

# A preliminary screening of antifungal activities from skin mucus extract of Malaysian local swamp eel (*Monopterus albus*)

<sup>1</sup>M. N. Nik Mohd Ikram and <sup>\*2</sup>B. H. Ridzwan

<sup>1</sup>Health Entrepreneurship Programme, Department of Tourism, Hospitality, and Wellness, Faculty of Entrepreneurship and Business, University of Malaysia, Kelantan karung berkunci 36, Pengkalan Chepa, 16100 Kota Bharu, Kelantan, Malaysia

<sup>\*2</sup>Department of Biomedical Science, Faculty of Allied Health Sciences, International Islamic University of Malaysia, Bdr Indera Mahkota, 10, 25200 Kuantan, Pahang DM, Malaysia

**A preliminary screening of antifungal activities of skin mucus extract from Malaysian local swamp eel, *Monopterus albus* was performed. The eel's skin mucus was extracted using two aqueous extraction solvents; distilled water and Phosphate Buffer Saline (PBS). In addition, pure mucus was also applied without any extraction process. Each extract was prepared in different concentration via 10-fold serial dilution and applied onto an agar containing cultured fungi using a blank diffusion disc. The method utilized was the disc diffusion sensitivity assay. The tested pathogenic fungi were *Candida albicans*, *Candida krusei*, *Cryptococcus neoformans* and *Fusarium* spp. PBS extract and pure mucus showed no zone of inhibition for all tested fungi. Water extract of skin mucus revealed an inhibitory effect on all the fungi whereby *Fusarium* spp. Was observed to be the most sensitive. The zone of inhibition varies from as low as 8 mm to as high as 20 mm but was still dependent on the incubation period. It was also observed that water acted as a more effective solvent to extract possible antifungal compound from the skin mucus of *M. albus*. Throughout, it is suggested that freshwater eel possess antifungal activity from its skin mucus and thus supported the claims among locals.**

**Keywords:** Malaysian swamp eel, *Monopterus albus*, skin mucus, antifungal activities.

## INTRODUCTION

The frequency of mycoses due to opportunistic fungal pathogens has increased significantly. Thus, there is a great need for researchers to find a possible new source in developing antifungal agents. Local freshwater eel, *Monopterus albus* is known to have been widely used in Malaysia as a source of nutrition as well as traditional medicines. Skin mucus of *M. albus* is being used as part of a skincare and in wound healing among locals. However, no interest was shown by researchers to prove these claims scientifically. Although there were some studies done but, on marine species of eel, *Anguilla japonica* found along the coastal area of Japan.

According to Tasumi et al. (2002), lectin produced by their skin mucus demonstrated agglutination activity and growth suppressive activity against *E. coli*.

Therefore, this study aimed to investigate the existence of antifungal activities in the skin mucus extract of *Monopterus albus* towards selected species of pathogenic fungi. Besides, to determine which aqueous extraction method is more effective in extracting antifungal compounds from the skin mucus.

## MATERIALS AND METHODS

### Chemicals and reagents

0.01 M Phosphate Buffer Saline (PBS) pH 7.0 powder (Sigma Chemical Co., St. Louis, USA), Sabouraud Dext-

rose Agar (SDA) (Difco™, Maryland, USA), Luria Bertani (LB) Broth (Scharlau Chemie S.A., Barcelona, Spain), Antibiotic Disc (Amphotericin-B and Fluconazole) were both purchased from Mast Group Ltd., Merseyside, UK.

### Tested fungi

*Candida albicans*, *Candida krusei* and *Cryptococcus neoformans* were all obtained from the stock culture in microbiology laboratory of Kulliyah of Science, International Islamic University Malaysia. *Fusarium* (*Fusarium* spp.) was obtained from the Institute of Medical Research (IMR), Malaysia.

### Samples

General species of local swamp eels used in this study was *Monopterus albus*. 4 Kg of eels which comprised of 16 adults were purchased from the wet market in Pasir Puteh, Kelantan and Selayang, Selangor with the help of dealers as well as the local folks. The eels sold at both wet markets were freshly captured from the nearby paddy fields. They were kept alive to be brought to the laboratory by putting them in a container filled with sufficient fresh water that is natural for the eels.

