercial reference libraries. All of the extracts and fractions were dissolved in methanol (v/v) just prior to performance of the assays.

Determination of total phenolic compounds

The total phenolic contents of Temulawak and Kunyit extracts were determined according to the FolinCiocalteu method. Briefly, FolinCiocalteu phenol reagent was added to the reconstituted samples and held for 3 min. Then 1 ml of 10% (w/v) sodium carbonate solution were added and the mixture allowed to stand at room temperature for 10 min. The absorbance at 760 nm was measured. The total phenolic content was calculated by a standard curve prepared with gallic acid and expressed as milligrammes of gallic acid equivalents (GAE) per gramme of solid of extract.

Preparation of Gtase

Streptococcus 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 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.

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Assay for GTase inhibitory activity

Insoluble glucan synthesized by GTase was measured tubidimetrically. GTase was incubated in 300l 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. 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.) IC50 means the sample concentration (g/ml) giving 50% inhibition of Gtase.

RESULT AND DISCUSSION

In this investigation *Curcuma xanthorrhiza* and *Curcuma domestica* showed inhibition of the glucosyltransferase activity. Figure 1 shows the effect of *Cx* extracts and fractions on the glucosyltransferase activity. It was found that all the extracts and fractions *Cd* had inhibitory effects on the glucosyltransferase activity, except water fraction. EA-*Cx* fraction exhibiting a stronger activity than other extracts on *Curcuma xanthorrhiza*.

Figure 2 shows the effect of *Cd* extracts on the glucosyltransferase activity. All the extracts and fractions *Cd* had inhibitory effects on the glucosyltransferase activity. EE-*Cd* extract exhibiting a stronger activity compared to other extracts and fractions on both *Curcuma xanthorrhiza* and *Curcuma domestica*. All of the *Cd* fractions also exhibited a strong activity than *Cx* fractions, except W-*Cd* fractions. The inhibitory effect increased with increasing extract concentration.

It was observed that the ability of glucosyltransferase was affected by the presence of crude *Curcuma xanthorrhiza* and *Curcuma domestica extracts*, the ME-*Cd* and EE-*Cd* exerting stronger activity than the ME-*Cx* and EE-*Cx* extract. The receptors on glucosytransferase may be modified by component in the crude extract, leading to reduction of GTase activity.

The GTase activity was reduced to 56.2%, 67% and 61% at 31.2 g/ml EE-*Cd*, H-Cd, and EA-Cd extract,respectively and a higher concentration of the ME-*Cd* (62.5 g/ml) was required for a similar reduction (60%).

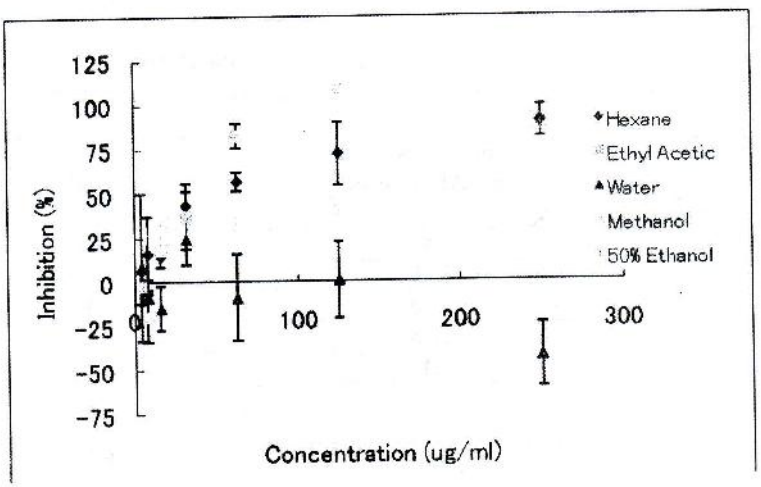

Fig. 1. Effect of *Curcuma xanthorrhiza* extracts and fractions on the glucosyltransferase Activity

