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Glycyrrhizin glabra as a bioenhancer for antibiotics

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ABSTRACT

Over usage of drugs in therapeutic treatments lead to the development of multiple drug resistance.So recently there has been a shift in global trend from Synthetic to Natural. Phytochemicals as medicines are esteemed all over the world as rich source of therapeutic agents. Combination of Glycyrrhizin glabra with commercial antibiotics exhibited promising bioenhancing activity by *in-vitro* Bioassay methods. Enhancement in antimicrobial activity of antibiotics like Ciprofloxacin, Gettifloxacin, Ofloxacin, Erythromycin (Antibacterial) and Flucanazole (Antifungal) was obtained. Butanol extract of glycyrrhizin glabra was enhancing antibacterial antibiotics and water extract, antifungal antibiotic. 10% Butanol extract of glabra enhanced antibacterial activity of Ciprofloxacin by 4mm and Gettfloxacin by 7mm against *Proteus vulgaris*. Incase of Ofloxacin enhancement was observed in all bacteria like *Bacillus subtilis* (2mm), *Proteus vulgaris* (3mm) *Staphylococcus aureus* (1mm) *Pseudomonas fluorescence* (7mm). Erythromycin showed enhancement against *Staphylococcus aureus* (5mm) and *Proteus vulgaris* (2mm) while for *Pseudomonas fluorescence*, only combination exhibited zone of inhibition of 7mm but not in antibiotic alone. Water extract of glabra showed enhanced antifungal activity in *C.lipolytica* (4mm) and *C.neoformens* (5mm), while for *C.albicans*butanol extract showed maximum enhancement of 11mm in Flucanazole antibiotic.

KEYWORDS

Bioenhancer;
Herbal extract;
Antibiotics;
Antimicrobial activity.

INTRODUCTION

India has an extensive rich heritage of herbal medicines since from the time of Ayurveda, this emphasizes on potential role of plants with medicinal properties[3]. For a long period of time plants have been a valuable source of natural producers for maintaining human health. Medicinal plants would be the best source to obtain a variety of drugs. In the last few years a number of studies have been conducted to prove efficiency of plant extracts with known antimicrobial properties[8,10,11,15]. Medicinal herbs have been known from millennium and are esteemed all over the world as a rich source of therapeutic agent for prevention of diseases and ailments (cow urine manual, 2002). Glycyrrhizin glabra is an ancient herb with active constituents

like Liquritin, isoliquritin, glycirrhizin and glycirrhizic acid. Commonly known as Atimadhura or Yestimadhu, used as a remedy for cough, to decrease allergic reactions and also to protect liver and other vital organs being damaged by oxidants[13].

Antibiotics are the drugs that kill or prevent the growth of microorganisms. Indiscriminate use of these lead to consequences like emergence of multiple drug resistance in microbes and side effects in consumers[1,2,6,9] So there is an intense need of methods which reduce dosage of antibiotics to minimize drug resistance, also enhance the activity of antibiotics. Hence present study was undertaken to evaluate enhanced bioactivity of antibiotics when combined with crude plant extracts[4,9,16,17].

MATERIALS AND METHODS

Microbial strains for antimicrobial activity were obtained from National collection of microorganisms industrially important (NCIM, Pune, India) Bacterial strains, *Bacillus subtilis NCIM-2010 and Staphyloccusaureus NCIM-2492*. Gram negative bacteria, *Pseudomonas aeruginosa NCIM-2200, and Proteus vulgaris NCIM-2027*.

Yeast strains, *Candida albicans NCIM-3100, Candida lipolytica NCIM-347, and Cryptococcus neoformens NCIM-3541*

Commercially available dried rhizoid part of stem of glabra was obtained, powdered and 10% concentration was prepared both in sterile water and Butanol solvent[5,14], along with 1% concentration of Antibacterial and antifungal antibiotics.

