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ANTIMICROBIAL ACTIVITY OF DIFFREENT EXTRACTS OF GLYCYRRHIZ GLABRA

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Abstract
The present study was done to analyze the antimicrobial activity of different extracts of *Glycyrrhiza glabra.* Medicinal plants serve as potent antimicrobial agents. Here, the ethanolic, methanolic, choloroform, ethyl acetate and aqueous extracts of *Glycyrrhiza glabra* were examined for their antibacterial activity against gram positive and gram negative bacterial strains using disk diffusion technique. Further, Minimum inhibitory concentrations (MICs) of the extracts were also determined using microbroth dilution test. It was observed that all the extracts of *Glycyrrhiza glabra* possess high antimicrobial potential against maximum test bacteria used. Maximum zone of inhibition was observed for chloroform extract of *Glycyrrhiza glabra* against *Enterococcus faecalis* ATCC 29212 (29 mm) with MIC 10.4 µg/ml and MLC 41.7 µg/ml among gram positive bacteria. Whereas, among gram negative bacteria maximum zone of inhibition was observed in case of methanolic extract against *Escherichia coli* ATCC 11840 (20 mm) with MIC 20.8 µg/ml and MLC 83.3 µg/ml.

Keywords: - Glycyrrhiza glabra , antimicrobial

Introduction
India is known for its biodiversity and knowledge on plant based drugs used for prevention of diseases as well as a curative medicines. The study of medicinal plants is important to promote the proper uses of herbal medicines and to determine their potential as a source of new drugs [Arora, 1997; Singh & Pandey, 1998; Shetty & Singh, 1993]. There is an increase in infections cases within recent years due to which antibiotic resistance is becoming a major therapeutic problem [Austin, 1999]. Infectious diseases are the second leading cause of death worldwide [Fazly-Bazzaz et al., 2005]. In recent years, multiple drug resistance has developed due to extensive use of existing antimicrobial drugs in treatment of infectious diseases [Service, 2000]. Use of antibiotics is also associated with side effects on the hosts like hypersensitivity. One of the way by which this emerging problem can be minimized is by using natural products of higher plants as a new source of antimicrobial agents with possibly novel mechanism of action [Barbour et al., 2004]. Therefore, researches on development of plant based alternative antimicrobial drug, with novel mechanism of action for the treatment of infectious disease are extensively going on. The storage organs of higher plants contains bioactive compounds like alkaloids, flavanoids and phenolic compounds [Buwa & Staden, 2006] which show great biological activities.

The awareness about the medicinal value of plants has increased to a great dimension in the past few decades due to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse variety of secondary metabolites which possesses antimicrobial potential and can be used as antibiotics [Dimayuga and Garcia, 1991]. In recent years numbers of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants [Gundidza and Gaza, 1993; Perumalsamy and Ignacimuthu, 2000]. A variety of compounds are accumulated in plants accounting for their constitutive antimicrobial activities [Callow, 1983].

Glycyrrhiza glabra, also known as licorice and sweet wood, is native to the Mediterranean and certain areas of Asia. Historically, the dried rhizome and root of this plant were employed medicinally by the Egyptian,

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Chinese, Greek, Indian, and Roman civilizations as an expectorant and carminative. In modern medicine, licorice extracts are often used as a flavoring agent to mask bitter taste in preparations, and as an expectorant in cough and cold preparations. It is used as Tonic, demulcent, expectorant, diuretic, mild laxative, anti-arthritic, antiinflammatory, anti-biotic, anti-viral, anti-ulcer, memory stimulant (being MAO inhibitor), anti-tussive, aphrodisiac, anti-mytotic, estrogenic, anti-oxidant, anti-caries agent, anti-neoplastic, anti-cholinergic, anti-diuretic, hypolipidemic activity, etc [Dhuke, etal., 2002; Kokate, et al., 2005; Vaidhyarataman, 1995; Kirtikar and Basu, 1987].

This study describes the antimicrobial effect of different extracts of *Glycyrrhiza glabra* against microorganisms.

2. Materials and Methods

2.1 Plant material

Dried spice of *Glycyrrhiza glabra* was collected from Patanjali Herbal Nursery, Haridwar, Uttaranchal, India.

2.2 Bacterial strains

Standard strains were procured from American Type Culture Collection (ATCC) and were maintained as per directions. Strains used were *Escherichia coli* ATCC11840, *Escherichia coli* ATCC 12632, *Pseudomonas flourescens* ATCC 13525, *Klebsiella pneumoniae* ATCC 9621, *Klebsiella aerogenes* ATCC 31488, *Proteus mirabilis* ATCC 29245, *Streptococcus lactis* ATCC 8043, *Staphylococcus aureus* ATCC 12600, *Bacillus cereus* ATCC 12826, *Enterococcus faecalis* ATCC 29212, *Aspergillus niger* ATCC 26603 and *Rhizopus oryzae* ATCC 8993

2.3 Culture preparation

A loopful of 24 h surface growth on a NA slope of each bacterial strain was transferred individually to 5 ml of luria burnetii broth (pH 7, Difco). After incubation at 37°C for 24 h, bacterial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match that of a 5 McFarland standard (10^8 CFU/ml). Then, a 1:10 dilution of the cell suspension was performed to give an inoculum concentration of 10^7 CFU/ml.