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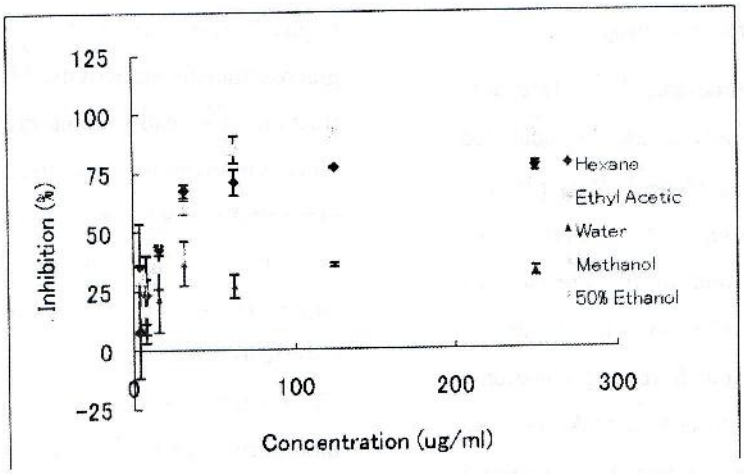

Fig. 2. Effect of *Curcuma domestica* extracts and fractions on the glucosyltransferase activity

It implies that the active component(s) are present in *Curcuma domestica* extracts are being more potent in 50% ethanol extract, hexane and ethyl acetate fractions than in the methanol extract.

Table 1 shows that total polyphenols of *Cx* and *Cd* extracts and fractions were ranged at 13.78 - > 250 mg GAE/g extract. These polyphenols contribute to the activity of *Curcuma xanthorrhiza* and *Curcuma domestica* on oral diseases. It has been reported that polyphenol compound could inhibited the glucosyltransferase activity[7,8] and antimicrobial on oral diseases[9].

The present sudy demonstrate that *Curcuma domestica* extracts and fractions, as compared with *Curcuma xanthorrhiza* extracts and fractions, exhibits a higher inhibitory effect on glucosyltransferase, despite a lower content of phenolic compounds.

The chromatographic results of HPLC of extracts and fractions of *Curcuma xanthorrhiza* (*Cx*) and *Curcuma domestica* (*Cd*) are shown in Fig. 3.

The EE-*Cd* has compounds that of in H-*Cd* and EA-*Cd* fractions. Both H-*Cd* and EA-*Cd* fractions has low concentration to inhibit glucosyltransferase on Ic_{50}.

Table 1. Contents of total phenolics and IC_{50} of Gtase activity of *Curcuma xanthorrhiza* and *Curcuma domestica* extracts

	Total phenolics extracts (mg GAE/g extract)	IC_{50} (µg/ml)
Curcuma xanthorrhiza		
EE-*Cx*	3.83	148.39
ME-*Cxd*	2.68	> 250
H-*Cx*	3.20	44.15
EA- *Cx*	2.98	37.27
W-*Cx*	1.49	n.d
Curcuma domestica		
EE-*Cd*	4.75	13.78
ME-*Cd*	3.14	46.76
H-*Cd*	2.15	20.21
EA- *Cd*	2.23	24.43
W-*Cd*	2.01	> 250

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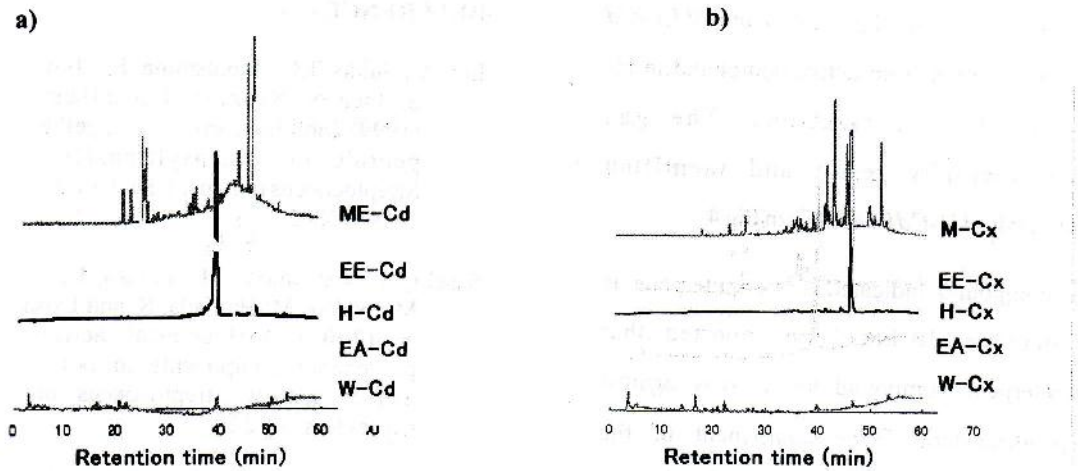

