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Research Article

Senna siamea Hexane Extract: Potent Antifungal Activity Against *Candida albicans*, *Candida krusei* and Identification of Its Chemicals Content

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ABSTRACT

Background: *Senna siamea* contains several chemical: flavonoid, steroids, terpenoids, alkaloid, and tanin which is as an antifungal againts of *Candida* sp because interfere function of the fungal cell membrane and inhibit syntesis of chitin. *Candida albicans* and *Candida krusei* could causing oral candidiasis, vulvovaginal infections, life threatening candidiasis, such as candidemia and internal organ infections. *S. siamea* is a medicinal plant which empirically used as antifungal. *S. siamea* leaves has been reported to exhibit activity against *Candida* sp but limited to ethanol extract. Thus, the evaluation of other extract- and identification of active compound(s) against *C. albicans* and *C. krusei* is needed to be explore. **Methods**: First, the microscopic morphology of *S. siamea* leaves were observed using Scanning Electron Microscope. The leaves were then extracted sequentially by hexane, ethyl acetate, and methanol solvent using the ultrasonic assisted extraction method, followed by its *in vitro* antifungal activity evaluation. The most active extract was further evaluated for its chemical(s) content by LC MS. **Results**: Scanning Electron Microscope identified the presence of oxalate in the leaves of *S. siamea*. Evaluation of the antifungal activity showed that the hexane extract had highest antifungal compared to others. **Conclusions**: *S. siamea* hexane extract leaf is prospective to be developed as an antifungal. Further in vivo research are needed.

Key words: *Senna siamea*, Hexane extract, Antifungal, Chemical content.

INTRODUCTION

The increase of immunocompromised patients also plays a role in the increase in fungal infections. Fungal infections which are often known as mycoses can be in the form of superficial mycoses and systemic/invasive mycoses.[1] The fungus that causes the highest mycosis is *Candida* sp. hereinafter referred to as candidiasis. Candidiasis is reported as the highest fungal disease in Indonesia.[2]

Candida sp. is a yeast fungus that is commensal in the dermis, mouth, gastrointestinal tract and vagina and is able to live in the environment. Due to the role of the environment (risk factor), *Candida* fungi which are initially commensal can turn into pathogens and infect susceptible individuals.[3] *Candida* sp. The most commonly reported causes of candidiasis in humans are *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusei*. *Candida* sp. The highest cause of candidiasis is *C. albicans*, but in the last twenty years there has been an increase in Candida non albicans as the cause of candidiasis.[4]

Thus, to overcome this situation, many researchers explore the potential traditional medicine for candidiasis drug development and discovery. *Senna siamea* is a green plant native to Asia which spread to Indonesian until Srilanka. *S. siamea* has been empirically used for treating malaria, fungi, itching, scabies, diabetes, fever, wounds. S. siamea is a plant with a hard tree trunk. The leaves contain compounds of alkaloids, saponins, flavonoids, tannins, phenols and anthraquinones. Moreover, every part of *S. siamea* are known to contain compounds which affected biological activity not only *in vitro*, such as anthraquinones, antrons, flavones, as well as some triterpenoids in which the active compounds citronellol and geraniol are included as well as alkaloids, including casadiamine. Further phytochemical screening of *S. siamea* extracts indicated the presence saponins, terpenoids for polar and non-polar extract, moreover flavonoid and alkaloids can be found at semi polar and polar extract.[5]

However, the information regarding the bioactive compound(s) responsible to its activity is still limited. Thus, in this study, we evaluated the activity of others extracts from *S. siamea* leaves against *Candica sp* and identified the chemical compound(s) which contained in most active extract.

MATERIALS AND METHODS

Sample and fungi preparation

Senna siamea were obtained from Karangbahagia Village, West Java, Indonesia. Leaves parts were used in this study. Prior to experiment, the leaves were determined by the Indonesian Institute of Sciences, Bogor, followed by drying, and grinded into powder form.

Candida albicans ATCC 90028 and *Candida krusei* ATCC 6258, obtained from the parasitology Laboratory Parasitology department, Faculty of Medicine, University of Indonesia. The *C. albicans and C. krusei* were maintained in *Sabouraud Dextrose Agar* (*oxoid*, UK) and incubated at 37°C aerobic incubator.

