ORIGINAL ARTICLE

A Comparative evaluation of *in vitro* anti-inflammatory and antifungal activity of *Ganoderma lucidum* strains DARL-4 and MS-1

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Abstract

Background: *Ganoderma lucidum* commonly known as *Reishi* is a lignicolous high value medicinal mushroom belonging to family Ganodermataceae. DARL-4 is an indigenous strain and MS-1 is an exotic Malaysian strain which is *in vitro* cultivated under sterile condition. The main aim of this study is a comparative evaluation of *in vitro* anti-inflammatory and antifungal activity of *G. lucidum* strains DARL-4 and MS-1. **Materials and Methods:** The hydroalcoholic extract of *G. lucidum* strains DARL-4 and MS-1 was screened for *in vitro* anti-inflammatory activity using inhibition of albumin denaturation technique at different concentration. Diclofenac (100 µg/ml) was used as standard reference drug. *In vitro* antifungal activity of hydroalcoholic extract of *G. lucidum* strains DARL-4 and MS-1 was evaluated by agar well diffusion method using *Candida albicans* as a fungal strain. Fluconazole was used as standard drug. **Results and Discussion:** The % inhibition of denaturation produced by hydroalcoholic extract of DARL-4 and MS-1 was comparable with that produced by diclofenac. MS-1 shows more significant anti-inflammatory activity than DARL-4. DARL-4 and MS-1 show moderate antifungal activity with a zone of inhibition of 19 ± 0.21and 21 ± 0.36 mm, respectively, as compared to the standard (fluconazole) having zone of inhibition of 30 ± 0.03 mm. **Conclusion:** MS-1 possesses more significant anti-inflammatory and antifungal activity as compared to DARL-4.

Key words: *Ganoderma lucidum*, Anti-inflammatory activity, antifungal activity, albumin denaturation, agar well diffusion

INTRODUCTION

Ganoderma lucidum commonly known as *Reishi* is a lignicolous high value medicinal mushroom belonging to family Ganodermataceae. *G. lucidum* (W.Curst.:Fr.) P. Karst. (Higher Basidiomycetes) is well known for nutraceutical and pharmaceutical properties for promoting human health. *G. lucidum* has been reported to show antitumor, hypotensive, cytoxicity, antioxidant, anti-allergic, antimicrobial, hepatoprotective, hypolipidemic, anti-diabetic, and anti-inflammatory effects.[1] *G. lucidum* contains bioactive components mainly triterpenoids, steroids, glycoproteins, and polysaccharides.[2-5] These bioactive components play a role in maintaining a good health and fulfill the nutritional requirements. Wild *G. lucidum* is less abundant in nature, and thus, it is not available sufficiently for nutraceutical product development so its' *in vitro* cultivation is developed for its easy availability for nutraceutical and pharmaceutical development. DARL-4 is an indigenous strain and MS-1 is an exotic Malayasian strain which is *in vitro* cultivated under sterile condition.

Inflammation is a normal protective response to tissue injury which involves enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair.[6] It is frequently associated with pain and involves the increase in vascular permeability, increase of protein denaturation, and membrane alterations.[7] Inflammation is a

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physiologic defense mechanism that helps the body to protect itself against infection, burn, toxic chemicals, allergens, or other noxious stimuli. Inflammations are mainly as acute and chronic inflammations.[8] The present non-steroidal anti-inflammatory drugs (NSAID's) are commonly used drugs for treating inflammation, and the long-term use of NSAID's causes severe side effects. For this reason, in recent time, a search for other alternatives seems necessary and beneficial.

Pathogenic fungi are fungi that cause disease in humans. Most of the incidence of fungal infections increase due to the weak immune system related to HIV, cancer, and other diseases.

Candida albicans is responsible for a wide range of superficial and systemic infections.[9] There has been an increase in resistance by *C. albicans* to conventionally produced antimicrobials recently, leading to the search of a new antifungal agent.[10,11]

The main aim of this study is a comparative evaluation of *in vitro* anti-inflammatory and antifungal activity of *G. lucidum* strains DARL-4 and MS -1.

MATERIALS AND METHODS

Collection of Fruiting Bodies of *G. lucidum* Strains DARL-4 and MS-1

In vitro cultivated samples of *G. lucidum* strains DARL-4 and MS-1 were collected from the polyhouse of DIBER, Pithoragarh, between April and May. The strains were authenticated by Mycology Department, DIBER, field station, Pithoragarh. Samples were then air-dried and grinded into powdered form.