2.4 Extractions of spices

The air-dried material was ground into fine powder and Soxhelet extracted using methanol, ethanol, ethyl acetate and chloroform (Merck). Aqueous extract of *Glycyrrhiza glabra* was also prepared. Solvent was removed from filtered extract under reduced pressure. The resulting extract was kept in a dark and cold place in sterile vials for further test.

2.5 Antimicrobial assay

Antibacterial activity of the extracts was investigated against 10 bacterial strains by the agar well diffusion. For agar well diffusion method, wells were prepared in the plates of Mueller-Hinton agar medium (Merck), previously inoculated with a bacterial suspension (0.5 McFarland ca. 10^8 CFU/ml), with the help of a cork-borer (0.85 cm). 25 µl, 50 µl, 75 µl and 100 µl of the test compound were introduced into separate wells along with controls. The plates were incubated overnight at 35 ± 1ºC for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. Antibiotic disks were used as positive control and DMSO containing disk was used as negative controls instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented. MIC and MLC of the extracts were also evaluated against test microorganisms.

Results and Discussions:

The *in vitro* antimicrobial activity, minimum inhibitory concentration and minimum lethal concentration of different extracts of *Glycyrrhiza glabra* are shown in table 1 to table 3.

The results of this study showed that the aqueous extract of *Glycyrrhiza glabra* was not efficient antibacterial agent and thus did not showed inhibition against any of the test microorganisms (Table 1). *Klebsiella pneumoniae* ATCC 31488 and *Klebsiella aerogenes* ATCC 9621 are not sensitive towards any extract of *Glycyrrhiza glabra* [Irani et al., 2010]. Methanolic extract of *Glycyrrhiza glabra* showed excellent antibacterial potential, inhibiting in the order of *S. aureus* ATCC 12600 > *Escherichia coli* ATCC 11840 > *Enterococus faecalis* ATCC 29212 > *Proteus mirabilis* ATCC29245 > *Streptococcus lactis* ATCC 8043 > *Pseudomonas flourescens* ATCC 13525 > *Bacillus cereus* ATCC 12826 (Fig 1). Evidences from study performed by Sultana et al., 2010 also shows highest antimicrobial activity of the methanolic extract of *Glycyrrhiza glabra* against *Staphylococcus aureus.* They also showed that *G. glabra* exhibited antimicrobial activity against *Escherichia coli, Bacillus subtilis* and *Salmonella sp.* Table 2 and Table 3 shows MIC and MLC values of different extracts of *Glycyrrhiza glabra* against test microorganisms.

In comparison to methanolic extract the ethanolic extract of *Glycyrrhiza glabra* was not so much sensitive against microorganisms. Fig 2 and Fig 3 shows the comparison of MIC and MLC of various extracts of *G. glabra* against test organisms.

Table 1. Antimicrobial activity of *Glycyrrhiza glabra* showing Zone of Inhibition against microorganisms

S.No	Solvent	Conc (µl)	*Escherichia coli* ATCC 11840	*Escherichia coli* ATCC 12632	*Pseudomonas flourescens* ATCC 13525	*Klebsiella pneumoniae* ATCC 31488	*Klebsiella aerogenes* ATCC 9621	*Proteus mirabilis* ATCC 29245	*Streptococcus lactis* ATCC 8043	*Staphylococcus aureus* ATCC 12600	*Bacillus cereus* ATCC 12826	*Enterococcus faecalis* ATCC 29212
1.	Aqueous	25	-	-	-	-	-	-	-	-	-	-
		50	-	-	-	-	-	-	-	-	-	-
		75	-	-	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-	-	-
2.	Methanol	25	12	5	8	-	-	9	5	11	10	11
		50	13	9	9	-	-	12	9	14	12	15
		75	17	11	11	-	-	15	13	19	14	17
		100	20	15	14	-	-	16	15	21	14	19
3.	Ethanol	25	9	11	-	-	-	10	12	6	-	-
		50	13	15	-	-	-	14	16	9	-	-
		75	14	17	-	-	-	16	19	12	-	-
		100	14	19	-	-	-	18	23	14	-	-
4.	Chloroform	25	10	9	-	-	-	-	11	8	7	20
		50	15	12	-	-	-	-	13	13	9	22
		75	18	15	-	-	-	-	15	15	11	25
		100	19	19	-	-	-	-	17	19	14	29
5.	Ethyl acetate	25	11	13	8	-	-	9	10	11	-	13
		50	12	15	10	-	-	11	14	13	-	17
		75	15	17	12	-	-	16	15	14	-	20
		100	17	19	14	-	-	17	17	19	-	22

