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INHIBITORY EFFECT OF NATURAL HERBAL EXTRACTS ON SYSTEMIC BACTERIA

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ABSTRACT

For centuries natural herbs are used as traditional remedies to treat a number of systemic infections. The inhibitory effect of three herbs: *Glycyrrhiza glabra* (liquorice, local name: Mulethi), *Camellia sinensis* (Green tea) and *Piper betel* (Betel leaves, local name: Paan) was checked in aqueous extracts against fifty clinical isolates mainly collected from patients suffering from respiratory tract and gastrointestinal tract infections. Extracts were prepared in the form of decoctions and infusions and then applied on test organisms by Well Diffusion Assay in single as well as mixed preparations showing a combined effect of all herbs. All the herbs showed a significant antibacterial potential which was increased in combinations (cocktails) against most of the isolates, however, maximum activity against respiratory tract bacteria was observed in case of *Acinetobacter* sp., followed by *Pseudomonas* sp., *Klebsiella* sp., *Streptococcus pyogenes*, *Corynebacterium* sp., and *Micrococcus* sp., respectively. Among gastrointestinal isolates maximum activity was found against *Shigella dysenteriae*, *Enterobacter* sp., *Escherichia coli* and *Proteus* sp. Hence, the aqueous extracts (both decoctions and infusions) can be fairly utilized to treat respiratory and gastrointestinal tract infections.

KEYWORDS: *Camellia sinensis*, *Piper betel*, *Glycyrrhiza glabra*, Antibacterial activity, Systemic bacteria.

INTRODUCTION

The use of plants and herbs has always been a tradition to treat a number of infections all over the world. Among a large number of medicinal plants, *Glycyrrhiza glabra* (liquorice, local name: Mulethi), *Camellia sinensis* (Green tea) and *Piper betel* (Betel leaves, local name: Paan) are widely used as traditional remedies against various infections.

Glycyrrhiza grows as perennial herbs or sub-shrubs growing to a height of 2 m with horizontal underground stems. It is native to the Mediterrananean and near East; distributed in the sub-tropical and warm temperature regions (Patil *et al*., 2009). Glycyrrhizin is the main biologically active compound of the liquorice root, having a sweet and refreshing taste. Medicinal use of *Glycyrrhiza* includes cough suppression (Andreson & Smith, 1961), gastric ulcer treatment (Krausse *et al*., 2004), treatment of early Addison disease (Cooper *et al*., 2007 and Ross 1970), protection against hepatotoxicity (Dhirman and Chawla, 2005 and Kim *et al*., 2009), antitumor promoting effect (Nishino *et al*., 1986). The most common use of Liquorice or *Glycyrrhiza* is to treat upper respiratory infections like cough, hoarseness, sore throat, bronchitis, etc. (Shibata 1994 and Yang *et al*., 1990). Moreover, antimicrobial and antioxidant activities are reported by many scientists (Demizu *et al*., 1988).

Among some other medicinally important plants *Camellia sinensis* (Green tea) is one of the most widely used drink all over the world and a number of reports are available on its antimicrobial activity (Tiwari *et al*., 2005; Kim *et al*., 2004; Taguri *et al*., 2004; Lee *et al*., 2003); Tea prepared from *Camellia sinensis* is used in three forms: fermented green tea, non- fermented black tea and semi fermented oolong tea. Green tea has got a diverse range of pharmacological importance to minimize the risks for cardiovascular disease, obesity, dental caries and stroke etc. (Chako *et al*., 2010; Schneider *et al*., 2009; Taylor *et al*., 2005). *Piper betel* (betel leaf) commonly known as Paan belongs to family piperaceae has got a sharp burning taste and reported to have activities such as antidiabetic, antiulcerative, antitumor, antifertility, cardiotonic, antimuragenic, respiratry depressant and antohelminthic. Moreover, it is used to treat alcoholism. dyspepsia, bronchitis, asthama and leporosy (Chakraborty & Shah, 2011).

