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Betel Leaf Bolus Mixed With Phenolic Compounds From Betel Leaf To Inhibit Candida Albicans

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ABSTRACT

The effect of phenolic compounds from betel leaf to inhibit *Candida albicans* can be separated into two experiments. The first phase experimented to consider the potential of phenolic compounds found in betel leaf to inhibit Candida albicans, utilizing Folin-Ciocalteu's reagent and a spectrophotometer to quantify the light absorption at 760 nanometers and a disk diffusion test to determine the extent of Candida albicans inhibition. The test was incubated at a temperature of 35–37 degrees Celsius for 48 and 72 hours while observing the area of inhibition (zone of exhibition) in the paper disc area and measuring the diameter of the inhibition area. During the second phase, the most effective phenolic extract from the first experiment was selected and blended with varying levels of honey (10, 12.5, and 15%). The investigated the physical properties of the bolus, including morphological attributes, water content, disintegration time, and weight variation. The amount of phenolic chemicals, 78.90 micrograms/ml, was determined in a phenolic extract from dried betel leaf with 95% ethanol by volume. It was the most effective in inhibiting *Candida albicans*, which measured a clear zone size of 24.5 millimeters in 48 hours. In the second phase, the most effective was selected based on its ability to inhibit *Candida albicans*, and the results showed that mixed with 10% honey, exhibits the best morphology, moisture content, disintegration time, and weight variation.

1.0 Introduction

Betel vine (*Piper betel* Linn.) is a herb that can inhibit the growth of several bacteria and fungi. For instance, betel oil can inhibit the positive and negative gram of *Bacillus megatherium, Bacillus subtilis, Diplococcus pneumoniae, Escherichia coli, Pseudomonas solanacearum, Salmonella typhosa.* Pathogens such as *Mycobacterium tuberculosis, and Staphylococcus aureus. Skin fungi diseases are Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum, and Microsporum gypseum* (Nuntakran, et al., 1998)

Fungi are the normal flora that can be found in humid environments without sunlight and are commonly found in oral, dermal, and birth canal regions. Its pathogenic impact surfaces when the host's immune system is compromised. While studies on human infectious diseases reveal fungal infections to be relatively minor compared to bacteria and viruses, the medical and dental realms increasingly acknowledge the peril posed by fungi, especially Candida species like *Candida albicans,* leading to conditions such as candidiasis. Symptoms manifest diversely, ranging from

widespread red rashes on the oral palate to removable white patches. Typically, patients with fungal infections exhibit compromised immunity, including cancer patients undergoing chemotherapy, those on immunosuppressive medication, and individuals with nutritional deficiencies.

Therefore, this research focuses on studying the levels of phenolic compounds found in betel leaf and the potential for inhibiting *Candida albicans* at a preliminary level. This data could be valuable information for use in both human and animal applications, enabling those interested in studying herbs to benefit in the future.

2.0 Literature review

The Folin-Ciocalteu method is a technique for determining the total phenolic content by comparing it with the standard solution of Gallic Acid in terms of the weight of the extracted sample. The absorbance can be measured at a wavelength of 765 nm. The procedure involves preparing samples from both the outer peel and seeds of fresh and dried mangosteen fruits. For each sample, weighing precisely 1.0 grams, the outer peel and seeds are finely ground. Subsequently, a solvent is added in a volume of 50 milliliters for extraction, and the mixture undergoes shaking using an Orbital Shaker for one hour. Following the shaking process, the mixture is filtered using Whatman No. 1 filter paper, and the solvent is then removed using a Rotary Vacuum Evaporator at a temperature of 50 degrees Celsius. This process continues until an extract with the desired consistency, containing both the outer peel and seed components, is obtained. Tatsanee and et al., (2017) Cultivation is carried out on solid Sabouraud Dextrose Agar (SDA) medium at 30 degrees Celsius for 48 hours to cultivate yeast and mold. The obtained pure colonies are then inoculated into liquid Sabouraud Dextrose Broth (SDB) medium at 37 degrees Celsius for 24 hours. This liquid medium is used for subsequent disk diffusion testing. Pramaporn Chiewpattanakul Kaewmanee et al., (2011)