Table 2. Minimum Inhibitory Concentration of different extracts of *Glycyrrhiza glabra* against microorganisms

S.No.	Bacterial Strain	Minimum Inhibitory Concenration (µg/ml)				
		Aqueous	Methanolic	Ethanolic	Chloroform	Ethyl acetate
1.	*Escherichia coli* ATCC11840	NA	20.8	41.7	20.8	20.8
2.	*Escherichia coli* ATCC 12632	NA	41.7	41.7	20.8	20.8
3.	*Pseudomonas flourescens* ATCC 13525	NA	83.3	NA	NA	83.3
4.	*Klebsiella pneumoniae* ATCC 9621	NA	NA	NA	NA	NA
5.	*Klebsiella aerogenes* ATCC 31488	NA	NA	NA	NA	NA
6.	*Proteus mirabilis* ATCC 29245	NA	83.3	41.7	NA	41.7
7.	*Streptococcus lactis* ATCC 8043	NA	83.3	20.8	41.7	41.7
8.	*Staphylococcus aureus* ATCC 12600	NA	10.4	41.7	20.8	20.8
9.	*Bacillus cereus* ATCC 12826	NA	41.7	NA	41.7	NA
10.	*Enterococcus faecalis* ATCC 29212	NA	41.7	NA	10.4	41.7

Table 3. Minimum Lethal Concentration of different extracts of *Glycyrrhiza glabra* against microorganisms

S.No.	Bacterial Strain	Minimum Lethal Concentration (µg/ml)				
		Aqueous	Methanolic	Ethanolic	Chloroform	Ethyl acetate
1.	*Escherichia coli* ATCC11840	NA	83.3	83.3	83.3	83.3
2.	*Escherichia coli* ATCC 12632	NA	83.3	83.3	83.3	83.3
3.	*Pseudomonas flourescens* ATCC 13525	NA	83.3	NA	NA	83.3
4.	*Klebsiella pneumoniae* ATCC 9621	NA	NA	NA	NA	NA
5.	*Klebsiella aerogenes* ATCC 31488	NA	NA	NA	NA	NA
6.	*Proteus mirabilis* ATCC 29245	NA	166.7	166.7	NA	166.7
7.	*Streptococcus lactis* ATCC 8043	NA	83.3	83.3	166.7	83.3
8.	*Staphylococcus aureus* ATCC 12600	NA	83.3	166.7	83.3	83.3
9.	*Bacillus cereus* ATCC 12826	NA	>166.7	NA	41.7	166.7
10.	*Enterococcus faecalis* ATCC 29212	NA	83.3	NA	41.7	41.7

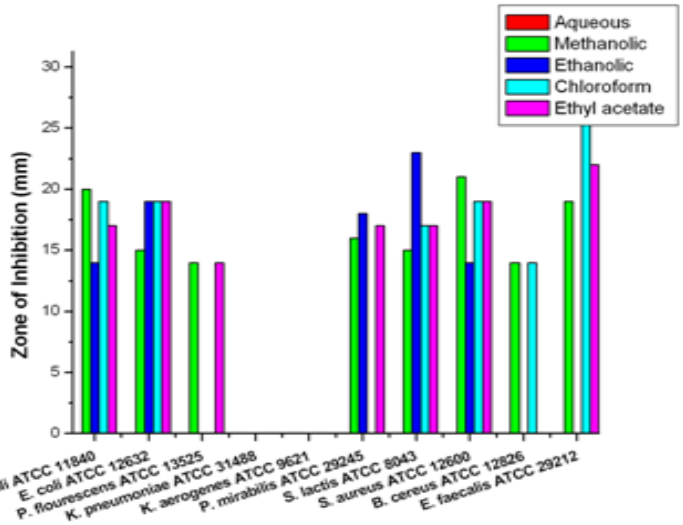

Fig 1. Comparison of Zone of Inhibition of different extracts of *Glycyrrhiza glabra* against microorganisms.

