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REGULAR ARTICLE

IN-VITRO EVALUATION OF THE ANTIFUNGAL ACTIVITIES OF EEL SKIN MUCUS FROM ASIAN SWAMP EEL (*MONOPTERUS ALBUS)*

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ABSTRACT

T Discovery and development of new drugs from marine and freshwater animal remain one of the most challenging areas in recent marine sciences field. Thus, the object of current study to examine the antifungal activity of Asian swamp eel (*Monopterus albus)* skin mucus. Eel skin mucus aqueous and methanol extracts were prepared with different extract concentrations from 0.49 to 1000 µg/mL against *fungus pathogens i.e. Aspergillus niger and Microsporum gypseum. T*he antifungal assay conducted using well diffusion method. The results showed a dose dependent decrease the fungal growth, at 100µl/well, the inhibition zone of methanol extract against *M. gypseum (*25.7±0.75) mm, while the aqueous one was (23.3±0.16) mm Whereas eel skin mucus methanol and aqueous extracts showed lower inhibition zone against *Aspergillus niger* at the same concentration which was (11.1±0.59) mm and (9.0±0.15) mm respectively. The methanol extract showed the highest inhibitory activity against *M. gypseum because M. gypseum* infect the upper layers of the skin and eel skin mucus protect eels from infections. The results were statistically significant with $p < 0.001$. In conclusion, the present study carried out to reveal the antifungal activities of eel skin mucus which might be use as a source of antifungal agent.

Keywords: *Monopterus albus, Aspergillus niger, Microsporum gypseum,* well diffusion method

INTRODUCTION

Asian swamp eel, *Monopterus albus* (*M. albus*) belongs to synbranchidae family under the order of synbranchiformes (Rossen et al., 1976). It is native to the tropical and subtropical areas of northern India, China, Malaysia, Thailand, Indonesia, Philippines and possibly north-eastern Australia (Collins et al., 2002). The Asian swamp eel commonly found in freshwater habitats such as paddy field, ponds, rivers, lakes, slow-flowing currents, canals, sluggish water, and shallow swampland. It is very hardy and adaptive to adverse environments compared to other fishes, it was reported that it is able to tolerate cold temperature even below freezing and with ice cover its pond habitat. The swamp eel also appears to tolerate brackish and saline conditions (Starnes et al., 1998). It has been documented that different extracts of *M. albus* extracts have higher anti-fungal activity as compared to ampicillin (Atif et al., 2015). It has been demonstrated that eel skin mucus extract from Asian local swamp eel, *M. albus* has antifungal activities against *Cryptococcus neoformans*, *Candida albicans*, *Candida krusei*, and *Fusarium* species and it was also observed that aqueous extract has higher antifungal activity than Phosphate Buffer Saline extract (Nor et al., 2013).

MATERIAL AND METHODS

Materials

Malaysian eels (*Monopterus albus*) were purchased from eel farm in Pekan, Pahang, Malaysia.
Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) (Oxid).
Microsporum gypseum (ATCC 24102) and *Aspergillus niger* (ATCC 16404) from the American Type Culture Collection (ATCC, Manassas, VA, USA).

Methods

Sample Extraction

Eel skin mucus (ESM) was homogenized with 2 volumes of distilled water (30 ml of ESM to 60 ml of distilled water), then centrifuged at 13,000 rpm for 30 min, the supernatant was lyophilized using freeze dryer. Dried substance was weighed and dissolved in distilled water to form aqueous extract and in methanol to form methanol extract (1 mg of eel skin mucus to 1 ml of the solvent), after that, the dissolved substance was filtered through 0.22µm (Sadakane et al., 2007).

Determination of antifungal activities of ESM extracts

Strains preparation

For fungal inoculum adjustment; the inoculum size was adjusted between 1×10^6 and 5×10^6 spores/ml by microscopic counting using hematocytometer. The turbidity of the suspension was achieved using spectrophotometer at 530 nm to obtain a final concentration between 1×10^6 and 5×10^6 CFU/ml. The adjusted suspensions were quantified on the agar plates, then the plates were incubated at 35°C for 48 hrs and after that, the colonies were counted after the observation of visible growth (Petrikkou et al., 2001).

Determination of antifungal activity using well diffusion method

In the present study, *Microsporum gypseum* and *Aspergillus niger* were used, which are spore-producing fungi so well diffusion method was approached for assessing the antifungal properties (Balouiri et al., 2016). The inoculum used was prepared from a cultured fungus on the dextrose agar, a suspension was made in a sterile saline solution (0.85%). 20 ml of agar were melted, poured into an agar plate and cooled before the agar solidifies. The agar was inoculated with 1 ml of adjusted spore from the fungus suspension then shake the suspension using shaker and allowed it to be solidified, then, four wells were cut out of the agar, and 20 µl of different concentrations (100, 50, 25, 12.5, 6.25 and 3.125) µg/mL of ESM aqueous and methanol extracts were placed into each well, ketoconazole was used as positive control. The plates were incubated at 35°C for 24 hrs, after incubation, the diameters of clear inhibition zones were measured (Magaldi et al., 2004).