Bioassay

a) Antibacterial activity

In-vitro antibacterial activity of glabra extract, antibiotics, and combination of glabra extract with antibiotics was tested by Well-in-Agar method (Dhingra, 1995). The inoculum suspension was spread uniformly over the agar medium to get uniform lawn of bacteria. Using a flamed cork borer Wells of 9mm diameter were made at equal distance in the Petri plates. 100µl of plant extract was aseptically filled into the Wells. Similar procedure was followed for antibiotics and combination of glabra extract +antibiotic. All these plates are incubated at 37^{0}C for 24 hours. Bioenhancing effect was assessed by measuring the diameter of inhibition zone in comparison with control plates.

b) Antifungal activity

Broth cultures of *C.lipolytica, C, albicans*and *C.neoformens* are swab inoculated on the surface of three sets of plates with SDA and MGYP medium. About 100 µl each of plant extract, antibiotics and combination of plant extract+antibiotics was aseptically filled in wells of all plates. Plates are incubated at 30^{0}C for 24-48 hrs.

RESULTS

Bioenhancing activity was seen in both the extracts of glabra. Maximum zone was in butanol extract compared to water extract. Butanol extract is more effective than water extract (TABLE 1).

Butanol extract of glabra enhances the antibacterial activity of Ciprofloxacin by 1mm in *P.flourescence*&B.subtilis, 4mm in *P.vulgaris*&3mm in *S.aureus* (TABLE 1& Figure 1). Incase of gettifloxacin enhancement was more in *P.vulgaris* (7mm), no enhancement in *B.subtilis* and *S.aureus*. For *P.fluorescence* zone of inhibition remained same (TABLE 1& ure 2). But extract inhibited the pigment formation in *Pseudomonas.* Enhancing activity was observed in Oflaxacin antibiotic against tested bacteria. Significant enhancement was in *P.fluorescence* (7mm), 1mm in *S.aureus* and 2mm in*B.subtilis* and *P.vulgaris* (TABLE1 & Figure 3). About 5mm enhancement in*S.aureus*, 2mm in *P.vulgaris*for Erythromycin antibiotic, and no enhancement in *B.subtilis*.Incase of *P.fluorescence* antibiotic alone was ineffective but the combination showed inhibition of 7mm (TABLE 1& Figure 4).

Enhancement of 4mm for *C.lipolytica* was observed in water extract where as butanol extract showed minimum inhibition (TABLE 2 & Figure 6). *In C.neoformens*butanolextrat showed 5mm enhancement (TABLE 2 & Figure 7). For *C.albicans* water extract was in-effective while butanol extract showed 8 mm enhancement (TABLE 2 Figure 8).

DISCUSSION

Bioenhancing potential of chemicals like silver

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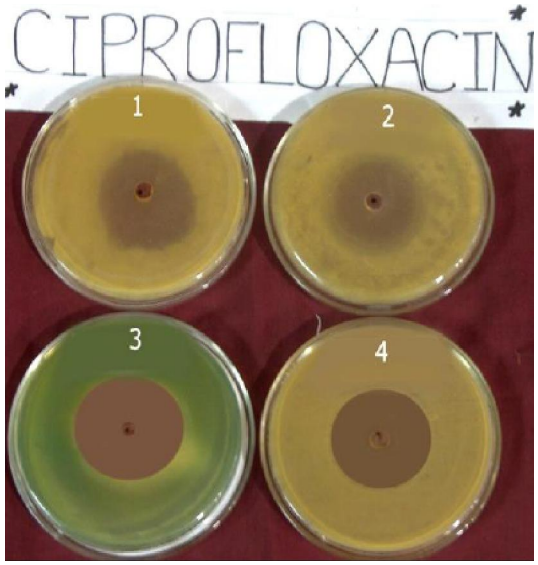
TABLE 1 : Bioenhancing efficacy of Antibacterial antibiotics with glabra extract. (FOR BACTERIA)

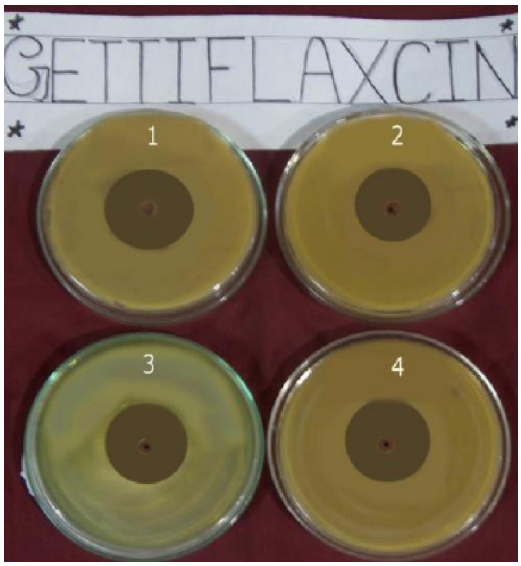
Name of Organism	Butanol extract	Zone of inhibition in mm							
		Ciprofloxacin		Gettiflaxcin		Oflaxcin		Erythromycin	
		Ab	Ab +BE	Ab	Ab+ BE	Ab	Ab + BE	Ab	Ab + BE
P. fluorescens	12	24	25	20	20	13	20	No Zone	7
P. vulgaris	11	12	16	15	20	12	15	9	11
B. subtilis	15	17	18	22	14	11	13	15	15
S.aureus	6	20	23	20	16	15	16	7	12