### Mucus collection and preparation

Only mucus from the dorsal part of the eel's body was collected daily on average 3 h per day for 7 consecutive days by scraping it carefully using a spatula. Ventral skin mucus was not collected to avoid intestinal contents and sperms contamination. The process of collecting in local term is called 'lurut'. During the one week process, fresh water from nearby lake being added to the container to maintain the eels' survival. 500 ml of pure mucus was collected. The mucus was immediately transferred into separate centrifuge tubes, labeled, and frozen at -4°C to prevent any bacterial contamination.

### Mucus extraction using water (Tasumi et al., 2002)

Prior to the extraction method, 1000 ml of distilled water was autoclaved at 121°C for 15 minutes. A 1:5 volumes homogenization was done by mixing 8 ml of thawed mucus sample with 40 ml of the sterile distilled water in a centrifuge tube. 18 tubes were prepared which altogether made up of approximately 144 ml mucus. The homogenates were then centrifuged at 9000 rpm at 4°C for 60 minutes. The supernatant obtained was then transferred into a glass petri dish to be placed in a deep freezer at -80°C overnight. Later, the frozen supernatant would be freeze dried overnight using the freeze dryer to

obtain a powdered form sample. The powder obtained was sealed and kept in the cold room.

### Mucus extraction using phosphate buffered solution (PBS)(Tasumi et al., 2002)

The protocol followed the water extraction method, but instead 10 mM PBS (pH 7.0) being used and prepared by mixing one packet of PBS powder into 1000 ml of distilled water in a Schott bottle.

### Fungi culture

Luria Bertani (LB) Broth was initially prepared by mixing 25 mg of LB powder in 100 ml distilled water. 10 ml of the broth was then distributed into different bijoux bottles that were later autoclaved at 121°C for 15 minutes. The sterile LB broth was kept at a room temperature for later used.

*C. albicans*, *C. krusei* and *C. neoformans* were all revived from the glycerol stock by suspending them in different LB broth. Meanwhile, *Fusarium* spp. was taken from the vial prepared by IMR and suspended them in different LB broth to be revived. They were all later being kept for 48 h at a room temperature for normal growth. After 48 h, a loopfull of each fungus was transferred into a new LB broth for second subculture. These new subculture were allowed to grow for another 24 to 48 h at room temperature.. Within that duration, the turbidity of the suspension was checked and adjusted to the required density of McFarland Standard. This was done by using a spectrophotometer with 1 cm light path at 530 nm of absorbance wavelength.

### Medium preparation

Soboroud Dextrose Agar (SDA) was used as the medium agar. SDA medium was prepared by suspending 650 g of SDA powder in 1000 ml of distilled water using a Schott bottle. It was then mixed thoroughly and autoclaved at 121°C for 15 minutes. Later, the liquid-form agar was poured in each agar plate for about 4 cm thick. It was later left opened in a laminar flow cabinet for the agar to solidify. Then, the solidified agar covered and stored in the cold room for later used.

### Serial ten-fold dilution

Different concentration of each extract was prepared prior to the sensitivity assay testing. A 1:1 homogenization was initially done by adding 10 mg of powdered PBS extract and 10 mg of powdered water extract with 10 ml of sterile PBS and distilled water, respectively. The solutions were then further diluted either in a sterile PBS or sterile

**Table 1.** Types and concentration of antibiotic disc used as positive control towards the tested fungi.

<b>Fungi</b>	<b>Antibiotic Disc (µg)</b>
<i>C. albicans</i>	Fluconazole (25)
<i>C. krusei</i>	Fluconazole (25)
<i>C. neoformans</i>	Amphotericin-B (20)
<i>Fusarium spp.</i>	Amphotericin-B (20)

distilled water by performing a serial tenfold dilution. The concentrations prepared were 1.0 mg/ml, 0.1 mg/ml, 0.01mg/ml, and 0.001 mg/ml of both extracts, respectively.

