POTENTIALS OF BETEL LEAF INFUSION (*Piper betle* L), LIME PEEL EXTRACT (*Citrus aurantifolia*) AND BUNDUNG EXTRACT (*Actinoscirpus grossus*) AS CANDIDIASIS THERAPY

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Abstract: Candidiasis is an infection caused by the fungus Candida, especially Candida albicans. *It often occurs in the mouth and sex organs, also in the nails, respiratory tract, digestive tract, and anus. Betel leaf stew is often used by people to gargle and clean the intimate organs. The nature of the betel leaf solution is easily oxidized and turns brown. Therefore, research needs to be done by mixing betel leaf (Piper betle L) with lime (Citrus aurantifolia) to maintain the color of betel leaf, coupled with natural ingredients that have antimicrobial properties, namely lime peel and bundung plants (Actinoscirpus grossus). With the hypothesis of the joining of three natural materials that have antimicrobial activity can strengthen the inhibitory activity of the fungus Candida albicans. This research was conducted by making a betel leaf infusion formulation, lime peel extract, and bundung extract with a concentration of 20%, 30%, 40%, and 50% which were then tested to determine the effect of the infusion formulation on the inhibitory activity of Candida albicans by the method spread on Saburoud Dextrose Agar media. Based on Zuraidah's research (2015), 80% and 100% betel leaf extraction has activity on Candida albicans. From the results of this study significant results were obtained at a concentration of 50% of a mixture of betel leaf infusion formulations, extracts of lime peel and bundung extract had the same inhibitory properties as the hand sanitizer antiseptic liquid against Candida albicans. Thus it can be concluded that the 50% test formulation of betel leaf infusion mixture with lime extract and bundung extract has greater potential than betel leaf extract alone as a candidiasis therapy.*

Keywords*: Piper betle L, Citruss aurantifolia, Actinoscirpus grossus, Antiseptik, Kandidiasis, Candida albicans*

INTRODUCTION

Candidiasis is an infection because of Candida fungi which has more than 20 types, but the most common is *Candida albicans*. Candidiasis can appear on various parts on human body, and the most common parts are on oral and sexual areas, and nails, respiratory tract, anus, and digestive tract.[1]

In normal condition, Candida itself has been already on human skin, but if it is over breed, especially on body part that is moist, it will lead to infection. Several factors that can increase infection risk is in baby and elderly, low immune system, chronic disease, and consuming cortisteroid and antibiotic for chemotherapy patients.[1]

Candidiasis become a scary scourge for female, adolescent, and housewives. This disease is caused by Candida albicans fungi. For sufferers who are less concerned with the initial appearance of this disease, it will cause sufferers to experience severe vaginal discharge. As a result, many sufferers have trouble in having children, and cause cysts on the wall of the uterus or in the cervical canal.[2]

Indonesia in producing chemical medicines still uses raw materials from India and China. This is a common challenge in making the herbal industry a mainstay of the Republic of Indonesia.[3]

The using of herbal is one of steps in back to nature style by utilization of natural ingredients in daily needs. Indonesia is rich in biodiversity; our ancestors also left the use of natural ingredients as medicine. Betel leaf is widely used by people as traditional herbal medicine or boiled it to be used in intimate organs. Betel leaf contains of essential oil that consists of alkaloids, tanine, terpenes, cinsele, cadinene, camphene caryophyllae, pineae, limonene, chavicol, allypyrocetechol, ally pyrocatechol, caevacrol, safrole, eugenol and chavibetol.[4] Betel leaf extract has been developed in several forms, for example toothpaste, soap and mouthwash. From research, betel leaf extract alone has inhibitory activity against *Candida albicans* at concentrations of 80% and 100%.[2]

Betel leaf solution is easily oxidized, the color of the solution turns brown. Therefore, researchers tried to combine several natural ingredients that are widely found in Indonesian, especially in South Kalimantan, such as lime (*Citrus aurantifolia*) and bundung (*Actinoscarpus grossus*).

