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Research Article
Antifungal Activity of Selected Malaysia's Local Medicinal Plants Against Sick Building Syndrome (SBS) Fungi

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Abstract

Background and Objective: Indoor air quality plays an important role in human health. One of the negative impacts of poor indoor air quality is sick building syndrome (SBS). The main factor that contributes to SBS is biological pollutants such as; fungi, bacteria and viruses. This study aimed to explore the antifungal potential of four medicinal plants in Malaysia namely *Vitex trifolia*, *Vernonia amygdalina*, *Cassia alata* and *Strobilanthes crispus* against 2 of the predominant SBS fungi (*Aspergillus niger* and *Penicillium oxalicum*). **Materials and Methods:** The plant samples were extracted by using the Soxhlet extraction technique. Antifungal screening was conducted by using disc diffusion sensitivity tests. **Results:** The results showed that *S. crispus* extract had the most effective antifungal activity against *A. niger* and *P. oxalicum*, with inhibition diameter of 11.1±0.0 mm ($\times 2$ (3) = 4.520, P = 0.034) and19.1±0.7 mm ($\times 2$ (3) = 8.520, P = 0.014), respectively. The *S. crispus* extract showed the minimum inhibitory concentration of 10 and 5 mg mL^{-1} for *A. niger* and *P. oxalicum*, respectively. Thin layer chromatography technique with solvent ratio 12:8:1 (hexane, chloroform, acetic acid) was applied to fractionate the active compounds from *S. crispus* extract and indicates the presence of flavonoids, alkaloids and terpenoids. However, the active compound efficacy test showed that single active compounds of *S. crispus* were unable to inhibit the fungi growth effectively. **Conclusion:** The *S. crispus* extract was the most effective antifungal agent against *P. oxalicum* and *A. niger*. However, the active compound separation did not exhibit any antifungal activity.

Key words: Sick building syndrome fungi, antifungal test, disc diffusion method, minimum inhibitory concentration (MIC), thin-layer chromatography

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Various types of bacteria and fungi grow on the walls of buildings[1], causing erosion and degradation of the building[2-4]. Failure in controlling the growth of microorganisms inside the building will cause deterioration of indoor air quality, hence leading to sick building syndrome (SBS)[5]. According to Ajam *et al.*[6], SBS is a major concern in Malaysia. In the United States, the World Health Organization (WHO) discovered that almost 30% of their residents have experienced SBS[7].

According to Syazwan *et al.*[8], the building occupants are usually unaware that they been exposed to this syndrome until they experience health issues, like breathing problems[9]. Thus, an indoor air quality assessment is crucial to identify buildings that have SBS[10]. Poor ventilation in the building may leads to the accumulation of spores, bacteria and virus on the surfaces of carpets, curtains, tablecloths or furniture, further polluting the indoor air environment of a building[7].

Several studies have been conducted to mitigate the presence of microorganisms in the indoor air[5]. One of the popular approaches is by using plant extracts containing antifungal properties[6]. Various types of medical plants contain antifungal compounds such as; alkaloid[11,12]. The selection of the plant species for an antifungal activity test should be based on the content of the secondary metabolite, as the secondary metabolites are species-specific[13]. Secondary metabolites play a role in supporting the plant defense mechanism against fungi and bacteria infections. For example, sesquiterpenoid alkaloid that works effectively inhibiting the reproduction of microorganisms[11].

This study, investigated the antifungal activity of four local medicinal plant extracts: *Vitex trifolia* (Lemuni), *Vernonia amygdalina* (Pokok Daun Afrika), *Cassia alata* (Ketepeng) and *Strobilanthes crispus* (Pecah Kaca) against two airborne fungal allergens, *Aspergillus niger* and *Penicillium oxalium*.

MATERIALS AND METHODS

Study area: Samples of *V. trifolia*, *V. amygdalina*, *C. alata* and *S. crispus* were collected from villages around Jeli, Kelantan, Malaysia. The samples were identified by Universiti Malaysia Kelantan's Botanist. This study was carried out from May-December, 2019 at the Faculty of Earth Science, Universiti Malaysia Kelantan.

Preparation of Sample: Table 1 indicates the 4 local medicinal plants that were chosen for the antifungal test[14-17]. Matured and infection-free (from diseases) leaves were selected to insure high concentration of alkaloid and phenolic compounds production[5]. The leaf samples were rinsed to remove dirt from the leaves. Then, the samples were air dried prior to being placed into an oven. The leaf samples were arranged layer by layer and dried at 60°C for 5 days. The dried leaf samples were blended by using a 1 mm sized blender blade. The purpose of this milling process was to increase the surface area of the leaves to ensure the effectiveness of the extraction of the leaf samples.

