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Screening Antibacterial Activity of Combination of Secang Extract (*Caesalpinia sappan*) and Clove oil (*Syzigium aromaticum*) Againts *Propionibacterium acnes* ATCC 11827 by diffusion method

Ni Luh Putu Vidya Paramita[1*], Cokorda Istri Sri Arisanti[1] and Ni Putu Linda Laksmiani[1]

[1] Department Pharmacy, Faculty of Mathematic and Natural Science, University of Udayana, Badung, Bali, Indonesia

[*]E-mail: vidya_paramita@unud.ac.id

Abstract. *Propionobacterium acnes* are common bacteria that cause an acne. It is populate in the skin and induce the inflammatory phase of acne. The methanolic extract of secang (Caesalpinia sappan) were proven that have an antiacne potency and the clove oil (Syzigium aromaticum) could inhibit the growth of propionibacterium acnes by disk diffusion method. The aim of this study is to investigated the potency of combination of both sample to inhibit Propionibacterium acnes by well diffusion method. The results showed that the antibacterial assay of the combination of methanolic extract secang and clove oil (1:1 v/v) was more effective than single samples. The combination could inhibit the growth of propionibacterium acnes with inhibition zone value is 19.3 mm. The inhibition zone value of methanolic extract of secang (8 mg/mL) and clove oil (20 µl/mL) respectively is 17.6 mm and 10.6 mm. This combination has the potential to be developed into an antiacne product so it requires further research to find the right formula combination from both samples to enhance their activity.

1. Introduction

Acne is a common dermatological condition that affects the psychological conditions of depression, anxiety, and others. Acne is a chronic inflammatory disease of pilosebaceous follicles[1,2]. The urgency of acne is the appearance of permanent scars both physically and emotionally[3]. Manifestations of acne are the presence of blackheads, papules, pustules and cysts. There are four main aetiology of acne, namely hypercornification of the pilocebaceus ductus, increased production of sebum, colonization of the *Propionibacterium acnes* and subsequently occurrence of inflammatory conditions[2]. Generally, the common therapy of acne are comedolytics and antibiotics. However, these terapies can produce side effects including antibiotic resistence to frequently used antibiotics[1].

Indonesia has many medicinal plants that have the potential as antiacne. Among them, *Caesalpinia sappan* and *Syzygium aromaticum* were a good potential as an antiacne. *C. sappan* was used as traditional usage including food, beverage, skin care and widely known as a medicinal plant. *C. sappan was* known as sappan wood. There is so many phenolic compounds was found in *C. sappan* including xanthones, coumarins, flavones, homoisoflavonoids, and brazilin[21]. The polifenol and flavonoid[8] compound have been isolated from the wood of *C. sappan* reported such as 4-O-

methylsappanol, protosappanin A[9], protosappanin B[10], brazilin[11], brazilein, caesalpin J[12], brazilide A[13], neosappanone A[6], caesalpin P, sappanchalcone, 3-deoxysappanone[7], 7,3′,4′-trihydroxy-3-benzyl-2Hchromene[14], and others. Brazilin and brazilein is the major phenolic compound in *C. sappan*[18]. Brazilin have been reported to posses pharmacological effects especially as anti-inflammatory[22], antioxidant[23], antibacterial[24] and antiacne[5,25]. Brazilein have been reported to have a cytotoxic effect[18]. Research on *C. sappan* extract as antibacterial againts *Propionibacterium acnes* has been widely proven. *C. sappan* methanol extract and 50 % Ethanol extract had the highest potency as an antiacne agent out of 80 plant extracts which had screened[4,5]. *C. sappan* had potency as an antiacne because their activity as antibacterial, lipase inhibitor and antioxidant. Braziliin have a potential antiacne because it's activity as an antibacterial, good lipase inhibitor activity, and antioxidant.

Clove oil is an essential oil and it was obtained 18% from flower buds of *S. aromaticum*. The buds of *S. aromaticum* were used in traditional medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment properties and condiment with carminative and stimulant activity[15]. Phenolic compound is a major cmponents in clove such as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenyl propens[27]. Eugenol is the main bioactive compound of clove oil (89%). A number of 5 to 15 % is eugenol acetate and β-cariofilenol was flound in clove oil[28]. Some biological activity were already proven especially antibacterial activity[2,3,4], anti-inflammatory[16] and potent antioxidant[26]. It was reported that clove oil have strongest activity as antibacterial againts five strain of *Propinoibacterium acnes* with inhibiton zones more than 20 mm[16]. *Propionibacterium acnes* contribute to the develompment of inflammation in acne because they release several proinflammatory products[2,3,4]. Clove oil were proven significantly to inhibit the increasing of several proinflammatory, modulated global gene expression and altered signalling pathways critical for inflammation[17]. The potential of clove oil as an antiacne was proven as their activity as antioxidant .