The extraction process was carried out using the ultrasound-assisted extraction (UAE) method. Extraction was performed sequentially using hexane,

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ethyl acetate, and methanol solvents. A total of 100 g of dried *S. siamea* leaf powder was extracted by 1000 mL of hexane solvent for 30 minutes at 40°C. The extraction was repeated 3 times to get the maximum yield. The filtrate was separated using filter paper, then evaporated using a rotary evaporator (Buchi Rotavator R-300, Germany) to obtain dried-hexane extract. Leave powder which extracted using hexane was dried, and further extracted sequentially by ethyl acetate and Methanol using same procedures as explained above. Prior to use, dried extract was difused by 1% DMSO and followed by 2-fold difusion by Aquadest.

Microscopic observation with light microscope and SEM

Microscopic observation of dried *S.siamea* leaves was performed using a light microscope (100 × magnification) and *Scanning Electron Microscope* (SEM) (750 x magnification) at Indonesian Institute of Sciences, West java, Indonesia.

Zona inhibitory test

The determination of antifungal activity is disc diffusion. A total of 10 µL of inoculum was added to a petri dish containing *Sabouraud Dextrose Agar* (SDA) (*oxoid*, UK) media and levelled using a spreader. Sterile paper discs were placed on the surface of the media and moistened with 1% dimethyl sulfoxide negative control and sample solution. The procedure was carried out in triplicate for each sample to a final concentration of 62.5µg/mL, 125µg/mL, 250µg/mL, 500µg/mL and 1000µg/mL. Commercial discs of Fluconazole 0.02µg/mL and Amphotericin B 0.1µg/mL were used as positive controls. This procedure was carried out on each of the johar plant extracts. The petri dishes were then incubated at 37°C for 24 hours. Interpretation of the results by measuring the diameter of the zone on media that inhibited fungi growth (indicated by clear-transparant yellow color). The experiment was performed thrid, each in duplicate.

Analysis of chemical compound by LCMS

Was the most active sample (ENH) out of antibacterial activity test of *P. acne* was analyzed for its active compounds using an LCMS (Liquid Chromatography Mass Spectrometry) instrument type QMicro QAA 842 and a Waters Quattro Micro MS-MS detector. The basic sample was dissolved in 5 ml of solvent, and 20 µl I was added to a reverse phase analysis column C18 with a particle size of 50 × 2.1 × 1.9 m at with flow rate of 0.2 ml/min. The temperature of the column used was 50°C and the final time was 20 min. The separation of the chemical compounds took place in column with the help of a pump using a pressure of 300 Bar. The sample was transferred The gas-phase becomes ionized in a vacuum. Ions are accelerated by an electric or magnetic field, by the mass to be charged (m/z) ratio. The results of the LCMS analysis were performed based on compound predictions based on the m/z profile existing secondary data and compared with the m/z profile results presented data from the sample.

RESULTS

Microscopic observation with light microscope and SEM

he light microscope and SEM observations of dried *S. siamea* leaves can1be1seen1in Figure11, respectively.

Extraction

In order to maximaze the phytochemical solution of the sample, the extraction process was perfomed sequentially, first by hexane solvent, to ethyl acetat solvent, and last by methanol solvent. The extraction was also performed using UAE instead of temperature increasing. The polarity of the solvent will affect the type and amount of chemical coumponen capacity, and biological activity of the1extract.[6] The results of the UAE sequential extraction were shown in Table 1, respectively. The result shown that hexane extract had the1highest1yield compare to others extract. This result1indicated1that *S. siamea*1contained1more non-polar components.

Zona inhibitory against *C. albicans and C. krusei* evaluation

The Zona inhibitory test was carried out using the diffusion method with cakram paper. The evaluation of the zona inhibition value was carried out visually. The result can be seen in table 3. The zona inhibition evaluation indicated that hexane (H) extracts shown inhibition growth at 22mm. Meanwhile for ethyl acetat (EA) extract and methanol (ME) extract zona inhibition value was observed at 17.6mm EA and 10.7mm ME. The result is shown in table 3, respectively.

