

Combination of Sandalwood and Ceremai Leaf Extracts as In Vitro Therapy for Canker Sores

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Abstract

[Combination of Sandalwood and Ceremai Leaf Extracts as In Vitro Therapy for Canker Sores] Recurrent aphthous stomatitis (RAS) or canker sores is a condition characterized by the presence of ulcerated lesions on the oral mucosa. Generally, the cause of canker sores is an infection of the bacteria *Staphylococcus aureus* and the fungus *Candida albicans*. The variety of flora that grows in Bali, has long been trusted by the community as an alternative to modern medicine. Based on Lontar Usadha Taru Pramana, the leaves of sandalwood (*Santalum album* L.) and ceremai (*Phyllanthus acidus* (L.) Skeels) can be applied as a treat for canker sore therapy. Therefore, this research aims to evaluate the combination of sandalwood and ceremai for their antibacterial and antifungal activities. The plant leaves were obtained from Karangasem-Bali. The fine powder mixture of both ingredients was extracted with 96% ethanol by maceration method, obtaining a thick extract. Antimicrobial activity test was done by Kirby-Bauer method for both microbes, *S. aureus* and *C. albicans* with extract concentrations of 25%, 50%, 75%, and 100%. Antibacterial and antifungal screening results showed that the inhibition zones formed were categorized as very strong, ranging from 25-30 mm and 20-27 mm. The maximum antimicrobial activity was obtained by 100% of the extract concentration, while the minimum inhibitory concentration was observed at 25%. Based on this study, further study should be focused on developing a herbal product using a combination of cendana and ceremai leaf extracts to treat canker sores.

Keywords: herbal plants, Recurrent Aphthous Stomatitis, bioprospecting, *Santalum album*, *Phyllanthus acidus*

INTRODUCTION

Recurrent aphthous stomatitis (RAS), often referred to as canker sore, is an ulcerated lesion on the oral mucosa without being followed by signs of other diseases (1). The initial symptom felt by sufferers is pain from single or multiple ulcers and this persistent painful oral ulcer lasting for several days to months (2). Recurrent aphthous stomatitis can be caused by bacterial infections, fungal infections, genetic factors, nutritional deficiencies, local trauma, and disorders of the immune and endocrine systems (1). RAS can be caused by both normal flora from within the oral cavity (such as *Candida albicans* fungus), and other

microorganisms from outside the oral cavity (such as *Staphylococcus aureus* bacteria) (3)(4).

This disease is classified as the most common disease in society, with a varying prevalence rate of around 5-66%. RAS most often occurs at the age of 20-29 years with a percentage of 36.28% (5). RAS ulcers are typically self limited and may recur several times a year. Unlike cold sores, which are caused by the Herpes Simplex virus (HSV), canker sores are not infectious (6). However, this disease causes a very disturbing symptom since it occurs around the patient's mouth. It can be distressing when the patient talks or eats.

Topical corticosteroids are the first line of RAS treatment. Furthermore, systemic steroids are generally indicated for more severe cases. Immunosuppressants are sometimes reserved to prevent the formation of new lesions and to decrease bad effects experienced with systemic steroids (2). Based on the etiology, therapy for canker sores caused by infections generally uses antibiotics or antifungals. However, currently, many of its uses are experiencing a resistance phenomenon (7). In this state, researchers need to improve antibiotics and antifungal efficacy, such as replacing modern medicine by alternating with herbs (7).

Indonesian people have long known and utilized various types of efficacious plants to treat medical problems. An example is the Balinese people, who have been utilizing surrounding plants with local cultural elements for generations. Various types of herbal plants from the Bali region are recorded in the Lontar Usada Taru Pramana (8). Several parts of the plant are said to be efficacious, including leaves, shoots, stems, roots, fruits, saps, and tubers. Plants that can be used as canker sore medicine in the study are sandalwood and ceremai (8).

Known for its fragrance wood and essential oil, sandalwood (*Santalum album* L.) is a plant widely traded from India and it is native to the tropics. The essential oil that can be extracted from the heartwood of this plant is usually used in various therapies, cosmetics, foods, and pharmacological industries (9). In previous publications, sandalwood essential oil was reported to possess antiviral, antifungal, and anticancer properties (10).

Ceremai plant (*Phyllanthus acidus* L. Skeels), known as gooseberry is an underutilized botanical plant. This plant has bioactive compounds, such as antimicrobial, antioxidant, immunomodulator, and acidifier which is a potential phytobiotic (11). Previous studies suggested that ceremai leaf extract might be a good antibacterial and antifungal herbal ingredient. Apparently, it has an antibacterial effect on oral mucosal wounds in Wistar rats (*Rattus norvegicus*) (12).