Table 1: Selected Malaysia's local medicinal plants and its local used

Scientific name	Local name	Local use
Vitex trifolia	Pokok Lemuni	Fever[14]
Vernonia amygdalina	Pokok daun afrika	Tonic source, diarrhea[15]
Cassia alata	Pokok ketepeng	Diabetes, skin problem[16]
Strobilanthes crispus	Pokok pecah kaca	Diabetes, diuretic[17]

The *A. niger* and *P. oxalicum* cultures were prepared according to the McFarland Standard[18]. The fungal spore suspension for each fungal species was prepared by using the 7 day old potato dextrose agar culture. Sterile cotton buds were used to transfer the fungal mycelium into the McCartney bottles containing 9 mL of sterile distilled water. The turbidity level of the distilled water that contained the spore was determined by using a spectrophotometer. The fungal inoculum was adjusted to 0.5 McFarland standards at fungi cell density, 1.5×10 raised to the eight-power cell/ml. The absorption value (*Attenuance*, *A*) of the spectrophotometer was set to 0.132 A.

Extraction of sample: For the extraction process, the weight of the sample needed depended on the ratio of one weight of sample to ten volumes (1:10) of solvent, 80% methanol. Soxhlet extraction method was used. Overall, the evaporation and condensation of the solvent-sample mixture took four to 5 days, depending of the plant samples. The change of solvent color in the Soxhlet tube indicated the completion of extraction process[19]. Next, the sample extract was evaporated by using a rotary vacuum evaporator to ensure the optimal separation of solvent and plant extract. The viscous extract was oven-dried at 60°C for one day. The range of extraction rate was 12.33-33.33%.

Antifungal test: The disc diffusion method was carried out following the description by Verma[5]. The PDA media in petri dish was punched by using a cork borer in the fume hood and each 4 hole was labeled. Next, 100 µL of fungal inoculum from the prepared sample was spread onto the surface of PDA media. Then, 50 µL of leaves extract was pipetted into the punched hole. Ninth replicates were prepared for this test. The plates were incubated at room temperature (23-25°C) for 4 days before the diameter of inhibition was measured.

Table 2: Significant different of fungi inhibition diameter

Types of fungi	Extract of sample	Average diameter of inhibitor	x^2	p-value	Significant level
Penicilium oxalicum	*Vitex trifolia*	12.3±0.06	8.520	0.01	Significant
	Vernonia amygdalina	11.7±0.45			
	Cassia alata	11.8±0.14			
	Strobilanthes crispus	19.1±0.74			
Aspergillus niger	*Vitex trifolia*	90.9±0.22	4.520	0.03	Significant
	Vernonia amygdalina	90.8±0.07			
	Cassia alata	90.4±0.02			
	Strobilanthes crispus	11.1±0.02			

Inferential statistic test (Kruskal-Wallis) was conducted to identify the significant differences of fungal diameter for each plant extract. The hypothesis of the statistic test is as follow:

H_o = Different types of extract are not significantly related to the diameter of fungi inhibitor

H_a = Different types of extract are significantly related to the diameter of fungi inhibitor

Based on the antifungal activity observed (Table 2) the *S. crispus* extract was used to determine the minimum inhibitory concentration (MIC). The MIC was determined by using amended PDA media containing several *S. crispus* extract concentrations[13]: 10, 5, 2.5, 1.25 and 0 mg mL^{-1}.

About 100 µL of fungi inoculum was pipetted into each prepared PDA media for MIC test and spread by using sterile cotton buds in aseptic conditions. The plates were incubated at room temperature for two days. The plate (representing one extract concentration) that did not have any fungi mycelium growth was considered as the required MIC.

Thin-layer chromatography: Separation of the active compounds was carried out by using the thin-layer chromatography method (TLC). The silica gel plate (F256) was cut into 5×10 cm. A straight line with 1 cm from the bottom and the top were pencil-drawn. After the separation process, the extract was dropped on the bottom and top line to indicate the limit of the organic solvent.

The mixture of the solvent should be tested first to obtain the best compound separation. As proposed by Harborne[20], the solvent system was determined based on the ratio of hexane, chloroform and acetic acid. There are 6 ratio solvents were tested in this study (16:4:1, 12:8:1, 10:10:1, 4:16, 1, 6:14:1, 8:12:1). About 0.2 g extract sample was dissolved with chloroform and dropped on the top line on the white area of *silica* gel plate by using a 5 µL capillary tube. Then, the TLC plate was inserted into a chromatographic tank that contained the mixture of solvents.

The compound separation was complete when the solvent absorption level reached the top part of the *silica* gel plate. Then, the silica gel was removed with forceps and dried. The isolate compound on the dried gel was observed under daylight and UV light (254 and 360 nm). The spot of colored separation observed on the surface of chromatogram was marked by using pencil. The color of the separated compound under UV light and normal light was recorded. Detection of the phytochemicals was conducted by comparison of retention factor (R_f) values and spot colours with literature data. The distance of the color spot from the bottom line was measured to obtain the value of R_f, calculated by using the formula below:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Antifungal activity of active compound: Antifungal activity of the active compound from the extract (bioautography) was conducted as proposed by Meyers and Smith[21]. The chromatogram plate was mounted onto the surface of PDA media and left overnight to enable the active compound to be absorbed onto the PDA surface. Next, the chromatogram was removed by using sterile forceps and 100 µL of fungi inoculum was spread onto the surface of PDA. The petri dish was incubated for two days at 27°C. The separation that showed the inhibition of bacteria growth was recorded.

