VITEX PUBESCENS AND *TERMINALIA CATAPPA* PLANT SPECIES FROM KALIMANTAN AS AN ANTI DENTAL CARIES AGENT

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Abstract: Methanol and 50% ethanol aqueous extract of two plants from East Kalimantan forest, *Vitex pubescens* and *Terminalia catappa* were tested for dental caries-inducing properties of *Streptococcus sobrinus* bacteria as well as for brine shrimp lethality. The stem wood, stem bark, and leaves were separately collected and investigated for their activities. The result showed that the extracts of these plant exhibited potency as an anti dental caries agents.

Keywords : *Vitex pubescens, Terminalia catappa, Streptococcus sobrinus,* Brine shrimp lethality

INTRODUCTION

Plant produce a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of medicinal plant and out of the 4000000 plant species on Earth only a small number has been systematically investigated for their antimicrobial activities. Additionally, there is a local ethnobothanical knowledge and bibliography describing the species most frequently used by population to cure diseases (Shokeen, 2009). Over 80% of the world's population, especially in the developing country, using plant extracts as traditional medicine to provide their health (WHO, 2002)

Vitex pubescens species belong to the family of Verbenaceae and some of species in this family has activity to treat the diseases. The genus Vitex consist of over 270 species, predominantly trees and shrubs, and is restricted to tropical and sub tropical regions. There are many species of Vitex with have medicinal and phytochemical importance. Genus *Vitex* is used as a traditional medicine in China, Indo-China, Indonesia and the Philippines. *V. pubescens* has been introduced to tropical Africa and South America. It is planted in Southeast Asia (Kalimantan) on trial for charcoal production. Tannin is extracted from the bark, leaves, roots, and fruit shell. A black dye is obtained from the bark, fruit, and foliage. The leaves and bark have a wide range of medicinal uses (Lex *et al.*, 2006). In traditional medicine, a decoction of the bark of *V. pubescens* is used to treat stomachache, and a poultice of its leaves is used to treat fevers and wounds.

Terminalia catappa L. is a Combretaceous plant whose leaves are widely used as a folk medicine in Southeast Asia. In traditional medicine, *T. catappa* leaf, bark and fruit are used in treating dysentery, rheumatism, cough and asthma. Some studies on medicinal properties of *T. catappa* roots and fruits have been also reported. The activity of five *Terminalia* species were reported as antibacterial against human pathogens *E. coli*, *P. aeruginosa*, *B. subtilis*, *S.aureus* and *S. epidermidis*. *Terminalia catappa* found to possess the compounds which are more antibacterial (Shinde *et al.*, 2009)

Even there were some studies about their potency as medicine, there is a little information about the potency of these plants as toothache medicine. Some investigation

about the activity for treat the tooth dieases have been reported in different species of *Vitex* and *Terminalia* (Padmalatha, 2009 and Kamal, 2009), but the anti caries potential of *T. catappa* and *V. pubescens* had not been mentioned in previous report.

Herbal remedies have long history of use for gum and tooth problems. The use of herbal chewing stick for relieving dental problems is common in many traditional cultures. Oral pain occurs as a result of bacterial activity in the pulp of a carious tooth. One of the bacteria types have been implicated in caries formation is *Streptococcus sobrinus*. There are several strategies to preventing the formation and development of dental caries, such as controlling the growth and cell adhesion of mutans streptococci.

The objective of this research is to determine the effect of methanolic and 50% ethanolic of *Terminalia catappa* and *Vitex pubescens* in different plant material (stem wood, stem bark, and leaves) on the growth and cell adhesion of *S. sobrinus*. The brine shrimp lethality bioassay was also carried out to investigate the cytotoxicity of extracts of medicinal plants.

MATERIAL AND METHOD

Plant material and preparation of extracts

Terminalia catappa and *Vitex pubescence* were collected from East Kalimantan. The plant materials were air dried to constant weight, grinded to powder and stored in container for further use. Two different solvent, methanol and 50% ethanol were used for extraction. Exactly 100 g of the plant material powder were extracted in methanol and 50% ethanol. The separated extracted were then filterded using filter paper (Whatman no 1). Furthermore the methanol and 50% ethanol filtrate were separately concentrated to dryness using a rotary evaporator to remove the solvent.