Ab – Antibiotic; BE-Butanolextract



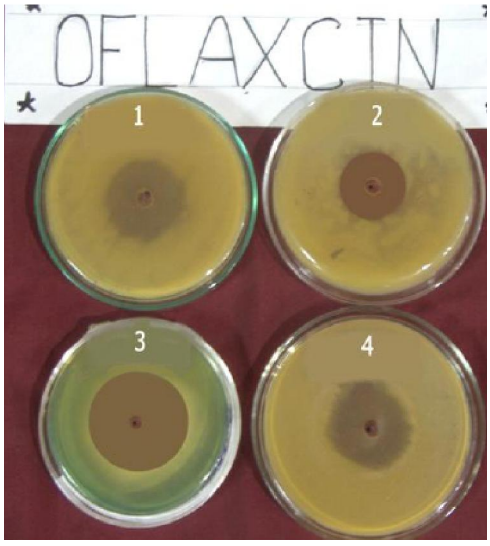
1. Staphylococcus aureus; 2. Bacillus subtilis; 3 Pseudomonas fluorescens; 4. Proteus vulgaris

Figure 1 : Bioenhacing activity of Ciprofloxacin antibiotic



1. Staphylococcus aureus; 2. Bacillus subtilis; 3 Pseudomonas fluorescens; 4. Proteus vulgaris

Figure 2 : Bioenhacing activity of Gettiflaxacin antibiotic



1. Staphylococcus aureus; 2. Bacillus subtilis; 3 Pseudomonas fluorescens; 4. Proteus vulgaris

Figure 3 : Bioenhacing activity of Ofloxacin antibiotic

1. Staphylococcus aureus; 2. Bacillus subtilis; 3 Pseudomonas fluorescens; 4. Proteus vulgaris

Figure 4 : Bioenhacing activity of Erythromycin antibiotic

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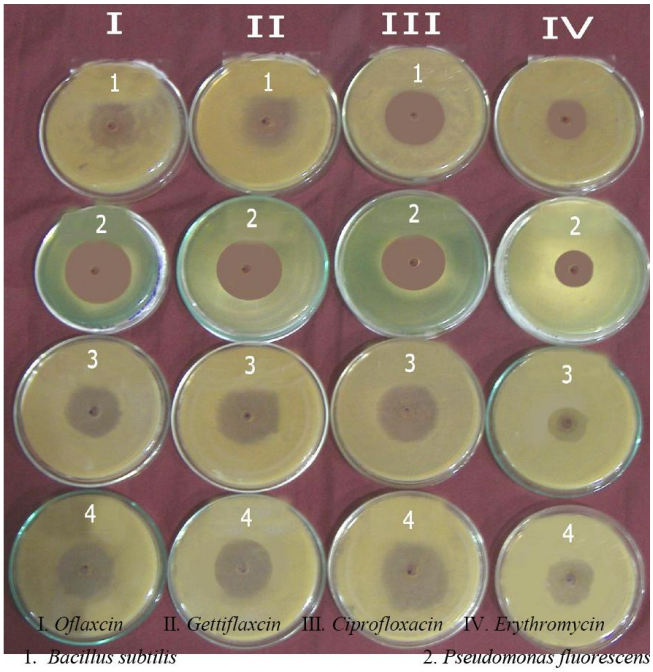
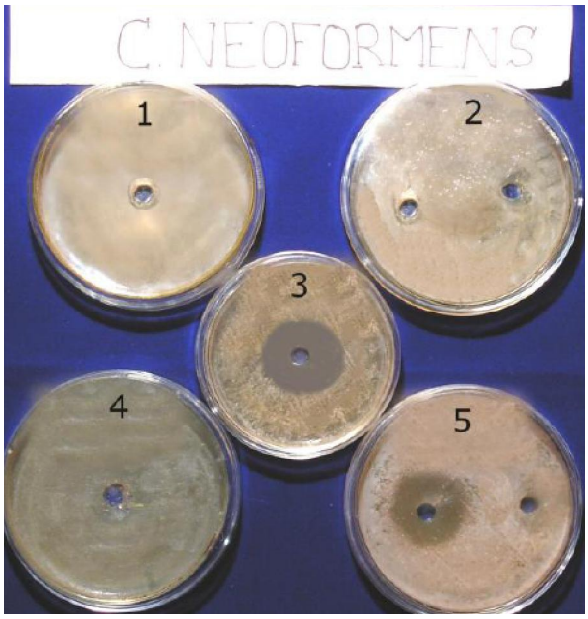


