Antifungal Activity Of Jukut Bau (Ageratum Conyzoides L. Leaves Extract On Candida Albicans In Vitro

Rida Oktorida Khashini, Irma Saraswati, Fatah Sulaiman, Alimuddin, Indah Juwita Sari

Abstract: Ageratum conyzoides L. or familiar as Jukut bau has been recognized as medicinal plant for many diseases. A highest use value of this plant species indicates the potential importance for Baduy community. The research aimed to evaluate antifungal activities of the aqueous and ethanolic leaves extracts of A.conyzoides L. in vitro against Candida albicans. The phytochemical screening revealed the presence of some phytochemicals contains alkaloids, flavonoid, saponins, tannins, triterpenoids and steroids. Antifungal potential of the extracts were evaluated quantitatively in vitro using disk diffusion method and compared with controls. The percentage yields of aqueous extracts were greater than that of ethanol extract. Both extracts showed a potentially good antifungal activity, however ethanol extract had higher activity and more efficient as compared to water extract. The MIC values of both extracts was 80 mg/ml. Microscopic observation showed that the treatment of aqueous and ethanol extract gave the inhibitory effect on the growth of C. albicans. The results from this study suggested that Ageratum conyzoides L. extracts and the chemicals content are likely to be promising medicine for human use against C. albicans.

Index Terms: Candida albicans, Jukut bau, leaves extracts, and phytochemical compounds

1. INTRODUCTION
Indonesia is a tropical country, has great potential in the biodiversity that can be utilized for the community welfare. It is about 30,000 species of plants originated from Indonesia and about 7000 of them have been utilized as food or medicinal. Nowadays, as the rising costs of health care people tend to use the use of medicinal plant as one element of complementary and alternative medicine. In addition, safety issues are also become a reason for medicinal plant consumption. It is believed that medicinal plant has small side effects compare to those chemical medicine. That is why research on medicinal plant and metabolite compound related to the plant is important as a scientific proof. Baduy tribe is a traditional community from Banten Province that still adheres to the rules of traditional customs (Pikukuh) which are inherited passes down between generations [1]. Baduy traditions and belief systems is in harmony with the nature. The value of local wisdom is used in in managing their land, resources for sustainable use. The Baduy utilize a diverse of plants for food, medicine and for customary ceremony. Ageratum conyzoides L. or familiar as Jukut bau for the Baduy tribe is one of the plants that has many benefits. This plant is easy to find in rice fields, gardens, yard, and road pavement because it is classified as a weed. Many Baduy people use this plant as traditional medicine. Based on the results of the previous study [2], Jukut bau has the largest Use Value index among other plants utilized by the Baduy people. The leaves and flowers of this plant are containing essential oils [3], and beneficial secondary metabolites [4]. Candida albicans is a dimorphic opportunistic microorganism that causes mycosis and is easily isolated especially from patients with HIV / AIDS [5] cancer patients undergoing chemotherapy [6], and patients with diabetes [7]. Among the C. albicans virulence factors, the formation of new hyphae begins with the presence of a germ tube which is very important for the survival of these microorganisms [8]. Infections caused by C. albicans can be treated with antifungal drugs from the azole, pollen and echinocandin groups [9]. The use of these drugs has limitations because it can cause increased drug resistance and toxicity to patients [10]. In addition, the types of antifungal used by the community are relatively less than other antimicrobials. Nowadays the interest of antifungal compounds researches derived from plants is increasing. Therefore it is necessary to find alternative medicines from traditional medicinal plants which have been frequently used empirically by the community, in this case by the Baduy community. This study was conducted with the aim of testing the antifungal activity of A. conyzoides L. plant extracts against the formation of C. albicans germ tubes in vitro as a basis for developing herbal-based Candida therapeutic ingredients.

2 MATERIAL AND METHODS
This research was conducted in April-June 2019 in the Laboratory of Biology Education, Faculty of Teacher Training and Education, University of Sultan Ageng Tirtayasa. The Glassware, incubators, microscope LEICA DM 500 used in this research, while the material used were C. albicans ATCC 10231 isolate, Potato Dextrose Agar (PDA) media, ethanol, chloramphenicol 250 µg/ml, ketonazole 2 µg/ml. Candida albicans ATCC 10231 from the Bogor Agricultural Institute Culture Collection (IPBCC) was cultured on PDA media. The culture then incubated for 24 hours until white round colonies formed. Jukut bau (A. conyzoides L.) plants were obtained from Cikeusik, Kanekes Village, Leuwidamar District, Lebak Banten. The process of identification and determination for the cross-referencing of plants was carried out at the Laboratory of Biology Education, Faculty of Teacher Training and Education, University of Sultan Ageng Tirtayasa by comparing the
herbarium with literature [11]. Voucher specimen is kept at the Department of Biology Education Faculty of Teacher Training and Education, University of Sultan Ageng Tirtayasa, Indonesia.

