Malaysian Journal of Microbiology, Vol 17(5) 2021, pp. 576-587
DOI: http://dx.doi.org/10.21161/mjm.211144

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Antifungal effects and phytochemical screening of *Andrographis paniculata* extracts on dermatomycoses

Tuan Kub Tuan Noorkorina[1,2]*, Farhaana Mohd Ab Aziz[1], Anis Amiera Muhamad Alojid[1], Nursaadatun Nisak Ahmad[3], Zeti Nurfidiyati Salmuna[1,2], Siti Asma' Hassan[1,2], Sabarisah Hashim[4] and Azian Harun[1,2]

[1]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia.
[2]Medical Microbiology and Parasitology Laboratory, Hospital Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia.
[3]Faculty of Business and Management, Universiti Teknologi Mara, Puncak Alam, Bandar Puncak Lama, 42300 Kuala Selangor, Selangor, Malaysia.
[4]Department of Neuroscience, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia.
Email: tnkorina@usm.my

Received 5 April 2021; Received in revised form 26 June 2021; Accepted 3 September 2021

ABSTRACT

Aims: *Andrographis paniculata* (AP), a medicinal herb was selected to investigate the antifungal activity on selected dermatophyte fungi. The phytochemical screening was also carried out to evaluate its chemical constituents.
Methodology and results: The potato dextrose agar (PDA) incorporated with aqueous, ethanol and methanol AP extracts at concentrations 0.99% (v/v), 1.96% (v/v) and 7.41% (v/v) were used for selected fungi culturing; *Trichophyton mentagrophytes*, *T. rubrum*, *T. interdigitale*, *Microsporum fulvum*, *M. nanum*, *M. gypseum*, *M. canis*, *Fusarium solani* and *Aspergillus fumigatus.* Phytochemical screening showed the presence of flavonoids, saponins and tannins in the ethanol extract and flavonoids alone in both aqueous and methanol extracts. Studies on antifungal effects indicated that the ethanol extract significantly increased the mycelial inhibition percentage of all tested fungi, especially at a concentration of 7.41% (v/v). All ethanol AP extract concentrations inhibited *M. gypseum* and *M. canis* (p<0.05) with at least 36.00% mycelial inhibition. In aqueous AP extract, it significantly increased the mycelial inhibition of *T. mentagrophytes*, *T. interdigitale* and *M. gypseum* (p<0.05), while the methanol AP extract significantly inhibited all fungi at a concentration of 7.41% (v/v) except for *T. rubrum*, *M. gypseum* and *F. solani* (p<0.05). No spore sedimentation was recorded for the fungal spores of *T. rubrum*, *M. nanum*, *T. mentagrophytes*, *M. gypseum* and *T. interdigitale* at 7.41% (v/v) ethanol AP.
Conclusion, significance and impact of study: It is concluded that the ethanol AP extract contained phytochemical constituents and showed the highest antifungal activity. In addition, this extract has a great potential to treat dermatophytes effectively.

Keywords: *Andrographis paniculata*, antifungal, mycelial inhibition, phytochemical, spore

INTRODUCTION

Medicinal plants are known to form an essential component of the rainforest biodiversity currently found in Malaysia. The medicinal plant sources are getting a significant expansion due to high interest among the public. As the plants contain diverse phytochemicals, it is more common now to consume drugs with medicinal herbs. Therefore, there is a need for studies evaluating the potential of herbal medicines concerning their quality and efficacy.

Andrographis is a shrub commonly found throughout India and other Asian countries. It is sometimes referred to as "Indian echinacea". This shrub grows to a height of 30-110 cm in moist shady places with glabrous leaves and white flowers with rose-purple spots on the petals (Verma *et al.*, 2019). *Andrographis paniculata* (AP) is one of the medicinal plants that seem to be found throughout Southeast Asia. The *Andrographis* tastes very bitter. The chemical compounds giving its bitter taste is related to the various pharmacological properties, such as anti-allergic, antibiotic, anticancer, anti-inflammatory, antimicrobial, antivenom, hypoglycaemic, immunostimulatory and antiviral activity (Sivananthan and Elamaran, 2013).

AP has a wide range of pharmacological effects. The principal medicinal component of the AP is

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 576-587
DOI: http://dx.doi.org/10.21161/mjm.211144

andrographolide, a diterpene lactone. Andrographolide has been reported for its anticancer (Malik *et al.*, 2021), cardioprotective and antioxidant properties (Adeoye *et al*., 2019). Other active components include 14-deoxy-11,12-didehydroandrographolide (andrographolide D), homoandrographolide, andrographosterin and stigmasterol (Yoopan *et al.*, 2007). The AP chemical compound was reported to have antimicrobial effect and greater potential as alternative antibiotics and antifungals. Hence, these alternative treatments have revolutionised the antibiotics and antifungal drugs treatment of various bacterial and fungal infections. For example, the methanol extract of AP has been reported to inhibit the growth of *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Enterococcus faecalis*, while the aqueous extract showed antibacterial effects on *S. aureus and Pseudomonas aeruginosa*. Acetone and alcohol extracts of AP recorded a maximum inhibitory effect on *Bacillus subtilis* (Geetha and Catherine, 2017). Traditionally, the plant's decoction is usually drunk to treat fever, flu, hypertension, tonsilitis and chest pain. To the best of our knowledge, there is a lack of established reports on the antifungal effect of AP on mould infections.

Among mould infections, dermatomycosis is a disease involving fungal infections to the skin or its appendages. This disease consists of three groups, which are systemic mycosis, subcutaneous mycosis and superficial mycosis. The main fungi groups causing superficial fungal infections are dermatophyte fungi, moulds and yeasts. Superficial fungal infections of the skin are common diseases found in clinical practice, with dermatophytosis as a common infection (Bitew, 2018). These fungi can produce keratinase, allowing them to live on human keratin, such as hair, nail and skin. The genus *Trichophyton*, *Epidermophyton* and *Microsporum* commonly cause these infections (Mercer and Stewart, 2019).