The main objective of the present study was to investigate the above mentioned herbs for their antibacterial activity against respiratory tract and gastrointestinal tract organisms.

MATERIALS AND METHODS

Plant material: Dried roots of liquorice, dried green tea and fresh betel leaves were obtained commercially and then used in decoction and infusion preparations.

Decoction of *Glycyrrhiza* was prepared by boiling 10 g of roots in 20 ml of distilled water for 5 minutes, it was then cooled and stored at room temperature until used. For green tea 5 g of dried and crushed leaves and for betel leaf, a whole leaf of medium size was boiled in 2 ml of distill water for 5 minutes. Infusions were made by overnight soaking of these herbs separately in 20 ml of distilled water. All the preparations were checked for antibacterial activity separately and in combinations (cocktails) which are abbreviated as follows: *Glycyrrhiza* (GLY), Green Tea (GT) and Betel leaf (B), *Glycyrrhiza* and Green Tea (GlyGT), *Glycyrrhiza*, green Tea and Betel leaves (GlyGTB), Green Tea and Betel leaves (GTB), *Glycyrrhiza* and Betel leaves (GlyB), while decoctions and infusions are denoted as (d) and (i), respectively.

Organisms used: A total of 50 strains of bacteria isolated from the patients suffering from respiratory tract and gastrointestinal tract infections were collected and used as test organisms against antibacterial activity of the decoctions and infusion preparations of all three herbs. Organisms used included clinical isolates of *Acinetobacter* sp., *Enterobacter* sp., *E. coli., Proteus mirabilis, Proteus* sp., *Bacillus cereus, Bacillus* sp., *Corynebacterium diphtheriae, Corynebacterium xerosis, Micrococcus* sp., *Staphylococcus aureus, Staphylococcus* sp., *Streptococcus pyogenes, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi B., Klebsiella* sp., and *Shigella dysenteriae*.

Antibacterial assay: The antibacterial activity of all the roots and leaves was checked by Agar Well Diffusion method. Nutrient agar plates were inoculated by 24 h old culture of test organisms uniformly spread all over the agar. On each plate 5 wells were cut with an aluminum borer of 6 mm diameter and 0.2 ml of herbal preparations were dispensed in each well. All the plates were incubated at 37°C for 24 hours. After the incubation period the mean diameter of zone of inhibition in mm obtained around the well was measured in each case, as shown in Table 1.

Table 1. Antibacterial activity of Glycyrrhiza, Green Tea and Betel Leaves against Gram positive bacteria.

S.No.	Gram positive	GT (d)	GT (i)	GLY (d)	GLY (i)	B (d)	B (i)	GLYB (i)	GLYGT (d)	GLYGT (i)	BGT (i)	BGT (d)	GLYBGT (i)	GLYBGT (d)
1.	*C.diphtheriae* (RT)	+	-	++	++	-	+	+	++	+	+	++	-	++
2.	*C.xerosis* (RT)	+	+	++	+	+	-	+	++	++	++	++	+	++
3.	*Enterococcus* sp. (GIT)	+	+	++	++	+	+	+	++	+	++	+	+	++
4.	*Micrococcus* sp. (RT)	+	+	+	+	+	+	+	++	+	++	+	+	++
5.	MRSA	+	+	+	+	+	+	+	++	-	+	+	+	++
8.	*S. pyogenes* (RT)	+	+	++	++	-	+	+	++	+	-	++	+	++
9.	*S. aureus* (a)	+	+	+	-	+	-	-	+	+	+	+	+	++
10.	*S. aureus* (b)	+	+	-	-	+	+	+	++	+	+	-	-	-
11.	*B. subtilis*	+	+	+	-	+	+	+	+	+	+	++	+	++
12.	*Staphylococcus* sp.	+	+	++	+	-	-	-	++	+	+	+	+	++
13.	*Staphylococcus* sp.	+	+	++	++	+	+	+	+	+	+	+	+	++
14.	*Staphylococcus* sp.	-	+	+	+	-	+	+	+	+	+	+	-	++
15.	*Staphylococcus* sp.	+	+	-	+	-	+	+	-	+	+	+	+	+
16.	*Staphylococcus* sp.	+	+	+	+	+	-	+	++	++	+	+	++	+
17.	*Bacillus* sp.	-	+	++	+	+	-	+	+	+	-	+	-	+
18.	*Bacillus* sp.	+	-	++	++	+	-	+	+	+	-	++	+	+++
19.	*B.cereus*	+	-	++	++	-	-	+	++	++	-	++	++	++

