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ESTIMATION OF ANTIMICROBIAL POTENTIAL OF *GANODERMA LUCIDUM* (LEYSS. EX FR.) KARST. EXTRACTS

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Abstract

Ganoderma belongs to a group of fungi with reported potent bioactive properties. Six different local isolates of *G. lucidum* were collected from Lahore. Ethanol extracts of the *Ganoderma lucidum* isolates were evaluated for antimicrobial potential. The extracts of the mycelia from G-1, G-3 and G-5 exhibited maximal potency against *Xanthomonas* sp., while G-2 and G-4 reduced the growth of *Escherichia coli* and *Pseudomonas* sp., respectively.

Introduction

Ganoderma lucidum (Leyss. ex Fr.) Karst. is a medicinal fungus in Polyporaceae. The fungus is saprobic in nature, growing alone or in groups on decaying hardwood logs and stumps (rarely on conifers). Its cap is about 4-20 cm, with a shiny, varnished surface often roughly arranged into lumpy "zones", and is light red to reddish brown when mature. Young basidiocarps have light yellow zones on the surface. At maturity the margins of the basidiocarp are whitish, with brown lemon-shaped basidiospores produced on the lower surface (Huie & Di, 2004).

The fungus has a wide range of potential hosts (*Acacia, Eucalyptus, Dalbergia* and many more), and is found both in urban and a forestry setting. The fruiting body has the form of a hoof-shaped bracket, which is normally located on the lower portion of a tree trunk (Cao & Lin, 2003).

Ganoderma lucidum is known as lingzhi and was first indexed in Shen Nong's Materia Medica (206 BC-8 AD) as a longevity-promoting and tonic herb of the non-toxic superior class. It has been used in traditional Chinese medicine (TCM) for more than 2000 years to prevent and/or treat diseases including hepatitis, chronic bronchitis, gastritis, tumor growth and immunological disorders (Lin & Zhang, 2004). Alcohol extractions have also been found to have various medicinal effects, including antiviral properties in a number of scientific studies. From a scientific perspective, lingzhi tinctures may be more effective than lingzhi teas for some diseases, despite the prevalence of teas in traditional Chinese medicine (Jung *et al.*, 2004). In truth, the fungus might be one of the most convincing examples of ancient folk remedies being translated into new drug development, and may represent an excellent paradigm for the general principle (Johnston, 2005).

Material and Method

Collection of the samples: Samples were collected from the Punjab University campus and from Changa Manga and Allama Iqbal Town (Fig. 1). The name of the host tree was also recorded at the time of sample collection. For this purpose mostly undisturbed areas were selected. Six samples of *Ganoderma lucidum* were assigned code numbers as **G-1** (growing on *Eucalyptus citriodora* from Quaid-e-Azam campus, University of the Punjab, Lahore), **G-2** (growing on *Eucalyptus citriodora* from Quaid-e-Azam campus, University of the Punjab, Lahore), **G-3** (growing on *Dalbergia sissoo* from Nishtar block, Allama Iqbal Town, Lahore), **G-4** (growing on *Acacia nilotica* from Changa Manga), **G-5** (growing on *Acacia nilotica* from Changa Manga), and **G-6** (growing on *Azadarichta indica* from Quaid-e-Azam campus, University of the Punjab, Lahore).

Fig. 1. Map of Lahore showing collection sites.

Preparation of the sample: About 250 g of each basidiocarp of *Ganoderma lucidum* were ground in liquid nitrogen with a mortar and pestle, then suspended in 500 ml of 70% ethanol. The suspension was air dried under shade for five days. Samples were further purified according to the procedure of Smith *et al.,* (2002). The material was partially dried in an oven dried for 48 hours at 55°C and then milled into a fine powder. The powder was further dried by lyophilization. The lyophilyzed sample was suspended in 1000 ml of distilled sterilized water, and stirred at 4°C overnight. The sample was filtered and the resultant liquid was used as the pure extract to assess the antimicrobial potential of *Ganoderma lucidum* metabolites.

