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Antioxidant and Antifungal Activity of Aqueous and Organic Extracts of Liquorice

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Abstract: Aqueous and organic extracts of liquorice were investigated for antioxidant and antifungal activities against four pathogens, *A. alternata, A. niger, F. solani, F. oxysporum* by agar dilution method. The antioxidant effect of aqueous and organic extracts was evaluated by using DPPH method. All the extracts exhibited significant inhibition in DPPH free radical formation which is comparable to BHT used as standard. The percentage DPPH radical scavenging was 66 ± 1.21,55 ± 1.09, 52 ± 0.98 and 57 ± 1.02 for BHT, aqueous, methanolic and ethanolic extracts respectively. MIC and MFC was evaluated by serial dilution method, the strongest antifungal activity was observed using the ethanolic extract against *F. oxysporum* (zone of inhibition: 33.37±0.38 mm; MIC: 0.078 mg/mL) while all the extracts had equal antifungal activity against *A. alternata* and *F. solani*. *A. niger* was more sensitive to methanolic extract (zone of inhibition: 29.27±0.14; MIC: 0.039 mg/mL) than the aqueous and ethanolic extract.

Key words: DPPH · Antioxidant · Antifungal · Zone Of Inhibition

INTRODUCTION

Many products of medicinal plants have recently become of great interest in reducing the adverse effects of many diseases and play a significant role in achieving positive health care system [1]. Now a day's vast numbers of plant species are being reported for their possible pharmacological properties. They are known to possess economically important compounds like vitamins, minerals, nitrogen containing and phenolic compounds. Although the concentrations of these compounds are lower but can be utilized to combat the disease causing pathogens [2-4]. Mycotoxins represent a critical problem to human health and one of the main causes of morbidity and mortality. Also grains and foodstuff during storage loss their nutritive value, organoleptic characteristics and limited shelf life rendering them unfit for humans. Main toxic effects like carcinogenicity, terratogenicity, hepatotoxicity, genotoxicity, immunosuppression and reproductive disorders are reported by more than 300 fungal metabolites [5-6]. The liquorice (*Glycyrrhiza glabra* L.; Family: Papilionaceae/ Fabaceae) native to southern Europe and parts of Asia. Below ground, glycyrrhiza glabra has an extensive root system with a main taproot and numerous runners, which is harvested for medicinal purpose [7]. Liquorice extracts to be effective for gastrointestinal health as a mild laxative, anti-inflammatory, prolong thrombin and fibrinogen clotting times. It is also investigated as certain immune functions such as interferon production, botanical boosting and preventing damage to genetic material that can eventually result in cancer [8, 9]. The aim of present study to investigate antioxidant and antifungal activities of aqueous and organic extracts of liquorice to highlight its potential as candidates for new drugs that may be of value in the treatment and prevention of human and livestock diseases.

Experimental

Collection and Identification of Plant Material: The liquorice was collected from local market of Lahore-Pakistan and authenticated by plant taxonomist from Institute of Agriculture Sciences, University of the Punjab, Lahore. A voucher specimen (HHC-963) was deposited at the Institute's herbarium.

Aqueous and Organic Extraction Procedure: Roots of plant material were thoroughly washed with tap water to remove debris and dust particles and then with distilled water and oven dried at 50°C for 24 h. The dried roots were pulverized by a mechanical grinder into powder form.

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50 g of milled sample was used for extracts preparations using Soxhlet apparatus. 500 mL distilled water for aqueous extract and 500mL of methanol and ethanol for organic extracts preparation was used respectively. Continuous extraction was done for 5 h. Aqueous extract was freeze dried while the organic extracts were dried using rotary evaporator and transferred to clean air tight vials, weighed and refrigerated at 4°C until further use.

