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ANTI-BACTERIAL PROPERTY OF THE ESSENTIAL OILS OF CLOVE AND PRONG FA AGAINST *STREPTOCOCCUS MUTANS* AND *STREPTOCOCCUS SOBRINUS*

Abstracts. In the article there are shown the antibacterial activity of two plants against bacteria causing oral problems, and the minimum concentration of exposure to these plants has been identified. On the disk diffusion test the clove oil was received by traditional method, showed the highest anti-bacterial activity against to bacteria *streptococcus mutans* and *streptococcus sobrinus*, the average zone of growth inhibition is respectively 16.78 and 16.68 mm, also the MIC value of the essential oil that can inhibit the growth of both bacteria is 2µg/ml. However, in the MBC, the result indicates that the clove oil is more effective against *S. mutans*.

Keywords: essential oil of clove, essential oil of prong fa, disk diffusion assay, minimum inhibitory concentration, minimum bactericidal concentration, streptococcus mutans, streptococcus sobrinus, anti-bacterial property.

Introduction. Oral diseases ranging from cavities to cancer are all serious threats to every people's oral health. The oral health is an important part of the overall health because there may be a link between oral disease and other health problems such as diabetes, heart disease and stroke as well as pre-term and low-birth-weight babies. The oral cavity is very convenient place for plenty of bacteria forming various associations in the mouth thereby residing more than 700 dissimilar species (Metwalli et al., 2013). The following bacteria are the most dominant form existing in the human oral cavity: *S. sanguis*, *S. mitis*, *S. mutans*, *S. salivarius*, *L. acidophilus*, *L. salivarius*, *L. casei*, *Staphylococcus spp*, *Eubacterium spp*, *Neisseria spp*, *Actinomyces spp*, *Peptostreptococcus spp*, *Micrococcus spp*, etc. One of the largest associations in the oral cavity is streptococci (Bhatia and Ichhpujani, 2003). *S. mutans* and *sobrinus* are the most prevailing species, high in rank than other streptococci. *S. sobrinus, S. mutans* plays a major role in tooth decay, metabolizing sucrose to lactic acid, it causes the highly mineralized tooth enamel to be vulnerable to decay. Oral health problems like dental caries caused by these bacteria are very common with children, early childhood caries is a source of pain and impaired quality of life, and for some it results in serious infection, hospitalization, and even fatality (Casamassimo, et al., 2009). Also, the disease may become refractory and irreversible for simple preventing methods of removing biofilms, like tooth brushing. Thus, ways on how to prevent or kill this type of bacteria is very relevant.

Since the use of synthetic chemicals is perceived to have a possible effect and may raise health concerns, microbial resistance, and environmental problems, extracts and essential oils of plants have been widely used and tested nowadays for their antimicrobial activity against different pathogens. Essential oils are complex mixtures of low molecular weight (usually less than 500 Daltons) compounds extracted by steam distillation, hydro distillation or solvent extraction (Nakatsu et al., 2000). EOs may constitute 20–100 different plant secondary metabolites belonging to a variety of chemical classes (Carson and Hammer, 2011). Most of the time the bioactivities of a particular EO is decided by either one of its main components (Bakkali, *et al.,* 2008). Naturally, their activity depends on the type, composition and concentration of the spice or the essential oils, the type and concentration of the target microorganism, the

composition of the substrate, the processing and the storage conditions (Skandamis and Nychas., 2000). Researchers from all over the world are trying to characterize a range of biological properties of EOs which includes antimicrobial, antiviral, antimutagenic, anticancer, antioxidant, anti-inflammatory, immunomodulatory, and antiprotozoal activities (Bakkali *et al.,* 2008).

In this study, the essential oil of two Thai herbs, Clove (*Syzigium aromaticum*) from the plant family Myrtaceae and Prong fa (*Murraya siamensis)* from the plant family Rutaceae, will be tested for its anti-bacterial activity against two oral disease causing bacteria *Streptococcus mutans* and *Streptococcus sobrinus.*

Materials and methods. *Plant extraction procedure.* Essential oils from two Thai herbs, Clove (*Syzygium aromaticum*) and Prong fa (*Murraya siamensis*) were used. One hundred grams of dried and ground clove buds in 500 ml flask was submitted to hydrodistillation for 4-6 hours and steam distillation for 8-10 hours.

The volatile distillate was collected until no oil drop out. The distillate was saturated with sodium chloride and added with ether. Then, the ether layer and hydro layer were separated by funnel. After dehydrated by anhydrous sodium sulphate, the ether was further heated in 60°C water bath to make oil to be concentrated and the ether to be recovered. The collected oil was refrigerated after the prior to use.

Microorganisms used. The used microorganisms were two oral disease causing, gram positive bacteria, *Streptococcus mutans* (DMST 48777) and *Streptococcus sobrinus* (DMST 35719). The bacterial cultures were obtained from the Department of Medical Sciences Thailand, Nontaburee, Thailand.