Antimicrobial activities against *S. sobrinus* by agar well diffusion

The *S. sobrinus* 6715 bacteria were first grown in a Nutrient broth for 18 h before use and standarized to 0.5 Mc Farland standard. The antimicrobial activities of the various plant extracts were evaluated by means of agar-well diffusion assay. Forthy five milliliters of Nutrient Agar media were poured into 100 x 100 mm sterile petri dishes. Standarized cell suspensions of *S. sobrinus* that prepared before then spread onto the surface using sterile swab sticks. Once the plates had been aseptically dried, seven 6 mm-diameter wells were bored in each dish by removal of the agar using a sterile cork borer, then immediately filled with the test and control materials (one well for each substance). Extracts and control (40µl) of different concentrations (3.0 – 8.0 mg/ml) were placed into the wells, left for one hour at room temperature for diffusion and the plates were incubated at 37^0C for 24 h. Chloramfenicol (0.5 mg/ml) and mouthwash containing hexetidine 1% were used as positive control. After incubation, the diameter of the zones of bacterial growth inhibition formed around the wells was measured in millimeters with a ruler under reflected light. The antimicrobial activity indicated by an inhibition zone surounding the well greater than 6 mm.

Determination of the cell adhesion of *Streptococcus sobrinus*

S. sobrinus culture was grown in a test tube containing 3 ml of Nutrient broth with 1% (w/v) glucose at 37°C and held at an angle of 30° for 18 h. After 18 h incubation, the adhering cells were collected by sonicating and washing the tube with saline (0.9% NaCl), and then determined turbidimetrically at OD 550 nm. This gave the total cell number and represented 100% adhesion. The experiment was repeated by incubating the *S. sobrinus* culture in 3 ml Nutrient broth with 1% (w/v) glucose and different concentrations (0.25 – 1 mg/ml) of plant extracts. This gave the total cell number adhering in the presence of different concentrations of the extract,and this was expressed as a percentage of the total cell number in the absence of the extract.

Brine Shrimp Lethality Assay

This assay was carried out to investigate the cytotoxity of *Terminalia catappa* and *Vitex pubescens* extracts. Brine shrimp eggs (*Artemia salina*) were hatched in artificial sea water. After 48 hours incubation at room temperature (25-29°C), nauplii (larvae) were collected by pipette and used for the assay.The plant extracts were tested at concentration 10, 100, 1000 ppm in sterile vials containing 3 ml of sea water and 10 shrimps. Survivors were counted after 24 hours, and the percentages of lethality at each concentration were recorded according to
Abbot's formula (McLaughlin, 1991) :

$$\%M = [(m_e - m_b) / (10 - m_b)]*100$$

Where m_e = dead shrimp in the sample and mb = shrimp dead in the blank
LC_{50} values were obtained from the best-fit line plotted concentration versus percentage lethality.

RESULTS AND DISCUSSION

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). Herbal preparations have been used by local practitioners to treat various ailment. Stems and leaves of certains herbs are commonly used in cleaning and treatment of dental diseases (Okafor, 2001).

S. sobrinus was choosen as test microorganism because it have been implicated in dental caries. The antibacterial assays against *S. sobrinus* in this study were performed by the agar well-difussion methods. The *in vitro* antimicrobial activities of the crude plant extracts of *Vitex pubescens* and *Terminalia catappa* are shown in Table 1. The susceptibility of the bacteria to the crude extracts on the basis of zones of growth inhibition varied according to plant material and extracting solvent. In this investigation most of the sample in this study showed inhibition of the growth of *S. sobrinus*, the sizes of zones of growth inhibitions vary from one plant material to another.

According to the findings of this study, the crude extracts from *V. pubescens* and *T. catappa* plants showed varying degree of antibacterial activities against *S. sobrinus*. Regarding inhibition, the most potent extract was the *T. catappa*-leaves-methanol extract with 10.6 mm zone inhibition at different concentration. The methanol extracts (inhibition zone 8 – 10 mm) was found to be more effective than the 50% ethanol extracts (inhibition zone 7 – 9 mm) against *S. sobrinus* bacteria. The antibacterial activities of the methanol and 50% ethanol extracts compared favourably with that of two positive control (chloramfenicol and mouthwash). This finding implies that the active compound have polar characteristics. Chloramfenicol and mouthwash containing 1% hexetidine which served as positive control produce zone inhibition measuring 13 mm and 9.5 mm, respectively. Highest activity was demonstrated by the control standard antibiotic (chloramfenicol), while the negative control (acetone) produced no observable zone. The high activity of chloramfenicol is because the antibiotic is in pure state and has refined processes that have established it as a standard antibiotic (Abubakar, 2009). Their effects on the growth of *S. sobrinus* were most likely due to the release of chemicals from the crude extracts into the medium. The different reactions of *S. sobrinus* to the different extracts indicated that each solvent extracted different chemical components of plants