Phenolic compounds, or phenolics, are natural substances found in various plants such as vegetables, fruits, spices, herbs, legumes, and seeds. They are synthesized by plants for growth and development benefits. Phenolic compounds have nutritional and pharmacological properties, with notable health benefits. They exhibit antioxidant properties, meaning they can counteract the effects of oxidative stress. Furthermore, these compounds are water-soluble. Phenolic compounds found in nature encompass a diverse array of types, exhibiting distinct chemical structural formulas. They range from simpler groups like phenolic acids to more complex structures such as lignin, a polymer. The most prevalent and extensive category encountered is the flavonoid group. Phenolic compounds in plants are often combined with sugar molecules in the form of glycosides. The most common sugar found in the molecular structure of phenolic compounds is glucose. It is observed that these compounds may amalgamate either among themselves or with other substances, such as organic acids, within protein molecules, alkaloids, terpenoids, and others. Pimpen Pholchaloemphong and Nithiya Ratthanapan (2003)

Candidiasis is a fungal infection that can affect various parts of the human body, including the mouth, skin, nails, or vagina. *Candida albicans* is the primary causative agent of this disease, and the manifestations of the disease can vary depending on the affected area. Certain environmental factors can promote the growth of *Candida albicans*, such as endocrine system disorders or compromised immune systems. Additionally, skin inflammation and complications during pregnancy can also arise from candidiasis. It is vital to take appropriate measures to manage the symptoms of candidiasis and prevent its recurrence. There is inflammation in the skin in pregnant women. Candida albicans is the yeast with an oval shape and 3.5-6 x 6-10 micrometers. *Candida albicans* haven't had a capsule. It can produce pseudohyphae, chlamydospores, and germ tubes. Additionally, it can grow at 25 and 37 degrees Celsius. *Candida albicans* is a commensal yeast species that is commonly found in environment and this organism can also act as an opportunistic pathogen in both humans and animals. Diagnosis of diseases caused by *Candida*

albicans in both human and veterinary medicine is required appropriate laboratory examination of the organism. There are many methods generally used for the identifcation of this organism in microbiological laboratory Khomson Satchasataporn and Duandaow Khunbutsri (2018). Summary report Paramaporn et al., (2021) the concentration of 0.1 mg/ml, Clove extract had the highest content of phenolic compounds followed by Turmeric, Ginger, Galangal, Centella, Black Galingale, Garlic and Holy basil, respectively. At a concentration of 150 mg/ml, Clove extract had the most antimicrobial activity against C. albicans followed by Ginger, Galangal, Centella, Black galingale, Garlic and Holy Basil, respectively. However, both concentrations of Turmeric extract (150 and 300 mg/ml) were not found to inhibit the growth of C. albicans. There was a greater inhibition zone at 48 than 72 hours. Conclusion that herbal extracts with high phenol constituents tend to have higher potent of inhibitory effects of C. albicans, exception of Turmeric. The phenolic compounds in the herb were effective against C. albicans, and the optimum inhibitory activity test was 48 hours.