A report recorded the dermatophyte, *Trichophyton rubrum* as the common isolated fungal in clinical practice. Kaul *et al.* (2017) reported that *T. rubrum* and *T. mentagrophytes* are common pathogens infecting skin and nail, while *T. tonsurans*, *T. violaceum* and *Microsporum canis* are most common in scalp and hair shaft infections. Other than that, non-dermatophyte moulds from the hyalohyphomycete family, such as *Aspergillus* species and *Fusarium* species, are the most common group of isolated fungi (Tzar *et al.*, 2014).

The major antifungal used to treat dermatophyte infections including tioconazole, clotrimazole, oxiconazole, econazole, miconazole, naftifine and terbinafine. As the treatment requires prolonged therapy, resistance occurs mostly when using terbinafine, fluconazole, itraconazole, amphotericin B and griseofulvin (Lopes *et al.*, 2017). Therefore, there is a potential to explore herbal medicine due to the demand for a new formulation without side effects and fungal resistance.

The use of plants as therapeutic agents remains an essential component of the traditional medicinal system, as 80% of the world's population rely upon medicinal plants (Tugume and Nyakoojo, 2019). The usage of medicinal plants in infection treatments attain success as they are cost-effective, eco-friendly and have fewer side effects compared to existing drugs. As AP is claimed to possess many antimicrobial effects, this study attempts to embark on the antifungal activity against dermatomycoses. Besides, limited studies have been carried out on antifungal property against dermatomycoses. Recent years have witnessed a renewed interest in exploring natural resources for developing such compounds.

The antifungal activities of aqueous, ethanol and methanol AP extracts have been evaluated against the selected group of fungi, as they showed many antimicrobial effects (Hossain *et al.*, 2021). Therefore, this study selected a few species from the genus of *Trichophyton* and *Microsporum*, namely *T. mentagrophyte*, *T. rubrum*, *T. interdigitale*, *M. fulvum*, *M. nanum*, *M. gypseum*, *M. canis*, *Fusarium solani* and *Aspergillus fumigatus*, as non-dermatophytes moulds. On the other hand, the phytochemical screening of AP extract was carried out to detect the presence of selected phytochemical components.

MATERIALS AND METHODS

Materials

Andrographis paniculata plants were obtained from a planting farm in Tendong, Pasir Mas, Kelantan. The fresh plant was cleaned using tap water, dried and blended into powder form. The powder was stored at 4 °C prior to extraction process.

The antifungal study used the fungi isolates were obtained from microbial stock cultures collection at the Mycology Laboratory, Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia. The cultures were maintained on PDA at 35 °C and further identified by microscopic examination using Lactophenol Cotton Blue (LPCB).

Preparation of AP extracts

Aqueous AP extract

An approximately 100 g of ground dried AP was extracted using the Soxhlet instrument and distilled water as a solvent. Two litres of distilled water were heated at 100 °C, and the extraction process was carried out for 20-35 h. The extract was filtered and evaporated using a rotary evaporator. The concentrated extract was then placed in universal bottles and chilled at –20 °C. After that, the extract was freeze-dried overnight (Adnan *et al.*, 2019).

Ethanol and methanol AP extracts

An approximately 100 g of ground dried AP was soaked in a flask containing one litre of ethanol and blended using a laboratory blender. Then it was kept in several flasks and agitated for about two hours on an orbital

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 576-587
DOI: http://dx.doi.org/10.21161/mjm.211144

shaker (120 rpm) at room temperature (27 ± 3 °C). The supernatant/extract was then strained through a layer of Whatman No. 2 filter paper. The filtrate was evaporated using a rotary evaporator followed by further evaporation in an incubator at 35 °C for a few days to get the paste-like extract (Alfa *et al.*, 2019).

The same procedure was carried out to obtain the methanol extract, in which methanol was used instead of ethanol.

Preliminary phytochemical screening

Both of the extracts were subjected to a preliminary phytochemical screening following the standard protocols (Edeoga *et al.*, 2005).

Test for alkaloids

A total of 20 mg extract was added to 10 mL of methanol and placed in a sonic bath to dissolve. Then, it was filtered using a Whatman No. 1 filter paper and 2 mL of the filtrate was taken and mixed with 1% HCl. Six drops of Mayer's reagent were added to 1 mL of this mixture. Within a few minutes, in the presence of the alkaloids, a yellow-creamish precipitate colour was expected.

Test for saponins

A total of 20 mg was added to 10 mL of methanol and placed in a sonic bath to dissolve. Then, it was filtered using a Whatman No. 1 filter paper and 0.5 mL of the filtrate was mixed with 5 mL of distilled water. The frothing persistence indicated the presence of saponins.

Test for flavonoids

An approximately 0.5 g of extract was mixed with 5 mL of distilled water and filtered. Then, a few drops of sodium hydroxide were added to the filtrate, forming an intense yellow colour and later becoming colourless upon addition of diluted acetic acid, indicating the presence of the flavonoids.

Test for terpenoids

A total of 10 mL chloroform was added into 20 mg of extract, followed by the filtration process. Then, 2 mL of acetic anhydride and concentrated sulfuric acid were added to the filtrate. A blue-green ring on top of the mixture indicated the terpenoids presence.

Test for tannins

To determine the presence of tannins, 20 mg of extract was mixed with 10 mL of 10% ammonia solution and then shaken. The emulsion formation indicated the existence of tannins.

Antifungal bioassay

Poisoned food technique

Concerning the antifungal bioassay, the poisoned food technique was employed as it is commonly used to observe antifungal activities as reduction in the mycelial growth of mould. Besides plant pathology, this methodology is also used in anti-dermatophytic studies in medical microbiology (Mahboubi and Kazempour, 2015).

