In vitro assessments of antibacterial potential of Commiphora wightii (Arn.) Bhandari. gum extract

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The potential antibacterial efficacy of guggul gum was checked against six Gram-positive and four Gram-negative bacterial strains. The antibacterial activity was assessed by agar well diffusion and two fold serial broth dilution methods. Gram-positive bacterial strains were found to be the most susceptible organisms compare to Gram-negative towards guggul gum extract once. The minimum inhibitory concentration (MIC) noticed in concentration range of 0.5 - 2 mg/ml. The bioautography was performed to localize active compound present in crude as well as eluted fraction. Compound 5(1-methyl, 1-amino ethyl)-5-methyl-2-octanone, identified which possessed antibacterial activity by various spectrometric method. The extract of guggul gum possesses significant antibacterial activity against Staphylococcus aureus.

Key words: Commiphora wightii, guggul gum, antibacterial activity, bioautography, 5(1- methyl, 1-amino ethyl)-5-methyl-2-octanone.

INTRODUCTION

The Commiphora wightii (Arn.) Bhandari (Burseraceae) plant is known as Indian bdellium. It is distributed in the arid, rocky tracts of Rajasthan, Gujarat, and Mysore States of India, and the Sindh and Baluchistan States of Pakistan. In Gujarat, this species is mainly found in Kachchh and in some parts of Saurashtra regions (Sabnis and Rao, 1983; Shah, 1978). Earlier the plant was found abundant in Rajasthan and Gujarat but currently threatened because of significant declines in population sizes (IUCN, 2004) and faulty extraction methods employed by traditional guggul resin collectors. It came in vulnerable list in the 1997 IUCN Red List as vulnerable (VU) and in the 2004 IUCN Red List as data deficiency (DD) (IUCN, 2004). The Government of India has recently banned the export of the gum (IUCN, 2004), due to its high market price in international trade.

This plant grows up to 1 - 2.5 m in height and has sharp spines and papery bark (Kumar and Shankar, 1982).

Leaves have aromatic smell. The guggul gum, extracted from the stem of C. wightii. It is important for various medicinal and pharmacological properties. Guggul also used in various formulation of ayurveda. Guggul gum is pharmacologically active in controlling rheumatoid arthritis, obesity and peptic ulcer (Atal et al., 1975). The pharmacological and clinical studies on its crude drug constituents and various extractives have revealed its significant hypocholesteremic, hypolipidimic, anti-inflammatory, antirheumatic and antifertility activities (Nityanand and Kapoor, 1971a,b; Kapoor et al., 1979; Kakrani, 1981a,b; Tajuddin et al., 1994; Singh, et al., 2001).

Guggulsterone – Z and E, the active constitute of resin are responsible for lipid lowering properties in human blood (Satyavati et al., 1969; Tripathi et al., 1968; Singh et al., 1994). Despite having so many activities no reports found on the antibacterial activity of guggul gum up to structural identification of active compound. So in this context, present study was under taken to study antibacterial activity and to identify active compound present in guggul gum.

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MATERIALS AND METHODS

Plant material

Guggul gum was obtained from Gujarat State Forest Development Corporation Ltd., Vadodara.

Preparation of extracts

Extract was prepared by cold extraction method in which 25 g of guggul gum powder was soaked in equal volume of dichloromethane and methanol (125 ml) for 24 h at room temperature and was shaken occasionally. The extract was filtered and then concentrated by evaporating the solvent at room temperature. The residue (5 g) was stored in the airtight glass bottle in a refrigerator. Different concentrations of extract (10, 50, and 100 mg/ml) were prepared in DMSO (Dimethyl Sulfoxide) for checking the antibacterial activity.

Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Micrococcus luteus, Enterococcus faecalis, and four Gram-negative (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi) bacterial strains were tested which were obtained from V.P. and R.P.T.P Sciences College, Vallabh Vidyanagar and Pramukh Swami Medical College, Karmasad, Gujarat. The bacterial cultures was maintained and grown in nutrient broth and nutrient agar medium (Hi media). The fresh bacterial cultures compared with 0.5 Mc Farland turbidity standard, which is equivalent to approximately 1x10^8 CFU/ml (Perilla et al., 2003) was used for bioassay.