Estimation of antimicrobial potential of the extract: Antimicrobial potential of the fungal extract was estimated by the method described by Zjawiony (2004). *Ganoderma lucidum* extracts were applied in various concentrations to fungal and bacterial cultures. The cultures were obtained from First Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Bacterial species were cultured in broth medium while fungal test species were grown on malt extract agar medium (MEA). After 3-4 days of incubation, inhibition of growth of the microbes was estimated.

Preparation of fungal cultures: Three different fungi, *Aspergillus niger, Alternaria alternata* and *Botrytis sp.* were grown on MEA for 3-4 days. Fungal plates were prepared by adding 500 µl of *G. lucidum* extract to the surface of a poured medium plate and evenly spreading the extract with a sterilized spreader. Three dilutions of extract (25%, 50%, and 75%) were used, while sterile distilled water was used as a control. The extract was allowed to get absorbed in the medium for 2.0-2.5 hours before a disc (5 mm) of pure culture of the test fungus was transferred aseptically on to the plates. Plates were incubated for seven days at 25±2ºC in the dark. The fungal mycelium was harvested with a clean spatula and air dried on a pre-weighed filter paper. The filter paper was reweighed to give the fresh mass of the test fungus. The filter papers with fungal mass were oven dried for 24 hours at 80°C for dry weight measurements. The experiment was conducted in triplicate.

Preparation of bacterial cultures: *Pseudomonas* sp.*, E. coli* and *Xanthomonas* sp. were selected as bacterial test samples. Varying amounts (0 µl, 20 µl, 40 µl, 60 µl, 80 µl and 100 µl) of *G. lucidum* extracts were added to 20 ml of liquid medium with bacterial inoculation. The potential of bio-active compounds against bacteria was determined by means of spectrophotometric analysis. Results for the bacterial cultures were recorded after 24 hours. The OD values of the bacterial cultures treated with different amounts (0 – 100 µl) of *G. lucidum* extracts were noted for three replicates at each concentration at 600 nm absorbance and the growth inhibition pattern of the bacterial cultures was observed. Growth medium without any bacterial inoculation was used as a control for comparison. The data were analyzed using Duncan's Multiple Range Test (Steel and Torrei, 1980).

Results and Discussion

Maximum growth inhibition (0.57 OD) against *Xanthomonas* sp. was observed from the 100 µl application of *G. lucidum* extracts G1, G3 and G5. In the case of *Pseudomonas*, maximum growth inhibition (0.65 OD) was observed when G4 extracts were applied at a volume of 80 µl. However, in the control without any extract, a high OD value (~ 1.50) was observed. The application of 100 µl of extract G2 had the maximum antibacterial activity against *E. coli* (0.33 OD). Extracts G3 and G5 had similar inhibition effects on *E. coli* with OD values ranging ~0.53 OD from 1.4. The maximum *E. coli* growth inhibition was observed when *G. lucidum* extracts were applied at the rate of 100 µl volume.

For the other volumes of extract (0 µl, 20µl, 40µl, 60µl, and 80 µl), there was a gradual trend of reduction in optical density (~1.4 OD to 0.54 OD) as the volume increased, indicating that inhibition was better at higher volumes of extract (Table 1).

For the fungal cultures, the fresh weight of the fungus was noted seven days after treatment with *G. lucidum* extracts to estimate their antifungal potential. The general trend was that the increasing concentrations of all the extracts showed inhibitory effects on fungal growth by reducing the fresh weight of the test fungi. In the case of *Aspergillus niger*, strong growth inhibition was recorded from extracts G1 and G3 at 100% concentration (Fig. 2). It was observed that in the case of *Alternaria alternata,* extracts G-3 and G-5 proved to be the best growth inhibitors (Fig. 3). The maximum growth reduction was observed in *Alternaria alternata* (85%). *Botrytis* showed some resistance to the extract and the rate of growth inhibition was less significant (Fig. 4). Extracts of *Ganoderma lucidum* contain ganoderic acid, which belongs to the saponins (Wen *et al.,* 2005; 2006).