Various diseases can cause illness in animals, and it is important to be aware of these conditions in order to prevent their spread. For example, avian thrush, respiratory and gastrointestinal diseases, pneumonia, and mastitis can all affect cattle. In kittens, the disease can lead to enteritis and stomatitis, while adult cats may develop pyothorax. Puppies are susceptible to mouth inflammation, while older dogs may develop infections in the reproductive tract. Ponies and pigs may experience ulcerative lesions in the stomach. (Quinn et al., 1994; Hirsh and Zee, 1999). From the reported of Arinee Chatchawanchonteera et al., (2005) garlic and onion water extracted were test in vitro to evaluate the inhibitory in Candida albicans by broth dilution technique together with ketoconazole, as a control drug. The inhibitory effect of *Allium sativum*, *Allium cepa* and ketoconazole shower the average IC_{5c} value as 1.26 mg/ml, 64 mg/ml and 0.039 µg/ml; and MFC value as 6.35 mg/ml, 322.54 mg/ml and 8µg/ml respectively. Both garlic and Onion at the highest concentration yielded a hundred percent of inhibitory effect. Vipada Kantayos, Yingyong Paisooksantivatana et al., (2012) Z. montanum, Z. officinale, Z. rubens, Z. cornubracteatum,Zingiber 'Plai chompoo', Z. zerumbet, Z. ottensii and Z. bisectum that are approximately 10-12 months old were made.Let it dry with a temperature control unit. By adjusting to the lowest temperature, which is approximately 37 degrees Celsius. and extracted withDissolve 95% distilled ethanol at room temperature for 1 day, then filter using Whatman filter paper. 4 Store at -20 degrees Celsius until analyzed by various methods. Quantitative analysis of total phenolic compounds By considering comparison with the standard graph. The highest total phenolic compounds were 9.56 mg gallic acid equivalent to water. Suwannee Saenthaweesuk et al., (2012) reported the total phenolics content, antioxidant and antimicrobial activities of some herbs. The result show that 70% ethanol extracts from Asiatic Penntwort had the highest content of total phenolics content (56.254 mg/g) ($P<0.01$) and 70% ethanol extracts from Asiatic Penntwort had the greatest the half maximal inhibitory concentration (IC_{50}) (2.510 mg/g) ($P<0.01$) In addition, 70% ethanol extracts from Asiatic Penntwort had the highest antimicrovial activity of *Escherichia Coli* (0157:H7) 79.690%) and *Staphylococcus aureus* (29.770%), respectively.

3.0 Methodology

The experiment is separated into two phases;

Phase I: A Study of phenolic extract and efficiency of inhibiting *Candida albicans* with different concentrations. The experiment followed a different concentration of solvents which are 50, 75, and 95 v.

Preparation of phenolic extracting process

i. Bringing betel leaves from the local market and cleaning all of them with water.

ii. Then, steam all of them at 70-80 degrees Celsius after that group them into 3 groups,

iii. raw betel leaves, dry betel leaves, and steamed betel leaves.

iv. Next, dry the group of dry betel and leave the group at 60 degrees Celsius for 24 hours.

v. All of the samples were ground.

vi. Prepare the solvent concentration which is Ethanol into 50, 75, and 95 by volume.

vii. Extract the phenolic compounds from betel leaves by the maceration process.

viii. This is followed by the different Ethanol concentrations which are 50,75 and 95 by volume and the difference in betel leaves. The ratio between betel leaves and the solvent is 1:5 by volume. Then, fermented for 5 days after that bring 9 samples to evaporate at 78 degrees Celsius

Figure 1.1: The process of preparing betel leaves

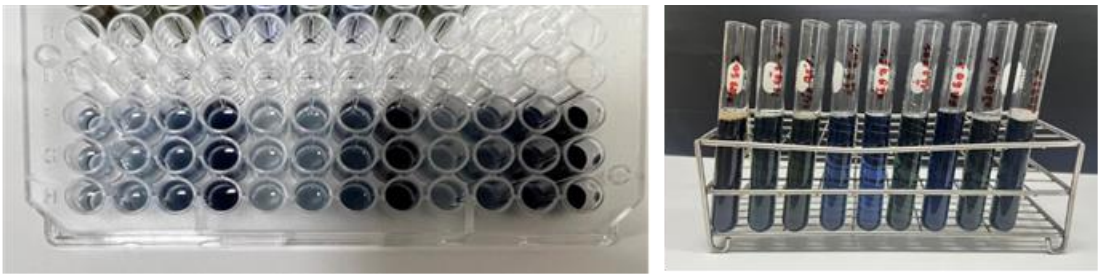

Figure 1.2: The process of analyzing phenolic compounds

After that, add 20 grams of glucose. Adjust the volume to 1000 millimeters. Divide into flask. Close the lid with foil. Put it in the autoclave for 20 minutes. Bring the petri dish to sterilize at 200 degrees Celsius for 2 hours. Put the potato dextrose agar in the petri dish and for 1/3 of the volume.