RESULTS AND DISCUSSION

The aqueous extracts showed a significant activity against 48% of respiratory pathogens and 45% of gastrointestinal pathogens. No significant difference was observed in biological activities of decoctions and infusions and both methods for aqueous preparations were found to be effective. Single preparations of all the herbs were found to be effective against both Gram positive and Gram negative bacteria, however, activity was increased significantly in cocktails with maximum activity against *Bacillus sp.* by decoction cocktail of *Glycyrrhiza*, Betel leaves and Green Tea (Tables 1 and 2).

Among the respiratory tract organisms higher activities were observed in case of Gram negative bacteria such as *Acinetobacter* sp., followed by *Pseudomonas* sp., *Klebsiella* sp (Fig. 1). In case of Gram positive respiratory bacteria, *Streptococcus pyogenes, Corynebacterium* sp., and *Micrococcus* sp., were found to be highly sensitive against various combinatations of extracts (Fig. 2).

In case of gastrointestinal bacteria activity profiles were high for *Shigella dysenteriae*, followed by *Enterobacter* sp., *E. coli* and *Proteus* sp. (Fig. 3).

Several reports are available on the antibacterial activity of *Glycyrhizza* against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, E. coli* (Nitalikar *et al*., 2010), while aqueous extracts of *Glycyrrhiza* were applied on *B. subtilis, S. aureus, P. aeruginosa* and *E. coli* and significant antibacterial potential was found in each case (Patil *et al*. 2009).

Antibacterial potential of Green tea has also been demonstrated against Methicillin resistant *S. aureus* and multidrug resistant *Pseudomonas* sp., (Radji *et al*., 2013). Green tea extracts were also tested against a number of clinical and peridontopahtic bacteria and found effective in case of *S. mutans, Aggregatibacter actinomycetecomitans* as reported by Araghizadeh *et al*., 2013. Another report (Shirazi *et al*., 2007) shows the inhibitory effect of Green tea against *S. typhi, S. paratyphi B, S. sonnei, S. flexneri* and ETEC *E. coli*.

Few other researchers revealed antibacterial activities in aqueous extracts of Betel leaves against *S. pyogenes, S. aureus, P. vulgaris, E. coli* (Chakraborti *et al*., 2011) and against *E. coli, P. aeruginosa, S. aureus* (Agarwal *et al*., 2012). Ethanol extracts of Betel leaves were also found to be effective against *P. vulgaris, S. aureus* (Datta *et al*., 2011) and *V. cholerae, E. coli, S. dysenteriae, S. aureus* (Hoque *et al*., 2011). However, to the best of our knowledge, this is the first report about the combined effect of aqueous extracts of *Glycyrrhiza*, Green Tea and Betel leaves against respiratory and gastrointestinal bacteria.

Table 2. Antibacterial activity of Glycyrrhiza, Green Tea and Betel Leaves against Gram negative bacteria.