**Extraction Process**

Firstly the leaves were separated from the plants and then washed with water. Then it was dried using an oven at 40 °C, crushed, and sieved to obtain a 100 mesh powder which is ready to be extracted. Water content and ash content of ash powder were measured using the AOAC method [12]. Leaf extraction process was conducted by maceration method using two types of solvents: ethanol and water. A 70% ethanol was used to soak the sample for 24 hours in a ratio of 1:10. The results of the homogenate are then filtered and the extract obtained is concentrated using a rotary evaporator at 40°C. The process of extracting plants with a water solvent was conducted by boiling leaves powder using distilled water with a ratio of 1:10 for 2 hours. The decoction then filtered and the filtrate was collected. The filtrate was then evaporated and concentrated using a rotary evaporator at 40°C.

**Phytochemical Screening**

Phytochemical screening of alkaloid, flavonoid, tannin, saponin, terpenoid was carried out to find out secondary metabolite compounds contained in ethanol extract and water extract of Jukut bau leaves [13].

**Antifungal Activity Test**

The antifungal activity test in this study was carried out by determining the minimum inhibitory concentration (MIC) of water and ethanol extracts against C. albicans through the Kirby Bauer disc diffusion method. A total of 10^8 CFU / ml of inoculum suspension was inoculated into a tube containing liquid PDA media, homogenized and poured in a sterile Petri dish. Paper discs 10 mm in diameter were soaked each in a sample solution of ethanol extract and water extract from Jukut bau leaves. Disc paper was placed on the media and incubated at 37 °C for 48 hours. The negative control was 10% DMSO while the positive control was 2 μg / ml ketoconazole. Inhibition zones formed as antifungal activity were observed. The extract which has antifungal activity, then used for determining the MIC concentration.

**3 RESULT AND DISCUSSION**

Proximate analysis results showed that the Jukut bau has a moisture content of 5.35% ± 0.02. This moisture content was obtained through the process of drying the leaves. The ash content of the leaf powder obtained through the ash process by heating above 450 °C was 8.16 ± 0.41%. Leaf extraction using 70% water and ethanol produced different yield values. The highest yield mass produced by water solvents followed by 70% ethanol solvent, each of which is 15.63% ± 0.43 and 9.34 ± 25. The water and ash content in A. conyzoides leaves powder will affects the quality during storage. High water content affects the growth of microorganisms and active ingredient content during the storage period. Based on the research, the water content of A. conyzoides leaf powder was 5.35% ± 0.02. Ash content is associated with minerals of a substance whose composition and amount depend on the type of food and the analytical method used. Based on the results, ash content was 8.16 ± 0.41%. This value has met the requirements of traditional medicine. The standard water content and maximum ash content of simplicia is 10% [14]. Jukut bau leaf extraction with distilled water, and 70% ethanol were analyzed qualitatively for phytochemical content based on the color reaction formed from the reagents used (Table 1). Phytochemical tests on water extracts Jukut bau leaves showed positive results in the presence of alkaloids, saponins, tannins and steroids while for flavonoid and triterpenoid compounds were not detected. On ethanol extraction of 70% showed positive results for flavonoids, saponins, triterpenoids, and steroids, while not detected for alkaloids and tannins.

**Table 1.**

Phytochemical Screening of A. conyzoides Leaf Extract

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>Water</th>
<th>Solvent 70%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcaloid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: + secondary metabolites present, - there are no secondary metabolites

Determination of MIC was carried out by a disk diffusion test of various concentrations of 50, 60, 70, 90, and 100 mg / mL of water extract and 70% ethanol extract (Figure 1). Aqueous extract and 70% ethanol extract did not show inhibition of C. albicans growth at concentrations of 50, 60, and 70 mg / ml. Both solvents showed clear zone formation activity at concentrations of 80 mg / ml with different inhibition zone diameters. The inhibition zone produced by ethanol extract is greater than that of water extract (Figure 1). The greater the concentration of the Jukut bau leaf powder, the greater the diameter of the inhibition zone produced.