Table 1. Antimicrobial assay of *Ganoderma lucidum* against bacteria.

Extracts	Volume (µl)	OD values (mean ± standard error)		
		***Xanthomonas* sp.**	***Pseudomonas* sp.**	***E. coli***
G1	0	1.16 ± 0.01 a	1.28 ± 0.01 ab	1.03 ± 0.02 a
	20	0.93 ± 0.01 cd	1.30 ± 0.01 a	0.92 ± 0.03 ab
	40	0.37 ± 0.01 cd	1.32 ± 0.02 a	0.93 ± 0.01 ab
	60	0.87 ± 0.005 bc	1.36 ± 0.02 a	0.89 ± 0.01 b
	80	0.74 ± 0.005 b	0.96 ± 0.02 bc	0.77 ± 0.02 bc
	100	0.57 ± 0.01 c	1.00 ± 0.01 b	0.65 ± 0.02 bc
G2	0	1.04 ± 0.02 a	1.38 ± 0.03 a	0.91 ± 0.01 a
	20	0.95 ± 0.01 bc	0.85 ± 0.03 c	0.86 ± 0.01 ab
	40	0.20 ± 0.01 bc	1.02 ± 0.01 ab	0.73 ± 0.02 ab
	60	1.02 ± 0.02 a	0.99 ± 0.02 b	0.63 ± 0.02 ab
	80	0.54 ± 0.02 b	0.97 ± 0.01 b	0.54 ± 0.01 b
	100	0.81 ± 0.03 ab	0.93 ± 0.03 bc	0.33 ± 0.02 bc
G3	0	1.01 ± 0.01 a	1.40 ± 0.01 a	1.44 ± 0.02 a
	20	0.13 ± 0.01 c	0.97 ± 0.01 ab	0.91 ± 0.01 b
	40	0.40 ± 0.01 bc	0.92 ± 0.02 b	0.87 ± 0.02 bc
	60	0.89 ± 0.02 ab	0.88 ± 0.02 bc	0.71 ± 0.01 c
	80	0.91 ± 0.01 ab	0.02 ± 0.02 c	0.79 ± 0.03 cd
	100	0.57 ± 0.01 b	0.94 ± 0.02 ab	0.53 ± 0.01 d
G4	0	1.89 ± 0.02 a	1.34 ± 0.01 a	1.41 ± 0.01 a
	20	1.63 ± 0.02 ab	1.04 ± 0.05 b	0.87 ± 0.01 b
	40	1.54 ± 0.02 b	1.08 ± 0.04 b	0.81 ± 0.01 c
	60	0.96 ± 0.02 bc	1.07 ± 0.02 b	0.85 ± 0.03 bc
	80	0.92 ± 0.01 bc	0.65 ± 0.01 c	0.66 ± 0.02 d
	100	0.76 ± 0.02 c	0.95 ± 0.03 bc	0.89 ± 0.07 b
G5	0	1.22 ± 0.01 ab	1.28 ± 0.04 a	1.52 ± 0.01 a
	20	1.32 ± 0.01 a	1.17 ± 0.02 ab	1.39 ± 0.01 b
	40	1.12 ± 0.01 b	0.93 ± 0.02 b	1.28 ± 0.01 bc
	60	0.82 ± 0.02 bc	0.82 ± 0.01 bc	1.22 ± 0.01 bc
	80	0.63 ± 0.01 c	0.71 ± 0.01 c	0.87 ± 0.02 c
	100	0.57 ± 0.02 c	0.54 ± 0.01 c	0.83 ± 0.03 c
G6	0	1.43 ± 0.02 a	1.32 ± 0.01 a	1.02 ± 0.02 a
	20	1.27 ± 0.02 ab	1.17 ± 0.01 ab	0.99 ± 0.01 ab
	40	1.15 ± 0.01 b	0.93 ± 0.02 b	0.79 ± 0.01 b
	60	1.09 ± 0.02 b	0.82 ± 0.01 bc	0.63 ± 0.01 b
	80	0.63 ± 0.01 bc	0.36 ± 0.01 c	0.54 ± 0.01 bc
	100	0.34 ± 0.01 bc	0.41 ± 0.01 c	0.65 ± 0.02 c

