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In Vitro Antifungal and Antibacterial Activities of Root Extract of *Glycyrrhiza Glabra*

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Abstract: Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against microbial infections. The aim of this study was to evaluate the antimicrobial activity of crude extracts and fractions of the *Glycyrrhiza glabra* roots which have been traditional used as demulcents and expectorants in western countries, in Japan and China. The decoction of root is used to treat allergic inflammation and microbial infections. Extracts and their fractions were tested against six bacteria and two fungal strains using well diffusion method and microdilution method. All extracts and fractions possessed antimicrobial effect. Two fungal strains, *Candida albicans* and *Trichophyton rubrum* showed interesting susceptibility profiles when evaluated using the extracts and fractions with MICs ranging from 0.8 to 200 mg/mL. In case of bacterial strains, *Staphylococcus aureus, Listeria monocytogenes* and *Escherchia coli* were significantly susceptible to the extracts and fractions with MICs ranging from 0.2 to 1.2 mg/mL. Comparative results were carried out using Gentamicin for bacteria and clotrimazole for fungi as standard antibiotics.

Key words: *Glycyrrhiza glabra*, antifungal, antibacterial

INTRODUCTION

A number of traditional healers have claimed the efficacy of Glycyrrhiza species for a variety of pathological conditions as a diuretic, choleretic and used as insecticide and indicated in traditional medicine for coughs, colds and painful swellings [1]. In literature, a variety of pharmacological activities have been reported for the plants of genus Glycyrrhiza, Glycyrrhizin isolated from Glycyrrhiza species were found to possess anti-inflammatory, antispasmodic, expectorant and as tonics [2]. In another study, glabridin showed that it is potentially active against *Mycobacterium tuberculosis* in a dose-dependent manner [3].

Glycyrrhiza glabra, belongs to genus Glycyrrhiza and is commonly called as licorice which is available india. In literature no considerable scientific work has been done on microbial infections. Locally it is used as a folk remedy for skin infections and painful conditions [1]. In the current study, we have tried to explore the scientific basis of its use in microbial infections and in folk medicine, which is not studied as in this plant.

MATERIALS AND METHODS

Plant Material: The root material was collected in and around Mysore in March 2008. A voucher specimen was deposited in the Department of Botany, University of Mysore, Mysore. The roots of the plant were air-dried under shade for six consecutive weeks at room temperature. The dried root material was later on chopped, finely ground and stored in a polyethylene bag under refrigeration for further experimentation.

Extract Preparation: General extraction procedure was adopted for preparing extracts [4-7]. The air-dried roots of the plant (3.25 kg,) of *Glycyrrhiza glabra* were percolated with 80% ethanol (10 L) twice at room temperature. The extract was concentrated in vacuum, yielded 385 g of crude extract. The extract (361.5 g) was suspended in water, and then fractionated successively with equal volumes of chloroform, ethyl acetate and n-BuOH, leaving residual water soluble fraction. Each fraction was evaporated in vacuum to yield the residues of chloroform soluble fraction (101 g, 27.93% w/w), ethyl acetate soluble fraction (12.5 g, 3.45% w/w) and n-BuOH soluble fraction (51.5 g, 14.24% w/w), the remaining water fraction was (196.5 g, 54.35% w/w). Each organic extract was then evaporated to dryness. Stock extract solutions were prepared at 200 mg/m in distilled water. Extracts were sterilized over a membrane filter unit of 0.2 µm of pore size (Minisart, Sartorius) and preserved at 4 ^{0}C until used.

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Fungal and Bacterial Strains: Tests were performed on two fungal and six bacterial reference strains. Bacterial starins were *Staphylococcus aureus* ATTC 12600, *Listeria monocytogenes* ATTC BAA-679, *Escherchia coli* ATTC 11775, *Salmonella typhimurium* ATTC 13311, *Vibrio parahemolyticus* ATTC 17802 and *Pseudomonas aeruginosa* ATTC 10145. Fungal strains include *Candida albicans* MTCC 183 and *Trichophyton rubrum* MTCC 269. They were maintained on agar slant at 4 ^{0}C. The strains were activated at 37 ^{0}C for 24 h on nutrient agar (NA) or Sabouraud glucose agar (SGA) respectively for bacteria and fungi, prior to any screening.

Well Diffusion Method: The antimicrobial tests were carried out by the well diffusion method using a cell suspension of about 1.5 X 10^6 CFU/mL obtained following Mac farland turbidity standard No. 0.5 [8]. The concentration of the suspension was standardized by adjusting the optical density to 0.1 at 600nm wavelength (SHIMADZU UV-vis spectrophotometer) [9]. Well of 6mm diameter were then made on the NA plate (8mm thick) and loaded with 150 µL of ethanolic extract, fractions or standard drug(s). The inoculated plates were incubated at 37 ^{0}C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition around the well. The assay was repeated three times and the mean diameter was recorded. Gentamicin img for bacteria and clotrimazole img for fungi were used as standard antibiotics for comparison with extracts and fractions.