Preparation of Crude Extract

50 g of DARL-4 and MS-1 were extracted with hydroalcohol by the cold maceration process. The extracts were filtered with the help of Whatman No. 1 filter paper and evaporated to dryness. The extracts were finally lyophilized and stored in a desiccator.

Inhibition of Albumin Denaturation

The anti-inflammatory activity of hydroalcohol extracts of *G. lucidum strains* DARL-4 and MS-1 were performed using inhibition of albumin denaturation method and shown in Figure 1[12-14] followed with slight modifications. The reaction mixture (2 ml) was containing test extracts of different concentrations (100–500 µg/ml) or 100 µg/ml diclofenac (SAID) and 1% aqueous solution of bovine albumin fraction. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, and after cooling the samples,

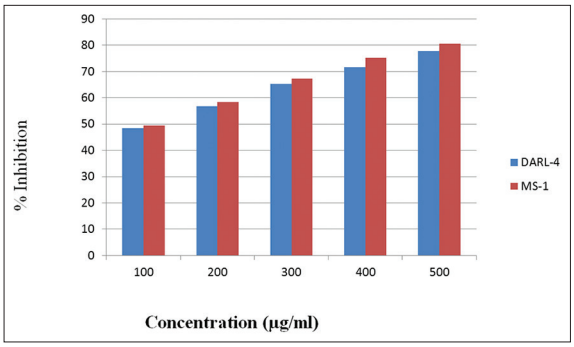

Figure 1: Percentage inhibition of hydroalcohol extracts of DARL-4 and MS-1 on albumin denaturation

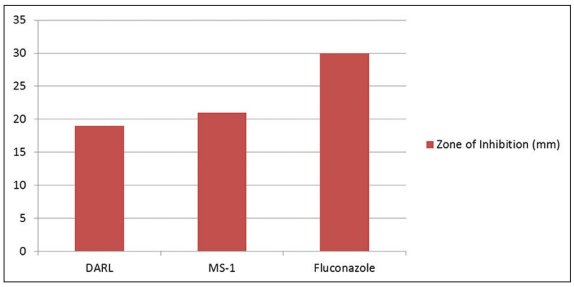

Figure 2: Evaluation of antifungal activity of hydroalcohol extract of *Ganoderma lucidum* strains DARL-4 and MS-1

the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. The experiment was performed in triplicate. The percentage inhibition was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\times 100 / \text{Abs control}}$$

In vitro Antifungal Activity of *G. lucidum* strains DARL-4 and MS-1

In vitro antifungal activity of hydroalcohol extracts of DARL-4 and MS-1 was performed using agar well diffusion method and shown in Figure 2.[14-17]

Preparation of Culture Medium

Sabouraud's dextrose agar media (Hi Media) were used for *in vitro* antifungal activity. For the preparation of media, dextrose (40 g), peptone (10 g), and agar (20 g) were accurately weighed, dissolved in distilled water, and autoclaved at 121°C for 15 min. pH of the media was maintained at 5.6.

Standard Preparation

Fluconazole was used as a standard antifungal agent and prepared in sterile distilled water to give a final concentration of 10 µg/ml.

Sample Preparation

The hydroalcohol extracts of DARL-4 and MS-1 were dissolved in dimethyl sulfoxide (DMSO) to give the final concentration of 20 mg/ml.

Preparation of Inoculum

The suspension of fungus was prepared by McFarland Nephelometer standard method. The culture of *C. albicans* was used for the preparation of fungal suspension. An inoculum was prepared by suspending the isolated colony in 2 ml of 0.9% w/v of normal saline solution and then mixed to form a smooth suspension.

Procedure for *In vitro* Antifungal Activity

The Sabouraud's dextrose agar media (Hi Media) were poured in sterile Petri plates and allowed to solidify. After that, the prepared inoculum was poured onto the surface of agar plates and spreaded by a glass spreader. The flamed sterile borer (21 mm in diameter) was used and the medium was bored, and then 0.1 ml of standard and test samples were added in each bore. A control having DMSO was also maintained. The above procedure was carried out in aseptic condition under Laminar Air Flow. The plates were then incubated at 28°C for 72 h. Finally, the zone of inhibition in each plate was measured and the test samples were compared with the standard. The experiment was run in triplicate.