Disk diffusion assay. A bacterial suspension was prepared in NaCl from an overnight-grown culture of the bacterial strains. These bacterial suspensions were adjusted to the 0.5 McFarland standard. A sterile swab was immersed to the bacterial suspension and was used to spread in the Mueller-Hinton Agar with blood (5 % of MHB) medium. Each plate was divided into four sections. 10 µl of each essential oil (diluting of EO 500 µg per 100 ml of tween 20-20%) was applied to the sterile 6 mm paper discs and was aseptically placed in the medium which was previously swabbed with bacteria. Erythromycin (15 µl) was used as the positive control and tween 20 (10 µl) for the negative control. After 24 hours of incubation in an anaerobic condition at 37°C, the zone of inhibition was measured in mm. All experimental set-ups were carried out in triplicate.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The bacterial strains were isolated into broth medium (MHB) with lysed blood (5% of MHB) and was incubated in an anaerobic condition for 24 hours at 37°C. A series of two fold dilution of each oil. Triplicate serial dilutions with broth for each essential oil was prepared in two cell culture plates. Fifty microliters of the test organisms with the concentration of 10^6 cells per ml was then inoculated in each of the broth dilution. After overnight incubation of the cell culture plates, MBC was carried out by 1 µl

Zone of growth inhibition for essential oils on two bacterial strains

Essential oil	Bacteria	Diameter of Zone of Inhibition, mm	Average diameter of zone of inhibition, mm	Anti-bacterial activity
Clove oil	Streptococcus mutans	16.30	16.78	Intermediate
		16.90		
		17.15		
	Streptococcus sobrinus	16.75	16.68	Intermediate
		16.05		
		17.25		
Prong fa oil	Streptococcus mutans	0	0	Not effective
		0		
		0		
	Streptococcus sobrinus	0	0	Not effective
		0		
		0		

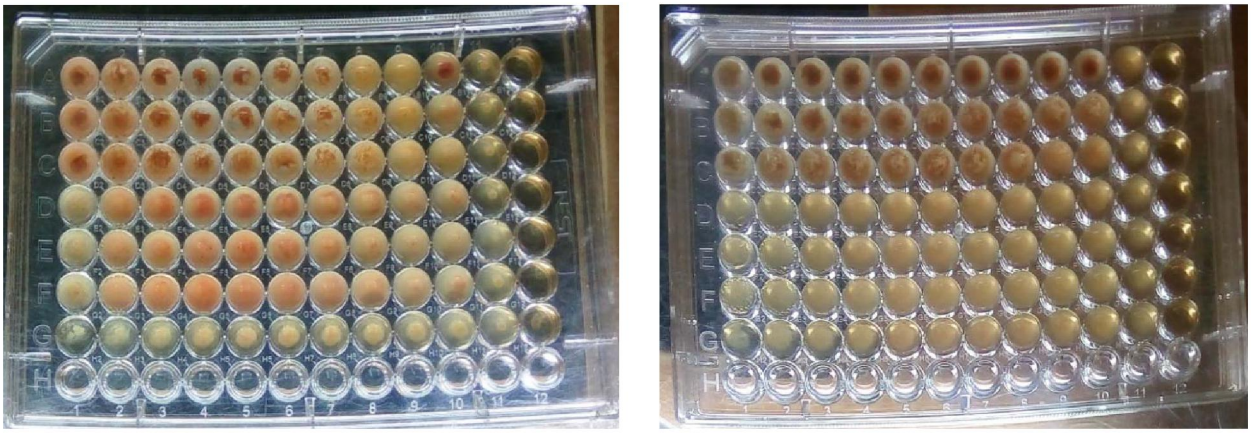
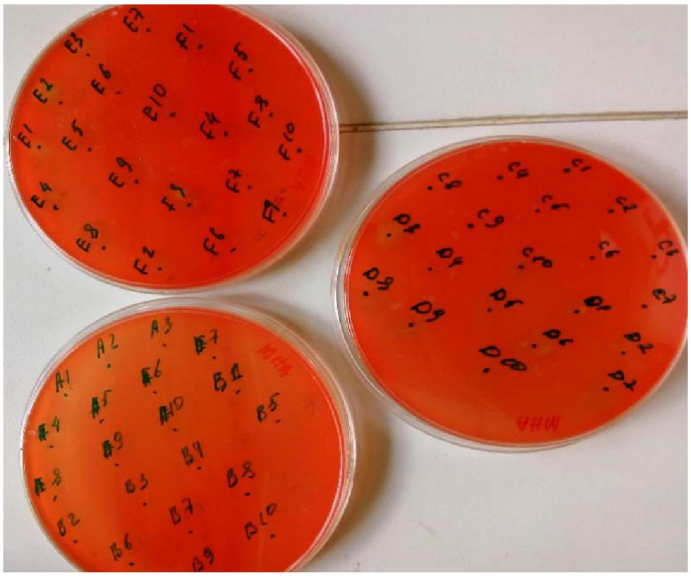


Figure 1 – Minimum Inhibitory Concentration (left) *Streptococcus sobrinus*; (right) *Streptococcus mutans*

Note: Rows A, B, C were the Clove oil; D, E, F were the Prong fa oil; and row G was the tween 20.



a. Streptococcus sobrinus

b. Streptococcus mutans

Figure 2 – Minimum bactericidal concentration (a) *Streptococcus sobrinus*; (b) *Streptococcus mutans*

sub culturing in agar plates (MHA) each of the well of the cell culture plates. The plates were then incubated in appropriate condition for 24 hours, while the cell culture plates were added with 50 µl of 0.2 mg/ml Iodonitrotetrazolium chloride (INT) and stayed for 30 minutes to see the coloration of the cells with bacterial growth. After the incubation of the cell culture in MHA, the result of the MBC was obtained through optical observation.

