# Anti-fungal capacity of Saga leaf (*Abrus precatorius l*) towards *Candida albicans* testing

**Muslim Paranto NA, Karlina Harjadinata, Warta Dewi**

**Department of Dental Material Science & Technology Faculty of Dentistry Universitas Padjadjaran**

**ABSTRACT**

The aim of this study was to evaluate the antifungal effect, by determining the Minimum Inhibitory Concentration (MIC) of saga leave extract againts *Candida albicans*, as a causal of oral candidiasis. The saga leave were extracted following masseration method. *Candida albicans,* as a tested fungal was isolated from patients at the Oral and Dental Hospital Faculty of Dentistry Universitas Padjadjaran. They were cultured on Sabouraud Agar plate, incubated at 37°C for 18-24 hours. The isolation and the identification of *Candida albicans* were based on colonial morphology and the fermentation of glucose, maltose, succharose, dan lactose at the Microbiology Laboratory. This experimental laboratory study was conducted according to a serial dilution method from 16 mg/ml up to 0,25 mg/ml of saga leave extract with two-fold dilution in three repetitions. The result showed that the Saga leave extract be able to inhibit *Candida albicans* at minimum 2 mg/ml concentration. As a conclusion, the concentration of 2 mg/ml or more of saga leave extract has antifungal effect againts *Candida albicans*.

**Key words:** *Saga* leave extract, *Candida albicans*, antifungal effect

## INTRODUCTION

Oral infection may be caused by various microorganisms including bacteria, virus and fungus. One of the fungus that is frequently found in the oral cavity is *Candida albican.*[1] *Candida* can be found on normal mucous membrane such as oral mucosa, vaginal mucosa and digestive tract mucosa. *Candida albicans* that is commensal is sometime found in the healthy human digestive tract.[2] In certain conditions, such as in cell immunity disturbance, such as in AIDS patients or the lost oral normal flora due to broad spectrum antibiotic therapy, *Candida* may become invasive and occurs in the form of various acute and chronic infection or localized lesion. This is caused by the lack of nutrition competition with other organisms that *Candida* can grow rapidly. Therefore, basically, the infection is very much influenced by the host condition, not only by the virulence of the fungus.

*Candida albicans* infection can be treated by oral *nystatin.*[3] For the digestive tract disease due to *Candida,* intravenous *amphotericin B* is used.[4] Besides, it can also be treated topically using *clotrimazole* and orally using *ketoconazole*.[3] The use of those drugs may have side effects including nausea, burning sensation, erythema, and disturbing edema. Another side effects that sometimes occur are headache, epigastric pain, photophobia, paresthesia, and gum bleeding.[5]

The prevalence of candidiasis is still high in

*Correspondence author: Muslim Paranto NA, Department of Dental Material Science & Tech. Faculty of Dentistry Universitas Padjadjaran. Jl. Sekeloa Selatan No. 1 Bandung, West Java-Indonesia, Tel./Fax: +6222-2504985/2532805*

Indonesia with an average of 41.66%.[6] Currently, the treatment given generally uses synthetic medicine. However, those drugs often trigger side effect which is a disadvantage for the patients. Synthetic drugs are also toxic for human who needs to neutralize the toxic nature in the heart. Another problem is the price of the synthetic drugs. The expensive treatment and the existing social economic condition of the population, especially the lower class population makes treatment and cure difficult. In relation with the above condition, other alternative treatments are started to be sought.

Herbal medicine that uses herbs as the main materials is now starting to be developed. In addition to the fact that the price is more affordable, the side effect of this herbal medicine is mild. People can grow the herbs in the area surrounding the house so that they can use it anytime. The term that is often used for the grown herbs in the house is *TOGA (Tanaman Obat Keluarga)* or family herbal plants.

One of the herbs that are potential to grow is *saga* (*Abrus precatorius*), a wild bush that is frequently seen in rural areas. The parts that are often used for herbal medicine is the leaves and seeds.[7] The leaves are often used as antibiotics.[8] It can also be used as flu and dysentery.[9] In addition, *saga* leaf can also be used to reduce mouth ulcers.