Preparation of Candida albicans fungus

Take a baby's teat that has Candida albicans fungus. Soak the baby's teat in a liquid culture medium. Then, it was grown in a culture medium at 37 degrees Celsius for 24 hours.

Testing the fungus-inhibiting effect using the disk diffusion method.

Take the three types of betel leaf extract and 50% volume ethanol, make a 50 microliter sample volume, drop it on a 6 6-millimeter diameter filter paper, and then sterilize it in a dryer. Make a sheet of filter paper and place it on the culture medium. Put it in a dryer at 30 degrees Celsius for 48 hours and measure the diameter of the zone of inhibition in millimeters.

Phase II: Betel leaf bolus testing

Preparation of bolus

1. Measure the ratio of honey into 3 groups, which are 10, 12.5, and 20 ml.

2. Measure the dry betel leaves, which is the best result from the first experiment into 10 grams.

3. Combine all of the ingredients and shape them into 0.5 grams small spheres per one.

4. Dry it for 6 hours and measure moisture content, disintegration time, and weight variation.

4.0 Discussion of analysis and findings

Phenolic compounds in betel leaves were formulated to contain 50, 75, and 95 volumes of Ethanol. The results show that betel leaves with 95% Ethanol by volume have the highest amount of phenolic compounds. According to Vipada Kantayos, Yingyong Paisooksantivatana et al., (2012) reported the amount of phenolic compounds in ginger by using 95% Ethanol by volume.

Table 1.1: Absorbance values of extracts at a wavelength of 760 nm

Type of extract	Absorbance (Abs) Average	Amount of phenolic compounds (microgram/milliliter)
Raw 50%v	0.4197	39.56
Raw 75%v	0.8585	70.86
Raw 95%v	1.2563	78.90
Steam 50%	0.2735	20.84
Steam 75%	0.5906	36.06
Steam 95%	0.9958	49.30
Dry 50%v	0.4033	37.47
Dry 75%v	0.8378	68.18
Dry 95%v	1.1864	70.96

Table 1.2, showed the best ratio is 10:10 which has a moisture content of 6.48% and a weight variation is 0.542 ml. Therefore, the disintegration time of 10:10 is less than 10:12.5 and 10:15, which is 1.30 minutes.

5.0 Conclusion and future research

The study of quantifying compound concentration. Phenolics in dried, fresh, and steamed betel leaves. From three samples: 1. Extraction of dried, fresh, and steamed betel leaves with 50% Ethanol by volume. 2. Extraction of dried, fresh, and steamed betel leaves with 75% Ethanol by volume. 3. Extraction of betel leaves, dried, fresh, and steamed with 95% Ethanol by volume. By creating a gallic acid standard curve. The concentration of phenolic substances (micrograms per milliliter). It was found that dry extraction of betel leaves with 95% Ethanol by volume had the highest concentration of all phenolic compounds examined. When tested with *Candida albicans*, it was found that dried betel leaf extract with an ethanol concentration of 95 percent by volume at 48 hours had the best antioxidant capacity. This research provided information. The basis for Betel leaves was developed into a bolus for use in treating *Candida albicans*.

In the second experiment, the result shows that bolus with the ratio between dry betel leaves and honey of 10:10 is the best ratio which has a moisture content of 6.48%, weight variation of 0.542 ml, and Disintegration time of 1.30 minutes.

In the future, the bolus will have to experiment more with contamination inspection. It can be developed to feed animals and protect them from fungus diseases. Additionally, boluses can be developed to increase the consumption rate of animals.

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