MIC Determination by Microdilution Method: Extracts (10 mg/mL) were dissolved in DMSO and serially diluted with sterile water. The same volume of an actively growing culture of the test bacteria was added to the different wells and cultures were grown overnight in 100% relative humidity at 37 ^{0}C. The next morning tetrazolium violet was added to all the wells. Growth was indicated by a violet color of the culture. The lowest concentration of the test solution that led to an inhibition of growth was taken as the MIC. The negative control DMSO had no influence on the growth at the highest concentration used. Gentamicin for bacteria and clotrimazole for fungi were used as controls for comparison.

Phytochemical Tests: The phytochemical analysis of these fractions as well as that of the crude ethanolic extracts was also performed following the classical methods described by Harbone [10].

RESULTS AND DISCUSSION

Phytochemical Tests: The extract and fractions were found to be positive for the presence of saponins, tannins, flavonoids, phenols, alkaloids and glycosides but volatile oils, steroid and balsam (gum) were absent.

Antimicrobial Activities: Well diffusion (Table I) and microdilution technique were used to evaluate the antimicrobial properties of crude extracts & fractions of *Glycyrrhiza glabra*. The resulting MIC values were found to be ranging from 0.2 to 1.2 mg/mL for bacteria and 0.8 to 200mg/mL for fungi. *Glycyrrhiza glabra* extracts (Chloroform fraction, Ethyl acetate fraction and crude) showed the lowest MIC values (Table) and thus they could be considered as a source of interesting antimicrobial compounds.

Antibacterial Activity: Ethanolic extract of *Glycyrrhiza glabra* (Cr), chloroform fraction of root (Cl) and ethylacetate fraction (E) exhibited the lowest MIC values and inhibit the bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherchia coli* (Table I and III). Ethyl acetate was the most active extract (MIC = 0.41, 0.29, 0.59, 0.61, 0.96 and 0.92 mg/mL for *S. aureus*, *L. monocytogenes*, *E.coli*, *S. typhi*, *V. parahemolyticus* and *P. aeruginosa* respectively). *S. aureus*, *L. monocytogenes* and *E.coli* were the most susceptible bacteria. With Low MIS values The water soluble fraction does not show any effect against all the strains.

Antifungal Activity: *Glycyrrhiza glabra* extracts and their fractions exhibited the most interesting inhibitory activities against *C. albicans* and *T.rubrum*. Among the fractions hexane and chloroform fractions of ethanolic extract did not show any effect against all the strains (Table II). Crude extracts and their fractions showed zone of inhibition in mm against these strains. The species of *T. rubrum* is strongly inhibited with MIC ranging from 0.8 mg/mL to 100mg/mL, followed by the *C. albicans* with MIC ranging from 1.6 mg/mL to 200mg/mL (Table IV). The water soluble fraction does not show any effect against all the strains.

Discussion: The ethanolic root extract along with their fractions was found to be active on the eight pathogens studied. This confirmed its traditional use for infectious diseases of skin. The MIC values of the extracts observed against the sensitive strains ranged from 0.2 to 1.2 mg/mL (for bacterial strains) and 0.8 to 200 mg/mL (for fungal strains). It was observed that the antimicrobial activity was gradually increasing as we moved from the crude extract to fractions in some cases. This was revealed with both well diffusion and microdilution techniques. However, in case of bacterial strains, crude and ethyl acetate fractions showed potent activity against the *P. aeruginosa* having MICs 0.31

Table I: Antibacterial activity at 5mg /ml (Zone of inhibition in mm)* of the extracts and their fractions of *Glycyrrhiza glabra*

	S. aureus	*L. mono*	*E. coli*	*S. typhi*	*V. para*	*P. aeru*
Std	17	20	25.6	22	23	16.5
Cr	9	11	8	7	6	14
H	10	--	--	8	--	--
Cl	8	11	–	9	--	--
E	18	17	20	17.5	16	14
B	15	12	10	10	9	10
W	--	--	--	--	--	--

* -- no activity, Std: Gentamicin, 7–9mm non significant, 17–18mm (or above) significant, Cr.crude fr., H.hexane fr., Cl.chloroform fr., E.ethyl acetate fr., B.butanol fr.,W.water fr.

Table II: Antifungal activity at 200mg/ml (Zone of inhibition in mm)* of the extracts and their fractions of *Glycyrrhiza glabra*

	C. albicans	*T. rubrum*
Std	28	23
Cr	22	15
H	--	--
Cl	--	--
E	19	13
B	12	10
W	--	--

* -- no activity, Std: clotrimazole, Cr.crude fr., H.hexane fr., Cl.chloroform fr., E.ethyl acetate fr., B.butanol fr.,W.water fr.

Table III: Antibacterial activity (MIC values in mg/mL) of the extracts and their fractions of *Glycyrrhiza glabra*.

