The active compound of soursop leaf extract (*Annona muricata*, L.) as anti-vaginal discharge (*Fluor albus*)

E Rustanti†,* and Z Fatmawati‡

† STIKES Husada Jombang, Peterongan, Jombang East Java, 61481 Indonesia

*E-mail: eilrose1211.er@gmail.com

Abstract. The aims of this study is to determine the effectiveness of antifungal Soursop leaf extract against the fungus *Candida albicans* and determine the class of compounds that act as vaginal discharge. Soursop leaf was extracted using maceration method with 96% ethanol solvent then concentrated with a rotary evaporator. The class determination of active compound by using multilevel composition fractionation method with n-hexane, chloroform, and ethyl acetate solvent. The effectiveness test of antifungal of each fraction toward *Candida albicans* by diffusion method is so that the researcher can look at the inhibitory zone diameter, then identify them by using UV-VIS and FTIR spectrophotometry to determine the active compound as antifungal. The highest antifungal effectiveness of soursop leaf active compounds was the n hexane fraction with inhibition diameter 23.7 mm which was categorized as strong, compared to the positive control of ketoconazole with inhibition zone 22.5 mm and nystatin 15.9 mm. The compound which is thought to have antifungal activity from the n-hexane fraction of soursop leaves is a terpenoid compound.

1. Introduction

Women have many problems in the vaginal area. Most cases that occur are vaginal discharge. *Leucorrhoea* or in medical language is called *fluor albus* is an excessive discharge from the vagina that is not some kinds of menstrual blood. According to the World Health Organization (WHO) in 2006, health problems regarding poor female reproduction have reached 33% of the total burden of diseases that attack women worldwide. This number is greater than the men reproductive problems which only reach 12.3% in the same age as women [2].

According to a study conducted by Ayuningtyas [3] that around 50% of Indonesian women experienced vaginal discharge in 2002, then in 2003 around 60% experienced vaginal discharge. In 2004 women who experienced vaginal discharge reached 70%, while in the notes from Ayuningtyas [3] it was estimated that throughout 2011 Indonesian women who experienced vaginal discharge were around 70% and in 2012 it would continue to increase. The cause according to WHO (2008), based on its prevalence, they were 25% - 50% candidiasis, 20% - 40% bacterial vaginosis and 5% - 15% trichomoniasis. Europe only had 25% of women experienced vaginal discharge caused by weather factors. Whereas, women in Indonesia are more prone to be experienced because it is triggered by humid weather so it is easily to be infected with the fungus *Candida albicans*. Besides, the increase of vaginal discharge is also caused by women's behavior in maintaining genital hygiene [4].

Treatment using antibiotics is one of the efforts to cure the disease. Whereas the use of antibiotics continuously can decrease normal flora in the vagina. As a result, unfortunately, the fungus replaces the normal flora. In Indonesia, there are several plants that are empirically used as a drug for leucorrhoea,
one of which is soursop (*Annona muricata* L.) leaves. Soursop leaves have many uses, including antibacterial, antifungal, antitumor, anticonvulsant, sedative, antiparasitic, and cardiodepressant properties. Soursop leaves contain alkaloids, polyphenols, terpenes, acetogenins, flavonoids, and lectins. The research of Rohadi [1] showed that ethanol extract of soursop leaves indicated antmicotic activity especially against Candida albicans. In the range concentration of 15% - 60%, the higher the extract concentration the greater the activity. Though in the study of Masloman et al [5] stated that soursop leaf extract (*Annona muricata* L.) has an inhibitory effect on the growth of the fungus *Candida albicans* with an average inhibition zone diameter of 12.5 mm.

