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Full Length Research Paper

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 it 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 hypocholestermic, 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 dichloro-methane and methanol (125 ml) for 24 h at room tempera-ture 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 and further identification and characterization of compound.

In the present study six Gram-positive bacteria (*Bacillus cereus, 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 Vidyanager 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 1x108 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 chroma-tography. 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 column and again eluted by n-hexane and ethyl acetate (7:3). Finally antibacterial compound was purified by preparative thin layer chromatography. The purified compound identified by using infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR) and gas chromatography couple with mass spectroscopy (GC-MASS).

Antibacterial activity

Bioassay was carried out by agar well diffusion method (Perez et al., 1990). Bacterial culture of 0.1 ml spread on the nutrient agar plate. A well of 10 mm diameter punched off with the help of sterile cork borer in the nutrient agar plate and then 100 µl of extract was carefully added. Plates were incubated at 4°C for pre-diffusion of extracts in a refrigerator. The minimum inhibitory concentration (MIC) was determined by two-fold serial broth dilution method in the concentration range of 0.125 - 8.9 mg/ml. Plates and tubes were incubated at 37°C in an incubator for 24 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition excluding the diameter of well. Streptomycin (Hi-Media, Mumbai) at a concentration of 30 µg/ml as positive control and 100% DMSO were used as positive control and negative control respectively. The experiment was performed twice in duplicate (Chattopadhyay et al, 1998).

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 at the Sophisticated and instrumentation Centre for Applied Research and Training (SICART), Vallabh Vidyanagar, Gujarat.

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 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.

NMR spectroscopy

1 H 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

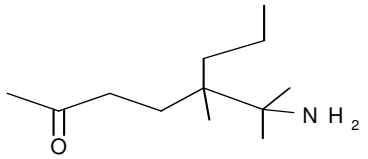
In the present study, the antibacterial activity assay of guggul gum extracts against selected the six Gram-positive and four Gram-negative bacteria (Table 1). The crude gum extracts was extracted using dichloromethane and methanol using equal volume and finally sample are prepared in DMSO used for antibacterial 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.

Organisms	Zone of inhibition			Streptomycin	DMSO	MIC
	100 mg/ml	50 mg/ml	10 mg/ml	(30 µl/ml)	100%	(mg/ml)
Gram - positive						
B. cereus	8	7	6	13	00	1
B. subtilis	8	7	6	14	00	0.5
B. megaterium	11	10	10	10	00	0.5
S. aureus	15	14	13	15	00	2
M. luteus	14	12	12	00	00	1
E. faecalis	15	12	12	13	00	0.5
Gram - negative						
E. coli	7	6	4	17	00	0.5
K. pneumoniae	8	7	6	14	00	2
P. aeruginosa	6	5	4	00	00	>2
S. typhi	4	4	4	14	00	>2

Table 2. IR spectra of *C. wightii*.

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



5-(1-Amino-1-methyl-ethyl)-5-methyl-octan-2-one

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

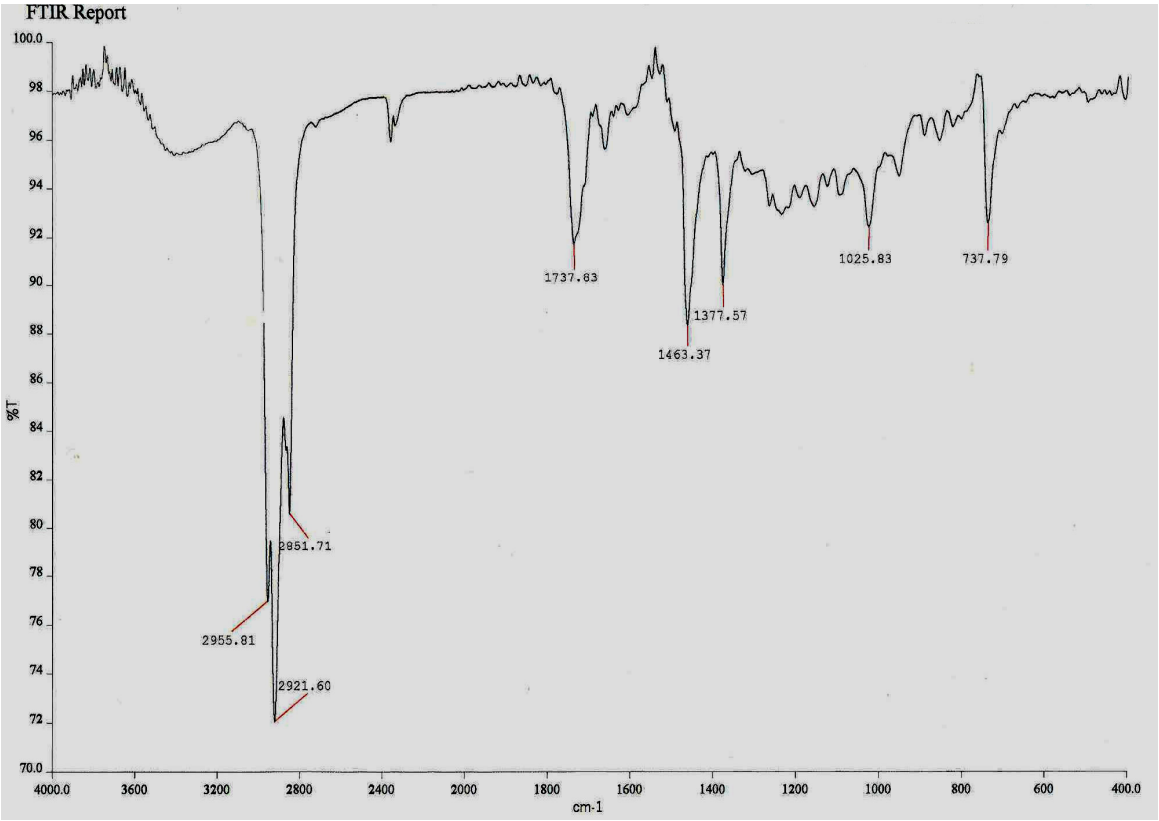


Figure 2. IR spectra of compound isolated from guggul gum extract.

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. aureaus*, *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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