Lime contains lots of vitamin C and citric acid ($C_6H_8O_7$) which can be used as an eco friendly cleaning subtance. Citric acid can prevent the oxidation of a solution such as betel leaf solution. Citric acid decomposes subtances such as sulfate, phosphate, and natrium that is potential in forming kindney stones. Citric acid is also a good and natural preservative, flavor enhancer, buffer solution to control the pH of the solution.[7] The flavonoid content of orange peel has anti-bacterial, anti-fungal, anti-diabetic, anti-cancer and anti-viral activity.[5]

Bundung (*Actinoscirpus grossus*) is an endemic plant from Kalimantan and is used by community as wound therapy because it is believed to contain antimicrobials. From the research of Noval et al (2019), it was found that 1% to 8% of Bundung plant extract levels had activity against *Staphylococcus aureus* and *Escherichia coli*.[6]

Based on those background, mixed formula of natural ingredients can turn betel leaf solution becomes more lasting and does not turn it into brown color. The combination of natural ingredients that have antimicrobial activity is unknown yet against fungi. In view of that fact, a research in finding the formula of lime and bundung combination which have inhibitory activity to *Candida albicans* as herbal for Candidiasis theraphy is should be carried out.

RESEARCH METHOD

This research begins by managing a

research license from LPPM no. 399.1 / ST-Research / LPPM / UNISM / 2020. This research is an experimental study with the method of spreading the fungus *Candida albicans*.

Materials and tools were prepared: *Candida albicans* fungi, SDA media (Saburoud Dextrose Agar), betel leaves, lime, bundung plants, infusion pans, gas stoves, glass jars, beaker glasses, stirring rods, filters and filter paper, analytical scales, ethanol 95%, distilled water, petri dish, magnetic stirrer, autoclave, refrigerator, evaporator, pH meter, viscometer, ose shaft, test tube, NaCl infusion, vortex, BSC (Biological Safety Cabinet), incubator, plastic wrap, tweezers, spreader triangle rod , scissors, knives, flakes, measuring cups, blenders, parchment paper.

Lime (*Citrus aurantifolia*) were bought from traditional market, Ahad Market Banjarmasin. There were 100 of purchased lime, which still had fresh and green peel, signing unripped lime. Preparation of lime peel extract by separating the lime peel from the fruit, washed clean, thinly sliced and dried indirectly for 3 days, into dry simplicia, reduced the simplicia size with a blender to become small simplicia and weighed, obtained 418.50 grams of dry simplicia, put in a glass jar and added 2100 ml of macerated 95% ethanol for 3 days with daily stirring. The results of maceration were filtered, and the volume measured was obtained 1110 ml, evaporated with an evaporator at 40 rpm, 50 ° C, the yield was 36.44 grams of thick extract.

The second extract, the bundung plant extract, was sought by people who know the typical Kalimantan bundung plant, cleaned, dried at room temperature until dry, cut into pieces then made small simplicia in a blender, the result is fibrous simplicia weighed 265 grams, put in a jar divided into three jars and added with 95% ethanol as much as 5720 ml, macerated for three days with daily stirring. The maceration results were filtered which obtained 4150 ml of macaration solution of bundung plant, and then was evaporated using evapotaror at 40 rpm 50°C. The yield was 7,51 grams of thick extract of bundung plants (*Actinoscirpus grossus*).

The filtration of the third ingredient was making infusion of betel leaves (*Piper betle* L). betel leaves were bought at traditional market, Ahad Market Banjarmasin. the chosen betel leaves were the leaves that were dark green colored and still in the whole form of leaves. The betel leaves were cleaned first and drained well, then measured with formulation: 20 gram, 30 gram, 40 gram, and 50 gram. Each portion were added with heated aquades and set into infusion pans, and stemed for 15 minutes. After 15 minutes, infusion pot was cooled, then the betel leaves infusion was filtered and obtained betel leaf infusion solution 20% w/v, 30% w/v, 40% w/v and 50% w/v. Each of these formulations was added with 8 grams of lime juice.