The various functions of *Saga* (*Abrus precatorius*) in medicine are due to the element in the plant. The sweet *saga* leaf is originally thought as containing of glychirrhizine only but further study reveals that it also contains glycoside including anti bacterial Abrucyde A-D and Abrusgenin and a small amount of toxic abrin.[10] The seed contains alkaloid compound including abrin, choline, trigoline.[8]

The studies performed[11], show that *saga* leaf extract can inhibit the growth of *Pasteurella multosida* and *Corynebacterium sp. Saga* seed extract has the ability to inhibit the growth of Moraxella sp. Prajogo and Dyatmiko[12], have performed a study using *saga* leaf extract using three types of solvents, i.e. methanol, ether, and butanol showing that the three solution is able to prevent *Candida albicans* growth through contact for 16 hours. From the results of this study, it has been proven that butanol fractioned *saga* extract has the best inhibitory power.

The aim of this study is to describe the presence antifungal property and determine Minimum Inhibitory Concentration (MIC) of *saga* leaf extract towards *Candida albicans* to establish *saga* leaf extract as the alternative medicine for oral and dental diseases, particularly those caused by *Candida albicans* because different locations of the disease can lead to different results.

## METHODS

The study performed is laboratory experiment to determine the antifungal ability of *saga* leaf extract (*Abrus precatorius*) through Minimum Inhibitory Concentration (MIC) towards *Candida albicans* based on the sensitivity test using serial dilution method. The study is performed in the period of July 2006 to February 2007 at the Microbiology Laboratory of Faculty of Dentistry Universitas Padjadjaran. The population is the *saga* plants grown in Bandung district with extracted wet *saga* leaf grown in Ciwidey area.

The extraction of *saga* leaf was performed using maceration method. Five hundred and fourteen grams of *saga* leaf powder was added to 2.5 litres of 95% methanol solvent and let still for 5 days with regular mixing every 24 hours. After 5 days, the mixture was filtered and the sediment was separated. The sediment was then placed in a tube to be re-extracted by flowing methanol from the upper part of the tube to dilute the residual active agents. This treatment was repeated three times so that the compound of the *saga* leaf can be extracted optimally.

The filtered extract was then treated by rotavapor producing dry extract. The methanol fractionated dry extract was then suspended with water and re-extracted using ether. The ether fraction was dried and the re-extracted using 70% butanol. The extract fraction was then treated with rotavapor to produce dry extract to be used as test material.

The test fungus was isolated *Candida albicans* from oral mucosa swab. The culture media used for sensitivity test was Saboraud Glucose Agar.

To get test fungus, one *oese* of oral mucosa swab. The swab was inserted to a tube containing bullion to be cultured in SGA and for further microbiology tests.

**Table 1. Serial dibullion of *Saga* leaf exract solution.**

| Tube Number | Bullion (ml) | *Saga* leaf extract (SLE) | SLE dilution (x) | SLE final concentration (mg/ml) | Candida suspension (Mc Fahrland 0.5) (ml) |
|---|---|---|---|---|---|
| 1 | - | 4 ml | 1 | 16 | 0.1 |
| 2 | 2 | 2 ml of tube 1 | 2 | 8 | 0.1 |
| 3 | 2 | 2 ml of tube 2 | 4 | 4 | 0.1 |
| 4 | 2 | 2 ml of tube 3 | 8 | 2 | 0.1 |
| 5 | 2 | 2 ml of tube 4 | 16 | 1 | 0.1 |
| 6 | 2 | 2 ml of tube 5 | 32 | 0.5 | 0.1 |
| 7 | 2 | 2 ml of tube 6 | 64 | 0.25 | 0.1 |
| 8 | - | 2 ml of tube 7 | - | KN | - |
| 9 | 2 | 2 ml of tube 8 | - | KP | 0.1 |

For microscopic test material (MTM) a direct preparation on object glass was made from the oral mucosa swab. After the preparation was fixated and dyed using Gram dye, it was observed under the microscope. In addition, one *oese* test material was cultured in SGA using lining method and then incubated for 18-24 hours in a temperature of 37ºC.

On the next day, the suspected colonies of *Candida albicans* on SGA were isolated and identified microscopically through Gram dye and biochemical tests based on fermentation test on carbohydrate: glucose, maltose, saccharose, and lactosa. As the pH indicator, red phenol was added. After the gas was formed, Durham tube is placed in an inversed position.