	S. aureus	*L. Mono*	*E. Coli*	*S. Typhi*	*V. Para*	*P. aeru*
Std	0.03	0.15	0.2	0.14	0.18	0.21
Cr	0.59	0.53	0.66	0.83	0.98	0.31
H	0.66	--	--	0.89	--	--
Cl	0.23	0.71	--	0.44	--	--
E	0.41	0.29	0.59	0.61	0.96	0.92
B	0.87	0.63	0.92	0.82	1.1	1.15
W	--	--	--	--	--	--

* -- no activity, Std: Gentamicin, Cr.crude fr., H.hexane fr., Cl.chloroform fr., E.ethyl acetate fr., B.butanol fr., W.water fr.

Table IV: Antifungal activity (MIC values in mg/mL) of the extracts and their fractions of *Glycyrrhiza glabra*.

	C. albicans	*T. rubrum*
Std	2.5	1.98
Cr	1.61	1.82
H	--	--
Cl	--	--
E	>200	0.82
B	>200	100
W	--	--

* -- no activity, Std: clotrimazole, Cr.crude fr., H.hexane fr., Cl.chloroform fr., E.ethyl acetate fr., B.butanol fr.,W.water fr.

and 0.92 mg/mL. Crude, ethyl acetate and n-BuOH fractions exhibited significant activity against the *Escherchia coli* having MICs 0.66, 0.59, and 0.92 mg/mL respectively. Similarly crude, ethyl acetate and n-BuOH fractions showed significant activity against the *Staphylococcus aureus* having MICs of 0.59, 0.41 and 0.87 mg/mL respectively. This antimicrobial activity can be due to alkaloid, saponins, flavonoids, tannin, glycosides and phenols found in crude extract and fractions. These phytochemical groups are known to posses' antimicrobial compounds [11-12]. Further purification and characterization of the active principles from the fractions crude, ethyl acetate and BuOH (for antibacterial and antifungal studies) will provide a better understanding of the antimicrobial mechanism and serves as a tool for potential lead compounds for microbial infectious diseases [13-14].

REFERENCES

1 Chopra, R.N., S.L. Nayar and I.C. Chopra, 2002. Glossary of Indian Medicinal Plants. New Delhi: NISCAIR, CSIR.

2. Rackova, L., V. Jancinova, M. Petríkova, K. Drabikova, R. Nosal, M. Stefek, D. Kostalova, N. Pronayova and M. Kovacova, 2007. Mechanism of anti-inflammatory action of liquorice extract and glycyrrhizin. Natural products Research, 14: 1234 - 41.

3. Gupta, V.K., A. Fatima, U. Faridi, A.S. Negi, K. Shanker, J.K. Kumar, N. Rahuja, S. Luqman, B.S. Sisodia, D. Saikia, M.P. Darokar and S.P. Khanuja, 2008. Antimicrobial potential of *Glycyrrhiza glabra* roots. Journal of Ethnopharmacology, 116: 377 - 80.

4. Lu, Y., Z. Wang, D. Wei and H. Xiang, 2007. Mechanism and inhibitory effect of galangin and its flavonoid mixture from *Alpinia officinarum* on mushroom tyrosinase and B16 murine melanoma cells. Journal of Enzyme Inhibition and Medicinal Chemistry, 22: 433-438.

5. Zhao, W., C. Gao, Y. Zhang and W. Tian, 2007. Evaluation of the inhibitory activities of aceraceous plants on fatty acid synthase. Journal of Enzyme Inhibition and Medicinal Chemistry, 22: 501- 510.

6. Atta-ur-Rahman., Khalid, A., Sultana, N., Ghayur, M.N., Mesaik, M.A., Khan, M.R., A.H. Gilani and M.I. Choudhary, 2006. New natural cholinesterase inhibiting and calcium channel blocking quinoline alkaloids. Journal of Enzyme Inhibition and Medicinal Chemistry, 21: 703-710.

7. Lodhi, M.A., J. Hussain, M.A. Abbasi, A.R. Jassbi, M.I. Choudhary and V.U. Ahmad, 2006. A New Bacillus pasteurii urease inhibitor from Euphorbia decipiens. Journal of Enzyme Inhibition and Medicinal Chemistry, 21: 531-535.

8. Berghe V.A. and A.J. Vlietinck, 1991. Screening methods for antibacterial and antiviral agents from higher plants. Meth Plant Biochem, 6: 47-68.

9. Tereschuck, M.L., M.V.Q. Riera, G.R. Castro and L.R. Abdala, 1997. Antimicrobial activity of flavonoid from leaves of Tagetes minuta. Journal of Ethnopharmacology, 56: 227-232.

10. Harborne, J.B., 1991. Phytochemical methods. 2nd ed. London:Chapman and Hall.

11. Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry, 30: 3875-3883.

12. Field J.A. and G. Lettinga, 1992. Toxicity of tannic compounds to microorganisms. Plants Polyphenols: Synthesis, Properties, Significance. Basic Life Science, 59: 673-692.

13. Bruneton, J., 1999. Pharmacognosie: Phytochimie, plantes M'edicinales, 3eme. Tec & Doc., Paris. pp: 1120.

14. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clini Microbiol Rev., 12: 564-582.