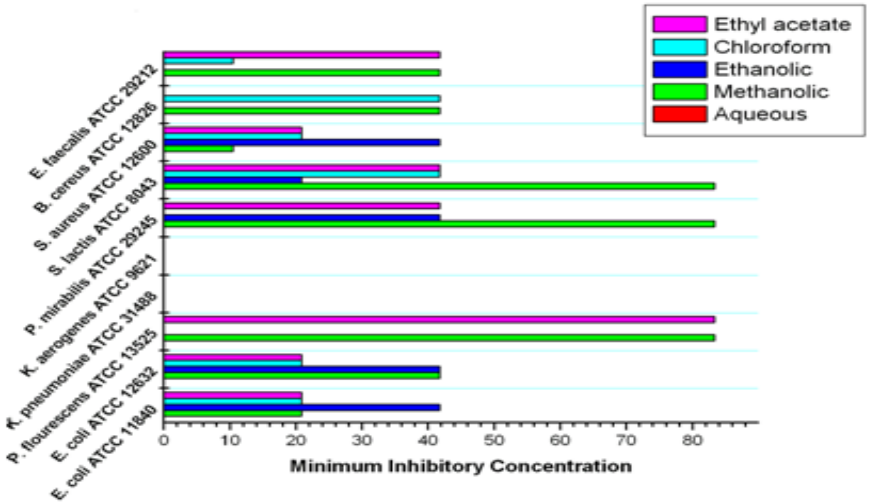

Fig 2. Comparison of Minimum Inhibitory Concentration of different extracts of *Glycyrrhiza glabra* against microorganisms

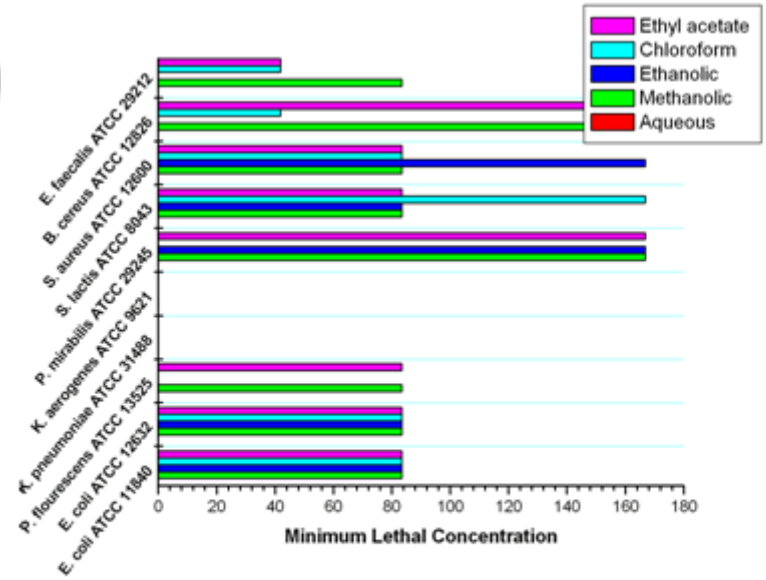

Fig 3. Comparison of Minimum Lethal Concentration of different extracts of *Glycyrrhiza glabra* against microorganisms

In comparison to methanolic extract the ethanolic extract of *Glycyrrhiza glabra* was not so much sensitive against microorganisms. Fig 2 and Fig 3 shows the comparison of MIC and MLC of various extracts of *G. glabra* against test organisms. It showed highest inhibition for *Streptococcus lactis* ATCC 8043. Inhibition against *Escherichia coli* ATCC 11840, *E. coli* ATCC 12632, *Proteus mirabilis* ATCC29245 and *Staphylococcus aureus* ATCC 12600 was also observed [Patil et al., 2009]. *Pseudomonas flourescens* ATCC 13525, *Bacillus cereus* ATCC 12826 and *Enterococcus faecalis* ATCC 29212 were found to be resistant for this extract [Ates et al., 2003].

The growth of *Enterococcus faecalis* ATCC 29212 was highly suppressed by the chloroform extract of *Glycyrrhiza glabra.* Microbes isolated from food samples also showed good inhibition against this extract. No inhibition was found for *Pseudomonas flourescens* ATCC 13525 and *Proteus mirabilis* ATCC 29245. Ates et al., 2003 also confirms our study. Studies performed by Nitalikar et al., 2010 also showed the antimicrobial potential of chloroform extract of *Glycyrrhiza glabra* against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli.* Our findings also support this result.

Glycyrrhiza glabra had an excellent antimicrobial potential in its ethyl acetate extract form against standard microorganisms. It inhibited all the microorganisms except *Bacillus cereus* ATCC 12826, *Klebsiella pneumoniae* ATCC 31488 and *Klebsiella aerogenes* ATCC 9621. Ates et al., also showed the resistance of *Bacillus subtilis* for chloroform extract of *Glycyrrhiza glabra.* Our study supports the results of the study performed by Patil et al., 2009 that the ethyl acetate extract of *Glycyrrhiza glabra* inhibits the growth of *S. aureus, Pseudomonas aeruginosa* and *E. coli.*

Conclusion

The above results obtained confirm the preservative potency of *Glycyrrhiza glabra* and suggests its use as antimicrobial agent In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation.

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