### McFarland 0.5 turbidity standard

A McFarland standard of the cultured fungi was prepared prior to the sensitivity assay. Each of the fungi cultured in the LB broth would be aliquoted and placed into cuvettes. The density of the fungi in the broth that would later be used in the sensitivity assay was checked by using a spectrophotometer with a 1 cm light path whereby the absorbance wavelength should be 530 nm. This is in the range of 0.5 – 2.5 O.D colony forming unit (CFU). Only cultures within this range would be used for the sensitivity assay.

### Sensitivity assays

100 µl of each fungus that had been revived in the LB broth earlier was inoculated using a micropipette and spreaded on the Sobaroud Dextrose Agar by using a sterile hockey-stick. This was done optimally 15 minutes after the density of the fungi had been adjusted to meet the 0.5 McFarland Turbidity Standard.

The method used in the detection of inhibition zone was a simple disc-diffusion sensitivity assay based on Moist Disc Absorption Kirby Bauer Technique 1959.

### Negative control

The negative controls for this study were the solutions used to dilute the extract based on the extraction method used. Thus, for the PBS extraction method, the negative control was the PBS itself. As for the water extraction method, the negative control used was distilled water. Those negative controls were prepared by immersing a 6 mm blank disc for two minutes and later placed on the similar agar media that were used for sensitivity assay. This step was also done for all of the fungi cultured.

### Positive control

The positive controls for this study were the antibiotics that have already been proven to inhibit the growth of tested fungi. The antibiotics used were in a form of a 6 mm disc purchased from Mast Group Ltd. Merseyside, UK. The types and concentration of the antibiotic discs are shown in Table 1.

### Statistical Analysis

The inhibition zones recorded for sensitivity assay were presented as Mean ± Standard Error of Mean (S.E.M).

## RESULTS

### General observation

The preliminary screening of antifungal activities from the skin mucus of *M. albus* was done via Kirby Bauer Moist Disc Absorption Sensitivity Assay on four species of chosen pathogenic fungi. The results were observed and recorded within three days interval of every 24, 48, and 72 h. The results recorded were the measurement of zone of inhibition in diameters. Basically, three different results of each time interval were taken. First result observed was the zone of inhibition by the skin mucus from the water extraction method. Second result was the zone of inhibition by the skin mucus from the PBS extraction method. The final result to be observed was the zone of inhibition produced by the pure skin mucus which was directly applied on the fungi without any extraction and dilution process.

In general, the sensitivity assay was done at four different concentrations of skin mucus extract. The concentrations were 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml.

### Sensitivity assay using water extraction method

The results after 24 h, 48 h and 72 h observations are summarized in Table 2.

**Table 2.** Zone of inhibition (mm)  $\pm$ S.E.M. of water extract from mucus of *Monopterus albus* on fungi by the moist disc absorption technique after 24 h, 48 h and 72 h incubation.

Extract (mg/ml) / Fungi	Concentration	Time (hour)	Serial 10-Fold Dilution				Pure Mucus	Negative Control	Positive Control
			1	0.1	0.01	0.001			
<i>C.albanicans</i>		24	9.5 $\pm$ 0.5	9.0 $\pm$ 1.0	8.5 $\pm$ 1.5	9.0 $\pm$ 1.0	-	-	25.0 $\pm$ 0**
		48	8.5 $\pm$ 1.5	8.5 $\pm$ 1.5	8.5 $\pm$ 1.5	8.5 $\pm$ 1.5	-	-	30.0 $\pm$ 0**
		72	8.5 $\pm$ 1.5	8.5 $\pm$ 1.5	8.5 $\pm$ 1.5	8.5 $\pm$ 1.5	-	-	30.0 $\pm$ 0**
<i>C.Kriuser</i>		24	-	-	-	-	-	-	29.0 $\pm$ 1.0**
		48	14.0 $\pm$ 1.0	13.5 $\pm$ 1.5	13.5 $\pm$ 1.5	13.0 $\pm$ 1.0	-	-	33.5 $\pm$ 1.5**
		72	14.5 $\pm$ 0.5	14.5 $\pm$ 0.5	14.0 $\pm$ 0	14.5 $\pm$ 0.5	-	-	31.0 $\pm$ 1.0**
<i>C.neofarmans</i>		24	No growth	No growth	No growth	No growth	No growth	No growth	No growth
		48	16.5 $\pm$ 1.5	12.5 $\pm$ 0.5	15.0 $\pm$ 0	12.5 $\pm$ 0.5	-	-	18.0 $\pm$ 0*
		72	14.5 $\pm$ 0.5	11.0 $\pm$ 1.0	15.0 $\pm$ 0	11.5 $\pm$ 0.5	-	-	17.0 $\pm$ 1.0
<i>Fusarium spp</i>		24	No growth	No growth	No growth	No growth	No growth	No growth	No growth
		48	13.5 $\pm$ 1.5	10.0 $\pm$ 0	19.5 $\pm$ 0.5	20.0 $\pm$ 0	-	-	17.0 $\pm$ 0*
		72	15.5 $\pm$ 0.5	10.0 $\pm$ 0	19.5 $\pm$ 1.5	20.0 $\pm$ 0	-	-	18.0 $\pm$ 1.0*
Note: The disc size was 6 mm; - no inhibitory effect, *Amphotericin-B,**Fluconazole; Negative control was distilled water or PBS									