Those researches indicated that the researcher don’t know yet which compounds affect effectively in treating vaginal discharge. For this reason, further research is needed to obtain the active compounds of soursop leaves and determine the group of active compounds that are most effective as antiputihan. The purpose of this study is the initial characterization of candidate drugs in preclinical studies which will later be developed appropriately in the form of health products such as soap and anti-vaginal discharge drugs. The results of this study are expected that the active compounds of soursop leaves can be used as new drugs in treating leucorrhoea especially those which is caused by the fungus *Candida albicans*. In addition, with the results of this study, further research can be developed towards standardized herbal medicine and phytopharmaca.

2. Methods

2.1. The isolation of active compounds of soursop leaf

Clean the soursop leaves from dust and dirt. Next is dry it in the oven at temperature 45o C for 72 hours. After that is blend it by using a blender and sieve it to obtain a fine powder. Next is soak 500 grams of soursop leaf powder into 1 L of ethanol solvent in the ratio of 1: 5 (b/v) and repeat it five times with the same ratio. After that, soak it 4 days while doing several stirring. The resulting extract was then filtered by vacuum and concentrate the filtrate by using a rotary evaporator vacuum.

2.2. The Fractionation of soursop leaf extract results

The fractionation is done by concentrating ethanol extract of soursop leaves, then dissolve with water, filter, and fractionate with n-hexane in a separating funnel with a ratio of 1: 1, then shake it sufficiently. After that, leave it until it forms 2 layers (the n-hexane and the water layer). Repeat this treatment several times until the n-hexane layer looks clear so that the n-hexane fraction is obtained. The treatment for the water layer is done similar with the n-hexane layer which is several repetitions by using chloroform, ethyl acetate, 1: 1 ratio. The results of the n-hexane, chloroform, ethyl acetate, and water fraction were evaporated by using rotary evaporator so that a thick fraction was obtained then tested for antifungal activity.

2.3. Antifungal Activity Test

The suspension of the fungus *Candida albicans* as much as 100 µl was put into a sterile petri dish, then put 10 ml of SDA medium which was still liquid, and let the media be condense. On top of the SDA medium, put sterile disk paper which had been soaked before with soursop leaf fractions at a concentration of 0.1 g / mL (10%) for 30 min. Then place disc paper on the surface of the media by using tweezers and pressed a little. After that, incubated it at 37°C for 24 hours. Positive control used was 10% ketoconazole and nystatin 10% while the negative control used was DMSO. After 24 hours, observe the presence of clear zone around the disc paper. The data is obtained from the inhibition zone results in the form of the ability of soursop leaf extract fractions to inhibit the fungus *Candida albicans*. The data was then being analyzed to test for the influence or difference between the treatment of sample variations from the fractions on fungal growth.
2.4. Identification of active compounds

The active fraction that provides the best antifungal effectiveness is then identified using a UV-VIS spectrophotometer in the wavelength range and FTIR spectrophotometer in the wave number range 4000-600 cm$^{-1}$. Data obtained from the results of the UV-VIS spectrum in the form of wavelength chromophore groups and the results of the FTIR spectrum in the form of information functional groups that compose a compound structure. The data is then identified by correlation tables which contain information which functional groups absorb.

3. Results and Discussion

3.1. Isolation of Active Compounds

At this stage, the extract of soursop (Annona muricata L.) leaves is done with the purpose to get extract from soursop leaves. Extraction method used in this study is maceration extraction. Maceration is an extraction of process simplicia by using solvents with several stirring at a specific room temperature. In this study the solvent used was 96% ethanol. Ethanol is used as a solvent because it is polar, universal and easy to obtain. Besides, ethanol is also a solvent for organic and inorganic substances [6]. The lowest purity ethanol solvent that can dissolve a metabolite compound secondary is 66%, so 96% ethanol is expected to be able to extract the compound secondary metabolites more. Because the higher the ethanol concentration will be the easier the process of separating secondary metabolites from samples [7]. Maceration process is carried out for 5 days and the extracts which are obtained before can be separated with the solvent by using a vacuum rotary evaporator and it can obtain thick ethanol extract which is dark green. After the concentrated extract is obtained then the next process is fractionation.