In this study, the poisoned food technique by Balamurugan (2014) was adopted. The aqueous, ethanol and methanol AP extracts were studied against the fungi, namely *T. mentagrophytes*, *T. rubrum*, *T. interdigitale*, *M. fulvum*, *M. nanum*, *M. gypseum*, *M. canis*, *F. solani* and *A. fumigatus*. The principle of this methodology is that the antifungal activity is observed when there is a reducing mycelial growth of fungi inside the 'poisoned plates' compared to the controlled plates.

PDA medium was prepared by autoclaving it at 121 °C and cooling it to 45 °C. Then, appropriate stock solution quantities of each extract were added to the PDA medium [(0.2 mL extract + 20 mL PDA), (0.4 mL extract + 20 mL PDA) and (1.6 mL extract + 20 mL PDA)] to get 0.99% (v/v), 1.96% (v/v) and 7.41% (v/v) concentrations of the extracts. This methodology was adapted from Tuan Noorkorina *et al.* (2018).

For the control measure, only PDA was used instead of plant extract. All fungi were inoculated onto the medium by placing the cultures at the centre of each Petri dish. All tests were performed in triplicates. The disc position was marked on the dish base using a marker pen and two orthogonal axes passing through the disc centre were marked to be used as references for growth recording. The inoculated plates were then incubated at 35 °C for seven days. The growth diameter along each line was recorded after seven days by using callipers. The mycelial inhibitions percentage was calculated as below:

$$\text{Mycelial inhibition (\%)} = [(dc-dt)/dc] \times 100$$

where, dc = colony diameter in control plate and dt = colony diameter in treatment plate

Spore sedimentation

Further study regarding the spore formation of aqueous and ethanol AP extracts were carried out to evaluate the relation of the fungal growth activity and the AP extract antifungal effect. The comparison among the two extracts was selected as the AP ethanol extract exhibited highest antifungal effect, while AP aqueous extract exhibited lowest antifungal effect in the earlier study.

The evaluation was carried out using the method described by Noorkorina and Noorfatiehah (2012). In order to prepare a conidial suspension, the seven-day-old culture plate was flooded with 10 mL of sterile distilled water. By then, all the fungal conidia and mycelia were

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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scraped using a sterile scalpel. The conidial suspension was then filtered using Whatman No. 1 filter paper into a test tube. The spore quantity in the conidial suspension was recorded by measuring the spore sedimentation height in millimetre unit. The significant difference of the results was compared to control. The spore sedimentation of the fungi inoculated onto the PDA plate without AP extracts were used as control.

Microscopy study using scanning electron microscope (SEM)

Under the SEM, the fungal colony from the plate with the extract that exhibited the highest antifungal activity was examined. In order to assess the ethanol AP extract effect on the fungal ultrastructure, the SEM was used to examine the specimen exterior features. Concerning the preparation of fungal tissue, the seven-day-old fungal cultures of *T. mentagrophytes*, *T. interdigitale*, *M. gypseum* and *M. canis* were manipulated in this study. These four fungi were selected as they produced significant difference of mycelial inhibition at least at 1.96% (v/v) of AP ethanol concentration.

Blocks of the culture of approximately 5 × 5 mm were cut from the sporulating colonies on the plate and positioned on a planchette using double-sided sticky tape. The planchette was placed in a Petri dish lined with a filter paper. Vapour fixation was completed by placing a few drops of 2% osmium tetroxide on the filter paper, and the Petri dish was instantly closed. The planchette was plunged into slushy nitrogen (–210 °C) and transferred onto the 'Peltier-cooled' stage of the freeze dryer and left to freeze-dried for about 10 h after storing in the fume hood for 1-2 h. Subsequently, the sample was sputtered with 5-10 nm of gold before viewing it under Field Emission Scanning Electron Microscope (FESEM) (Carl Zeiss Leo Supra 50 VP Field Emission equipped with Oxford INCA – X energy dispersive microanalysis system).

Statistical analysis

Analysis of data was performed using the Statistical Package for Social Science (SPSS), version 25.0 software by general linear model, repeated measures analysis of variance (ANOVA) for each group. A value of $p<0.05$ was considered as statistically significant.

RESULTS

Preliminary phytochemical screening

In the present study, preliminary phytochemical screening has been done on aqueous, ethanol and methanol AP extracts and being compared with one another. It exhibited the various presence of phytochemical constituents (Table 1). It showed that the ethanol AP extract contained flavonoids, saponins and tannins, while aqueous and methanol AP extracts only contained flavonoids.

Table 1: Analysis of phytochemicals in *Andrographis paniculata* extracts.

Phytochemicals	Aqueous extract	Ethanol extract	Methanol extract
Alkaloid	-	-	-
Saponin	-	+	-
Terpenoids	-	-	-
Flavonoids	+	+	+
Tannins	-	+	-

+ = Presence; - = Absence.

Antifungal bioassay

Poisoned food technique

The results of AP extract antifungal activity are summarised in Table 2. The extracts produced different antifungal activity levels against the fungi tested. Among all fungi tested, *M. gypseum* was found to be the most susceptible towards all tested extracts.

The results indicated that ethanol extracts significantly increased the mycelial inhibition percentage of all fungi tested, especially at a concentration of 7.41% (v/v). It also significantly increased the mycelial inhibition of *M. canis* and *M. gypseum* ($p=0.001$) at all concentrations. The aqueous extract significantly increased the mycelial inhibition of *T. mentagrophytes*, *T. interdigitale* and *M. gypseum* ($p<0.05$) at all concentrations. However, the remaining six fungi showed no significant difference in mycelial inhibition effects compared to control.

The highest inhibition of the ethanol AP extract mycelial growth was observed with *T. mentagrophytes* (97.41%) at a concentration of 7.41% (v/v), while the lowest inhibition was observed with *A. fumigatus* (4.5%) at a concentration of 0.99% (v/v).