## 24 h result

After 24 h of incubation at room temperature, it was observed that only one fungus, *C. albicans* found to be sensitive to the skin mucus extract. The extract for each concentration yielded a positive result against *C. albicans*. The mean diameter  $\pm$  S.E.M. zone of inhibition observed for skin mucus extract at 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml concentrations were  $9.5 \pm 0.5$  mm,  $9.0 \pm 1.0$  mm,  $8.5 \pm 1.5$  mm, and  $9.0 \pm 1.0$  mm, respectively. Based on this result, *C. albicans* was most sensitive towards 1 mg/ml concentration of skin mucus extract. There was no zone of inhibition observed for other fungi at all different concentrations while there was also no growth of fungi observed for *C. neoformans* and *Fusarium* spp.

## 48 h result

After 48 h of incubation at room temperature, all the fungi species were susceptible to the skin mucus extract. The mean diameter  $\pm$  S.E.M. Zone of inhibition observed for skin mucus extract towards *C. albicans* was  $8.5 \pm 1.5$  mm at all different concentrations. The mean diameter  $\pm$  S.E.M. of zone of inhibition for *C. krusei* were  $14.0 \pm 1.0$  mm,  $13.5 \pm 1.5$  mm,  $13.5 \pm 1.5$  mm, and  $13.0 \pm 1.0$  mm at 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml concentrations, respectively, thus showing some decrement as the concentrations were diluted.

As for *C. neoformans*, skin mucus extracts at 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml concentration yielded a zone of inhibition of  $16.5 \pm 1.5$  mm,  $12.5 \pm 0.5$  mm,  $15.0 \pm 0$  mm, and  $12.5 \pm 0.5$  mm, respectively. Meanwhile, skin mucus extracts on *Fusarium* spp. at 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml concentration yielded a zone of inhibition of  $13.5 \pm 1.5$  mm,  $10.0 \pm 0$  mm,  $19.5 \pm 0.5$  mm, and  $20.0 \pm 0$  mm, respectively.

After 48 h, it was also observed that *Fusarium* spp. to be more sensitive towards the skin mucus extract but at a lowest concentration. The results for both *C. neoformans* and *Fusarium* spp. revealed inconsistent inhibitory effects with respect to the concentrations of the skin mucus extract.

## 72 h result

Overall, result after 72 h incubation showed not much of a different or fluctuation compared to the reading after 48 h. The mean diameter  $\pm$  S.E.M. Zone of inhibition observed for skin mucus extract towards *C. albicans* were still  $8.5 \pm 1.5$  mm at all different concentrations. The mean diameter  $\pm$  S.E.M. Zone of inhibition towards *C. krusei* were  $14.5 \pm 0.5$  mm,  $14.5 \pm 0.5$  mm,  $14.0 \pm 0$  mm, and  $14.5 \pm 0.5$  mm at 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and

0.001 mg/ml concentrations, respectively. There was a slight increased of the zone diameter compared to the readings recorded after 48 h. However, inconsistent inhibitory effects were also observed with respect to the concentration when the zone diameter at the lowest concentration increased higher than at the previous dilutions.