S.No.	Gram negative	GT (d)	GT (i)	GLY (d)	GLY (i)	B (d)	B (i)	GLYB (i)	GLYGT (d)	GLYGT (i)	BGT (i)	BGT (d)	GLYBGT (i)	GLYBGT (d)
1.	*Acinetobacter* sp. (RT)	+	+	++	++	+	-	+	++	+	+	+	++	+++
2.	*Acinetobacter* sp.(a) (RT)	+	+	++	++	+	-	+	+	++	+	+	++	+++
3.	*E. coli* (GIT)	+	+	+	-	+	+	+	++	+	+	+	+	+
4.	*E. coli* (GIT)	+	-	++	+	-	-	+	++	++	-	+	-	++
5.	*E. coli* (GIT)	+	+	+	+	+	-	+	+	+	-	+	++	++
8.	*Enterobacter* sp. (GIT)	+	+	++	++	-	+	++	++	+	+	+	++	++
9.	*Enterobacter* sp. (GIT)	-	-	++	+	-	+	+	+	+	-	-	+	-
10.	*P. aeruginosa* (RT)	+	+	+	++	-	-	+	++	++	+	+	++	++
11.	*P. mirabilis* (GIT)	+	+	++	-	+	+	+	+	+	+	+	+	++
12.	*P. mirabilis* (GIT)	+	+	++	-	+	+	+	++	+	+	+	+	++
13.	*Proteus* sp. (GIT)	+	+	+	++	+	+	++	+	+	+	+	++	++

Abbreviations: Glycyrrhiza (GLY), Green Tea (GT) and Betel leaf (B), Glycyrrhiza and Green Tea (GlyGT), Glycyrrhiza, green Tea and Betel leaves (GlyGTB), Green Tea and Betel leaves (GTB), Glycyrrhiza and Betel leaves (GlyB), decoctions (d) and infusions (i)