The determination of minimum inhibitory concentration is performed based on serial dilution method in reaction tube. The *saga* leaf extract was weigh, 80 mg, placed into 5 ml sterile bullion to get standard solution with a concentration of 16 mg/ml. Using pipettes, 4 ml of standard solution is placed into the tube. Reaction tube number 2-7 and 9 is filled with 2 ml of sterile bullion. In tube 2, 2 ml of the solution of tube 1 was added and then mixed until homogenous and a solution with 2 x dilution of 8 mg/ml. Furthermore, 2 ml of mixture from tube 2 was removed to tube 3, mixed to homogenous until 4 x dilution was gained or a concentration of 4 mg/ml. This dilution technique was applied to the next tubes to reach a concentration of 2; 1; 0,5; 0,25 mg/ml in tube 4, 5, 6, and 7. The residual 2 ml of tube 7 is inserted to empty tube 8 as negative control.

The *Candida albicans* suspension was made with a turbidity that matched Mc Fahrland 0.5 standard and was placed into tube 1-7, each 0.1 ml. 0.1 ml of the suspension was removed using pipettes to tube 9 that only contained sterile bullion as positive control.

All cultures in the tube were incubated in a temperature of 37°C for 18-24 hours. The next day, the cultures in the tube were observed for its turbidity. The tube that showed clear culture showed that the test material was able to inhibit the growth of the microorganism. On the contrary, the turbid solution showed that the test microorganism grew because the test material was unable to inhibit it. The Minimum Inhibitory Concentration is the minimum concentration of the test material that is able to inhibit the microorganism growth in the tube.

To assure that the microorganism growth in each tube was actually presence, one *oese* was taken and growth in sectors on SGA plate. The SGA was then incubated aerobically in a temperature of 37° C for 18- 24 hours. The area that did not have *Candida albicans* showed the *saga* leaf extract concentration that has antifungal property. The smallest extract concentration that shows the lowest *Candida albicans* growth is the Minimum Inhibitory Concentration of *Saga* leaf extract.

## RESULTS

### Saga leaf extraction result

The extraction of *saga* leaf was performed using maceration method with 95% methanol solvent that produces thick solution that, if it is left still, will produce sediment with water on it. To

remove the water produced during the procedure, the extract was treated using rotavapor. After the extraction using ether solvent was performed, drier extract was gained with dark green color. From 514 grams of dry *saga* leaf, 40 grams of *saga* leaf extract was gained.

The *saga* extract test with ether fraction towards *Candida albicans* does not show good result because *Candida albicans* can still grow in the concentration of 16 mg/ml. On the contrary, *saga* extract can be re-extracted using 70% butanol solvent that produces good inhibitory property towards *Candida albicans*.

**Fungus isolation and identification result**

The microscopic examination towards MTM collected from oral mucosa swab using Gram dye shows that the fungus colony is oval, Gram positive with a size that is bigger than the bacterial size. Although fungal colonies are more dominant, the colonies are still mixed with other bacterial colonies, especially Gram Positive Rod bacteria.

The Sabouraud's Glucose Agar (SGA) cultures from the MTM and which are incubated in a temperature of 37ºC for 18-24 hours show a colony growth with yellowish white characteristic, yeast like odor, smooth and slippery colony surface that is suspected that the colonies are *Candida albicans* colonies. On SGA, there are also other bacterial colonies with a characteristic that is similar to *Candida* but produces phlegm.

The microscopic examination results from the SGA cultures show an oval fungal image with a size bigger than bacterial size, purple and Gram positive. The fermentation test for colonies suspected as *Candida albicans* towards carbohydrates show a fermentation reaction for glucose, maltose and sucrose. The reactions are marked by the change in the color of the red phenol indicator to yellow. In the tube containing glucose and maltose, gas as a result of the fermentation is produced as an addition of change in indicator color. In sucrose, no gas is formed. Fermentation with lactose does not show any reaction, the color is still red and no gas is formed.

Based on the identification, both from the morphological examination of the colonies, microscopic examination and fermentation test, the colonies grown on SGA can be diagnosed as *Candida albicans*.