As for *C. neoformans*, skin mucus extracts at 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml concentration yielded a zone of inhibition of  $14.5 \pm 0.5$  mm,  $11.0 \pm 1.0$  mm,  $15.0 \pm 0$  mm, and  $11.5 \pm 0.5$  mm, respectively. Compared to the result after 48 h incubation, the zone of inhibition decreased in diameter. Meanwhile, skin mucus extracts at 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml concentration gave a zone of inhibition of  $15.5 \pm 0.5$  mm,  $10.0 \pm 0$  mm,  $19.5 \pm 0.5$  mm, and  $20.0 \pm 0$  mm on *Fusarium* spp., respectively. There was not much change in the diameter, except for the slight increased at the highest concentration compared to the 48 h result.

It was also observed that *Fusarium* spp. was still the most sensitive towards the skin mucus extract, but still at its lowest concentration. The results for both *C. neoformans* and *Fusarium* spp. also revealed inconsistent inhibitory effects irrespective of the skin mucus extract concentrations.

## Sensitivity assay using PBS extraction method

There was no zone of inhibition observed on all fungi species tested at different concentration of skin mucus PBS extract. The result remained constant for each time interval of 24, 48, and 72 h of incubation at room temperature.

## Sensitivity assay using pure mucus

The direct application of pure mucus revealed no inhibitory effect on the growth of all fungi species tested at different concentrations after 24, 48, and 72 h incubation at room temperature.

## Positive and negative controls

In this study, two different antibiotics were used as positive controls; Fluconazole 25  $\mu$ g and Amphotericin-B 20  $\mu$ g. These two antibiotics were applied at assigned fungi. The Fluconazole was used for the *Candida* species since some study had reported that *Candida* spp. might be resistant to Amphotericin-B (Holly et al., 2001). Fluconazole yielded a zone of inhibition in the range of 25 mm to 35 mm. As for, Amphotericin-B, it yielded a zone of inhibition towards the assigned fungi; *C. neoformans* and *Fusarium* spp. in the range of 17 mm to 18 mm.

Meanwhile, as expected, the negative controls (PBS or distilled water) did not yield any zone of inhibition towards all of the fungi. This showed that both of these solvents did not interfere with the result.

## DISCUSSION

In this preliminary screening study, Malaysia local swamp eel (*Monopterus albus*) was chosen as a possible source for a new antifungal agent. *M. albus* skin mucus is being used traditionally by the locals to treat skin diseases or infections as well in cosmetic. Therefore, this study aimed to screen for a possible antifungal activity from the skin mucus of *M. albus*. The fact that *M. albus* is within the fish group in animal kingdom, its skin mucus may possess certain bioactive antimicrobial compound such as glycoproteins (Concha, 2002), lectins (Arason, 1996), or lysozymes (Ellis, 2001). This is probably the scientific explanation behind the use of its skin mucus as wound healing agent and skincare product among the locals.

The screening was done on four chosen pathogenic fungi namely *C. albicans*, *C. krusei*, *C. neoformans* and *Fusarium* spp. These fungi were chosen based on the fact that they are currently the most common pathogenic fungi that were said to be opportunistic and resistant towards some antifungal agents in the current market.

Aqueous extractions using water and PBS solvents were utilized in this study to extract the unknown antimicrobial constituents in the skin mucus of *M. albus*. The method of extraction chosen was based on the study by Tasumi et al. (2002) on the skin mucus of marine Japanese eel. An aqueous extraction method was chosen based on the fact that most antimicrobial activities observed by previous studies were the one resulted from the extract from aqueous solvent. A study conducted by Ridzwan et al. (1995) showed that PBS extract of sea cucumber (*Holothuria atra*) was the only effective solvent that yielded inhibitory effects on certain microbes. An antimicrobial activity was also observed from the PBS extract of skin mucus of *Anguilla japonica* (Tasumi et al., 2002).