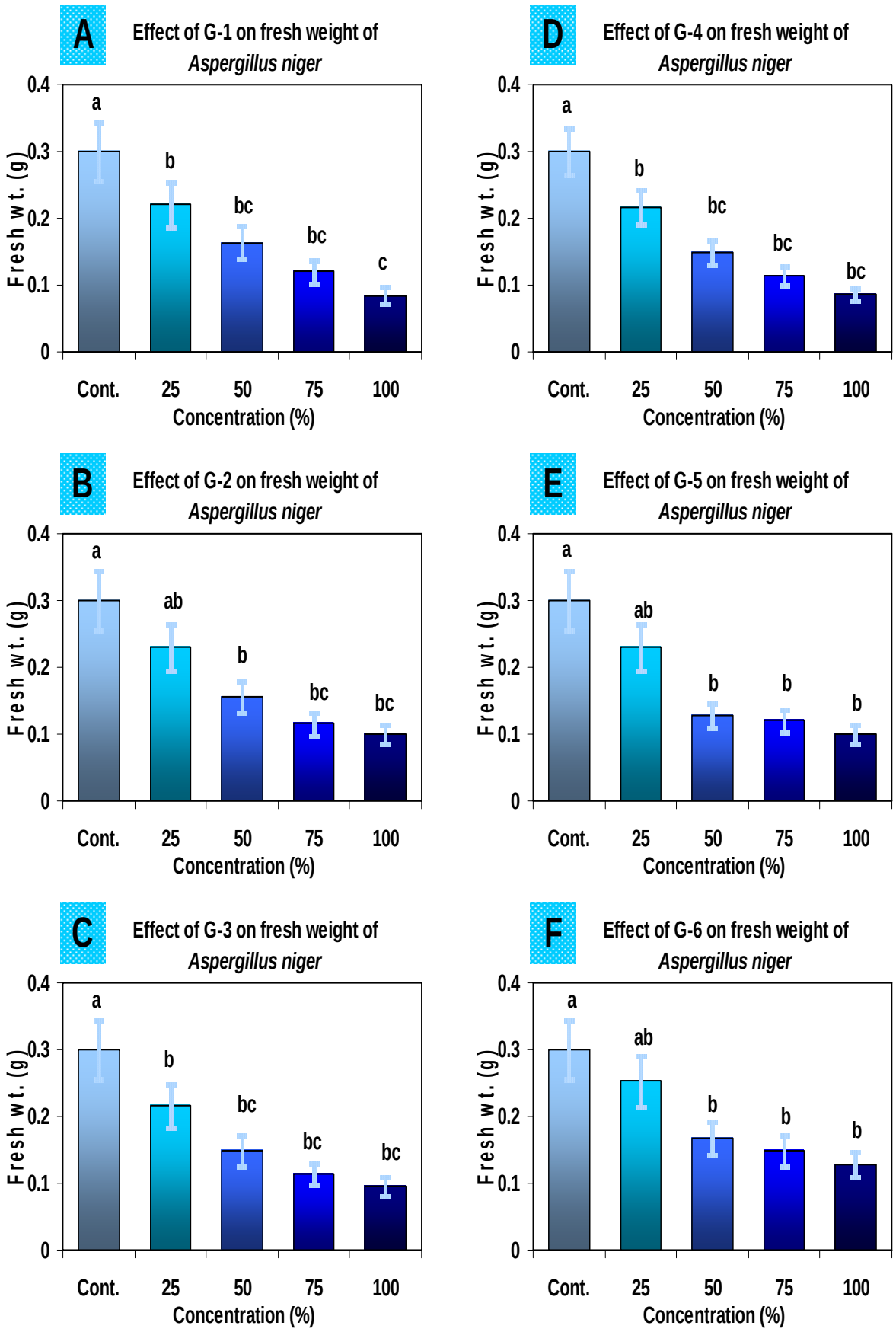

Fig. 2 A-F. Effect of *Ganoderma lucidum* extracts on *Aspergillus niger*.