In addition to the Diffusion study, the test was also carried out to observed fungal activity of each extract. The result shown that methanol exhibit MBC at concentration 500ppm and ethyl acetate extract exhibit MBC at concentration 250ppm. However, the hexane shown MBC at lower concentration 125ppm. The MBC evaluation figure are shown at Figure 2, respectively.

Based on the research test results on antifungals that cause candidiasis, it shows that extract NH provides a good activity profile in inhibiting the growth of *S. siamea* fungal; the next step is the analysis of compounds with using LC-MS. LC-MS can be used to determine the secondary metabolite profile of extract NH by observing the ion spectrum product in the form of compound retention time and compound fragmentation based on m/z. The compound identification process was based on the literature available in the LC-MS database and other supporting literature based on: m/z compounds such as Pubmed for phytochemicals.

DISCUSSION/ CONCLUSION

Johar is a plant species native to southeast Asia that spreads from Indonesia to Sri Lanka, especially in Asian countries, to prevent and treat diseases. Various parts of the *S. siamea* plant contain biological compounds responsible for its antibacterial, and antifungal activities. Several studies have shown that the *S. siamea* plant has antimicrobial activity. *S. siamea* leaf extract has the best antifungal activity, which confirms the strength of the bioactive compounds and proves the use of *S. siamea* plants in health care.[7] However, the effectiveness of *S. siamea* leaf extract against *Candida sp* has not been explore yet.[8] Further, morphological identification of the leaves of *S. siamea* was also carried out. The results of the light microscope and SEM, showed the presence of parasitic stomata, calcium oxalate, and vascular bundles found in *S. siamea* leaf plants as indicated in (Figure 1).

The ultrasonic extraction method is faster and more efficient than for extracting secondary plant metabolites1than1conventional1methods such as maceration or *soxhlation.*[9] Ultra power high audio (20 to 25 kHz) can change the permeability of the cell wall and increased solvent penetrates through plant cells, increasing the solubility of phytochemicals. Extraction using UAE indicated that the extracts obtained from *S. siamea* leaves H extracts had the highest yields than EA and ME extracts as shown at (Table 1). The non-polar content in *S. siamea* leaves is soluble in the extraction process using N-hexane as a solvent. UAE extraction of neem leaves yielded 6.64% hexane1extract, 5.98% ethyl acetate extract, and 5% methanol extract. In addition, the different extraction methods used can cause differences in the oil content produced. Sonication is the most effective method of extracting natural materials. Sonication resulted in high yield, fast extraction time, and good selectivity. In addition, the sonication extraction method is very effective for extracting thermolabile compounds because it can reduce exposure to high temperatures.[10]

The results of *C. albicans* and *C. krusei* the Diffusion value test on the H, EA, and ME extracts as shown in (Table 3) indicated that, the