Purification of compound

The guggul gum extracted in a mixture of dichloromethane and methanol (1:1) and than partitioned with n-hexane and ethyl acetate. The n-hexane phase, which showed antibacterial activity, was subjected to silica gel (60 - 125 mesh) column chromatography. The fractions eluted by passing through increasing polar gradient of n-hexane and ethyl acetate. Fractions, possessed antibacterial activity were pooled together and reloaded on silica gel column was performed to check to localize the antibacterial compound. Linomat 5 sample applicator (Camag, Switzerland) was used for application of extract on 0.25 mm thick precoated silicagel (60F254, Merck, Germany). First plate developed in solvent system of n-hexane and ethyl acetate (7:3) and than overlaid by nutrient agar medium seeded with S. aureus.

Bioautography

The bioautography of crude gum extract and fractions collected from silica gel column was performed to check to localize antibacterial compound. Linomat 5 sample applicator (Camag, Switzerland) was used for application of extract on 0.25 mm thick precoated silicagel (60F254, Merck, Germany). First plate developed in solvent system of n-hexane and ethyl acetate (7:3) and than overlaid by nutrient agar medium seeded with S. aureus.

Fourier transformer infra red (FTIR) spectroscopy

A thin film of guggul gum active eluted fraction in methanol was applied on the glass and IR spectra were recorded by using Perkin Elmer spectrophotometer, Spectrum Instrument (Germany) with FTIR paragon 1000 PC software. The GC-MS analysis was clone by electron impact ionization (EI) method on auto system XL gas chromatography (Perkin Elmer Instrument, Germany) coupled to a Turbo Mass Spectrophotometer (Perkin Elmer Instrument, Germany) at SICART, Vallabh Vidyanagar, Gujarat. The column was fused silica capillary column, 30 x 0.25 mm ID; coated with D-I, 0.25 μm film thickness. The temperature of column was programmed at 70 to 250°C at the rate of 10°C /min increase, injection port temperature at 250°C. Helium was used as carrier gas at constant pressure of 100 kpa and flow rate of 20 ml/min. Samples which dissolved in methanol was run fully at range of 60 - 550 amu and the results were compared by using NIST 107 Spectral library search programme.

Gas chromatography-mass spectroscopy (GC-MS)

The GC-MS analysis was clone by electron impact ionization (EI) method on auto system XL gas chromatography (Perkin Elmer Instrument, Germany) coupled to a Turbo Mass Spectrophotometer (Perkin Elmer Instrument, Germany) at SICART, Vallabh Vidyanagar, Gujarat. The GC-MS analysis was clone by electron impact ionization (EI) method on auto system XL gas chromatography (Perkin Elmer Instrument, Germany) coupled to a Turbo Mass Spectrophotometer (Perkin Elmer Instrument, Germany) at SICART, Vallabh Vidyanagar, Gujarat.

NMR spectroscopy

1H NMR spectra were recorded in CDCl3 using a HITACHI, R-1500, and 60 MHz for proton NMR spectrometer at the Department of chemistry, Sardar Patel University, Vallabh Vidynagar, Gujarat, India.

RESULTS

In the present study, the antibacterial activity assay of guggul gum extracts against selected the six Gram-positive and four Gram-negative bacteria (Table 1). The crude gum extracts was extracted using dichloromethane and methanol using equal volume and finally sample are prepared in DMSO used for antibacterial assay. The zone of inhibition of 12 mm was recorded against M. luteus at 10 mg/ml concentration where as 12 and 14 mm zone of inhibition was shown at 50 and 100 mg/ml concentration respectively (Table 1). In Gram-positive organisms, B. megaterium, M. luteus and E. faecalis were found to be most susceptible organisms (10 - 15 mm zone of inhibition)
Table 1. Antibacterial activity of guggul gum extract.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Zone of inhibition (100 mg/ml)</th>
<th>Streptomycin (30 μl/ml)</th>
<th>DMSO 100%</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram - positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. cereus</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>B. megaterium</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>S. aureus</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>M. luteus</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>00</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Gram - negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>00</td>
</tr>
<tr>
<td>S. typhi</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 2. IR spectra of C. wightii.