The phytochemicals of *Terminalia catappa* include tannins), flavanoids and triterpinoids. Probably, the bioactivite flavonoids and other phenol compound and its derivates present in *T. catappa* are involved in its biological activity. Tanin and flavonoids have been reported to be responsible for the antibacterial activities of the extracts (Heminway and Karchesy, 1991). The inhibitory effect of the extracts in this

study as antimicrobial against *S. sobrinus* can introduce these plants as a potential candidate for antimicrobial agent development for the treatment of dental caries.

Table 1. Antibacterial activities profile of *Terminalia catappa* and *Vitex pubescens* extracts against *S. sobrinus*

Plant species	Part	Control	Zone of inhibition (mm)					
			3 mg/ml		6 mg/ml		8 mg/ml	
			MeOH	50% EtOH	MeOH	50% EtOH	MeOH	50% EtOH
Vitex pubescense	Stem		8.2 ± 0.1	-	8.6 ± 0.3	7.2 ± 0.3	9.2 ± 0.3	8.2 ± 0.1
	Bark		-	8.2 ± 0.2	8.2 ± 0.3	8.2 ± 0.1	8.2 ± 0.3	8.2 ± 0.3
	Leaves		-	9.2 ± 0.3	9.2 ± 0	9 ± 0.4	10.0 ± 0.2	9.6 ± 0.3
Terminalia catappa	Stem		9 ± 0.1	8.4 ± 0	9 ± 0.2	8.8 ± 0.1	10 ± 0.0	9.6 ± 0.2
	Bark		-	-	8.6 ± 0.5	9 ± 0.3	9 ± 0.1	9.2 ± 0.2
	Leaves		9.6 ± 0.5	8.6 ± 0.1	9.8 ± 0.2	9 ± 0.4	10.6 ± 0.1	9.4 ± 0.2
Mouthwash		9.5 ± 0.5						
Chloramfenicol (0.5 mg/ml)		13.0 ± 0.2						

Figure 1a and 1b show the effect of plant extracts on the adhesion of *S. sobrinus* to a glass surface. It was found that the *T. catappa* and *V. pubescence* extracts could reduce the cell adhesion of *S. sobrinus*. The *T. catappa* extracts exhibiting a stronger activity than the *V. pubescence* extracts. The inhibitory effect increased with increasing the concentration.

It is clearly shown that all the extracts tested in this study affected the cell adhesion of *S. sobrinus*. The extract from *T. catappa* can reduce the cell adhesion at range 17 – 85.5 %, while V. pubescens show lower activity to reduce the cell adhesion (12 – 84.3%). It was observed that the ability of *S. sobrinus* to adhere to a glass surface was affected by the presence of crude extracts. The receptors on the cell *S.sobrinus* may be modified by components in the crude *T. catappa* and *V. pubescence* extracts, leading to reduction of adhesion ability. The adhesion was reduced to 69.7% at 0.25 mg/ml *T. catappa*-bark-methanol extract, and a higher concentration of the other extracts were required for a similar reduction.

The highest activity for reducing the cell adhesion of *S. sobrinus* come from *T. catappa* – stem – 50% ethanol extracts at 1 mg/ml concentration, followed by *V. pubescens* – bark – methanol extracts in the same concentration. The reduced adhesion of *S. sobrinus* cells to the tooth surface would disrupt the colonization of the tooth surface by the microorganisms, and this could affect plaque accumulation.

Brine shrimp larvae have been used as a bioassay for a variety of toxic substances. The method has also been applied to plant extracts in order to facilitate the isolation of biologically active compounds. Cytotoxixity activity of extracts was tested in order to evaluate a limited toxicity. Brine shrimp nauplii have been used in a number of bioassay systems (McLaughlin *et al.*,1990) and the crude extracts of the plant extracts of *T. catappa* and *V. pubescence* were subjected to this evaluation.

The extracts were tested at concentrations of 10, 100 and 1000 ppm (data not shown) and data LC_{50} value shown in Table 2. The result obtained the variety of toxicity value on each extracts.