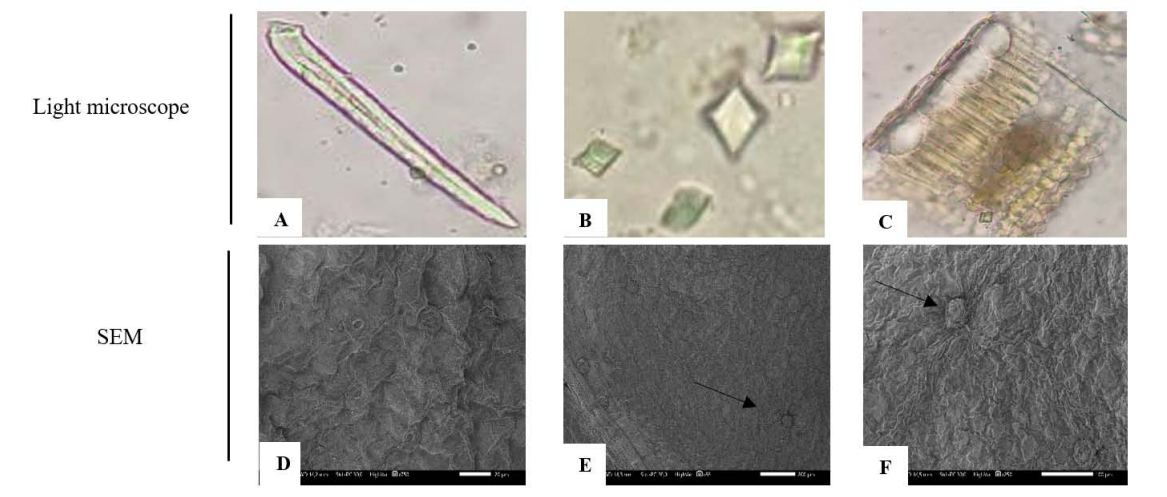

Figure 1: Light Microscopic and SEM results of *S. siamea* leaves. Light microscopic observation indicated oil cell (A) and calcium oxalate In *S. siamea* leaves (B), Epidermis, palisade and cuticula (C). The SEM evaluation was further confirm the presence of D) stomata, E) and F).

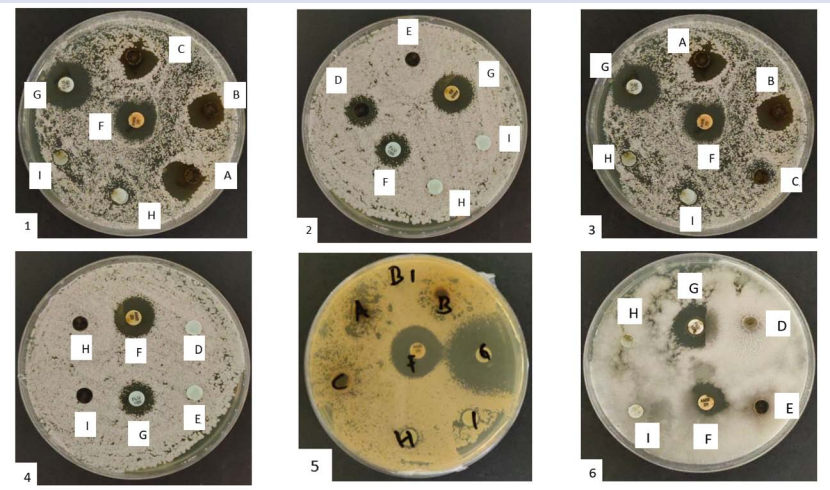

Figure 2: Diffusin test results of *S.siamea*, A. 1000ppm, B. 500ppm, C. 250ppm, D. 125ppm, E. 62.5ppm, F. Amfotericin B, G. Fluconazole, H. Positive control, I. Negative control (1 and 2) Hexane Extract: (3 and 4) Ethyl Acetate Extract: (5 and 6) Methanol Extract: independent experiment each in duplicated ware performed .

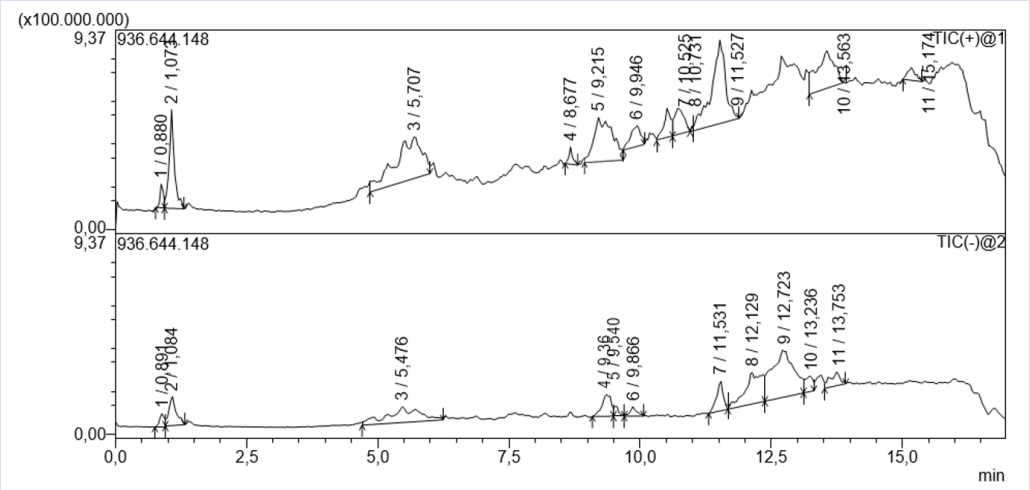

Figure 3: LCMS test identification results.