RESULT

In vitro anti-inflammatory activity of hydroalcohol extracts of DARL-4 and MS-1 was investigated by albumin denaturation method. The results are indicated in Tables 1 and 2. The hydroalcohol extracts of DARL-4 and MS-1 were used in the concentration range of 100–500 µg/ml, and it showed a concentration-dependent inhibition of albumin denaturation. Diclofenac, a SAID, showed the maximum inhibition 81.84% at the concentration of 100 µg/ml. MS-1 and DARL-4 show 80.52 ± 0.24% and 77.89 ± 1.02% maximum inhibition at concentration of 500 µg/ml. MS-1 shows more significant anti-inflammatory activity in concentration-dependent inhibition of albumin denaturation than that of DARL-4.

The results of antifungal activity of hydroalcohol extracts of DARL-4 and MS-1 against the fungal strains *C. albicans* are shown in Table 3. The hydroalcohol extracts of MS-1 (21 ± 0.36 mm) produced the highest zone of inhibition against *C. albicans* as compared to DARL-4 (19 ± 0.21 mm). The standard antifungal control used (fluconazole 10 µg/ml) formed a desirable zone of inhibition of 30 ± 0.03 mm. There were no zones of inhibitions formed by the negative control. The hydroalcohol extract of MS-1 possesses more significant antifungal activity.

DISCUSSION

Fungi are responsible for many infectious diseases.[18] *G. lucidum* and other *Ganoderma* species have been used

Table 1: Effect of hydroalcohol extract of DARL-4 on albumin denaturation

Treatment	Concentration (µg/ml)	Absorbance (660 nm)	% Inhibition
Control	-	0.19±0.24	-
DARL-4	100	0.098±1.23	48.42±0.79
DARL-4	200	0.082±0.29	56.84±0.98
DARL-4	300	0.066±0.59	65.26±0.74
DARL-4	400	0.054±1.20	71.57±0.78
DARL-4	500	0.042±0.035	77.89±1.02
Diclofenac	100	0.034±0.42	81.84±1.24

Table 2: Effect of hydroalcohol extract of MS-1 on albumin denaturation

Treatment	Concentration (µg/ml)	Absorbance (660 nm)	% inhibition
Control	-	0.19±0.24	-
MS-1	100	0.096±0.90	49.47±0.90
MS-1	200	0.079±0.28	58.42±0.28
MS-1	300	0.062±1.04	67.36±1.02
MS-1	400	0.047±0.059	75.15±0.59
MS-1	500	0.037±1.12	80.52±0.24
Diclofenac	100	0.034±0.42	81.84±1.24

Table 3: Antifungal activity of hydroalcohol extract of *G. lucidum* strains DARL-4 and MS-1

Plant extract	Zone of inhibition (mm)
DARL-4 (hydroalcohol extract)	19±0.21
MS-1 (hydroalcohol extract)	21±0.36
Fluconazole	30±0.03
Control	-

G. lucidum: Ganoderma lucidum

to treat various bacterial and fungal diseases. This might be due to the presence of rich phytochemical constituents such as polysaccharides, phenol, triterpenoids, and flavonoids. There has been an increase in resistance by fungal strains to conventionally produced antifungal agents recently, leading to the search of a new antifungal agent. The agar well diffusion method had shown that the tested hydroalcohol extracts of DARL-4 and MS-1 have moderate antifungal activity against the tested *C. albicans.*

Denaturation of proteins is a well-known cause of inflammation. When proteins are denatured, they lose their biological functions. Production of autoantigen in certain arthritic disease is due to denaturation of protein.[19,20]

In the present study, the hydroalcohol extracts of DARL-4 and MS-1 are capable of inhibiting albumin denaturation. MS-1 shows more significant anti-inflammatory activity as compared to that of DARl-4.

CONCLUSION

The results from the present study reported that *in vitro* cultivated *G. lucidum* strains DARL-4 and MS-1 used as an ideal bio-pharmaceutics. The hydroalcohol extracts of DARL-4 and MS-1 possessed significant anti-inflammatory and antifungal activity. This might be due to the presence of rich phytochemical constituents such as polysaccharides, phenols, flavonoids, and terpenoids. MS-1 which is an exotic Malaysian strain was found to have more significant anti-inflammatory and antifungal activity when compared with DARL-4 which is an indigenous strain. This study is suggested that *G. lucidum* can be used as anti-inflammatory and antifungal agent in the development of new drug.

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