

# Evaluation of the Antifungal Activity of the Extracts of the Rhizome of *Alpinia Nigra*

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**Abstract**— The study was aimed to investigate the bioactivity in the fresh extract of the rhizome of *Alpinia nigra* using antifungal activity against human skin infections causative fungi *Malassezia furfur* and *Microsporum gypseum*. Fresh rhizome of *A. nigra* was subjected to steam distillation and the essential oil was separated using solvent extraction. The antifungal activity of the extract was evaluated using disk diffusion method. Highest inhibition for *M. furfur* was observed at the dose of 400 µg with a mean diameter of the inhibition zone ( $2.1 \pm 0.09$ ) cm and for *M. gypseum* the diameter was ( $2.4 \pm 0.05$ ) cm. The results confirmed that the extract of *A. nigra* shows antifungal activity on the growth of *M. furfur* and *M. gypseum*. Hence it is confirmed that *A. nigra* have the potential to cure the dermal infections.

**Keywords**— Antifungal, *Alpinia nigra*, Disk diffusion, *Malassezia furfur*, *Microsporum gypseum*

## I. INTRODUCTION

MEDICINAL plants are the richest natural sources of medicinal products used in traditional and orthodox medicine [1]. The search for medicinal values of different plants has attracted increasing interest in the past couple of decades, presumably because of their potential as sources of potent pharmacological activities, convenience to users, economic viability, as well as low toxicity [1]. *Alpinia nigra* in Sinhalese “*Kaluwa Ala*” is a plant, which used in traditional medicinal system in Sri Lanka and belongs to the Zingiberaceae family. It is an herbaceous plant that grows well on riverside and can also grow on moist land. The underground stem is rhizome and aerial stem is pseudo-stem which consists of leaf sheath, approximately 3.08 meters in height. Leaves are simple, alternate, oblong, entire and acute at base and apex of the plant with very short petiole and very long leaf sheath with small ligule (Figure 1) [2]. The rhizome of *A. nigra* represents largely untapped resource of bioactive compounds such as antifungal activities as playing important medicinal role. In Sri Lanka, the fresh extract of this rhizome is used to treat the fungal infections in the skin such as Pityriasis versicolor. *Malassezia furfur* and *Microsporum gypseum* are some of the major causative fungi infected on human skin and known as cutaneous fungi [3]. A thorough

literature survey revealed that the antifungal activities of *A. nigra* for such an infectious diseases have not been studied extensively and only a handful investigations have been found regarding the phytochemical and biological properties of the plant [1],[2],[4],[5]. The present study was carried out in order to investigate the bioactivity in the essential oil of the rhizome of *A. nigra* using antifungal activity against human skin infections causative fungi.



Fig 1: The plant of *A. nigra*

## II. MATERIALS AND METHODS

### A. Sample collection and extraction

The rhizomes of the plant *A. nigra* were collected from Haldummulla, Sri Lanka. The specimens of the plant were taxonomically identified and authenticated. The collected rhizomes were washed, cut into small pieces and dried in the shades of sun for about a week. The dried smaller parts were subjected to steam distillation and extracted aqueous layer with essential oil was separated into dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) using separatory funnel. The organic solvent was evaporated and the remained essential oil was used for the antifungal bioassays.

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### B. Antifungal Assay

Antifungal effects of these extracts were tested against two species of human skin infectious fungi, *Malassezia furfur* and *Microsporum gypseum* using disk diffusion method [6]. These fungi were cultured in a medium of Sabouraud Dextrose Agar (SDA). Spore suspensions of *M. furfur* and *M. gypseum* were prepared using seven days old pure cultures. Two drops (0.1 ml) from the suspension were inoculated into sterilized petri dishes contained SDA and evenly distributed using sterilized glass spreader. Sterilized filter paper disks (5 mm diameter) were cut and four of them were placed on agar plates containing fungal suspension. Dimethyl Sulphoxide (DMSO) was used to dissolve the fungal extract. To prepare 1 mg/ml solution, 1 mg of fungal extract was dissolved in 1 ml of DMSO. Filter paper disks contained in agar plates were soaked with 50  $\mu$ l, 100  $\mu$ l, 200  $\mu$ l, 300  $\mu$ l and 400  $\mu$ l of each dissolved fungal extract separately. Parallel experiment was conducted with Fluconazole (50  $\mu$ l), a commercial fungicide recommended by the Department of Health, SL as positive control, and with DMSO (50  $\mu$ l) as negative control for comparison purposes. Each sample was replicated five times and arranged according to a Complete Randomized Design (CRD). All Petri dishes were sealed and kept under room temperature and observed the fungal growth daily.