**Minimum inhibitory concentration of *saga* leaf extract towards candida albicans**

Sensitivity test of *Candida albicans* towards *saga* leaf based on serial dilution method shows that the cultures in tube 1-3 are clear, only dark green sediment from *saga* leaf extract is seen. Cultures in tubes number 4-7 are changed into a turbid culture, almost as turbid as the positive control tubes. From all repetation, the negative control tube is still clear and positive control is always turbid.

The changes in cultures in the tube to a turbid solution show that fungus have grown. On the contrary, the clear culture shows lack of growth. From this visual observation, it can be estimated that the minimum concentration that can inhibit *Candida albicans* is in tube 4. The result of the observation can be observed in the below table.

The result of sectored cultures on SGA shows that there is no growth in sector 1 (16 mg/ml); 2 (8 mg/ml); 3 (4 mg/ml), and negative control. This shows the presence of *saga* extract inhibitory property towards *Candida albicans* growth. In sector 4, which is collected from culture in tube 4 with a *saga* extract concentration of 2 mg/ml, shows fewer *Candida* growth compared to fungal growth in sectors 5-7. This shows that the minimum concentration that can inhibit *Candida albicans* is 2 mg/ml.

**DISCUSSION**

From the performed extraction, it turns out that *saga* leaf contains a lot of water. From 14 kg of wet *saga* leaf, only 514 grams dry *saga* leaf is collected. This shows a high water content of *saga* leaf, more or less 95%. From the overall weight of dry *saga* leaf 40 grams of *saga* leaf extract is collected. This amount shows that the active content of *saga* leaf is 7.8% of its dry weight.

The change in red phenol indicator into yellow is caused by decreased solution pH. Basically, fermentation is a process of carbohydrate molecule break down, which in this study glucose, maltose, sucrose and lactose are used, that creates acid and sometimes followed by hydrogen or carbon dioxide gas as the side product of the fermentation. The gas formed will be seen as air bubble in the tube. The effect of acid production

**Table 2. Results of the determination of minimum inhibitory concentration towards *Candida albicans* in Tubes.**

| Sample | Repeat | SLE concentration (mg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 16 | 9 | 4 | 2 | 1 | 0.5 | 0.25 | KN | KP |
| 1 | 1 | - | - | - | ± | + | ++ | ++ | - | ++ |
| | 2 | - | - | - | ± | + | ++ | ++ | - | ++ |
| 2 | 1 | - | - | - | ± | + | ++ | ++ | - | ++ |
| | 2 | - | - | - | ± | + | ++ | ++ | - | ++ |
| 3 | 1 | - | - | - | ± | + | ++ | ++ | - | ++ |
| | 2 | - | - | - | ± | + | ++ | ++ | - | ++ |

± : slightly turbid; + : turbid; - : clear; ++ : very turbid.

during fermentation process is lower pH. As the indicator of pH change, the red phenol will turn to yellow. On the contrary, if there is no fermentation reaction, the indicator will stay red.[13]

When determining minimum inhibitory concentration visually, from all repeats, the negative control tube stays clear because it only contains *saga* extract suspension in bullion, without *Candida albicans* suspension. This shows that *saga* extract used as test material does not contain bacteria. In positive control tube, only *Candida albicans* solution in bullion is found, without *saga* leaf extract leading to turbid appearance in every repeat. This means that the test microorganism is able to grow in the media used.

The sectored culture in SGA shows a minimum concentration of *saga* extract in solution that has an inhibitory property towards *Candida albicans* as 2 mg/ml. The inhibitory property owned by *saga* is caused by the presence of glycirrhizine, glycocide compound that makes *saga* leaf sweet. In addition, *saga* leaf also contains flavanoid compound and lypolytic enzyme. Those compounds have anti bacterial activity.