3.2. The fractionation of soursop leaf extract results

The fractionation is the process of separation between liquid and liquid. Fractionation is carried out in stages based on the level of polarity, namely non polar, semi-polar, and polar. Non-polar compounds will dissolve in non-polar solvents and something which has polar character will dissolve into polar solvents [8]. This fractionation process is carried out using a separating funnel. In this fractionation, the solvent which has a higher density will be in the layer bottom, and which have a smaller density will be in the upper layer. Compound contained in the extract will be separate according to the level of polarity of the solvent used. The first fractionation was between the ethanol extract water filtrate with a 1: 1 ratio of n-hexane solvent and formed two layers namely the top layer of the fraction n-hexane and lower layer of water fraction. The difference in solvent density causing the n-hexane fraction to be above because of the density of n-hexane (0.655 g / ml) is smaller than the density of water (1 g / ml). The second fractionation between fractions water with chloroform solvent is formed in two phases namely the upper phase of the water fraction and the phase under the chloroform fraction. The position of chloroform fraction is below because chloroform has a density (1.498 g / mL) that is greater than the density of water. On last fractionation between water fraction and ethyl acetate solvent in comparison (1: 1), it was formed two layers, namely the upper layer of the ethyl acetate fraction and the lower layer of the fraction water. The position of ethyl acetate fraction is above because ethyl acetate has a density 0.894 g / mL which is smaller than the density of water. The obtained fractions are then evaporated using a rotary evaporator until a concentrated fraction is obtained then the inhibitory must be tested against the fungus Candida albicans.

3.3. Inhibition Test of Soursop leaf fraction

This research was conducted to test the antifungal activity of the soursop leaf fraction toward Candida albicans in vitro. If the fungus is given a certain substance that is antifungal, their growth will be inhibited. The inhibition zone is a clear zone around the disc paper on the media that has been inoculated by fungus Candida albicans or zones where there is no growth of Candida albicans. On this study, Candida albicans will be inhibited by the fraction by using disc paper. Because the extract
used is too thick which causes unable to diffuse into the media inoculated with test fungi, the extract needs to be dissolved to a concentration of 10% [10]. Here are the results of inhibition zones of soursop leaf fraction:

![Image of inhibition zones](image)

**Figure 1.** The inhibition zone of soursop leaf fraction

Based on the figure 1, the inhibition of each fraction of soursop leaves gives different inhibition zone diameters that can be seen in Table 1

| Group treatment          | The diameter of Inhibition zone (mm) |
|--------------------------|--------------------------------------|
| N-Hexane fraction        | 23.70                                |
| Chloroform fraction      | 11.00                                |
| Ethyl Acetate fraction   | 10.75                                |
| Water fraction           | 10.35                                |
| Control + (Nystatin)     | 15.9                                 |
| Control + (Ketokonazol)  | 22.5                                 |
| Control – (DMSO)         | 1.88                                 |

Based on Figure 1. and Table 1., the fraction that gave the highest inhibition zone was the N-hexane fraction which was effective in inhibiting the growth of the fungus *Candida albicans* with an inhibition diameter of 23.7 mm. The results are greater compared with the positive control of ketoconazole with inhibition zone of 22.5 mm and nystatin with inhibition zones of 15.9 mm. This is possible because The test extract used contained active compounds which acted as antifungal, so it can inhibit the growth of test fungi.

According to Hermawan *et al* [14], the interpretation of the inhibition area of Antimicrobial growth refers to the general standards issued by The Department of Health which mentioned that microbes are said to be sensitive against active compounds that are as antimicrobials of a plant if it has a resistance diameter of 12-24 mm. The research of Masloman *et al* [5] stated that soursop leaf extract (*Annona*...
muricata L.) has inhibitory effect on the growth of the fungus Candida albicans with a mean inhibition zone diameter of 12.5 mm. The size of the inhibition zone formed by testing the antifungal activity depends on the high or low active substances contained in the extract. Meanwhile, the inhibition zone is formed or not around the paper disc, it depends on the presence or absence of active compounds in the extract. Inhibitory zones are large may be caused by the high active substance in the extract. The inhibitory zones don’t form at certain concentrations may be caused by the small concentration of the active substance so that it has not been able to inhibit microbes. The formation of the inhibition zone around the disc paper shows that there are compounds that are antimicrobial which is inside extracts from plants.