Therefore, although water and PBS are a form of aqueous solvent, both were chosen to determine whether the bioactive substances for antimicrobial activity are found in strong polar water-soluble fraction or in less polar compounds. In theory, water extraction method should extract most polar compounds while PBS extraction method is extracting less polar compounds. In addition to the extract, the pure or crude mucus was also applied to screen for an initial antifungal activity. Initial screening of potential antibacterial and antifungal compounds may be performed with the pure substances or crude extracts (Freiburghaus et al., 1996) (Cited by Nur Baiti Anum, 2007).

The method of antifungal screening utilized in this study was the disk diffusion assay or better known as

Moist Disc Absorption Kirby Bauer Technique 1959. It is reported that agar-based techniques have been used extensively by a few laboratories because they are simple, economical, and easy to perform simultaneously on large numbers of organisms (Rex et al., 1993). In this study, water extract of skin mucus of *M. albus* was the only extract that was observed to yield an antifungal activity.

Based on the results, *C. albicans* was the only fungus found to be sensitive to the water extract of skin mucus of *M. albus* after 24 h incubation. The extract from highest to lowest concentration was observed to yield an inhibitory effect on the growth of the fungus. At 1.0 mg/ml concentration, the zone of inhibition observed was  $9.5 \pm 0.5$  mm in diameter. The size slightly decreased to  $9.0 \pm 1.0$  mm at 0.1 mg/ml and  $8.5 \pm 1.5$  mm at 0.01 mg/ml. However, the size increased to  $9.0 \pm 1.0$  mm at the lowest concentration of 0.001 mg/ml. The size of zone of inhibition was later observed to be slightly decreased after 48 h incubation. The size decreased to  $8.5 \pm 1.5$  mm for all levels of concentration and the result remained static after 72 h incubation.

The inhibitory effect on *C. krusei* could only be observed after 48 and 72 h of incubation. At 48 h, it could be suggested that the size of inhibition was directly proportional to the concentration of skin mucus extract whereby the size decreased as the concentration lowered. Hence, it could be said that the inhibition zones was according to the dose-dependent manner. There was not much change in size of inhibition zone after 72 h incubation compared to the 48 h incubation as the size averaged 13 to 14 mm. However, the results seemed inconsistent at the 72h with respect to the concentration of skin mucus water extract. Similar to the *C. albicans*, the inhibition size slightly increased at the lowest concentration.

As discussed above, it is evident that the skin mucus extract of *M. albus* only exert a weak inhibition towards *C. albicans* and *C. krusei*. However, it should be taken into account that the comparatively weak inhibition found in this study could be influenced by the ability of the antifungal compound to diffuse uniformly through the agar (Nur Baiti Anum, 2007).

As for *C. neoformans* and *Fusarium* spp., there was no result to be discussed after 24 h of incubation at room temperature since there was no growth observed for both fungi. It has been reported that *C. neoformans* and certain mould type fungi could only grow after 48 h (Holly et al., 2001). Indeed, the growth of both fungi was observed after 48 h onward. There were inhibitory effects observed for both fungi at all concentrations of skin mucus extract after both 48 and 72 h incubation.

The zone of inhibition towards *C. neoformans* was slightly larger than that of *C. krusei* after 48 and 72 h incubation whereby the sizes were in the range of 11 to 16 mm at different concentrations. However, the inhibition zone towards *C. neoformans* at 1.0 mg/ml concentration

of skin mucus extract after 48 h incubation was  $16.5 \pm 1.5$  mm closed to the zone of inhibition produced by the positive control, Amphotericin-B i.e.  $18.0 \pm 0$  mm. Besides the interesting finding, there was also inconsistency in the zone of inhibition at both 48 and 72 h with respect to the concentration of the skin mucus extract. The zone of inhibitions decreased at 0.1 mg/ml, increased at 0.01 mg/ml, and decreased again at 0.001 mg/ml.