On average, *saga* leaf contains 15% glychirrhizine of all active contents in *saga* leaf.[14] During extraction, glychirrhizine can be hydrolized into sugar compound. Strong interaction between sugar molecule and water molecule in solution leaves a small amount of water to support microorganism life. Sugar level in *saga* will make the environment surrounding bacteria becomes hypertonic. The high osmotic pressure will cause liquid movement from bacterial cell to the environment leading to bacterial cell shrinkage (plasmoptysis) and microorganism cell death.[15]

The flavanoid compound in *saga* leaf, such as abrectorin, also influences the inhibitory property towards *Candida*. In general, flavanoid compound is a lypophylic compound. The more lypophilic the flavanoid the more destruction it brings to the cell membrane leading to cell component lysis that in turns will cause cell death. Flavonoid binds with cell wall lipid layer and destroy lipid structure that will disturb metabolism, nutrient transport, and increase cell wall permeability.[16]

Other agent found in *saga* leaf is lypolytic enzyme[14], that also plays a role in inhibitory property of *saga* leaf despite the relatively small amount of this enzyme is seen in the leaf. The mechanism of this enzyme is not too different from the flavanoid compound, i.e. destructing lipid layer leading to disturbance in fungal metabolism and nutrient transport.

From this study, it is proven that *saga* leaf has inhibitory property towards *Candida albicans*. This implies that *saga* leaf can be developed as an alternative medicine in therapy of *Candida albicans* infection.

Until recently, *saga* leaf has been used by the people to cure various diseases. In addition to the antifungal property, glychirrhizine found in the leaf can also work as expectorant.[17] This is the reason why *saga* leaf is often used as coughing cure.

The use of *saga* leaf as a medicine does not make it necessary to use it in the extract form. It can be use in a simpler form such as in the form of traditional mouthwash. Dry *saga* leaf can be ground and then mixed with warm water and used as mouthwash. It can also be chewed to heal mouth ulcer or stomatitis caused by *Candida albicans*. Further research is very important because this leaf has many beneficial use that it can be used as alternative medicine in the medical world.

**CONCLUSION**

Based on the study results using serial dilution, it can be concluded that the Minimum Inhibitory Concentration (MIC) of *saga* leaf extract (*Abrus precatorius L*) towards *Candida albicans* is 2 mg/ml. To inhibit *Candida albicans,* it is better to use a *saga* leaf extract concentration of 2 mg/ml or more.

**REFERENCES**


1. David B. Bacteriology and immunology for students of medicine. London: William Clawes; 1989.
2. Schmid J. Molecular microbiology of candida albicans. Massey University; 2003.
3. Eversole LR. Oral medicine. A pocket guide. Philadelphia: W.B. Saunders Co.; 1996.
4. Bhattacharyya I, Cohen DM, Sol S. Red and white lesions of the oral mucosa. In: Burket's oral medicine diagnosis & treatment. 10th ed. Ontario: BC Decker Inc.; 2003.
5. Ganiswarna SG. Farmakologi dan terapi. Jakarta: Gaya Baru; 1995.
6. Soenartyo H, Astuti ER. Pengaruh penurunan kadar Fe dan kebersihan rongga mulut wanita hamil terhadap terjadinya kandidiasis. Maj Il Kedokt Gigi FKG Usakti 1999; Edisi Khusus FORIL VI.
7. Simpson. Herbal botany. Singapore: Fang & Song Printers.; 1986.
8. Wistpal E, Jensen PM. Plant resource 07. Prosea; 1989.
9. [cited 2006 Oct] Available from:http://www.bsienvis.org.htm.
10. Dwivedi SK, Sharma MC. Therapeutic evaluation of an indigenus drugs formulation againts Scabies. Indian J Med 1985.
11. Prajogo B, Dyatmiko W. Aktivitas antimikroba daun Abrus precatorius. Warta Tanaman Obat Indonesia 1993.
12. Cappuccino JG, Sherman N. Microbiology: A laboratory manual. 6th ed. New York: Benjamin Cummings; 2001.
13. Sundari D, Wien M, Pudjiastuti. Informasi penetian khasiat keamanan dan fitokimia tanaman Saga. Warta Tumbuhan Obat Indonesia 1993.
14. Burton G, Engelkirk PG. Microbiology for the health sciences. 5th ed. New York: Lippincott; 1996.
15. Jawetz E, Melnick EA, Adelberg. Mikrobiologi kedokteran. Edisi ke 20. Jakarta: EGC; 1996.
16. Society of Japan Pharma. Japanesse pharmacopeia. 14th ed. Tokyo: Yakuji Nippo, Ltd.; 2001.