The methanol AP extract showed a high mycelial inhibition percentage at a concentration of 7.41% (v/v) for all tested fungi except for *M. gypseum*. At the lowest concentration of 0.99% (v/v), no significant difference in the mycelial inhibition of all tested fungi was observed. Significant mycelial inhibition was seen at the highest concentration of methanol AP extract [7.41% (v/v)] for all tested fungi except for *M. gypseum*, *T. rubrum*, *M. nanum* and *F. solani* ($p<0.05$). However, at the highest concentration of ethanol AP extract [7.41% (v/v)], it showed a significant difference for all tested fungi.

Figure 1 shows the colonies on PDA and aqueous, and ethanol AP extracts in which the best inhibitory effects were observed for all fungi treated with the ethanol AP extract at a concentration of 7.41% (v/v) [Figure 1 (a–i); e-3].

Spore formation

The earlier study revealed the good antifungal results of the ethanol AP extract compared to the least antifungal effect of the aqueous AP extract. Therefore, the AP extract spore formation study was carried out concerning aqueous and ethanol AP extracts.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 576-587
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Table 2: Antifungal activity of *Andrographis paniculata* extracts on fungi growth using poisoned food technique.

Fungi	Percentage of mycelial growth inhibition (%)								
	Aqueous extract concentration (v/v)			Ethanol extract concentration (v/v)			Methanol extract concentration (v/v)		
	0.99%	1.96%	7.41%	0.99%	1.96%	7.41%	0.99%	1.96%	7.41%
T. mentagrophyte	19.40*	8.20*	22.10*	16.64	36.86*	97.41*	0.30	37.28	88.47*
T. rubrum	20.60	31.10	42.30	12.58	59.62	97.07*	8.33	4.17	80.56
T. interdigitale	20.00*	22.70*	51.40*	19.80	57.10*	92.20*	3.99	42 .89	96.00*
M. fulvum	21.80	45.90	32.00	8.63	17.26	90.38*	31.12	42.60	93.35*
M. nanum	13.40	21.80	32.70	17.04	45.33	96.46*	23.37	32.18*	85.44
M. gypseum	33.50*	28.90*	56.80*	79.70*	79.20*	88.80*	0.13	1.75	7.39
M. canis	1.00	5.20	13.70	36.00*	60.40*	73.30*	16.23	43.82*	90.52*
F. solani	7.70	4.90	1.40	8.86	25.17	92.08*	6.35	10.06	67.49
A. fumigatus	1.20	1.60	1.40	4.50	9.70	80.20*	8.84	46.42*	90.77*

* denotes significant when value of $p < 0.05$ compared to control (0.00% inhibition).

Table 3: Antifungal effect of *Andrographis paniculata* extracts on spores' sedimentation.

Fungi extract concentration (v/v)		Spore sediment height (mm)								
		MF	TR	MN	MC	FS	TI	TM	MG	AF
AE	0.99%	1.70 (2.02)	1.23 (2.17)	1.13 (1.28)	1.17 (1.33)	1.57 (2.51)	1.80 (2.12)	2.88 (3.14)	2.14 (2.81)	3.02 (3.21)
	1.96%	1.50 (2.11)	0.97* (3.16)	0.93 (1.48)	0.97 (1.59)	1.43 (2.35)	0.53 (2.23)	2.96 (3.59)	2.56 (2.95)	2.98 (3.31)
	7.41%	0.93* (4.10)	0.90* (4.21)	0.90 (1.33)	1.03 (1.72)	1.53 (1.68)	0.77 (2.45)	1.28* (3.72)	1.32* (3.03)	2.76 (2.94)
EE	0.99%	3.13 (4.02)	3.03 (3.19)	1.00 (1.64)	1.13 (1.91)	1.27 (1.85)	0.73 (2.34)	1.76 (2.81)	0.14 (2.31)	2.15 (2.89)
	1.96%	1.33 (2.23)	0.90 (2.75)	1.17 (1.51)	0.99 (1.38)	0.97 (1.99)	0.30 (2.18)	1.37 (2.68)	0.32 (2.38)	1.99 (2.56)
	7.41%	0.83 (2.01)	0.00* (4.18)	0.00* (1.99)	0.32* (2.67)	0.67* (3.08)	0.00* (2.38)	0.00* (2.93)	0.00* (2.94)	1.84 (2.61)

* denotes significance difference compared to control when value of $p < 0.05$. The value in bracket represents control data.
Abbreviations: TM, *Trichophyton mentagrophyte*; TR, *Trichophyton rubrum*; TI, *Trichophyton interdigitale*; MF, *Microsporum fulvum*; MN, *Microsporum nanum*; MG, *Microsporum gypseum*; MC, *Microsporum canis*; FS, *Fusarium solani*; AF, *Aspergillus fumigatus*; AE, aqueous extract; EE, ethanol extract.

There was a significant inhibition ($p<0.05$) of sporulation by the aqueous AP extract on *M. fulvum*, *T. mentagrophytes* and *M. gypseum* at concentration of 7.41% (v/v) and *T. rubrum* at concentration of 1.96% (v/v) and 7.41% (v/v) (Table 3).

The significant difference ($p<0.05$) was also detected for the ethanol AP extract of the fungi tested, such as *T. rubrum*, *M. nanum*, *F. solani*, *T. mentagrophytes*, *M. gypseum*, *M. canis* and *T. interdigitale* at a concentration of 7.41% (v/v). From the results, it shows that that both extracts did not affect *A. fumigatus* ($p>0.05$). The highest spore precipitation was recorded in the ethanol AP extract for *M. fulvum* (3.13 mm), while the lowest was for *T. interdigitale* (0.30 mm).

The higher concentration of the aqueous AP extract showed a decrease in the fungal spore precipitation for *M. fulvum*, *T. rubrum*, *T. mentagrophytes and M. gypseum.* In the case of the ethanol AP extract, it showed the same for all fungi except for *M. fulvum* and *A. fumigatus*.

Microscopy study of fungi ultrastructure

The micromorphological features complemented the mycelial growth inhibition results on SEM. Scanning electron microscopy findings showed that the treatment of the fungal strains with the ethanol AP extract at the highest concentration (7.41%) had a harmful effect on their structure. Electron micrographs of all tested fungi showed a stark contrast in their structures compared to those without the ethanol AP extract exposure.

