INHIBITORY EFFECT OF NATURAL HERBAL EXTRACTS ON SYSTEMIC BACTERIA

Mahrukh Zaidi, Komal Tayyab, Ayman Wajahat, Maryam Shafique and Sehar Afshan Naz*

Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi- Pakistan.
*Corresponding author e-mail: seharafshan@fauast.edu.pk

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 Mediterranean 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 it grows as perennial herbs or sub-shrubs growing to a height of 2 m with horizontal underground stems. (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.

ABSTRACT
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., 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.

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.

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 Glycyrrhiza 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. aeruginos 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 peridontopathic bacteria and found effective in case of S. mutans, Aggregatibacter actinomycetemcomitans 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.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Gram negative</th>
<th>GT (d)</th>
<th>GT (i)</th>
<th>GLY (d)</th>
<th>GLY (i)</th>
<th>B (d)</th>
<th>B (i)</th>
<th>GLYB (d)</th>
<th>GLYB (i)</th>
<th>GLYG (d)</th>
<th>GLYG (i)</th>
<th>BGT (d)</th>
<th>BGT (i)</th>
<th>GLYBG (d)</th>
<th>GLYBG (i)</th>
<th>BGT (d)</th>
<th>BGT (i)</th>
<th>GLYBG (d)</th>
<th>GLYBG (i)</th>
<th>BGT (d)</th>
<th>BGT (i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Acinetobacter</em> sp. (RT)</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td><em>Acinetobacter</em> sp. (a) (RT)</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td><em>E. coli</em> (GIT)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td><em>E. coli</em> (GIT)</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td><em>E. coli</em> (GIT)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td><em>Enterobacter</em> sp. (GIT)</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td><em>Enterobacter</em> sp. (GIT)</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td><em>P. aeruginosa</em> (RT)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td><em>P. mirabilis</em> (GIT)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td><em>P. mirabilis</em> (GIT)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td><em>Proteus</em> sp. (GIT)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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*. 
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.
REFERENCES


(Received May 2013; Accepted July 2013)