<table>
<thead>
<tr>
<th>Absorption (cm⁻¹)</th>
<th>Absorption range(cm⁻¹)</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2955.81</td>
<td>2962-2853</td>
<td>Hydrogen Chromophore-C-H stretching– Alkene</td>
</tr>
<tr>
<td>2921.60</td>
<td>2962-2853</td>
<td>Hydrogen Chromophore- C-H stretching – Alkene</td>
</tr>
<tr>
<td>2851.71</td>
<td>2962-2853</td>
<td>Hydrogen Chromophore- C-H stretching – Alkene</td>
</tr>
<tr>
<td>1737.83</td>
<td>1750-1735</td>
<td>Ester stretching vibration – Saturated, acyclic</td>
</tr>
<tr>
<td>1463.37</td>
<td>1485-1445</td>
<td>C-H bending- Alkene</td>
</tr>
<tr>
<td>1377.57</td>
<td>1410-1310</td>
<td>O-H bending and C-O stretching vibration-Phenols</td>
</tr>
<tr>
<td>1025.83</td>
<td>1220-1020</td>
<td>Amines, C-N vibration-Aliphatic</td>
</tr>
<tr>
<td>737.79</td>
<td>~ 750</td>
<td>C-H bending, Aromatic, for adjacent hydrogen atoms</td>
</tr>
</tbody>
</table>

Figure 1. Antibacterial activity of compound from guggul gum extract.

to gum extract where as S. aureus was shown resistance to gum extract. Guggul gum displayed moderate inhibitory activity towards gram-negative bacteria. The observed MIC of gum was in range of 0.5 to 2 mg/ml (Table 1). The MIC value for B. subtilis, B. megaterium, E. coli and E. faecalis was 0.5 mg/ml where as for B. cereus and M. luteus was 1 mg/ml, which is comparable to antibiotic streptomycin (Table 1). The MIC value of crude extract obtained for the organism S. aureus, K. pneumoniae, P. aeruginosa and S. typhi was 2 mg/ml (Table 1).

The results of bioautograpy revealed that inhibitory compound showed blue florescence at Rf 0.31 at 254 nm on control plate. The study of infrared spectra revealed the presence of amino as a major functional group (Table 2, Figures 1 and 2). The peak showing maximum percentage area at RT 7,3.000 in GC-Mass analysis and scan 9.56 e4 through mass spectrophotometer, revealed
the presence of 5(1-methyl, 1- amino ethyl)-5- methyl-2- octanone and have molecular weight of 213, pK is 16.696 (Figure 3) (Saeed and Sabir, 2004; Bajaj and Sukhdev, 1982; Newton et al., 2002; Asres et al., 1998).

**DISCUSSION**

The guggul gum extract was effectively inhibited the growth of selected the six Gram-positive and four Gram-negative bacteria (Table 1). As general observation, there is increment in inhibitory efficacy with increase concentration of extract or compound. But in the present investigation, the inhibitory activity was not steadily increased at higher concentration of extracts. This result are compare with different species of Commiphora sp. Commiphora abyssinica were found to be active against Proteus mirabilis, B. Subtilis, Pseudomonas sp., S. aureus, E. coli and Klebsiella sp. (Ranganathan and Habte, 1982). The observed MIC of gum was in range of 0.5 to 2 mg/ml. The MIC value for B. subtilis, B. megaterium, E. coli and E. faecalis was 0.5 mg/ml where as for B. cereus and M. luteus was 1 mg/ml, which is comparable to antibiotic streptomycin. Similar antibacterial activity is also found in other species of Commiphora sp. The guggul gum extract displayed lower inhibitory activity towards S. typhi and P. aeruginosa. Newton et al. (2002) reported antibacterial activity of guggul gum extract against Mycobacterium aurum and Mycobacterium smegmatis. The essential oil of C. tenuis exhibited antibacterial activity against S. aureus, P. mirabilis and E. coli with MIC of 5 – 10 mg/ml (Asres et al., 1998).

The results of bioautograpy revealed that inhibitory compound showed blue florescence at Rf 0.31 at 254 nm on control plate. Similar bioautograpy is also found in other species of Commiphora sp. Calson et al. (1992) isolated sequiterpene T- cardinol from Commiphora guidottii and found to be active against S. aureus and Trichopyton mentagrophytes.

**Conclusion**

The results obtained from the current study suggest that extract of guggul gum possesses significant antibacterial activity against Gram-positive bacteria and moderate activity against Gram-negative once. M. luteus and E. faecalis were found to be most susceptible organisms where as S. aureus and S. typhi were shown resistance.
Figure 3. MS spectra of compound isolated from guggul gum extract.

*S. aureus* was shown more resistance to gum extract. The observed MIC of gum was 2 mg/ml against *S. aureus*. *S. aureus* against elucidation the active compound. The Rf. value of effective compound was 0.31. All spectroscopic analysis showed the presence of 5(1-methyl, 1-aminoethyl)-5-methyl-2-octanone, which possesses antibacterial property.

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