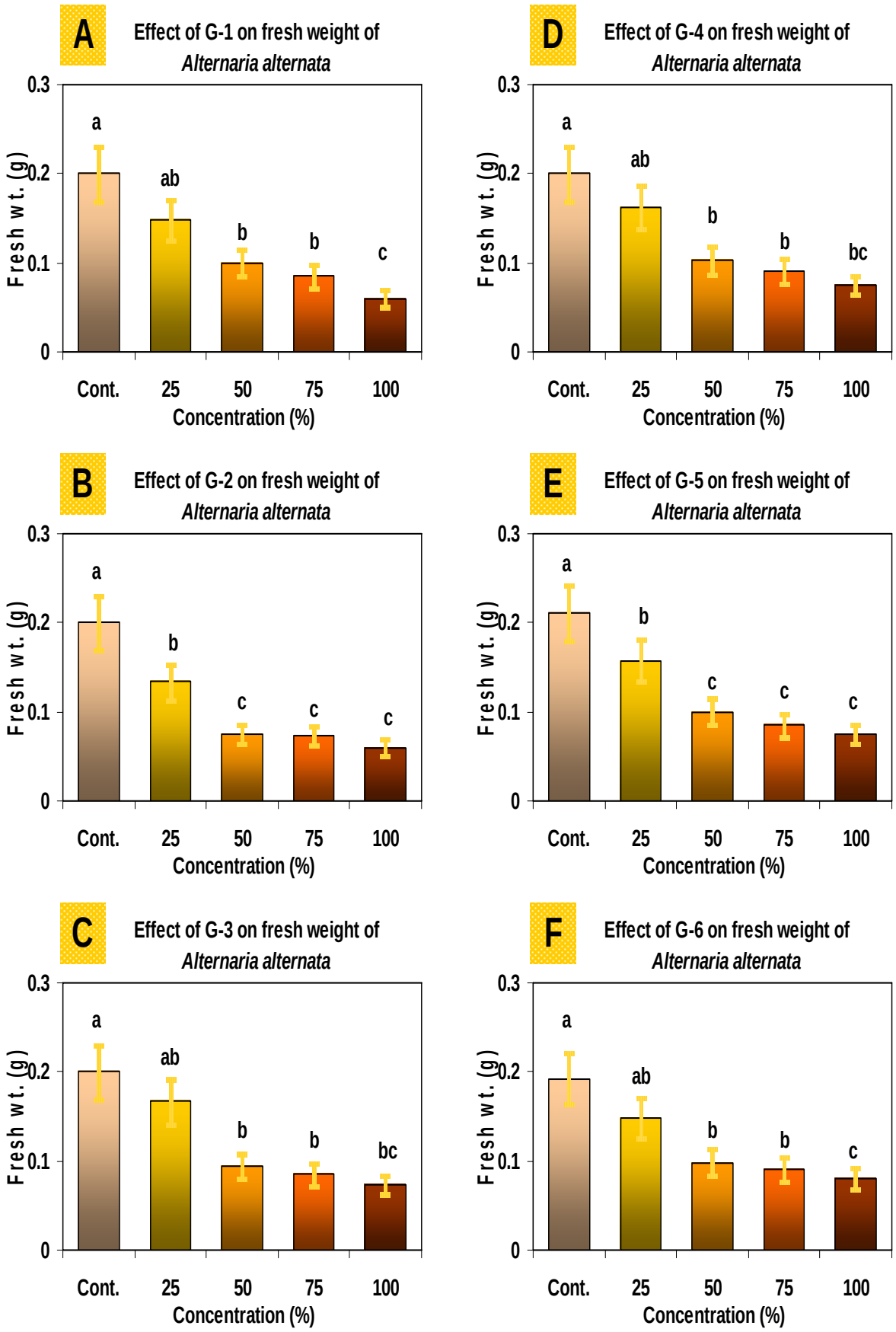

Fig. 3 A-F. Effect of *Ganoderma lucidum* extracts on *Alternaria alternata*.