The design for the formulation of the three materials made above began with preparing the ingredients by weighing the lime peel extract for 4 design formulations of 8 grams each, the bundung plant extract weighing 1 gram each for 4 times in weighing. The lime peel extract was dissolved with betel leaf infusion solution, likewise the bundung plant extract was diluted with betel water assisted by a magnetic stirrer. Mix those ingredients into one solution then filtrated and collected in Erlenmeyer and labeled as formulation 20% w/v, 30% w/v, 40% w/v and 50% w/v.

Table 1 Formulation design of mixed betel leaves infusion, lime peel extract, and bundung extract solution test

No.	Materials	F20% b/v	F30% b/v	F40% b/v	F50% b/v
1	Betel leaves infusion	20%	30%	40%	50%
2	Lime extract	8g	8g	8g	8g
3	Lime peel extract	8g	8g	8g	8g
4	Bundung extract	1g	1g	1g	1g

Making SDA (Sabouraud Dextrose Agar) media by weighing 22.75 grams of SDA media material on parchment paper with analytical scales, put it in a glass beaker and dissolved with 350 ml of heated aquades. Dissolving process was assisted well by magnetic stirred 300-400 rpm for 30 minutes, then put it into Erlenmeyer tube whach had been sterilized in autoclave 121°C for 15 minutes. Prepared 10 petri dish 35 ml which had been sterilized in autoclave for 15 minutes and put the media on it. SDA media was ready to be tested for the inhibitory activity of *Candida albicans*.

Planting process of *Candida albicans* and solution formulations test of 20%, 30%, 40%, 50%, positive control of antiseptic hand sanitizer and negative control of distilled water was conducted in the BSC (Biological Safety Cabinet). Starting with preparing tools and materials into the BSC. Preparation of *Candida albicans* suspension, by taking *Candida albicans* with a sterilized ose stalk and put it in a test tube that already has 2 ml of sterile NaCl, homogenized by rotating a vortex device. Take 1 ml of the bacterial suspension with a micropipette and then spread it on SDA (Saboroud Dextrose Agar) media. Besides that, the disc immersion in the formulated solution of 20% w/v, 30% w/v, 40% w/v, 50% w/v has been prepared, positive control of hand sanitizer and negative control soaked in aquades for 15 minutes. The lid of the media petri dish was labeled with 20% w/v, 30% w/v, 40% w/v, 50% w/v, negative control and positive control. The soaked discs were taken one by one with sterile tweezers and planted in SDA media with *Candida Albicans* according to the label attached on the lid. Then the petri dishes are wrapped in plastic wrapping and put in an incubator cabinet at 37 ° C for 24 hours.

The last method was observation of the inhibitory activity of *Candida albicans* after 24 hours incubation. Petri dishes were opened with plastic wrapping, observed whether there was inhibition of the discs that had been implanted with the test solution with positive control and negative control against *Candida albicans*, the diameter of the inhibition was measured with a ruler.

RESULTS AND DISCUSSION

In this research, the betel leaf extract was made by infusion / boiling the fresh cleaned betel leaf. This was carried out as an approach in a way which commonly done by the community. The results of lime peel extraction using 95% ethanol gave 8.7% yield. While the results of the bundung plant extract using 95% ethanol gave 2.83% yield. The less resulted yield depends on particle size, type of solvent, methode, and extraction period. Using ethanol 95% in extracting is considering that fungi and bacteria are hard to grow, non-toxic and having good absorbance. Ethanol can blend with water in every proportion and only requires a little heat for concentrating. Ethanol can solve alkaloids, essential oils, glicosides, curcumine, anthraquinone, flavonoid, steroid, resin, and chlorophyll; while less for lipid, wax, tanine, and saponins.[8-10]

Table 2. Yield of Ethanol 95% and lime (*Citrus aurantifolia*) peel extract and bundung (*Actinoscirpus Grossus*) extract

Materials	Simplicia (g)	Liquid extract (ml)	Thick extract (g)	Yield (%)
Lime peel	418,53	1110	36,44	8,7
Bundung	265,46	4150	7,51	2,83

The determination of formulation test activity used the activity of fungi *Candida albicans* growth with spread method based on the size of resulted inhibiting zone. Test sample was the formulation 20% w/v, 30% w/v, 40% w/v and 50% w/v with negative control aquades and positive control hand sanitizer antiseptic. Each sample resulted different diametre of inhibiting zone.