3.4. The identification of Soursop Leaf Compounds

The fraction that gives the best antifungal activity is the n hexane fraction. The following is spectrum of identification result of n hexane fractions of soursop leaves using UV-VIS spectrophotometry:

![Figure 2. Spectrum UV-VIS Fraction](image)

Based on the UV spectrum, it showed that the fraction of N-hexane soursop leaves in the ethanol solvent with the absorption at 203 nm wavelength and an absorbance value of 3.243 indicates that there is a compound with a non-conjugated double bond. The special uptake for terpenoid compounds has no conjugated chromophore. Furthermore, the soursop leaf active compound was identified by FTIR spectrophotometer. The following is the spectrum of the identification results of soursop leaf compounds by using FTIR spectrophotometry:

![Figure 3. Spectrum FTIR Fraction](image)
Based on the results of the FT-IR spectrum, the fraction of N-hexane soursop leaves showed the existence of functional groups including the presence of absorption bands in the region of numbers wave 3474.08 cm\(^{-1}\) which indicates the existence of stretching vibrations in the hydroxy group (OH) which is strengthened by the presence of O-H uptake at wave number 668.21 cm\(^{-1}\). C-H cluster aliphatic alkane at wave number 2924.50 cm\(^{-1}\) which indicates vibration aliphatic C-H stretches on the alkanes are strengthened at wave numbers 1459.66 cm\(^{-1}\) and 1384.11 cm\(^{-1}\) which is the bending vibration of the alkane C-H aliphatic. Other than that, there is aliphatic C-H buckling vibrations Alkenes at wave numbers 800.10 cm\(^{-1}\), then on wave number 1648.66 cm\(^{-1}\) indicates the presence of C = C alkenes. Carbonyl group or ketone (C = O) indicated wave number absorption 1736.96 cm\(^{-1}\). Besides, there is vibration C-O alcohol buckling in the wave number absorption area of 1263.18 cm\(^{-1}\) and 1082.93 cm\(^{-1}\). According to Atmoko [9], from the results of FT-IR spectrum compound analysis has a hydroxyl function O-H, C-O alcohol, C aliphatic C-H, C = C aliphatic, and C = O indicated as terpenoid compounds.

Based on the results of antifungal tests and identification of compounds with UV-VIS and FT-IR, fractions of N-hexane soursop leaves contain terpenoid compounds which have very strong antifungal properties. The mechanism action of terpenoid compounds in inhibiting fungal growth is the damage of cell membranes by antifungal active substances. The damage of cell membranes will disrupt the integrity of cellular components and result the process of fungal respiration does not occur. In the end, it results in insufficient energy for active transport of nutrients so that the growth of the fungus is disrupted. Triterpenoid has antifungal activity by influencing membrane permeability cells which can eventually cause cell membrane lysis [15].

4. Conclusions

Soursop leaf extract which has the highest activity as anti-vaginal discharge especially against the fungus Candida albicans is the N-Hexane Faction with inhibition zone of 23.7 mm in the strong category which is greater than ketoconazole and nystatin. Based on the results of identification with UV-VIS and FTIR, the active compounds that are suspected to have antifungal activity from the n-hexane fraction of soursop leaves are terpenoid compounds. So the soursop leaf terpenoids compounds can be used as new drugs in dealing with vaginal discharge, especially those which is caused by the fungus Candida albicans. It is hoped that further research can do research on the effect of dose variations, as well as assessing their toxicity. The development of similar research can be done by comparing the effectiveness of inhibition of soursop leaf fraction with other antifungals.

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