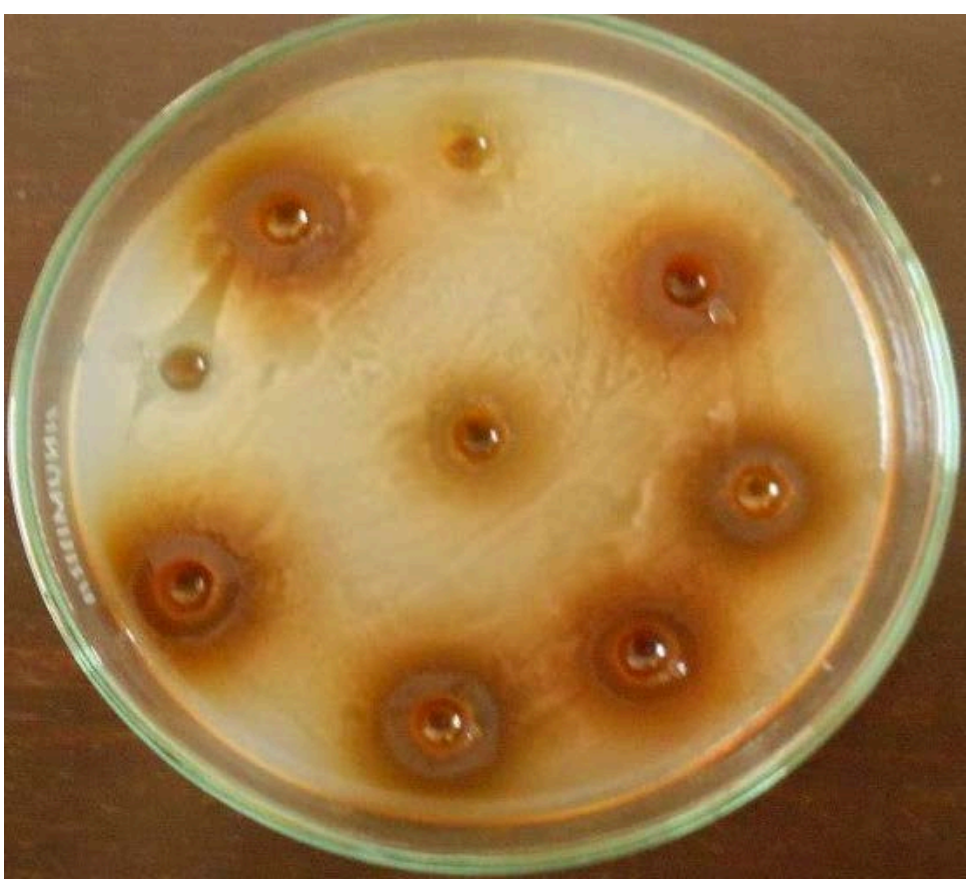

Fig. 1. Antibacterial activity of herbal extracts against *Klebsiella pneumoniae.*

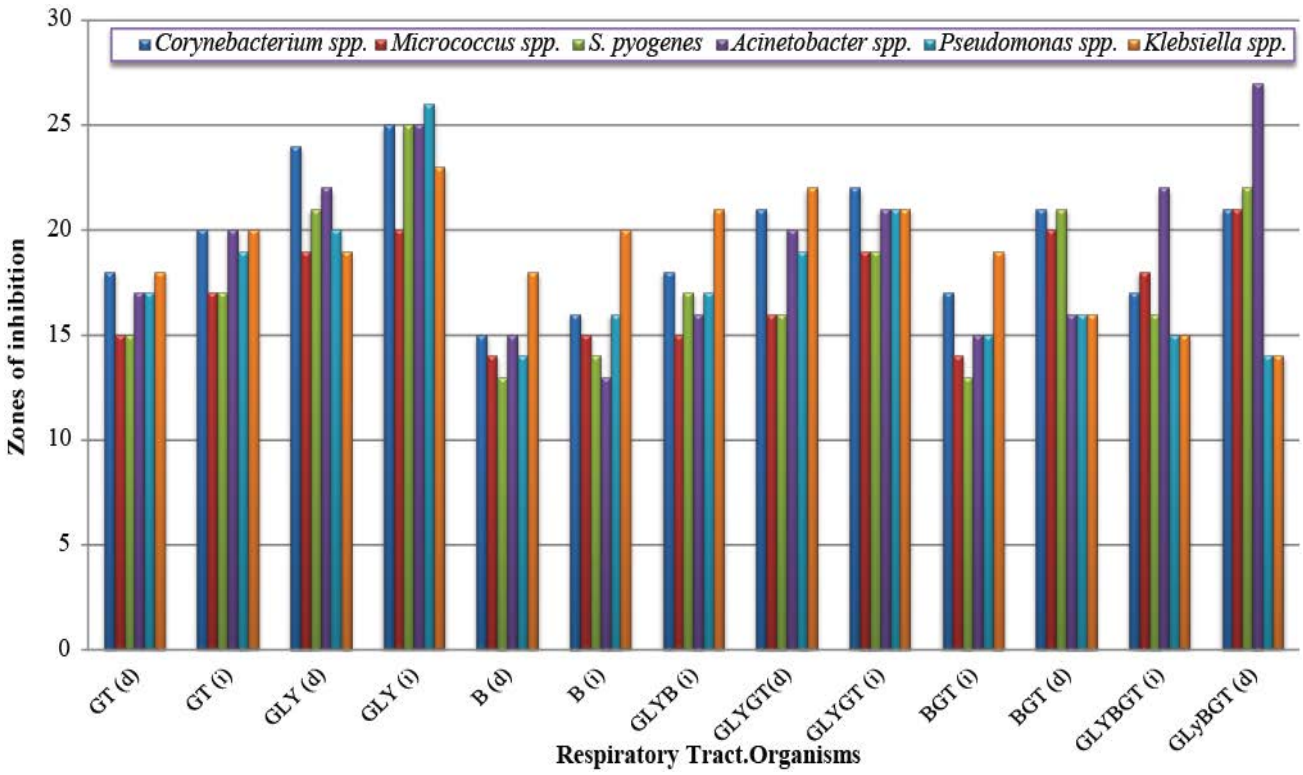

Fig. 2. Antibacterial activity of herbal extracts against Respiratory tract (RT) isolates.