Figure 5: Comparison of bioenhancing activity of antibiotics



1. Water extract; 2. Control; 3. Butanol + Ab; 4. Butanol extract5. Flucanozole

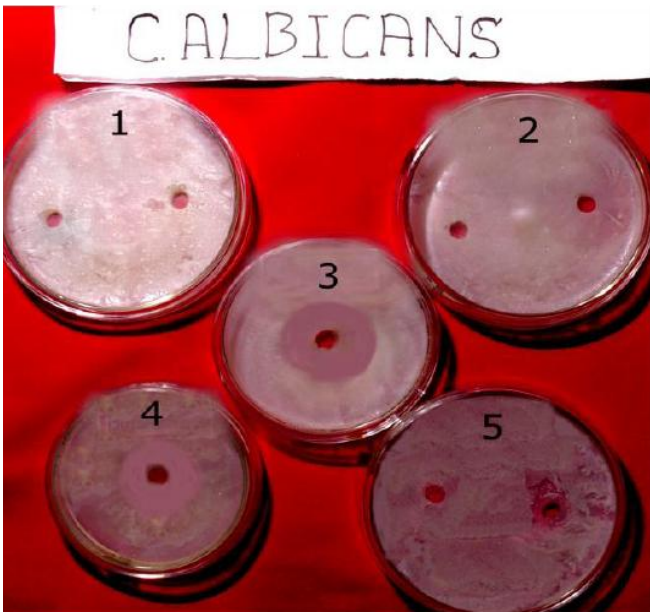
Figure 7

TABLE 2 : Bioenhancing efficacy of Antifungal antibiotic with glabra extract (FOR FUNGI).

Name of organism	Zone of inhibition in mm		
	Flucanozole antibiotic	Flucanozole + water extract of glabra	Flucanozole + Butanol extract of glabra
Candida lypolytica	11	15	-
Cryptococcus neoformans	10	-	15
Candia albicans	7	-	15

1. Water extract; 2. Control; 3. Water + Antibiotic; 4. Butanolextract; 5. Flucanozole

Figure 6



1. Water extract; 2. Control; 3.Butanol + Ab; 4. Butanolextract; 5. Flucanozole

Figure 8

nirate, showed enhancing activity when combined with 9 antibiotics against 7 microbial strains[4]. Bio-enhancing property of natural products was studied by using cow urine which showed bioenhancing potential against anticancer and antituberculosis drugs[16].

Influenced by this work where natural product was showing enhanced bactericidal potential, present study was concentrated on herbal extracts as they are having ancient history as green medicines and excellent therapeutic agents. Here crude extract itself was effective when combined with antibiotics minimizing the risks associated usage of antibiotics alone in large doses. The purification and characterization of active components of glabra and its combination with antibiotics in different ratios can gave out the best optimum concentration of glabra as effective Bioenhancer.Butanol extract of glabra showed maximum activity. So extraction of glabra in different solvents may also give fruitful results.

To overcome the difficulties associated with resistance in pathogens by the long term usage of antibiotics,natural products like herbal extracts indicates towards "come back to nature" where nature itself providing the remedy to overcome the hurdles created by man made environment.

CONCLUSION

The present work was a Preliminary screening test for bioenhancing potential of Glycyrrhizin glabra.

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