Meanwhile, *Fusarium* spp. seemed the most sensitive amongst other tested fungi towards the skin mucus based on the zone of inhibition yielded by 0.01 mg/ml and 0.001 mg/ml concentration of the extracts at both 48 and 72 h. 0.01 mg/ml concentration of the extract yielded a  $19.5 \pm 0.5$  mm and  $19.5 \pm 1.5$  mm zone of inhibition at 48 and 72 h incubation, respectively. The lowest concentration, 0.001 mg/ml yielded a  $20.0 \pm 0$  mm zone of inhibition for both 48 and 72 h incubation. These two readings were significance if they were compared to the zone of inhibition yielded by the positive control. Amphotericin-B at 20 µg yielded  $17.0 \pm 0$  mm and  $18.0 \pm 0$  mm zone of inhibition towards *Fusarium* spp. after 48 and 72 h incubation, respectively. Thus, this result shows that the zone of inhibition yielded by skin mucus extract of *M. albus* at 0.01 mg/ml and 0.001 mg/ml concentration had surpassed the zone of inhibition yielded by Amphotericin-B. However, there were still doubts on the result obtained. Firstly, inconsistency of the zone of inhibition with respect to the concentration was also noted. Secondly, it was as if the zone of inhibition was inversely proportional to the concentration of the extract. In other words, the lower the concentration, the larger the zone of inhibition. Thirdly, similar to *C. neoformans*, the shape of the inhibition zone produced was not uniformed.

Compared to the inhibition size yielded by the skin mucus extract to the positive controls Fluconazole and Amphotericin-B, there was a huge gap of size zone of inhibition. Fluconazole produced between 25 to 33 mm zone of inhibition after 24, 48, and 72 h incubation towards *C. albicans* and *C. krusei*. Whereas Amphotericin-B produced on average 17 to 18 mm zone of inhibition after 48 and 72 h incubation towards *C. neoformans* and *Fusarium* spp.. However, the main aim of this study was just to detect if there was any antifungal activity yielded by the skin mucus of *M. albus*.

In general, it is observed that the skin mucus from *M. albus* does possess an active substance that can inhibit the growth of certain fungi. However, the only positive result came from the skin mucus which was extracted by using water as its extraction solvent. The other aqueous solvent utilized (PBS) and pure mucus did not yield any inhibitory effects towards all fungi. Although the PBS extract did not show any positive reaction, it does not mean that the active substances that could inhibit fungi activity do not exist. The reason might be that the amounts of active substance are too low to exert any inhibitory effects (Ridzwan et al., 1995). It is known that water is higher in polarity compared to PBS. Therefore,

the difference of effect by water and PBS extracts might be due to the fact that PBS extracted less polar compounds which do not possess or have little antibacterial activities (Nur Baiti Anum, 2007). This might also applied to antifungal agents. A report suggested that antimicrobial activity was correlated with the polarity of extracts (Hayouni et al., 2007). This was based on the fact that the extracts obtained using higher polarity solvents were more effective radical-scavengers and microbial inhibitors than were those obtained using less polar solvents.

This scientific finding helped to explain why the locals do used skin mucus of *M. albus* or swamp eel as part of their traditional skincare product as well as in wound healing. Just like any other fish, the skin mucus of *M. albus* acts as their first line integumental defense against pathogens or parasite.

## CONCLUSION

The preliminary screening of antifungal activity of skin mucus from *Monopterus albus* showed the presence of such activity towards certain pathogenic fungi. It is suggested that the skin mucus of *M. albus* possess an antifungal agent that can produce an inhibitory effect on *Candida albicans*, *Candida krusei*, *Cryptococcus neoformans*, and *Fusarium* species (spp.) Only water extract of skin mucus yielded an antifungal activity whereas the PBS extract and pure mucus failed to produce any positive result. *Fusarium* spp. was found to be the most sensitive towards the skin mucus extract whereby the largest zone of inhibition was observed on this species at the lowest concentration. It can also be stated that antifungal activity of skin mucus extract was most evident on the mold fungus in comparison to the other yeast-type fungi. Therefore, it is concluded that the skin mucus of *M. albus* does produce an antifungal activity and thus further research should take place to further support the theory.

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