Table 3. Diametre of Inhibiting Zone on *Candida albicans*

No.	Formulation	Diametre of Inhibitory Power
1	20%	0 mm
2	30%	28 mm
3	40%	33 mm
4	50%	53 mm
5	Positive control	52 mm
6	Negative control	0 mm

The observation of inhibitory power on betel leaf infusion, lime peel extract, and bundung extract to *Candida albicans* results no inhibitory power from formulation 20%, as the result given from negative control group. Formulation 50% w/v has the biggest inhibiting zone, 53 mm, bigger than control positif group (antiseptic and hand sanitizer).

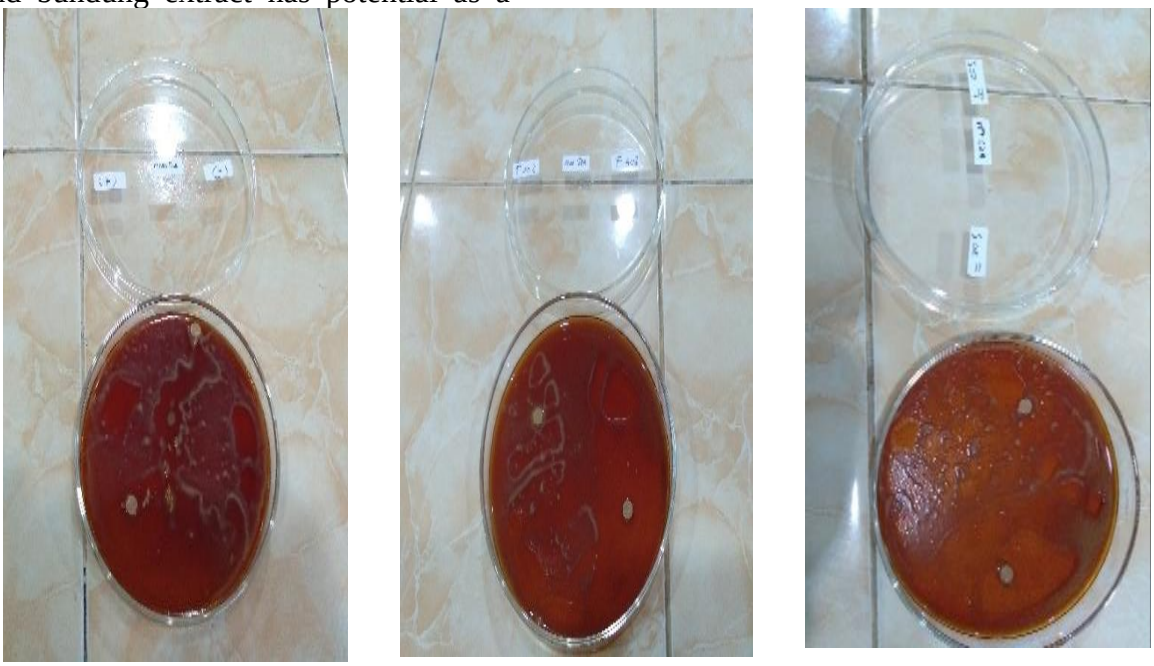
Table 4. The Power of microba activity based on Davis and Stout Criteria

Code	Inhibiting Zone
Weak	≤ 10
Moderate	11-15
Strong	16-20
Super Strong	>20

Based on table 4, it shows that the effect of the concentration of betel leaf (*Piper betle*) infusion formulation, lime peel extract (*Citrus aurantifolia*) and bundung (*Actinosciprus Grossus*) on the growth of *Candida albican* fungi marked by the formation of an inhibition zone. The inhibition zone is formed at concentrations ranging from 30% w / v, 40% w / v and 50% w / v. The concentration of 20% w / v did not provide inhibition against *Candida albican* fungi. Aquades negative control did not have inhibitory power against fungi, the zero-inhibition zone was *Candida albican*. Meanwhile, positive control and concentrations of 30% w / v, 40% w / v and 50% w / v had very strong inhibition of *Candida albican* fungi, getting stronger with increasing concentration. The formulation with a concentration of 50% w / v has a very strong inhibitory power, much stronger than the inhibition of positive control antiseptic, 2% stronger than the inhibition of *Candida albican* fungi than the positive control antiseptic.