![Fig. 1. Anti fungi activity from Jukut bau Leaves extract](image)

C. albicans is the most common species residing as or microflora or pathogen caused Candidiasis. There are several antifungals that effective used for candidiasis management. But there will be a resistance during therapy. Product derived from natural component such as herbs or medicinal plant can be used as and alternative agents for controlling fungal diseases. Jukut bau showed antifungal activity and strongly inhibited the growth of C. albicans. The efficacy of extract Jukut bau on antifungal mechanism was due to many potential metabolite compounds. Phytochemical analysis were conducted qualitatively aimed to determine the potential...
metabolite compounds as antimicrobial compounds. Phytochemical screening showed different results in extracts with different solvents depending on polarity, because polar compounds were bound (extracted) by polar solvents, as well as non-polar compounds bound to non-polar solvents. The results of research conducted by [15]. Different solvents based on polarity (petroleum ether, chloroform, methanol, and water) are very effectively used for the extraction of antimicrobial activity. Based on the research results obtained information that alkaloid compounds were detected in water extracts, but undetectable in 70% ethanol extract which is indicated by the absence of white sediment in Meyer reagent, brown sediment in Wagner reagent, and red sediment in Dragendoff reagent there is only ethanol extract marked by the formation of brownish red filtrate. This is in accordance with research conducted by [16]. Positive saponin compounds in both extracts are characterized by the formation of foam. Saponins are widespread in higher plants although saponins are one of the largest classes of natural plant products, biological functions in plants are not fully understood. However, saponins also have biological activity functions such as anticancer [17], anti-inflammatory [18] and antifungal [19]. According to [20], the antifungal mechanism that occurs is through the formation of complexes between saponins and sterols found in fungal plasma membranes, then destroying semipermeable cells and causing death of fungal cells. Positive tannin compounds in extracts with a water solvent are characterized by the formation of greenish black filtrate, but not detected in ethanol extracts. Tannins are anti-microbial [21]. The mechanism of tannin as an anti-fungi influences the composition of Candida cell membrane ergosterosol [22]. Positive triterpenoid compounds are present in ethanol extract but not in water extracts. Instead the steroid component is detected in the water extract but not detected in the ethanol extract, which is characterized by the formation of a green layer on steroids and a purple layer on triterpenoids. Triterpenoid and steroid compounds are known to have antimicrobial activity [23]. The extraction process of A. conyzoides leaf powder was carried out through maceration method using water solvent and 70% ethanol solvent. Solvents diffuse into cells through cell walls and cell membranes during the extraction process and dissolve bioactive compounds. The water extract was obtained by the boiling method at 100°C. The selection of this method is adjusted to the habits of the Indonesian people in taking traditional medicines, namely through the boiling process [24]. Based on the results of the study, the yield of water extract was lower than the extract with 70% ethanol solvent. The difference in yield is due to differences in polarity between water and ethanol 70%. Based on its polarity value, water has a polarity index of 9.0 and ethanol 70% has a polarity index of 5.2 [25]. This shows that the secondary metabolite compounds in smelly leaves are thought to be more polar in nature. Antimicrobial activity test in this study used the diffusion method. Inhibitory concentrations used as antifungal starts from concentrations of 80 mg / mL, 90 mg / mL, and 100 mg / mL. The difference in the ability of water extracts and ethanol 70% to inhibit the growth of C. albicans is related to the content of chemical compounds contained in the extract. In this study ketonazole was used as a positive control because it was commonly used in the treatment of candidiasis. The results showed that ketonazole had a large inhibitory effect on the growth of C. albicans when compared to water extract and ethanol extract. Ketoconazole is a broad spectrum synthetic anti-fungal drug. According to [26], ketoconazole inhibits endogenous and exogenous respiration in C. albicans cells and at the same time ketonazole inhibits NADH oxidase activity at the mitochondrial level. The process of inhibiting the growth of C. albicans spores in forming germ tubes in the presence of ethanol extract and water extract treatments was observed within a certain interval. Figure 2a. observation of C. albicans without treatment showed growth of sprout tubes in spores and developed into C. albicans mycelia after incubation for 24 hours. Another case in Figure 2b-c shows the growth inhibition in the treatment of the addition of water extract or 70% ethanol extract with a concentration of 80 mg / ml respectively.

There has been a recent urgency of interest in the use of medicinal plants in diseases management because of the health effects issue of chemical medicine. This study illustrates the potential medicinal plant may leads for the source of novel metabolite and bioactive compound further.

CONCLUSIONS
This research describes on antifungal activity Ageratum Conyzoïdes L. leaves extract on C. albicans in vitro. The water extract and ethanol extract of Jukut bau leaves have the ability to inhibit the growth of C. albicans. Concentration of 80 mg/mL is the minimum inhibitory concentration which is able to inhibit the growth of C. albicans with inhibition zone diameters of 1.55 mm and 1.59 mm for water extract and ethanol extract of leaves. It can be said that Jukut bau leaves extract has the potential to inhibit growth but is relatively weak when compared to ketonazole. The results of phytochemical screening showed the presence of alkaloid compounds, saponins, tannins, steroids, flavonoids, and triterpenoids in the extract of Jukut bau leaves. In vivo further testing is needed regarding the antifungal activity of extracts of water extracts and ethanol extracts of Jukut bau leaves, identification isolation and quantitative tests to analyze compounds which are thought to function directly against growth inhibition of C. albicans

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