METHOD

a. Plant Collection and Simplicia Preparation

The leaves of *S. album* (L.) and *P. acidus* (L.) Skeels were collected from Pempatan Village, Rendang District, Karangasem Regency, Bali Province. The two ingredients were sorted by size, washed clean, dried under shade and in the oven, then ground into a fine powder with a blender. The powder was sieved with a 40-mesh sieve, producing simplicia (4).

b. Simplicia Extraction

Obtained simplicia were mixed (50:50), then extracted with 96% ethanol by maceration method. Ethanol was used because it could attract more active compounds compared to any other types of organic solvents (13). First maceration was done in a closed container for 24 hours with occasional stirring. Then, the filtrate was taken from the filtered maceration results, while the dregs were macerated again in 24 hours using the same method. The filtrate resulting from the last maceration was thickened with a rotary evaporator (14).

c. Antimicrobial Test

Antibacterial and antifungal activities were tested by Kirby-Bauer Disc Diffusion Antimicrobial Susceptibility Procedures. The details are in Table 1.

Tabel 1. Test Procedure Comparison for Antibacterial and Antifungal Activity (15)(16)(17)(18)

Antimicrobial Test	Antibacterial	Antifungal
Microbe	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Medium	NA	NA
Extract Concentration	20µL	25µL
Positive Control	Chloramphenicol	Nystatin
Negative Control	Aquadest	Aquadest
Procedure	NA medium was solidified onto ten plates. Bacterial and fungal suspensions were added onto the medium surface in each plate before placing discs. A certain amount of extract or control substances were dropped onto the discs. Incubation was carried out at 37°C for 2 times 24 hours. The diameter of inhibition or clear zone (mm) was measured by a caliper.	

d. Data Analysis

The data analysis technique used is descriptive analysis to observe the test results of antibacterial and antifungal activity (19). The data of each test result were analyzed by one-way analysis of variance (ANOVA) (4).

RESULT & DISCUSSION

Plant collection and simplicia preparation was conducted independently by the researchers. In order to obtain good condition leaves, 700 grams of sandalwood (*S. album L.*) leaves and 600 grams of ceremai (*P. acidus (L.) Skeels*) leaves were slowly picked and sorted, removing any dirt brought in at the same time. Leaves prepared were dried under the shade and in the oven at 50-60°C. The dried leaves, both sandalwood and ceremai were ground by a blender to form 2 jars of fine powder (Figure 1). Sandalwood and ceremai leaf powder, each weighing 50 grams, were mixed and became simplicia.



Figure 1. Sandalwood & Ceremai Leaf Powder

In this research, the simplicia was extracted by a maceration method, which was done by dissolving the mixture of sandalwood and ceremai powder in ethanol 96 at the ratio of 1:1 (Figure 2). This maceration was done in a closed container, wrapped by a black plastic bag, and kept safe in a closed cupboard to avoid direct sun exposure. First maceration was carried out in 24 hours. The macerate was filtered using a cloth and the result was stored in a refrigerator. The dregs obtained from the first maceration are remacerated again for 24 hours with the same method.

All of the maceration results combined, as much as 1L, was evaporated by rotary evaporator to form 2,435 grams of thick extract. The extract was stored in a urine pot and labeled with its name and weight (Figure 3).



Figure 2. Maceration Method Storage Process



Figure 3. Condensed Ethanol Extract of Sandalwood and Ceremai Leaves

The antibacterial screening of *S. album L.* and *P. acidus (L.) Skeels* leaves extract showed that the inhibition zone formed was categorized as very strong (Figure 4). The four concentrations had inhibition zones with the diameter of 25-30 mm. Then, the positive control, namely the antibiotic chloramphenicol, formed an inhibition zone of 40 mm. Meanwhile, the negative control in the form of distilled water did not form an inhibition zone at all. The inhibition zones produced in the antibacterial screening were classified into groups: weak level: 0-5 mm; moderate level: 5-10 mm; strong level: 10 - 20 mm; and very strong level: > 20 mm (20). The antibacterial inhibition zones of concentrations of 25%, 50%, 75%, 100% are listed in Table 2.