Antioxidant Potential (DPPH Free Radical Scavenging Activity) of Different Extracts: The antioxidant activity of aqueous and organic extracts of liquorice and the standard was evaluated on the basis of the free radical scavenging effect by decrease in absorbance of the stable methanolic 1, 1-diphenyl-2-picrylhydrazyl (DPPH) solution. 0.2-1.0 mg/mL each of extract and standard Butylated hydroxytoluene (BHT) was prepared in methanol. A stock solution of DPPH (20 mg/L) was prepared in methanol and 1 mL of this solution was added into each 1 mL solution of extracts and standard. At 520 nm the optical density was measured after keeping the solution mixture in dark for 30 minutes using spectrophotometer. The percentage scavenging activity of DPPH radical was calculated according to the following equation.

DPPH scavenging activity (%) = {1-(Abs_{520}sample / Abs_{520}DPPH solution)} × 100

Fungal Strains and Growth Conditions: *Alternaria alternata (A. alternata), Aspergillus niger (A. niger), Fusarium solani (F. solani), Fusarium oxysporum (F. oxysporum)* were obtained from first fungal culture bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore. Fungal strains were cultured on malt extract agar (MEA) medium slants and the agar plates were incubated at 25°C for 5 days.

Preparation of Plant Extracts for Antifungal Activity: Each of powder extract was dissolved in dimethyl sulphoxide (DMSO) (10-30 mg/mL) and filtered through 0.45 µm membrane filter. The filter solutions were sterilized in autoclave at 15psi and 121°C for half an hour and stored in the refrigerator at 4°C until used for testing antifungal sensitivity.

Agar Well Dilution Assay: Antifungal activities of the extracts were examined against growth of the fungi using the agar well method on MEA plates. 100 µl of each fungal suspension with cell density of 10^5 cfu/mL was spread on MEA medium with L shaped glass spreader. Single well (8mm in diameter) was prepared at the centre of the plate using corkborer. 60 µL each extract was dripped into the respected well and then left in a clean bench. The inoculated plates were incubated at 37°C for 72 h. Antifungal activity was evaluated by measuring the zone of inhibition in mm. Studies were performed in triplicates and zone of inhibition was calculated with the mean ± SD values.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC: The minimal inhibitory concentration (MIC) was reported at level of the absence of growth while the minimal fungicidal concentration (MFC) was the killing of 99.9% of the test organism (Fungi) by lowest concentration that was prepared by serial dilution sequence. Ten screw-capped test tubes (13mm x 100 mm) were sterilized and numbered individually. Tube 1 was filled with 2 mL of malt extract broth including the extract stock solution. 1.0 mL of malt extract broth was introduced into tubes 2 and diluted with 1mL malt extract broth and repeated the procedure upto tube10. Extract concentration used for MIC value was 20-0.039 mg/mL obtained by two fold serial dilution technique. The tubes were incubated at 25°C for 72 h. After incubation the tubes were examined fungal growth. The tubes with the lowest concentration of the plant extract at which no growth or turbidity was observed was reported as the MIC against the organism.100 µL contents from the tubes containing no turbidity were cultured on the malt extract agar medium and incubated at 37°C for 24 h determine the minimum fungicidal concentration (MFC).

RESULTS AND DISCUSSION

The antioxidant activity of plant extracts were determined by DPPH stable free radical method. The antioxidant profile of standard BHT, aqueous and organic extract of liquorice is presented in Table 1. All the extracts with their maximum concentration showed antioxidant properties but ethanolic extracts showed the strongest DPPH radical scavenging activity, than the

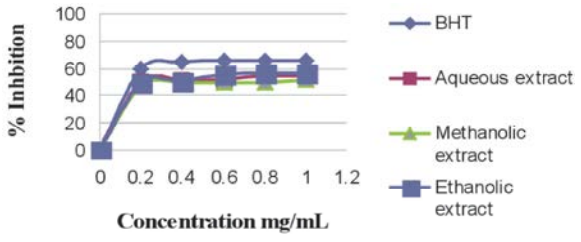

Fig. 1: DPPH free radical scavenging activity of standard BHT, aqueous and organic extracts