Scanning electron micrographs of *M. canis* can be seen in Figure 2. The comparison between normal and treated hyphae showed a little swollen appearance along the hyphae. The morphology of treated hyphae was also found shrivelling, blistering and lysed (Figures 2B and 2C).

In *T. mentagrophytes*, unlike the normal structures (Figure 3A), the exposure to the extract led to the deformation of cellular structure, as shown by the hyphal shrivelling and shrunken conidia. There were some

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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DOI: http://dx.doi.org/10.21161/mjm.211144

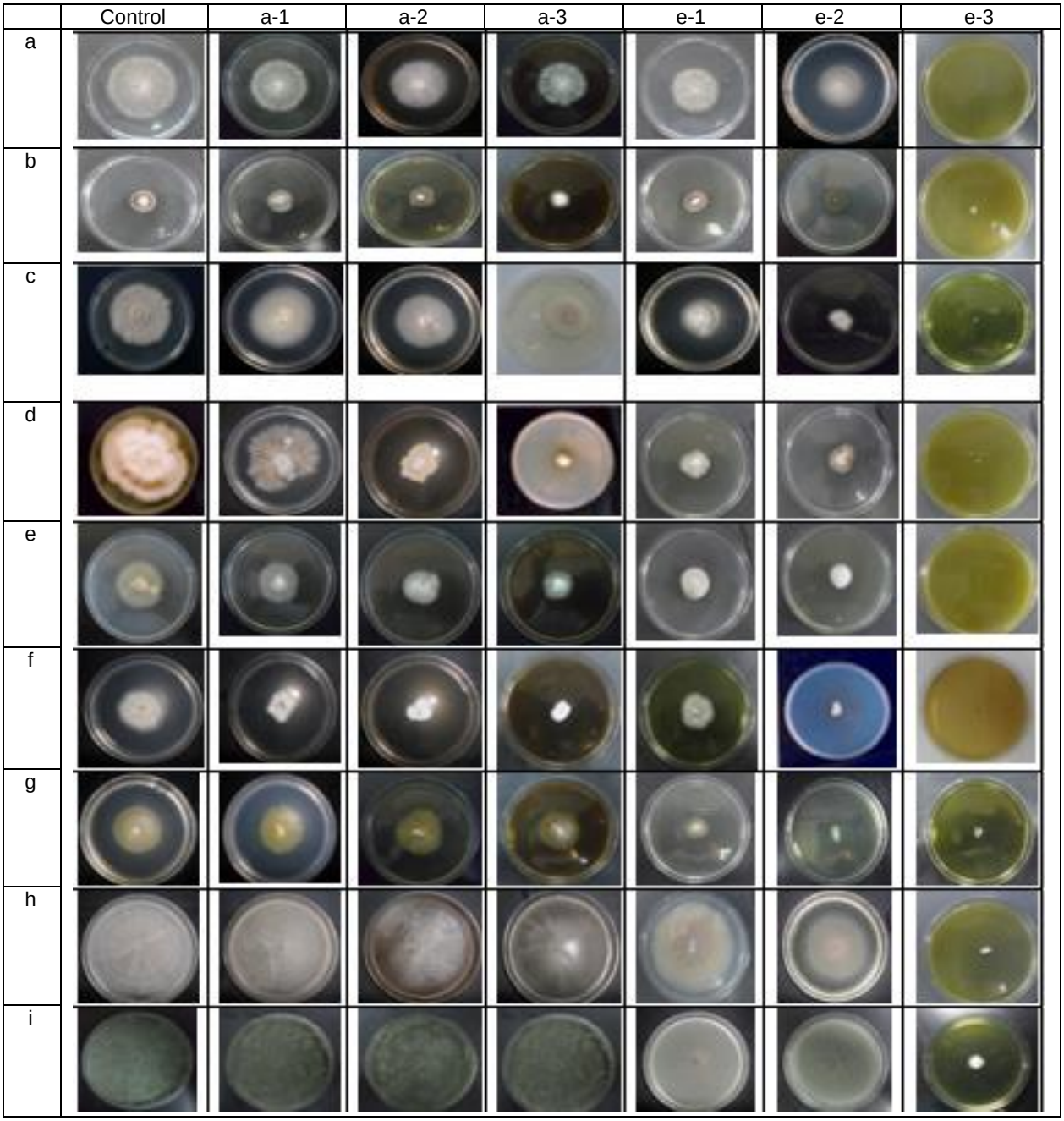

Figure 1: Fungal colonies on PDA and *Andrographis paniculata* extract at 30 °C after 7 days incubation. (a) *Trichophyton mentagrophytes*, (b) *Trichophyton rubrum*, (c) *Trichophyton interdigitale*, (d) *Microsporum fulvum*, (e) *Microsporum nanum*, (f) *Microsporum gypseum*, (g) *Microsporum canis*, (h) *Fusarium solani* and (i) *Aspergillus fulvum*. a-1: AP aqueous extract at 0.99% (v/v), a-2: AP aqueous extract at 1.96% (v/v), a-3: AP aqueous extract at 7.41% (v/v), e-1: AP ethanol extract at 0.99% (v/v), e-2: AP ethanol extract at 1.96% (v/v), e-3: AP ethanol extract at 7.41% (v/v).

abnormalities, such as hyphal and conidia blistering, as well as the visible spore formation (Figures 3B–3E).

In *T. interdigitale*, ethanol AP caused the wrinkled hyphae with the abnormal shape of conidia, such as shriveling and breakage of conidia (Figure 4B and 4C). In *M. gypseum*, the non-exposed culture showed normal structure of tubular hyphae, smooth cell walls and the hyphae were produced in large amounts (Figure 5A), while the extract-exposed culture produced the hyphae with rough surface (Figure 5B and 5C).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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Figure 2: Scanning electron microscopy of *Microsporum canis* exposed to ethanol AP extract (scale bars = 2 µm; original magnification: 5000×). (A) Healthy hyphae in control Petri plate (arrow), (B and C) Effects of AP on hyphal morphology. Note alterations in hyphal morphology including little swelling along the hyphae, hyphal shrivelling and blistering (arrows) in Figure B and wrinkle and lysis (arrow) in Figure C.