Altough the antiacne activity of *C. sappan* methanol extract and clove oil already known, the combination activity of both of them have not yet been investigated. In this study, the combination *C. sappan* and clove oil were screened as antibacterial activity againts *Propionibacterium acnes* ATCC 11827.

2. Material and Methods

2.1. Material

Flower buds of *S. aromaticum* were collected from Bali, Indonesia. Heartwoods of *C. sappan* were collected from Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (BBPPTOOT) in Tawangmangu, Indonesia. Identification of both sample were collected at Departement of biology pharmacy, Faculty of Pharmacy, Gadjah Mada University. The *Propionibacterium acnes* ATCC 11827 were obtained from Faculty of Pharmacy, University of Sumatera Utara

2.2. Preparation of C. sappan extract and clove oil

The heartwood of *C. sappan* and flower buds of clove were grounded and dried before being extracted. The extraction method of *C. sappan* was performed by following the method of Laksmiani, et al[18]. The powdered of *C. sappan* extracted with methanol at volume ratio 1:10, room temperature. The methanol extract was then concentrated by rotary evaporator[18]. The essential oil of clove were obtained by steam destillation.

2.3. Antibacterial assay

This experiment was performed by agar well diffusion method. The Propionibacterium acnes ATCC 11827 were growth in mueller hinton broth for 20h before standardized to 0.5 Mc. Farland. Two hundred microliter (200 µL) of inoculum suspensions were introduced into 20 mL of mueller hinton agar. Wells were then bored into the agar using a sterile diameter 6 mm borer. Control positive in this

study is doxyxycline 0.75 mg/mL. C. sappan methanol extract and clove oil were diluted with DMSO. Ten microliter (10 µL) of C. sappan extract, clove oil, their combination (1:1 v/v), DMSO and control positive were loaded into the wells. The plates were incubated at 37 0C for 18 - 24 h. All of agar well diffiusion were performed in triplicate. Antibacterial activity was showed as the mean of their diameter of inhibition zones (mm).

3. Results and Discussion

Propionibacterium acnes is a microbial flora in the skin was significantly triggering acne condition. The number of bacteria did not correlation with the type and the severity of acnes[1,2]. Since, colonization of *P. acnes* will lead the inflammation because they secrets several inflammation product. So that, P. acnes is the target way to treatment the acne. Inhibiton of the growth of *P. acnes* is a parameter effectiveness of acne therapy[2]. In this study we screened antibacterial activity of *C. sappan* methanolic extract, clove oil, and the combination of both of them (1:1 v/v).

3.1. Antibacterial assay of C. sappan methanol extract

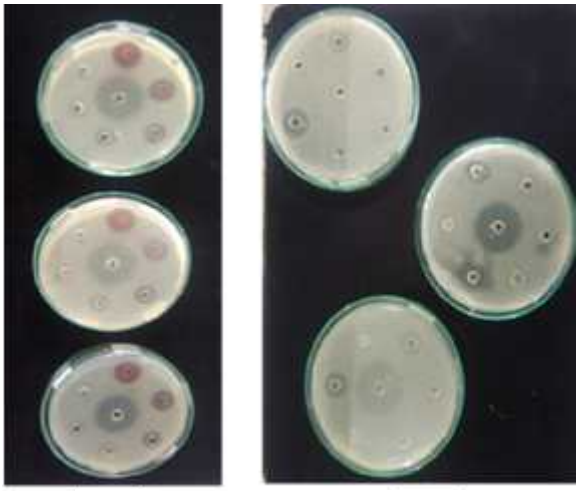
Methanolic extract of *C. Sappan* was reported had strongest activity as antiacne. Brazilin, sappanone B and protosappanin A was isolated from methanolic extracts. Brazilin is a compound which isolated from methanolic extract of *C. Sappan* had best actvity as antibacterial agent againts *Propionibacterium acnes* with MIC 0.5 mg/mL. The MIC value of protosappanin A and sappanone B respectively 1.00 mg/mL and > 2.00 mg/mL. Lipase inhibition activity of brazilin was strongest than protosappanin and chloramphenicol. Brazilin have been strong antioxidant activity and it's antioxidant activity was higher than sapponene B, but was not different with protosappanin A and cathecin[4]. Based of that, in this study we performed 5 concentration (0.5; 1; 2; 4; 8 mg/mL) of methanolic *C. sappan* extract in agar-well diffusion method for screening the antibacterial activity. The result was showed in table 1 and figure 1. The highest concentration (8 mg/mL) of methanolic *C. sappan extract* appear the highest diameter inhibition zones in inhibition of the growth of *P. acnes* (17.6 mm).