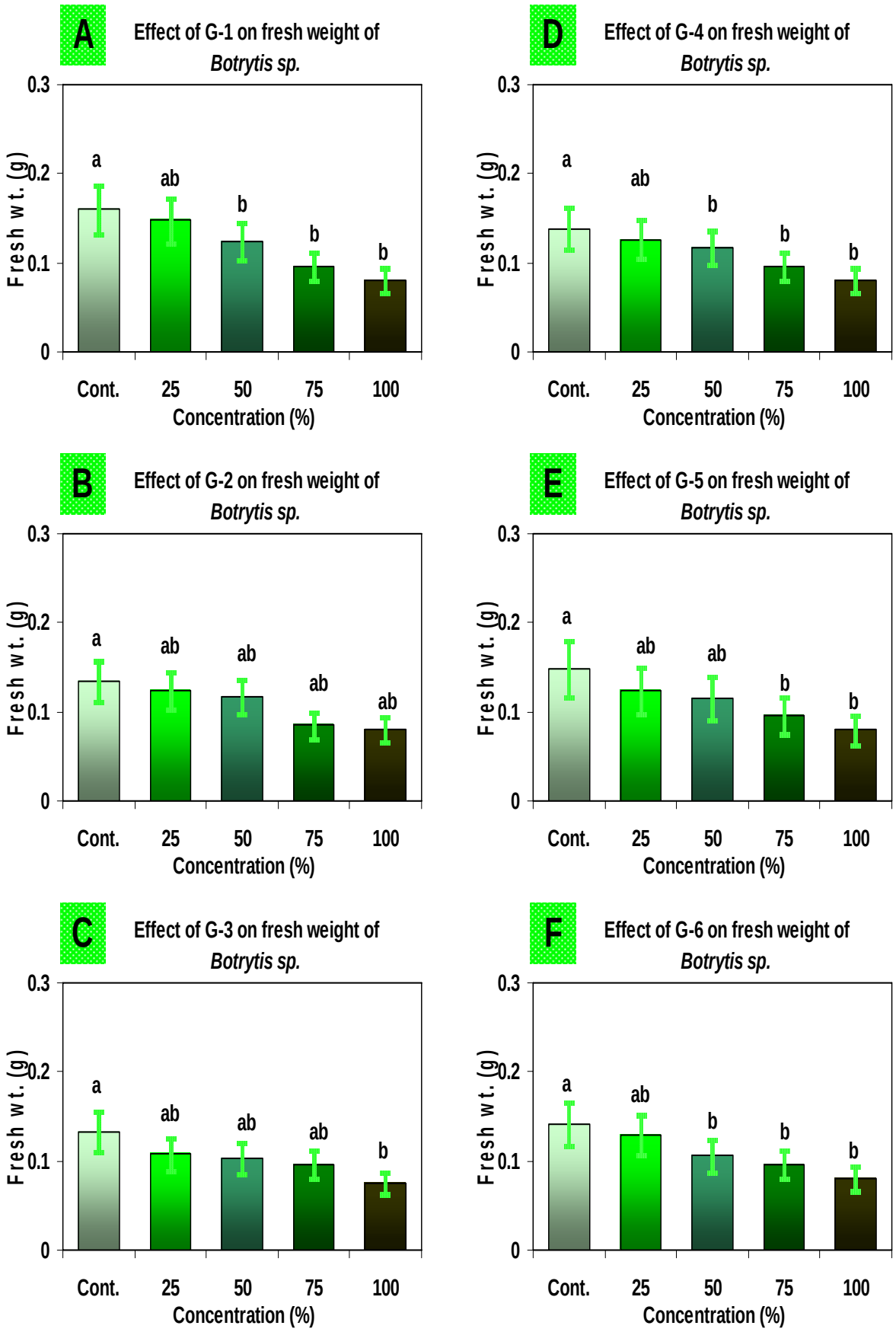

Fig. 4 A-F. Effect of *Ganoderma lucidum* extracts on *Botrytis* sp.

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