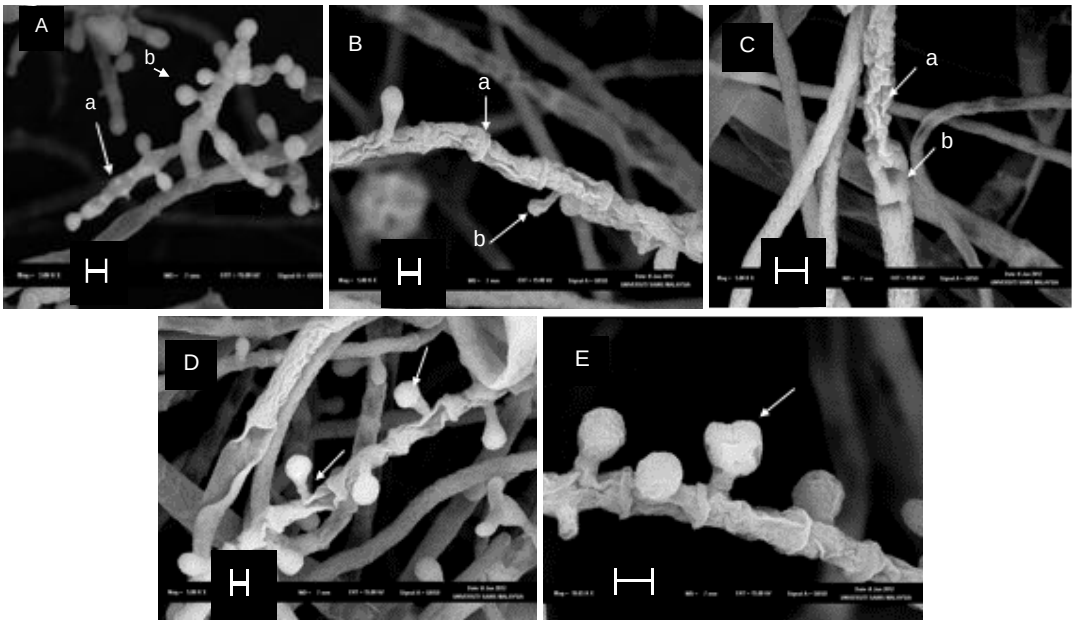

Figure 3: Scanning electron microscopy of *Trichophyton mentagrophyte* exposed to ethanol AP extract (Scale bars = 2 µm; original magnification: 4000×). (A) Healthy hyphae and conidia in control Petri plate; (a) healthy hyphae and (b) healthy conidia. (B-E) Effects of AP on hyphal and conidial morphology. Note the alterations in hyphal and conidial morphology including: (a) hyphal shrivelling and (b) shrunken conidia in Figure B, (a) hyphal blistering and (b) formation of pores in Figure C, conidial shrivelling (arrows) in Figure D, and conidial blistering (arrow) in Figure E.

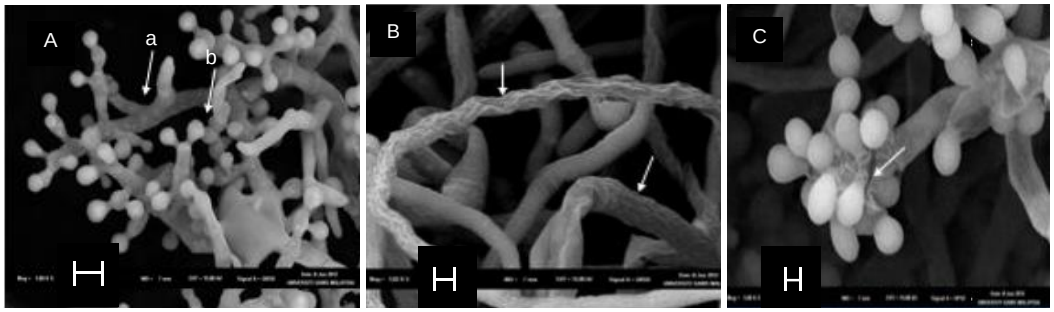

Figure 4: Scanning electron microscopy of *Trichophyton interdigitale* exposed to ethanol AP extract (Scale bars = 2 µm; original magnification: 4000×). (A) Healthy hyphae and conidia in control Petri plate; (a) healthy hyphae and (b) healthy conidia. (B and C) Effects of AP on hyphal and conidial morphology. Note alterations in hyphal and conidial morphology including the appearance of wrinkled hyphae (arrows) in Figure B and conidial breakage (arrow) in Figure C.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 576-587
DOI: http://dx.doi.org/10.21161/mjm.211144

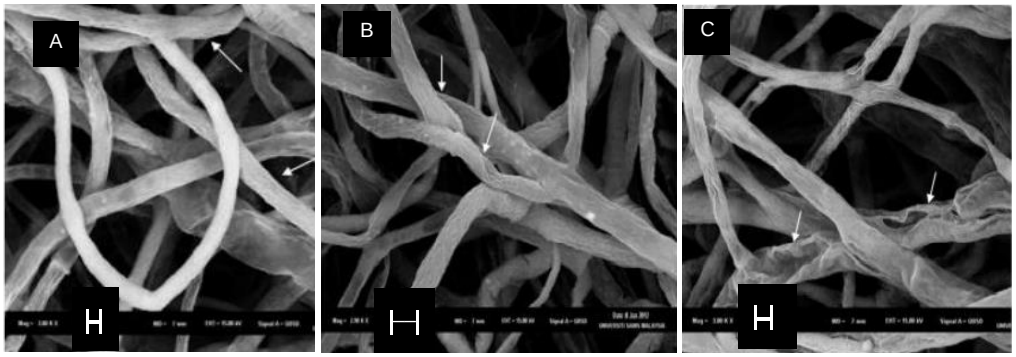

Figure 5: Scanning electron microscopy of *Microsporum gypseum* exposed to ethanol AP extract (Bar = 2 µm; original magnification: 5000×). (A) Healthy hyphae in control Petri plates. (B and C) Effects of AP on hyphal morphology. Note alterations in hyphal morphology including rough hyphae (arrows) in Figure B and hyphal blistering (arrows) in Figure C.