### C. Data Analysis

The data were analyzed using minimum concentration of the extract at which the fungus started to grow again was taken as the MIC and the minimum concentration at which the fungus did not show any growth again was taken as the MLC. All the data were statistically analyzed using MINITAB 14 statistical package.

## III. RESULTS AND DISCUSSION

Since there was no effect of DMSO (50  $\mu$ g) on the growth of *M. furfur* and *M. gypseum* it was used as the negative control and growth of *M. furfur* and *M. gypseum* were completely inhibited for Fluconazole, a synthetic antifungal compound (50  $\mu$ g) and used as the positive control in the antifungal assays carried out with the extracts.

The result of the antifungal activity of against *M. furfur* revealed that diameter of inhibition zone was increased with increasing dose of the extract. The mean diameter of the inhibition zones at the doses of 50  $\mu$ g, 100  $\mu$ g, 200  $\mu$ g, 300  $\mu$ g were (0.8  $\pm$  0.6) cm, (0.9  $\pm$  0.06) cm, (1.4  $\pm$  0.08) cm, (1.7  $\pm$  0.3) cm respectively. Highest dose of inhibition of *M. furfur* was observed at the dose of 400  $\mu$ g of the extract with a mean diameter of the inhibition zone (2.1  $\pm$  0.09) cm. MIC and MLC values were 200  $\mu$ g and 300  $\mu$ g respectively (Fig 2).

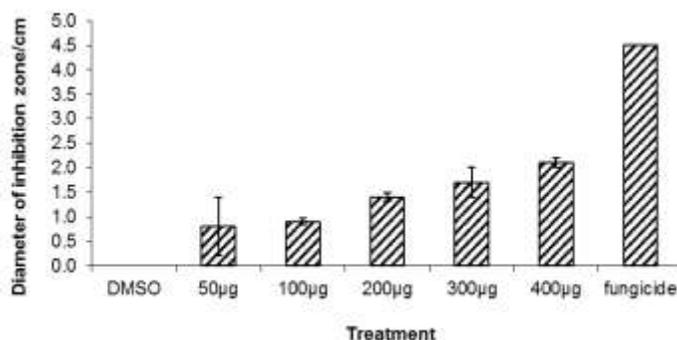


Fig 2: Antifungal activity of the extract of *A. nigra* against *M. furfur*

The graph displays in figure.3, shows the antifungal activity of the extract of *A. nigra* against *M. gypseum*. Observations of antifungal assay revealed that diameters of inhibition zones were increased with increasing the dose of the extract. The lowest mean diameter of the inhibition zone was (0.6  $\pm$  0.5) cm at 50  $\mu$ g. The diameter of inhibition zone at the dose of 400 $\mu$ g was (2.4  $\pm$  0.05) cm. MIC and MLC values for the extract were 100  $\mu$ g and 200  $\mu$ g respectively.

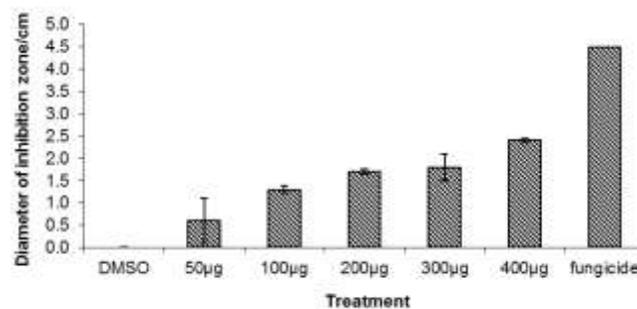


Fig 3: Antifungal activity of the extract of *A. nigra* against *M. gypseum*

In order to investigate the phytochemicals that playing antifungal activity, it is important to find out the major constituents of the *A. nigra* rhizome. *A. nigra* has two flavone glycosides, astragalin and kaempferol-3-*O*-glucuronide [7].

Even though there have been several researches conducted to evaluate the biological and phytochemical activities including antibacterial [8],[9], antioxidant [10],[11],[12], antiprotozoal [13], hepatoprotective [14] effects of the *A. nigra*, the responsible constituents were not identified yet. The above results confirmed that the extract of *A. nigra* shows antifungal effects on the growth of *M. furfur* and *M. gypseum*. Also it has been supported to verify the traditional uses of the rhizome of *A. nigra* to cure the fungi infected human skin as home remedies. Therefore, this study will be further extended in order to investigate the bioactive constituents encountered with the antifungal activity.

#### IV. CONCLUSION

Essential oil extract of the rhizome of *A. nigra* have the potential to inhibit the growth of *Malassezia furfur* and *Microsporum gypseum*, which cause dermal diseases in human.

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