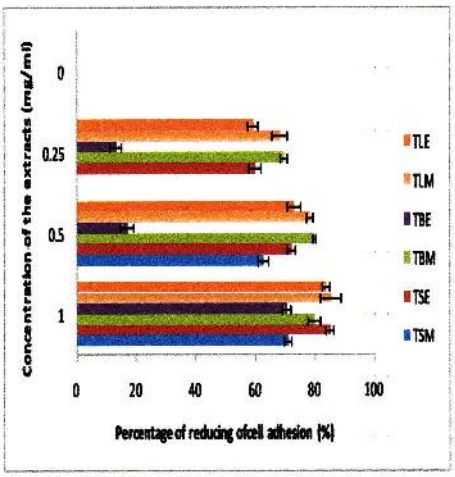

Figure 1a Effect of crude *T. catappa* extracts on the reducing cell adhesion of *S. sobrinus*

T : Terminalia catappa, L : Leaves, S : Stem, B : Bark, M : MeOH, E : 50% EtOH

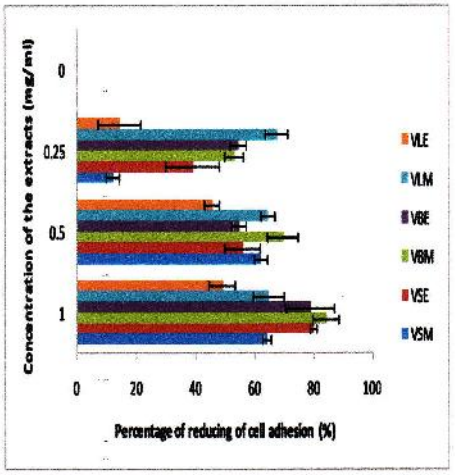

Figure 1b Effect of crude *V. pubescence* extracts on the reducing cell adhesion of *S. sobrinus*

V : Vitex pubescence, L : Leaves, S : Stem, B : Bark, M : MeOH, E : 50% EtOH

The toxicity showed that there were differences in LC_{50} values for type of plant extracts and plant species.

Table 2. Brine shrimp toxicity value (LC_{50}) of plant extracts of *Vitex pubescens* and *Terminalia catappa*

		Vitex pubescens (ppm)	*Terminalia catappa* (ppm)
Stem wood	MeOH	305.5 ± 0.27	17.78 ± 1.1
	50% EtOH	543.3 ± 0.21	0.44 ± 0.2
Stem bark	MeOH	298.5 ± 0.15	59.2 ± 0.2
	50% EtOH	10 ± 0.2	317.7 ± 1.2
Leaves	MeOH	2.9 ± 0.1	0.01 ± 1.4
	50% EtOH	40 ± 0.9	0.43 ± 0.75

In this study, the methanol extracts from *T. catappa* leaves showed very toxic activity (LC_{50} value = 0.01 ppm), followed by 50% ethanol extract of *T. catappa* leaves, 50% ethanol extracts of *T. catappa* stem wood, methanol extracts of *V. pubescense* leaves, 50% ethanol extracts of *V. pubescense* stem bark, and methanol extracts from *T. catappa* stem wood with LC_{50} value 0.43 ppm, 0.44 ppm, 2.9 ppm, 10 ppm and 17.78 ppm respectively. Other plant extracts in this study, however, showed moderate brine shrimp lethality or toxic, where the LC_{50} were found to be at lower than 1000 ppm. Toxicity of extract has classified by the value of $LC_{50} \leq 30$ ppm = very toxic; $31 \leq LC_{50} \leq 1000$ ppm = toxic; $LC_{50} > 1000$ ppm = practically no toxic (Meyer et, al, 1982).

The variation in BSLA results (Table 2) may be due to the difference in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the crude extracts.

Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 100 ppm or µg/mL) to brine shrimp is indicative of the presence of potent cytotoxic which warrants further investigation. BSLA results may be used to guide the researchers on which crude plant extracts/fractions to prioritize for further fractionation and isolation of these bioactive compounds. (Nonita, 2010). From this study the bioactivity of *T. catappa* and *V. pubescens* indicating the presence of compounds with possible biological activity.

CONCLUSION

The extracts of both species of *Terminalia catappa* and *Vitex pubescens* from different plant material proved to exert in vitro antibacterial and cell adhesion reduction against *S. sobrinus*. The ability of the crude extracts to inhibit the growth and reduce the cell adhesion of *S. sobrinus* used in this study is an indication that *T. catappa* and *V. pubescens* are a medicinal plant and can be used as an agent for developing broad spectrum antibiotics, which further validates its use in traditional herbal medicine to treat dental caries. The phytochemicals can further be isolated and undergo further pharmacological evaluation

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