DISCUSSION

Dermatomycosis infections are the common parasitic diseases affecting hair, nail and skin caused by fungal infection such as dermatophyte fungi of the genera *Microsporum* and *Trichophyton*, and non-dermatophyte such as *Candida* sp., *Fusarium* sp., *Aspergillus* sp. and *Alternaria* sp. However, dermatomycoses treatment becomes challenging since the emergence of antifungal drug resistance. In searching for new antifungal agents, various antifungal drugs have been tested currently, including the use of natural products from higher plants (Aldholmi *et al.*, 2019). In this study, the potential antifungal activity of AP was investigated.

The result from the present study indicates that, in the AP extract, flavonoid compounds are better extracted with aqueous, ethanol and methanol, while tannin and saponin compounds are better extracted with ethanol. Not all secondary metabolites are present in one solvent because some of the metabolites can dissolve in methanol, while some cannot; the same goes with water. For example, flavonoids can dissolve in water, but anthraquinones cannot (Tsao and Deng, 2004). Premanath and Devi (2011) reported that phenolic acids and flavonoids are generally extracted using alcohol, water or a mixture of alcohol and water, while Tang *et al.* (2012) reported that phenolic compounds and flavonoids are better extracted with the ethanol than with other solvents.

The results obtained from the present study may differ from the earlier report, which has shown flavonoids compound found in both aqueous and ethanol AP extracts (Tang *et al.*, 2012). However, a study by Igbinosa *et al.* (2009) indicated that the AP recorded a low amount of total flavonoid content. In the AP, some isolated flavonoids are 7-O-methylwogonin, apigenin, onysilin and 3,4-dicaffeoylquinic acid, which are anti-atherosclerotic. Polyphenols and flavonoids are plant secondary metabolites and are very important due to their antimicrobial activities (Jimoh *et al.*, 2011). Different chemical constituents of plant extracts are likely due to genotypic and environmental differences, choice of parts tested, time of samples taken and determination methods (Tang *et al.*, 2012). The AP extract antifungal activity in this study can be said to be due to the presence of these compounds since flavonoid, tannin and saponin are known to exhibit antimicrobial activity (Polash *et al.*, 2017).

Interestingly, a study by Khanna and Kannabiran (2008) reported that many saponins are known to be antimicrobial, especially to inhibit mould and protect the plant itself from insect attack. It is because saponins have high toxicity against fungi (Khanna and Kannabiran, 2008). The comparison of antifungal activity of saponin fractions with that of standard antibiotics revealed that the saponin antifungal activity was more significant than the commercial antibiotics (Soetan *et al.*, 2006). The antifungal activity of pure saponin fraction was more pronounced when compared to Amphotericin-B, showing *A. niger* as more susceptible to inhibition followed by *A. flavus* and *A. fumigatus* (Soetan *et al.*, 2006). A study conducted by Hendra *et al.* (2011) also revealed that saponin present in the plant, *Sorghum bicolor*, a popular cereal consumed worldwide, has useful antimicrobial properties. These studies supported our investigation that the saponins isolated from the ethanol AP extract possess unusual toxic activity against all fungi tested and may assume pharmacological importance.

With regards to our study, it clearly showed that AP aqueous and methanol extract have less antifungal activity compared to the ethanol AP extract due to in ethanol AP there is contains saponins and tannin rather than flavonoids alone (Table 1). This result is comparable with another study by Liu *et al.* (2008), suggesting that *Phaleria macrocarpa* extract isolates flavonoids compound and indicates no activity to weak inhibitory activities against all fungi tested. Based on the review of literature, there is no previous research concerning the antifungal activity of the AP extract on dermatophytes except for *Epidermophyton floccosum* and *T. rubrum*. This study's results indicated antifungal activities of AP extracts against certain dermatophytes. In Malaysia, this plant has been used mostly to treat diabetes and hypertension; however, studies conducted concerning the antimicrobial effect on superficial fungal infections are minimal.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Our findings were consistent with some previous reports in which the antibacterial effect of various AP extracts was demonstrated against skin infections caused by bacterial strains (Arifullah *et al.*, 2013). It revealed the scientific basis of the traditional usage of the AP on superficial fungal infections. We were prompted to choose and confirm this plant for further evaluation to ascertain its antifungal potential to treat skin infections caused by some pathogenic fungi from the evidence that the traditional clerics widely used it in treating some skin infections (Okhuarobo *et al.*, 2014).

The lowest mycelial inhibition value of 1% was obtained by *M. canis* at the lowest concentration of the aqueous AP extract, while *T. mentagrophytes* obtained the highest mycelial inhibition value of 97.51% at the highest concentration of the ethanol AP extract. The results obtained in the present study indicated that the ethanol extract is active against the pathogenic fungi and has a broad-spectrum activity. The mycelial inhibition percentage of AP extracts against fungi tested showed that fungi vary widely in the degree of their susceptibility to antifungal agents. The lack of antifungal activity of the extract in this study may be attributable to a lesser concentration of the extract used.