Figure 3. HPLC chromatogram of extracts and fractions on a) Curcuma domestica and b) Curcuma domestica

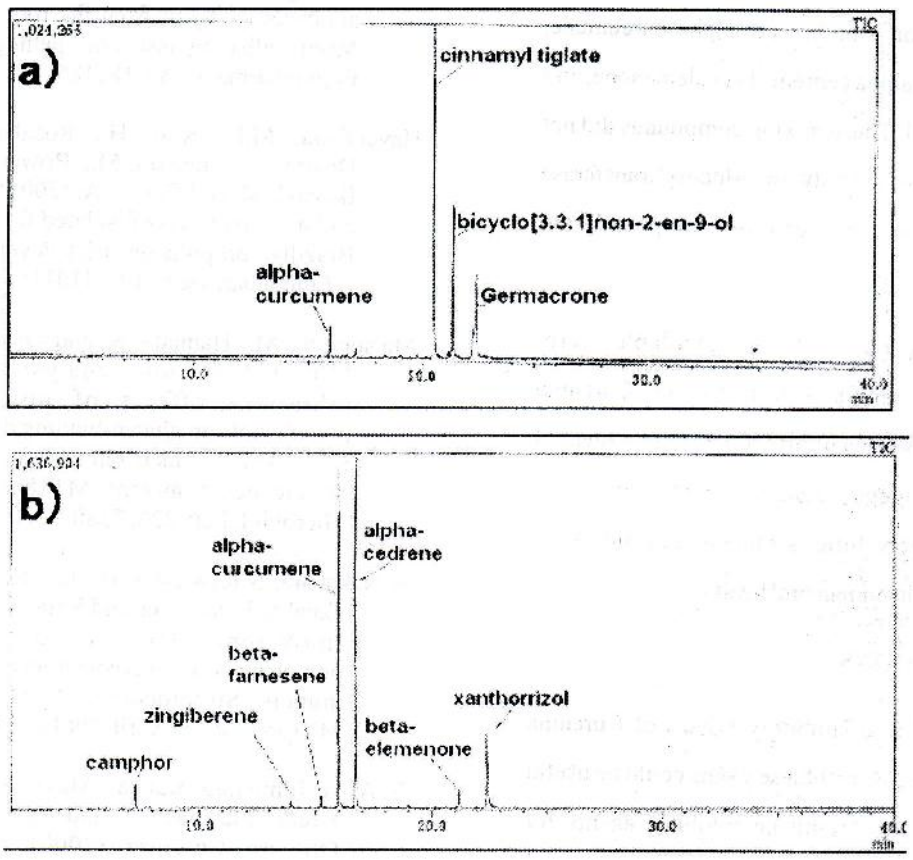

Figure 4. GC chromatogram of a) *Curcuma domestica* and Curcuma xanthorrhiza hexane fraction

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It implies that the inhibitor compound of EE-*Cd* was combination from active compound in H-*Cd* and EA-*Cd* fractions. The gas chromatography result and identified compounds of H-*Cd* fraction is in Fig 4.

One compound indicated as sesquiterpene is Germacrone. It has been reported that sesquiterpene compound has activity against glucosyltransferase[10].The component of the essential oil in hexane soluble fraction of *Curcuma domestica* was identified as - curcumene. Curcumin is known for its anti-tumor [11,12] and antioxidant properties[13]. Curcuma xanthorrhiza compound from hexane fraction were camphor, zingiberene, alpha curcumene, beta farnese, alpha cedrene, beta elemenone, and xanthorrhizol. These mixing compounds did not exhibit strong activity on glucosyltransferase than that of *Curcuma domestica* compound from hexane fractions.