The formulations of 30% w / v, 40% w / v and 50% w / v have very strong inhibiting power against the growth of *Candida albican*. The 50% w / v formulation had a higher inhibitory activity than the positive control with liquid antiseptic hand sanitizer. The positive control in this study used antiseptic solutions because liquid antiseptic is widely used as a treatment for

vaginal discharge and for cleaning female sex organs and are also used for mouthwash. Positive control of liquid antiseptic hand sanitizer has very strong inhibitory activity against *Candida albicans*, this proves that the infusion formulation of betel leaf, lime peel extract and bundung extract has potential as a Candidiasis therapy with non-systemic use, such as antiseptic fluids, mouthwash preparations, topical or ointment preparations and emulgel or liquid soap and solid bar soap for use on the skin, mouth and intimate organs.



(a) (b) (c)

Figure 1. The Inhibition Power on Candida albicans: (a)Negative Control and Positive Control; (b)Formulation 20% and 40%; (c) Formulation 30% and 50%

Anti-fungal substances work in various ways, including causing damage to cell walls, changes in cell permeability, changes in protein and nucleic acid molecules and proteins, inhibition of enzyme work and inhibition of nucleic acid and protein synthesis. Damage to one of these sites can initiate changes that lead to the cell's death.[11]

According to Effendi and Hertiani, 2013, the compounds that have anti-fungal activity from the ethanol extract of ant nest are phenol compounds.[12] According to Raharjo, 2012, compounds that have anti-fungal activity from the ethanol extract of Moringa leaves are flavonoids and saponins.[13]

Analysis of the chemical content of green betel leaf extract conducted by Ni Putu Rahayu Kusuma Pratiwi, 2016, found 31 compounds contained in betel leaf extract, the majority of active compounds from green betel leaf extract are phenolic groups that have antibacterial activity.[14]

Phytochemical analysis of lime peel extract conducted by Nindriya Kurniandari, 2015, showed that orange peel extract showed good potential for free radical antioxidants through the content of flavonoids such as quercetin, hesperidin and naringin. Naringin showed good protective activity against kidney. Naringin is known to have anti-carcinogenesis and antitumorigenesis properties.[15] The results of the phytochemical test showed that the ethanol extract of the Bundung plant

contained secondary metabolite compounds, they are flavonoids, tannins, saponins, phenolics, steroids and terpenoids.[6]

The number of chemical compounds from the three herbs in the betel leaf infusion formulation, lime peel extract and bundung extract allows it to have anti-fungal activity, this is proven in this study that the betel leaf infusion formulation, lime peel extract and bundung extract have inhibitory power against fungi *Candida albican*.

Based on Zuraidah's research, 2015, a study of betel leaf extract against *Candida albican* fungi provided inhibition at concentrations of 80% and 100%. From the betel leaf infusion formulation, lime peel extract and bundung extract with concentrations of 30%, 40% and 50% have inhibitory power against *Candida albican* fungi. Thus, these three types of herbs mutually reinforce the inhibition of the *Candida albican*.