The larger mycelial inhibition effects of all fungi tested could be because of the higher concentrations of active compounds found in the extracts. The reason why the fungi were sensitive towards the ethanol extract was most probably due to the interference by the active compounds of the extracts. Consequently, it leaked into the microconidia cell wall, losing its rigidity and causing cell death (Duraipandiyan *et al.*, 2006). The bioactive phytocomponents present in the plants have also been widely observed and accepted as the medicinal value of plants (Sadhana *et al.*, 2020). Kuiate *et al.* (2006) reported that every extract containing effective active phytochemicals is responsible for the elimination of microorganisms responsible for skin diseases in the antibacterial activity of the polar and non-polar extracts of the whole AP plants. This statement is consistent with our results because the study not only showed the inhibitory activities by the ethanol extract, but the aqueous AP extract has also pronounced the inhibitory activities against *T. mentagrophytes*, *T. interdigitale* and *M. gypseum*. However, the aqueous AP extract is less effective on other fungi tested compared to the ethanol AP extract due to the synergistic action of different active compounds groups contained in the different extracts (Zhang *et al.*, 2008).

The results obtained in the study by Tang *et al.* (2012) indicated that the ethanol AP extract is more active against the pathogenic bacteria; *Pseudomonas aeruginosa* and *Staphylococcus aureus* and has a broad-spectrum activity than other solvents used for extraction. They reported that the ethanol AP extract was found to be more susceptible to *E. floccosum* with 74.6% mycelial inhibition and *T. rubrum* with 70.9% mycelial inhibition for antifungal study. The results of our study also showed mycelial inhibition activity on *T. rubrum* was at the highest concentration of the ethanol AP extract. However, the value might vary from this report, showing that *T. rubrum* recorded mycelial inhibition of 97.07%.

In the ethanol AP extract, only at a concentration of 0.99%, it is enough to inhibit *M. gypseum* and *M. canis*. At a concentration of 1.96%, it inhibited *T. mentagrophytes*, *T. interdigitale, M. nanum*, *M. gypseum M. canis* and *F. solani*, while at 7.41%, it inhibited all the tested fungi. These results showed that a low concentration of the ethanol AP extract is good enough to retard the fungi growth. The highest concentration used (7.41%) was considered low because a study by Tang *et al.* (2012) showed that they used the ethanol AP extract at a concentration of 50% (v/v) for antifungal study on dermatophyte.

Most of the tested fungi demonstrated a decrease in the spore sedimentation when exposed to the ethanol AP extract concerning the effects on spore production. Several studies have reported the antifungal effects of herb extract, in which inhibition of spore germination was also demonstrated. These take into account a study by Tian *et al.* (2013), revealing the antifungal effect of nerol (compound isolated from neroli oil) with evident inhibitory effect on *A. flavus* spore germination. Besides, a study by Vitale *et al.* (2004) on *Fusarium sambucinum* reported that several salts completely inhibited the mycelial growth and spore germination of the fungi. In a study by Nidiry *et al.* (2015), the AP compound, andrographolide, at a concentration of 500 mg/L, exhibited 64.8% *Alternaria solan*i spore germination inhibition. These findings agree with the present study, showing that the ethanol AP extract with significant mycelial inhibition also showed inhibition of the spore sedimentation formation.

The microscopy observation results of the antifungal effect showed that the ethanol extract had shown growth inhibition activity against the tested fungi with a possibility of extract containing powerful components against *T. mentagrophytes* and *M. canis* infections. Marked morphological alteration of the hyphal wall was observed at high concentrations of the ethanol AP extract used in the test. The wall was changed and probably disrupted in which it was missing in some regions. The changes in the cell wall surface suggested that a modification in the normal assembly of the wall components had occurred (Zhang *et al.*, 2008). The ethanol AP extract presence changed the permeability of the fungal cell wall and subsequently resulted in inevitable changes in various structural and biochemical properties. The cell deformation also occurred as the impact of the loss of water, electrolytes and vital intracellular components, which are important for the cell's survival. It is suggested that in the development of a potential antifungal agent from a medicinal plant, the cell wall is the preferential target as its integrity is vital to survival and fungal growth (Liu *et al.*, 2017). Nowadays, the mechanisms interfering with the cell wall integrity are usually adopted for several antifungal drug developments.

Various reports on fungal structure alteration in other antifungal studies suggested the damage and suppression of the fungal growth. Shao *et al.* (2013) conducted an observation using SEM onto *Botrytis*

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

cinerea treated with tea tree oil. They found the shrivelled or flatted empty hyphae, ruptured of plasmalemma and leakage of cytoplasmic, compared to untreated *B. cinerea* with uniseriate, uniform hyphae and smooth surface.

Barlian *et al.* (2011) studied the effect of lyophilisate onto dermatophytes and reported the cellular deformation and pores formation, such as shrinkage of hyphae and microconidia of *M. gypseum* and *T. mentagrophytes*. Meanwhile, Radhakrishnan *et al.* (2018) studied the effect of silver nanoparticles onto *Candida albicans* and revealed the alteration of surface morphology, membrane microenvironment and fluidity. Kim *et al.* (2019) suggested that a bacterium isolated from soil, *Streptomyces blastmyceticus* as an antifungal agent onto *F. oxysporum,* damaged the plasma membrane of the fungal spores.

Based on this study's findings, ethanol can be concluded to be the best solvent for extracting antifungal bioactive compounds from AP plants. Thus, this study discovers the value of medicinal plants, which can be of considerable interest in developing new drugs on superficial fungal infections.

CONCLUSION

The ethanol AP extract can be concluded to be exceptionally significant to most of the fungal tested, compared to aqueous and methanol AP extracts. The ethanol AP extract has the potential to be used as a topical application for the treatment of dermatophyte infections. Further work on *in vivo* study will enable the production of antifungal agents from the AP extract. Besides, the gene expression related to cell wall synthesis in the fungi following exposure to AP treatment will be good research to validate the antifungal effect of AP. It is also vital to study further the benefits of the antifungal effect of AP in terms of its chemical composition, molecular mechanism, chemical biology and medical application to the community.

The use of herbal medicine still plays an important role in developing countries nowadays. Therefore, this study reveals a natural alternative treatment in combating antimicrobial resistance by utilising herbal extract and conserving human health.

ACKNOWLEDGEMENTS

This work was funded by Short Term Grant (304/PPSP/6315341) from Universiti Sains Malaysia.

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538