Curcuma species are an available herb, inexpensive and shown non-toxic. Curcuma rhizome have been investigated as potential theurapic agents against several diseases. However, very little is known regarding their application in human oral health.

CONCLUSIONS

The remarkable inhibitory effects of Curcuma species, suggest that these plants could be useful source for the promising inhibitor agents for preventing dental plaque through the suppressing activity of glucosyltransferase of *S. Sobrinus*.

REFERENCES

Eto, A., Saido, T.C., Fukushima, K., Tomiokai, S., Imai, S., Nisizawa, T. and Hanada, N. (1999) Inhibitory effect of a selfderived peptide on glucosyltransferase of Streptococcus mutans. J. Biol. Chem. 274, 1579715802.

Sasaki, H., Matsumoto, M., Tanaka, T., Maeda, M., Nakai, M.,Hamada, S. and Ooshima, T. (2004) Antibacterial activity of polyphenol components in oolong tea extract against Streptococcus mutans. Caries Res. 38, 28.

Park, Y.K., Koo, M.H., Abreu, J.A.S., Kegaki, M.I., Cury, J.A.and Rosalen, P.L. (1998) Antimicrobial activity of propolis on oral microorganisms. Curr. Microbiol. 36, 2428.

Park, K.M., You, J.S., Lee, H.Y., Baek, N.I. and Hwang, J.K. (2003) Kuwanon G: an antibacterial agent from the root bark of Morus alba against oral pathogens. J. Ethnopharmacol. 84, 181185.

Hayacibara, M.F., Koo, H., Rosalen, P.L., Duarte, S., Franco,E.M., Bowen, W.H., Ikegaki, M. and Cury, J.A. (2005) In vitro and in vivo effects of isolated fractions of Brazilian propolis oncaries development. J. Ethnopharmacol. 101, 110115.

Matsumoto, M., Hamada, S. and Ooshima, T. (2003) Molecular analysis of the inhibitory effects of oolong tea polyphenols on glucan-binding domain of recombinant glucosyltransferases from Streptococcus mutans MT1848. FEMS Microbiol. Lett. 228, 7380.

K Nakahara, S Kawabata, H Ono, K Ogura, T Tanaka, T Ooshima, and S Hamada (1993) Inhibitory effect of oolong tea polyphenols on glycosyltransferases of mutans Streptococci. Appl Environ Microbiol. 1993 April; 59(4): 968973

Y. Akio, Tomomasa Kanda, Masayuki Tanabe, Fumio Matsudaira and Jose´ Geraldo Oliveira Cordeiro (2000) Inhibitory Effects of Apple Polyphenols and Related Compounds on Cariogenic Factors of Mutans Streptococci. *J. Agric. Food Chem48,* 566.65671

ISBN No. 978-979-25-

Harumu Katsura, Ryo-Ichi Tsukiyama, Akiko Suzuki and Makio Kobayashi (2001) In Vitro Antimicrobial Activities of Bakuchiol against Oral Microorganisms. Antimicrobial Agents and Chemotherapy, p. 30093013 Vol. 45, No. 11

Hyun Koo, Pedro L Rosalen, Jaime A Cury, YK Park, W.H. Bowen. 2002. Effects of compounds found in propolis on Streptococcus mutans growth and glucosyltransferase activity. Antimicrob Agents Chemother. 2002 May ;46 (5):1302-9

Aggarwal, BB.; Shishodia S. (May 2006). "Molecular targets of dietary agents for prevention and therapy of cancer". *Biochemical Pharmacology* **71** (10): 1397421. Elsevier.

Choi, Hyunsung; et al. (July 2006). "Curcumin Inhibits Hypoxia-Inducible Factor-1 by Degrading Aryl Hydrocarbon Receptor Nuclear Translocator: A Mechanism of Tumor Growth Inhibition". *Molecular Pharmacology* **70**: 166471. American Society for Pharmacology and Experimental Therapeutics.

Kawanishi, S; Oikawa, S; Murata, M; (2005). "Evaluation for safety of antioxidant chemopreventive agents". *Antioxidants & Redox Signaling* 7 (11-12): 172839

ISBN No. 978-979-25-1207-6