Table 1: Results extraction of S. siamea leaf using UAE method.

	H	EA	ME
Extract (g)	6.64	5.98	5
Yield (%)	6.64	5.98	5

Table 2: Results of diffusion test on *Candida albicans*.

Extract	Visual observation				
	Concentration (µg/mL)				
	62.5	125	250	500	1000
H	-	11.8mm	16.1mm	18.4mm	22mm
EA	-	-	-	12.2mm	17.6mm
ME	-	-	-	-	10.7mm
Concentration (µg/mL)					
0.1			0.02		
H	22.3mm	Antibiotic Fluconazole	H	35.6mm	
EA	25.6mm		EA	33.4mm	
ME	26.3mm		ME	34.9mm	

Description: - means clear-transparent (no turbid growth)

Table 3: Results of diffusion test on *Candida krusei*.

Extract		Visual observation				
		Concentration (µg/mL)				
		62.5	125	250	500	1000
	H	-	11.8mm	16.1mm	18.4mm	22mm
	EA	-	-	-	12.2mm	17.6mm
	ME	-	-	-	-	10.7mm
	Concentration (µg/mL)					
Antibiotic Amphotericin B	0.1			0.02		
	H	14.9mm	Antibiotic Fluconazole	H	10.4mm	
	EA	22.2mm		EA	14.5mm	
	ME	20.2mm		ME	15.3mm	

Description: - means clear-transparent (no turbid growth)

turbidity was seen at a concentration 500ppm and 1000ppm for Ethyl acetate and concentration 1000ppm for methanol, concentration 125ppm, concentration 250ppm, concentration 500ppm and 1000ppm for hexane looks clear. Then, it was confirmed again by the showed that the hexane extract in lower concentrations could inhibit the growth of *C. albicans* and *C. krusei* compared to the EA and ME extracts of *C. albicans* and *C. krusei* as shown (Figure 2). This showed that the hexane extract had better activity compared to other extracts.[11] It was reported that several chemical compounds in the hexane extract work synergistically so that the antifungal activity produced is more effective than the antifungal activity of a single compound. The Diffusion test on antibiotic was still overgrown with *C. albicans* and *C. krusei* fungal because of the fungal properties of Amfetericin B and Fluconazole.[12]

Based on previous reported research, it's known that the hexane extract contains terpenoid and steroid compounds.[13] Terpenoids are known to inhibit the growth or kill bacteria by interfering with forming membranes or cell walls so that they are not formed or formed imperfectly.[14] In addition, terpenoids alter porins (transmembrane proteins) on the outer membrane of the bacterial cell wall to form strong polymer bonds, destroying the porin.[15] Damage to the porin, which is the entrance and exit of the compound, it will reduce the permeability of the fungal cell wall. This cell wall permeability will interfere with the entry and exit of nutrients and other compounds, inhibit or dead fungal growth. Meanwhile, the mechanism of action of terpenoids as antifungals, according to,[16] is because these terpenoid compounds are fat-soluble so that they can penetrate fungal cells membranes, affect their permeability and cause disturbances in the structure and function of cell membranes.[17]

While the activity in ethyl-acetate and methanol extracts, it is possible that phenol compounds can form hydrogen bonds with fungal cell proteins, which cause damage to the fungal cell protein structure resulting in protein denaturation. Protein denaturation disrupts the permeability of the cell wall and cytoplasmic membrane, resulting in an imbalance of macromolecules and ions in the cell. As a result, the cell becomes lysed.[18]

The data in (Figure 3) show1that the UNIFI LC-MS1analysis is predictive that the compounds were steroid, phenolic, saturated acid, amino acid and terpenoids derivatives. The analysis of1identification of1chemical1compounds1using the LC-MS method with [M+H]+ mode on extract NH from *S. siamea* leaves showed that extract NH on average had a high predictive chemical content of steroid compounds. The1results1of1chemical content1screening1based1on the LC-MS1approach provide1an overview of the compounds contained in extract NH.