Table 2. Antibacterial Inhibition Zone of *S. album L.* and *P. acidus (L.) Skeels* Leaves Extract

Concentration	Inhibition Zone Diameter	Description
25%	25 mm	Very Strong
50%	26,7 mm	Very Strong
75%	28 mm	Very Strong
100%	30 mm	Very Strong
Positive Control (+)	40 mm	Very Strong
Negative Control (-)	0 mm	Weak

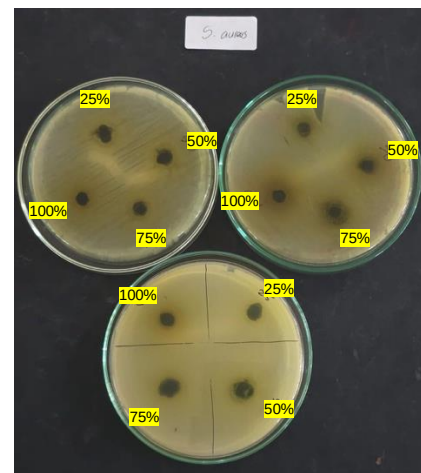


Figure 4. Antibacterial Screening of *S. album L.* and *Ps acidus (L.) Skeels* Leaves Extract against *S. aureus*

S. album L. and *P. acidus (L.) Skeels* contain active compounds such as flavonoids, tannins, alkaloids, saponins, and essential oils that contribute to antibacterial activity (10)(11). The active compounds in these extracts interact with bacterial cellular components which result in bacterial death. Death is caused by active compounds such as flavonoids and tannins that inhibit the activity of enzymes that play a role in bacterial metabolism (21). Disruption of this metabolism causes disruption of DNA replication and RNA transcription that

inhibits the growth and division of bacterial cells (22).

In addition, alkaloids and saponins can also interact with lipids in the bacterial cell membrane. This interaction results in a decrease in the supply of lipids, resulting in damage to the membrane structure and leakage of cell contents (23). Several other studies have also shown that the active compounds in this extract can stimulate the production of ROS in bacterial cells, resulting in oxidative damage to cellular components (24). This damage causes disruption of the metabolism of proteins, lipids, and bacterial DNA.

Antifungal screening of *Santalum album* L. and *Phyllanthus acidus* (L.) Skeels leaves extract showed that the clear zone formed was categorized as very strong (Figure 5). The four concentrations had clear zones with diameter of 20-27 mm. Positive control used, namely antifungal nystatin formed clear zone with a diameter of 40 mm. Meanwhile, the negative control in the form of distilled water did not form a clear zone at all. The clear zones produced in the antifungal screening consist of several groups, including: none: ≤ 10 mm; weak level: 11-15 mm; moderate level: 16-20 mm; and strong level: > 20 mm (25). The antifungal clear zones from concentrations of 25%, 50%, 75%, 100% are listed in Table 3.

Table 3. Antifungal Inhibition Zone of *S. album* L. and *P. acidus* (L.) Skeels Leaves Extract

Concentration	Inhibition Zone Diameter	Description
25%	20 mm	Moderate
50%	25 mm	Strong
75%	26 mm	Strong
100%	27 mm	Strong

Positive Control (+)	40 mm	Strong
Negative Control (-)	0 mm	Weak

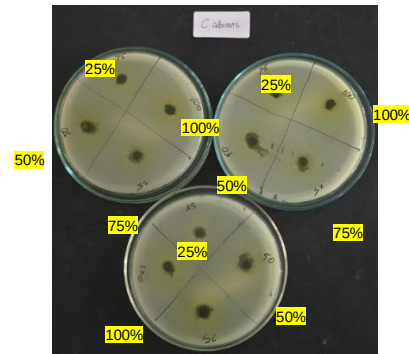


Figure 5. Antifungal Screening of *S. album* L. and *P. acidus* (L.) Skeels Leaves Extract Against *C. albicans*

S. album L. and *P. acidus* (L.) Skeels leaves extract performed an antifungal activity due to the mechanism of disturbing structure and function of fungal cells. Some active compounds in the extract were suspected as ergosterol inhibitors. Ergosterol is an important constituent in a fungal cell. Therefore, the inhibition occurring can cause any disruption of the cell membrane. The disruption can be worsened if a component, such as a flavonoid starts to precipitate the increased production of reactive oxygen species (ROS) in fungal cells. This might stimulate oxidative damage in protein and lipid metabolism that can interfere with any other cell dysfunctions. Further, active compounds in this extract were also responsible for the disruption of carbohydrate and nucleic acid synthesis in fungal cells. (17).

CONCLUSION

In conclusion, this study provides a preliminary finding that combination of *S. album* L. and *P. acidus* (L.) Skeels leaves extract showed as strong inhibition against *S. aureus* and *C. albicans*, the two causative agent of canker sores. Phytochemicals

compositions of = *S. album L.* and *P. acidus (L.) Skeels* leaves extracts contained flavonoids, tannins, alkaloids, saponins, and essential oils, which play an important role in the observed antibacterial and antifungal activities. Based on this results, further study should be focused to explore the development of herbal product to overcome cake sores based on the combination of these two plants.

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