Results. In the two essential oils, the clove was found to have an antibacterial property against the two bacterial strains, while the prong fa was ineffective.

On the disk diffusion test the clove oil showed intermediate anti-bacterial property against both bacteria, 10 µl of essential oil dissolved with tween 20 can effect to the growth of bacteria.

The results of MIC and MBC are shown in Figure 1 and 2 respectively. Only the clove oil showed antibacterial activity on *S. mutans* and *S. sobrinus*. The MIC value of the essential oil that can inhibit the growth of both bacteria is 2 µg/ml.

However, in the MBC, the result indicates that the clove oil is more effective against *S. mutans* than *S. sobrinus* because the former only has spots of bacterial growth on one of its least concentration while the latter has bacteria in the last two concentrations for all the replicates.

Discussion. The oral microorganisms present in dental biofilm are considered to be crucial for the initiation and progression of caries. It is known that the frequent consumption of carbohydrates, mainly sucrose, can result in the appearance of cariogenic microorganisms, such as *mutans* and *sobrinus* streptococci (Hamada *et al.*, 1984). The ability of *mutans* streptococci to produce extracellular polysaccharides, mainly glucans, has been described as a critical factor in the pathogenesis of dental caries and plaque formation and accumulation (Loesche, 1986). In this case, *S. mutans* and *S. sobrinus* should be a prime target for any therapeutic agent aimed at preventing dental caries. Therefore, the antibacterial activity of clove oils against these bacteria could play an important role in preventing the formation of dental plaque and caries.

Conclusion. Clove oil was found to have important antimicrobial activity against the two oral disease causing bacteria. In this regard, the use of clove as an addition to dental products are valuable to decrease the build-up of dental plaque and caries. It could also be a possible substitute with chemical and synthetic antimicrobial agents.

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ҚАЛАМПЫР МЕН PRONG FA ТАБИҒИ МАЙЛАРЫНЫҢ *STREPTOCOCCUS MUTANS* ЖӘНЕ *STREPTOCOCCUS SOBRINUS* МИКРОАҒЗАЛАРЫНА ҚАРСЫ АНТИБАКТЕРИАЛДЫҚ ҚАСИЕТІ

Аннотация. Мақалада қалампыр мен prong fa өсімдіктерінің ауыз қуысының мәселелерін туғызатын бактерияларға қарсы белсенділігі қарастырылып, минималды әсер ету концентрациясы анықталды. Дисперсионды талдау кезінде, қалампыр және prong fa өсімдіктерінен дәстүрлі жолмен алынған табиғи майлардың ішінде, *streptococcus mutans* және *streptococcus sobrinus* микроағзаларына қарсы жоғары белсенділікті қалампыр табиғи майы көрсетті. Әсер ету аумағы орташа есеппен сәйкесінше 16.78 және 16.68 мм тең, ал екі бактерия үшін минималды ингибирлеу концентрациясы 2 млг/мл мәнге ие, сонымен қатар минималды бактерицидті концентрациясын анықтау барысында, қалампыр табиғи майының *Str.mutans* микроағзасына әсері жоғары екені байқалды.

Түйін сөздер: қалампыр табиғи майы, prong fa табиғи майы, дисперсионды талдау, минималды концентрация, минималды бактерицидті концентрация, *streptococcus mutans, streptococcus sobrinus,* антибактериалдық қасиет.

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АНТИБАКТЕРИАЛЬНЫЕ СВОЙСТВА ЭФИРНЫХ МАСЕЛ ГВОЗДИКИ И PRONG FA ПРОТИВ *STREPTOCOCCUS MUTANS* И *STREPTOCOCCUS SOBRINUS*

Аннотация. В статье показана антибактериальная активность двух растений против бактерий, вызывающих оральные проблемы, а также была выявлена минимальная концентрация воздействий этих растений. При дисперсионном анализе, среди полученных традиционным способом, эфирное масло гвоздики и prong fa, высокую активность против штаммов *streptococcus mutans* и *streptococcus sobrinus* показало эфирное масло гвоздики. Средний масштаб воздействия, соответственно, равна 16.78 и 16.68 мм, а минимальная ингибирующая концентрация для двух бактерий является 2 мгл/мл. Однако анализ минимальной бактерицидной концентрации показывает, что гвоздичное масло более эффективно против *S. mutans*.

Ключевые слова: эфирное масло гвоздики, эфирное масло prong fa, дисперсионный анализ, минимальная ингибирующая концентрация, минимальная бактерицидная концентрация, *streptococcus mutans, streptococcus sobrinus,* антибактериальное свойство.