RESULTS

Antifungal activities of ESM extracts against *Microsporum gypseum*

The highest clear inhibition zone was observed in ESM methanol extract against *Microsporum gypseum* at 100 µl/well which was 25.72 ± 0.75 mm, while the aqueous extract was 23.31 ± 0.16 mm as shown in table 3.1. The values were highly significant with $p < 0.001$compared with the positive control (ketoconazole).

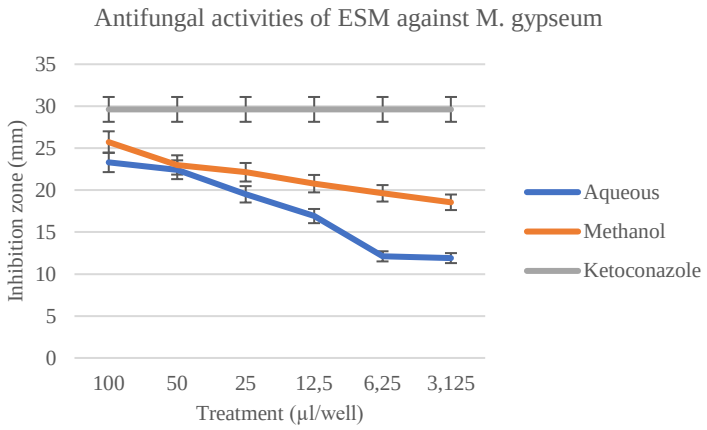

Figure 1 Antifungal activities of ESM aqueous and methanol extracts against *M. gypseum*. Ketoconazole was used as a positive control

Antifungal activities of ESM extracts against Aspergillus niger

ESM methanol and aqueous extracts showed lower inhibition zone against *Aspergillus niger* at a concentration of 100 µl/well which was 11.21±0.59 mm and 9.0±0.15 mm respectively as shown in table 3.2.

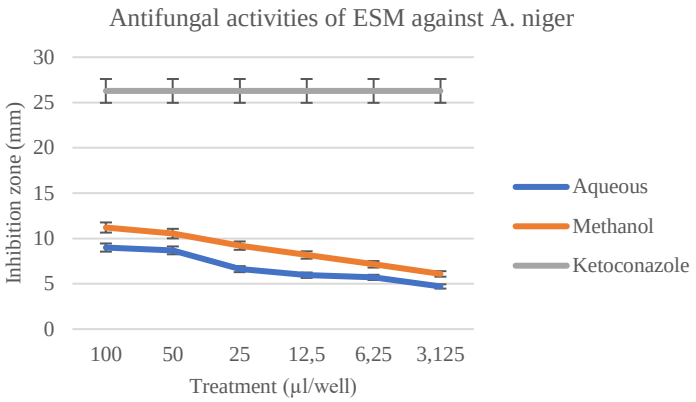

Figure 2 Antifungal activities of ESM aqueous and methanol extracts against *A. niger*. Ketoconazole was used as a positive control.

Table 1 Antifungal activities of ESM against *M. gypseum* using well diffusion method (mm). Results were expressed by mean ± SD (n=3).

Treatment (µl/well)	100	50	25	12.5	6.25	3.125
Aqueous	23.31±0.16	22.43±0.59	19.55±0. 26	16.91±0.71	12.11±0.14	11.9±0.92
Methanol	25.72±0.75	23.0±0.12	22.13±0.54	20.77±0.62	19.62±0.64	18.55±0.01
Ketoconazole	29.62±0.36	29.62±0.36	29.62±0.36	29.62±0.36	29.62±0.36	29.62±0.36

Table 2 Antifungal activities of ESM against *A. niger* using well diffusion method (mm). Results were expressed by mean ± SD (n=3).

Treatment (µl/well)	100	50	25	12.5	6.25	3.125
Aqueous	9.0±0.15	8.69±0.86	6.62±0.63	5.95±0.92	5.71±0.1	4.71±0.41
Methanol	11.21±0.59	10.54±0.7	9.21±0.04	8.18±0.11	7.15±0.27	6.09±0.38
Ketoconazole	26.28±0.59	26.28±0.59	26.28±0.59	26.28±0.59	26.28±0.59	26.28±0.59

DISCUSSION

At 100 µl/well concentration, ESM methanol extract showed higher antifungal activity against *M. gypseum* than *A. niger*; as it showed (25.7±0.75) mm inhibition zone against *M. gypseum* which was almost the same as the commercial antibiotic (ketoconazole) which was (29.5±0.36) mm, while *A. niger* showed (11.3±0.59) mm. This can be explained, because *M. gypseum* infect the upper layer of the skin of mammals, while *A. niger* affects certain vegetables and fruits and the mucus covering the outer layer of eel skin which is important to protect eels from skin infections; this explains the reason of high antifungal activity against *M. gypseum.* As it has been proven the antifungal properties in epidermal mucus of different fish species (Hellio et al., 2002).

CONCLUSION

In conclusion, the results revealed that the antifungal activities were higher against *M. albus* because Asian swamp eel stay always in shallow water, so their skin are more likely to be affected with fungus pathogens like *M. albus.* This presenting study clearly shows that ESM extracts have effective antifungal activities and it could be used as a potent antifungal agent.

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