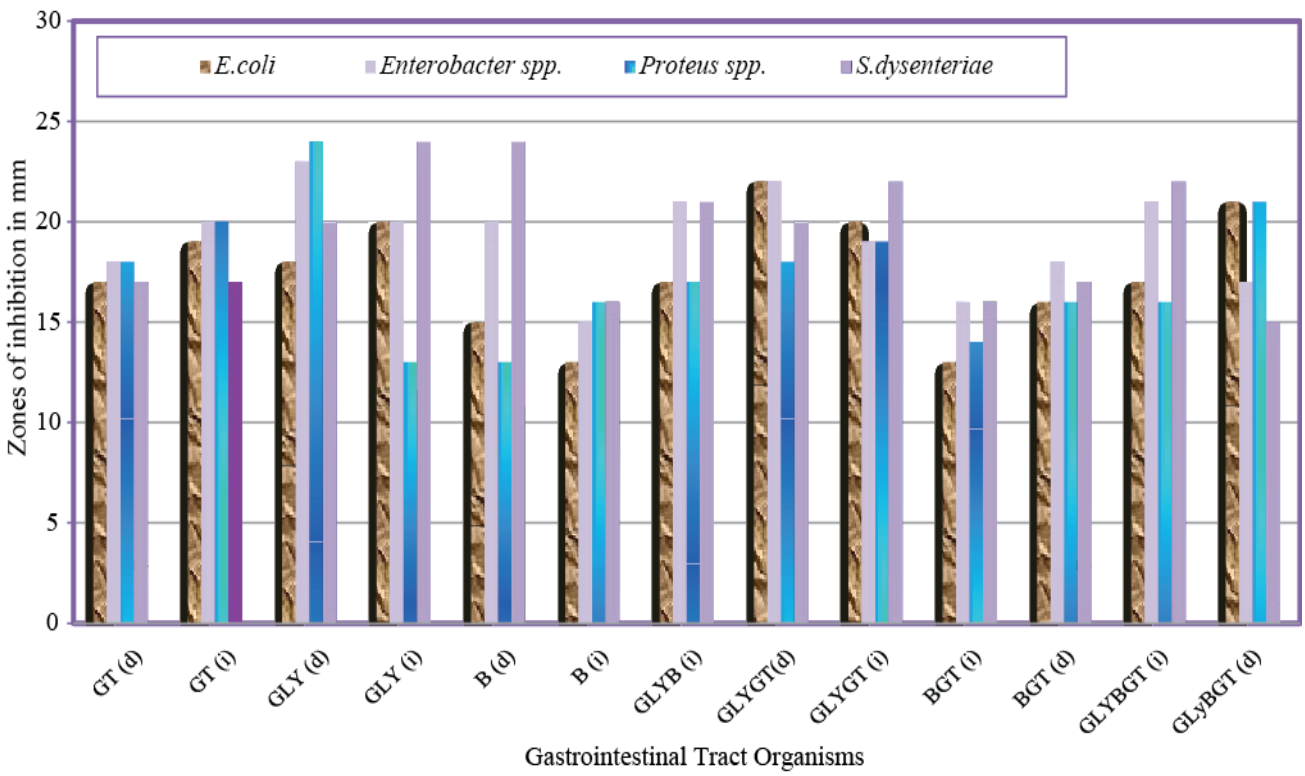

Fig. 3. Antibacterial activity of herbal extracts against gastrointestinal tract (GIT) isolates.

CONCLUSIONS

Glycyrrhiza glabra, Green Tea and Betel leaves possess antibacterial activities against a wide range of Gram positive and Gram negative bacteria in separate preparations and cocktails. This potential can be explored in future to determine MIC, MBC and further characterization.

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