Table 1. Inhibition zones (d) of *C. sappan* methanolic extract and clove oil.

C.Sappan methanolic extract		Clove oil		d (mm)	d (mm)
Concentration (mg/mL)	d (mm)	Concentration (mg/mL)	d (mm)	Doxycycline 0.75 mg/mL	DMSO 100%
0.5	0	2.5	0	25	0
1	8	5	0		
2	11.6	10	0		
4	14.8	20	10.6		
8	17.6				

3.2. Antibacterial assay of clove oil

Clove oil were essential oil which already known as an antibacterial agent againts P. acnes [16]. The phenolic major compound of that essential oil was eugenol (89%)[28]. By scanning electron microscopy, eugenol was damage the envelope of the organisms tested that is *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Escherichia coli*. Eugenol was responsible to cellular lysis because this component can reduce the viability of cell and induce the release of substance which suppose cell lethality[19]. The phenolic volatile oils that is eugenol and acetyl eugenol was potential as radical scavenger. Antimicrobial activity of clove oils have been proven widely[21]. Essential oil of clove was reported has antibacterial activity againts P. acnes at inhibition zones more than 20 mm with MIC value is 0.25% v/v. So that, we performed 4 concentration of clove oil in this experiment. These are 2.5; 5; 10; 20 µL/mL. The results were showed at table 1 and figure 1.



(a) (b)

Figure 1. agar well diffusion plates in triplicate of antibacterial assay of *C. sappan* methanolic extract (a) and clove oil (b)

3.3. Screening antibacterial activity of combination C. sappan methanol extract and clove oil

Based on both sampel activity at table 1. in this experiment, we were screened the potency of antibacterial activity of the combination of them. The combination ratio were 1:1 (8 mg/mL *C. sappan* methanolic extract and 20 µL/mL clove oil). This preeliminary study is used to find their combination potency before we study to evaluate the interactions of both of them by a checkerboard method. The results showed at table 2 and figure 2.

Table 2. Comparison of inhibition zones (d) of C. sappan methanolic extract, Clove oil and their combination

C. sappan methanolic extract (Sc) 8 mg/mL	Clove oil (Co) 20 µL/mL	Combination Sc (8 mg/mL) and Co (20 µL/mL)
16.7 mm	11.3 mm	19.3 mm

There is a positive correlation to combine both of sample being an formula of antiacne. For further investigated we need to evaluate the interaction by checkerboard method. This method can predict the synergism interaction, zero interaction or antagonist between of two agents to obtain the fixed concentration ratio of the mixture[20].

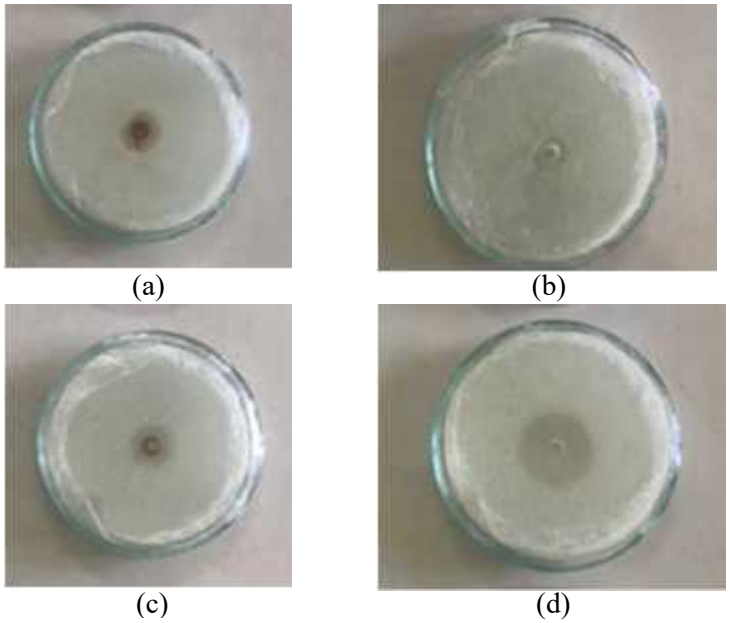


Figure 2. Agar well diffusion plates of antibacterial assay of *C. sappan* methanolic extract (a), clove oil (b), combination *C. sappan* methanolic extract and clove oil (c) and doxycycline (d)

4. Conclusion

The combination of C. sappan methanolic extract and clove oil has the potential to be developed into an antiacne product so it requires further research to find the fixed concentration ratio from both of them to enhance their activity by checkerboard method.

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