Table 1: Antioxidant profile of BHT, aqueous and organic extract of liquorice

Sample	Concentration (mg/mL)	DPPH radical scavenging (%)
BHT	1.0	66 ± 1.21
Aqueous extract	1.0	55 ± 1.09
Methanolic extract	1.0	52 ± 0.98
Ethanolic extract	1.0	57 ± 1.02

Table 2: MIC and MFC of aqueous and organic extracts of liquorice (mg/mL)

		Extracts		
Microorganisms		Aqueous	Methanolic	Ethanolic
A. alternata	MIC	0.0390	0.0390	0.0390
	MFC	0.1562	0.3125	0.3125
A. niger	MIC	0.0780	0.0390	0.3125
	MFC	0.3125	0.3125	0.1562
F. solani	MIC	0.0390	0.0390	0.039
	MFC	0.3125	0.3125	0.1562
F. oxysporum	MIC	0.3125	0.3125	0.0780
	MFC	1.250	0.6250	0.1562

Table 3: Antifungal activity of aqueous and organic extracts of liquorice

	Zone of inhibition (mean ± SD) (mm)								
	Aqueous extract (mg/mL)			Methanolic extract (mg/mL)			Ethanolic extract (mg/mL)		
Microorganisms	30	20	10	30	20	10	30	20	10
A. alternata	25.13±0.14	15.76±0.11	7.76±0.15	22.30±0.25	14.23±0.12	6.83±0.14	24.20±0.25	15.20±0.25	7.27±0.15
A. niger	21.47±0.13	13.73±0.40	6.56±0.12	29.27±0.14	18.63±0.38	9.10±0.25	21.73±0.14	14.77±0.13	6.51±0.13
F. solani	27.26±0.37	17.17±0.14	8.47±0.11	25.13±0.12	15.43±0.12	7.83±0.13	24.50±0.25	16.37±0.12	7.43±0.39
F. oxysporum	21.13±0.12	9.60±0.25	4.23±0.14	24.27±0.14	15.30±0.25	7.53±0.39	33.37±0.38	20.77±0.11	10.33±0.12

aqueous and methanol extracts but comparable with standard BHT. The scavenging free radicals activities of these extracts indicate their potential effect against the free radical related diseases. DPPH free radical scavenging of different concentration of aqueous, organic extracts and BHT standard presented in Figure 1 which determine the antioxidant activity. MIC and MFC of aqueous, methanolic and ethanolic extracts of liquorice are summarized in Table 2. The aqueous, methanolic and ethanolic extract showed the same activity against the *A. alternata* and *F. solani* (MIC: 0.039 mg/mL). The strongest activity was observed against *A. niger* using methanolic extract (MIC: 0.039 mg/mL) while ethanolic extract was more sensitive to *F. oxysporum* (MIC: 0.078 mg/mL). The strongest fungicidal (MFC) activity was seen against all the microorganisms using ethanolic extract and aqueous extract demonstrated weaker activity against all tested microorganisms. Results for antifungal activity of all the extracts of liquorice are presented in Table 3, both aqueous and organic extract possess significant antifungal potential against the entire studied microorganism. *F. oxysporum, A. niger* and *F. solani* were found more sensitive to ethanolic (33.37±0.38), methanolic (29.27±0.14) and aqueous (27.26±0.37) extracts respectively in terms of zone of inhibition (mm) using concentration of 30mg/mL of each extract. Furthermore, *A. alternata* was found be equally sensitive to all the extracts with comparable zone of inhibition.

CONCLUSION

All the extracts gave a wider spectrum of activity against all the pathogens. This work ultimately concluded to understand some molecular basis of therapeutic properties of liquorice in traditional medicine. Detail work by using different methods will be the aim of further investigation for pharmacological properties and alternatives of food preservations.

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