RESULTS AND DISCUSSION

Antifungal test: The *S. crispus* extract showed significant inhibition on the growth of both fungi, *P. oxalicum* (P = 0.01) and *A. niger* (P = 0.03) compared to the other plant extracts (Table 2). The results also showed that the average inhibition diameter for *P. oxalium* was wider (19.1±0.74) compared to that of *A. niger* (11.1±0.02 cm). This difference may stem from the ability of *A. niger* to resist the antifungal activity from the active compound extracts compared to *P. oxalium*[22,23].

According to Rahmat *et al.*[24], *S. crispus* extract contains volatile compounds such as; phytol (46.01%), alpha canidol (3.47%), tau-muurolol (2.49%), ledol (1.81%) and eugenol (1.08%). Phytol is a colorless compound and makes up the largest percentage in *S. crispus* compared to other

Table 3: Minimum inhibitory concentration of *S. crispus* extract towards Mycelium of *A. niger* and *P. oxalicum*

Minimum inhibitory concentration (mg mL^{-1})	Inhibitory rate of mycelium (%)	
	A. niger	*P. oxalicum*
0.00	0.00	0.00
1.25	39.27	45.79
2.50	55.67	60.98
5.00	65.79	100.00
10.00	100.00	100.00

Table 4: Rf values of thin layer chromatography

Solvent ratio	No. of spot	Rf values
16:4:1	2	0.14
		0.20
12:8:1	7	0.11
		0.15
		0.27
		0.37
		0.43
		0.51
		0.58
10:10:1	2	0.33
		0.41
4:16:1	4	0.17
		0.23
		0.43
		0.69
6:14:1	2	0.45
		0.54
8:12:1	2	0.26
		0.48

compounds. Phytol is an alcohol from acyclic diterpene at the highest boiling point. Diterpene is produced naturally by most trees as a secondary metabolite and is an effective antifungal compound[25].

Among all the tested plant extracts, *S. crispus* produced the most significant inhibition diameter on both fungal species. Therefore the *S. crispus* extract was tested to obtain the MIC value. Table 3 shows that MIC value of *S. crispus* extract was 10 mg mL^{-1} for *A. niger* and 5 mg mL^{-1} for *P. oxalicum*. The diterpenoid compound in *S. crispus* leaves is an active compound that inhibited the growth of fungi mycelium like *Aspergillus brasiliensis* on PDA[26]. Diterpenoid is terpenoid compound that has antifungal properties affecting cell walls and membranes and disrupts cell permeability. Due to its effect on cell membrane, terpenoid also affects electron transport, nutrient movement, protein production and enzyme activity[27,28], hence causing death to fungi and impairing their growth.

Thin-layer chromatography: The thin layer chromatography method was chosen to separate the active compounds in the extract. According to Panda *et al.*[29], as compared to other chromatographic techniques, TLC is the most simple, economical, rapid and flexible technique allowing sensitive parallel processing of many samples on one plate. Therefore, TLC was used as a "fingerprint" method to characterize the *S. crispus* extracts. This study found out that the ratio of organic solvents at 12:8:1 (Hexane: Chloroform: Acetic Acid) produced the most spot which is 7 spots that were visible under UV light (254 and 360 nm). The retention factor (Rf) values recorded were ranging between 0.11-0.69 (Table 4). Mostly spots of yellow, brown and purple colours were observed on TLC plates under UV, which indicates the presence of flavonoids, alkaloids and terpenoids, respectively[28].

Antifungal activity of active compound: The chromatogram plate from the ratio of organic solvents at 12:8:1 has been selected for antifungal activity. However, there was no inhibition activity of fungi recorded. According to Nurraihana and Norfarizan-Hanoon[30], even though various types of chemical compounds have been identified from *S. crispus*, research reports on isolated compound and the bioactivity and the mechanism of action of the isolated compounds are limited. This finding suggests that the antifungal activity in the plant extract may involve the mixture of more than two active compounds.

CONCLUSION

In conclusion, the *S. crispus* extract was the most effective antifungal agent against *P. oxalicum* and *A. niger*. The minimum inhibitory concentration showed that the antifungal activity of the *S. crispus* extract was more effective against *P. oxalicum* compared to *A. niger*. The active compound separation did not exhibit any antifungal activity, indicating that the antifungal activity needed the combination of more than one compound to be effective.

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SIGNIFICANCE STATEMENT

This study discovered the potential of local medicinal plant in Malaysia, *S. crispus* as natural biocide against SBS fungi that can be beneficial for society because the natural

biocide is harmless and nontoxic to human. Further studies need to be conducted to explore the synergistic reaction of multiple active compounds in *S. crispus* leaf extract. This study will help the researchers to uncover the critical areas of antifungal activity of medicinal plant against SBS fungi that many researchers were not able to explore. Thus, it's opens new opportunities to enhance the school of thought for understanding a new paradigm on antimicrobial research.

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