This study provides information that *S. siamea* leaves have an antifungal activity that causes *Candida sp*, allowing this plant to be developed as traditional medicine. The best result in inhibiting the growth of *C. albicans* fungal was n-hexane extract which in a concentration of 125ppm could inhibit fungal growth better than other extracts. In addition, the compounds contained in *S. siamea* leaves suspected of using LC-MS also have antifungal effects. Therefore, our research has prospects for development.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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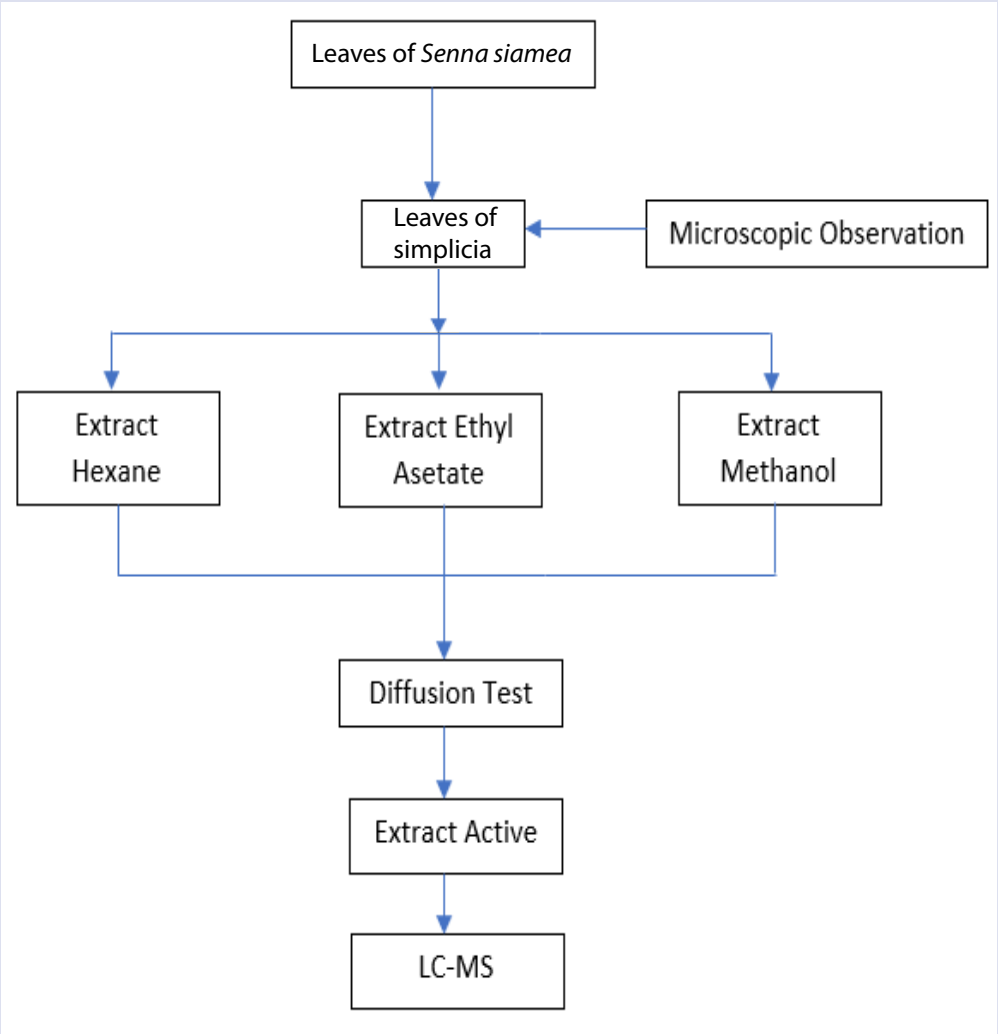
GRAPHICAL ABSTRACT



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