Identification of flavonoids from betel leaf, lime peel and bundung was carried out by separating with Thin Layer Chromatography method then sprayed with $FeCl_3$ liquid. The betel leaf infusion is evaporated on a vaporizer, an amount of the extract was dissolved with an amount of aquades. The lime and bundung extract were also dissolved with aquades. As a comparison, a small amount of quercetin solution was taken. The board for TLC was pre-activated by preheating 100 ° C for 15 minutes. Each solution of betel leaf, lime peel solution, bundung solution and quercetin solution were placed on the marked places on the board. Besides that, the mobile phase had been prepared in a 500 ml glass beaker. The mobile phase used was n-butanol: acetic acid: aquades with a ratio of 4: 1: 5, made 20 ml of n-butanol: 5 ml acetic acid and 25 ml of aquades. The TLC board which has been given the marking of the test sample is put into the glass beaker containing the mobile phase, awaiting the movement of the sample to the upper limit that has been marked. The boards were taken from glass, then dried with a hairdryer, and viewed with UV light, and by spraying $FeCl_3$. The result is that all the test solutions for betel leaf, lime peel, bundung and quercetin are greenish yellow, indicating that there are flavonoids in the betel leaf, lime peel and bundung formulations. Flavonoids are compounds that contain two aromatic rings with more than one hydroxyl group. Reduction of flavonoids with $FeCl_3$ produces red, yellow or orange colors. According to Maruti, 2011, flavonoids have antibacterial, anti-fungal, anti-cancer, anti-diabetic and antiviral activity.[16] It is proven that the betel leaf infusion formulation, lime peel and bundung extraction contain flavonoids and have inhibitory activity against the fungus *Candida albicans*. Flavonoid compounds have two ways of killing microbes, by destroying the microbial cell membrane, and forming complex compounds with extracellular proteins so that the microbial cell membrane is damaged and followed by uncontrolled entry of water into the microbial cell, this causes swelling and finally the microbial cell membrane breaks (Black and Jacobs, 1993). In addition, flavonoid compounds have the ability to denature microbial cell proteins by forming complex hydrogen bonds with microbial cell proteins, so that the structure of the cell walls and microbial cytoplasmic membranes that contain proteins become unstable and lose their biological activity, as a result the function of microbial cell permeability is disrupted and microbial cells will be undergo lysis which results in the death of microbial cells.[18]

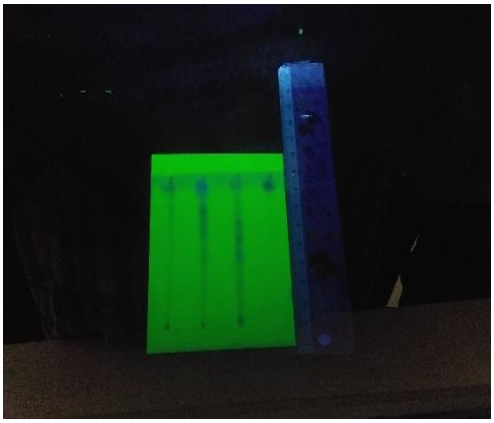


Figure 2. Flavonoids Test from Samples of Betel Leaf, Lime Peel and Bundung Test Formulations Using A Thin Layer Chromatographic Plate

Table 5. PH measurement with PHmeter obtained results as shown in

No	Formulation	pH
1	20%	3,2
2	30%	3,5
3	40%	3,6
4	50%	3,7
5	Liquid antiseptic	6
7	Aquades	7

The increasingly acidic pH is not good for skin health because it can cause irritation. The safe pH for topical use is between 4,5 – 6. The formulation 50% has a very strong inhibitory power and is higher than the positive control for antiseptic hand sanitizer. To be used as a topical product such as liquid soap, bar soap, mouthwash, antiseptic liquid or ointment and emulgel, the formulation can be added with additional ingredients suitable with the product and with the addition of a base such as NaOH until the pH is safe for topicals.

CONCLUSION

From the results of the study it is known that with the inhibition test method of *Candida albicans* fungI and identification of flavonoid compounds and Ph measurement, it can be concluded that: (1)Formulation 30%, 40% and 50% of betel leaf infusion, lime extract and bundung extract have very strong inhibition power againts *Candida albicans*; (2)Formulation 50% of betel leaf infusion, lime extract and bundung extract have stronger antiseptic antivity againts Candida albicans than liquid antiseptic or hand sanitizer; (3) Formulation of betel leaf infusion, lime extract and bundung extract contains flavonoid compound which have antibacterial, antifungal, antidiabet, anticancer, and antivirus activities; and (4) Formulation of betel leaf infusion, lime extract and bundung extract still have an acidic PH and need to be made a safe PH for topical preparations as a non-systemic therapy.

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