# Antimicrobial activity of *Commiphora wightii* gum (Guggul gum) extract against gram positive and gram negative bacteria

BHOJ RAJ SINGH[1], MAHTAB ZAKIRA SIDDIQUI[2]

[1]*Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar, India;* [2]*Processing and Product Development, ICAR-Indian Institute of Natural Resins and Gums, Ranchi, India*

*Received 10 September 2015*
*Accepted 26 October 2015*

## ABSTRACT

**Objective:** To determine the antimicrobial activity of guggul gum samples on clinically important bacteria of animal health significance.
**Methods:** Four samples of guggul gum (GG) and their methanolic extracts (GGE) were tested for antimicrobial activity through incorporation in to growth medium and using discs (5 mg) diffusion assay, respectively. Minimum inhibitory concentration was determined by the agar well dilution method. A total of 128 strains belonging to 39 species of bacteria were tested.
**Results:** From four samples of guggul gum (25 g each), 4.069 (±0.231) g of methanolic extract (GGE) was obtained. None of the gums or GGEs inhibited >23.4% of the strains tested. No significant difference was observed between Gram+ve (58) and Gram-ve (70) bacteria for their sensitivity to GGs/GGEs. Cotrimoxazole resistant strains had a higher probability of being resistant to GGs/ GGEs (p, 0.01). The minimum inhibitory concentration (MIC) of GGE for bacteria ranged between 0.5->10 mg/ml. MIC was minimum (0.5 mg/ ml) for *Staphylococcus intermedius* and *Streptococcus pyogenes* strains, but no specific trend of MIC for different groups of bacteria was evident. *Escherichia coli* strains had a slightly higher probability of being sensitive to GGs/ GGEs than of *Burkholderia* strains (p, 0.09).
**Conclusions:** Guggul gum had a broad spectrum antimicrobial activity but limited to only a few strains. Guggul gum may retain its antimicrobial activity even after autoclaving after incorporation in to growth medium.

**KEY WORDS:** **Herbal Antibacterial Extract Guggul gum *Commiphora wightii***

## Introduction

Guggul gum (GG), from *Commiphora wightii*, is known for its therapeutic value in rheumatoid arthritis, obesity, peptic ulcer and infections [1] due to its hypocholestermic, hypolipidemic, anti-inflammatory, anti-fertility, antilipidemic [2] and antimicrobial [3] properties. Major antimicrobial activity of GG is reported in its methanolic extract [1,3,4]. However, antimicrobial activity has so far been tested on a limited number of reference strains.

## Materials and methods

For testing antimicrobial potential of four Guggul gum (GG) samples (2 from Rajasthan, R1, R2, and two from Gujarat, G1, G2) on 128 strains of 39 species of 19 genera of health significance (Table 1), bacterial strains of animal origin available in Epidemiology Division, IVRI, Izatnagar, were revived. All strains were tested for identity and purity on blood agar plates and were used for assessing the antimicrobial activity of GGs. Methanolic guggul gum extracts (GGE) were prepared by cold extraction [1,4]. Discs (6 mm) containing 5 mg of GGE were prepared for disc diffusion assay [5].

*Correspondence to: Dr Bhoj Raj Singh*
*Email: brs1762@gmail.com*

For determining MIC, different concentrations of GGE (10, 8, 6, 4, 2, 1, 0.5, 0.2 and 0.0 mg/ 50 ul) were made in DMSO and the agar well diffusion method [5] was used. All bacteria but *Streptococcus*, *Bordetella*, and *Brucella* were tested on Muller Hinton agar plates, for these three groups - brain heart infusion agar plates were used [5].

**Table 1.** Sensitivity of bacterial strains of animal health importance to guggul gum (GG) and guggul gum extract (GGE)

| **Bacteria tested** | **Number of strains resistant to** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **(Number of strains tested)** | **GG 8** | **GG 9** | **GG 10** | **GG 11** | **GGE 8** | **GGE 9** | **GGE 10** | **GGE 11** | **Co** | **G** | **MIC** |
| *Aeromonas sobria* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | >10 |
| *Bacillus marcerans* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Bacillus polymyxa* (2) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 1 | >10 |
| *Bacillus subtilis* (1) | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 8 |
| *Bordetella bronchiseptica* (1) | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 6 |
| *Brucella abortus* strain 19 (1) | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 4 |
| *Burkholderia cepacia* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | >10 |
| *Burkholderia gladioli* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | >10 |
| *Burkholderia pseudomallei* (2) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | >10 |
| *Burkholderia spp.* (7) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 2->10 |
| *Citrobacter freundii* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | >10 |
| *Enterobacter agglomerans* (6) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 0 | 1 | >10 |
| *Erwinia chrysanthemi* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Escherichia coli* (27) | 22 | 23 | 22 | 22 | 23 | 23 | 22 | 23 | 7 | 2 | 2->10 |
| *Klebsiella pneumoniae* (5) | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 2 | 0 | >10 |
| *Pasteurella multocida* (2) | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 0 | >10 |
| *Pragia fontium* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | >10 |
| *Proteus mirabilis* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | >10 |
| *Proteus vulgaris* (4) | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 0 | >10 |
| *Pseudomonas aeruginosa* (4) | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | >10 |
| *Pseudomonas fluorescens* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | >10 |
| *Salmonella enterica* ser *Gallunarum*(1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | >10 |
| *Serratia fonticola* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | >10 |
| *Staphylococcus capitis* ssp. *urealyticus* (2) | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 0 | 1 | 2->10 |
| *Staphylococcus epidermidis* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Staphylococcus haemolyticus* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | >10 |
| *Staphylococcus hyicus* (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | >10 |
| *Staphylococcus intermedius* (10) | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 3 | 0 | 0.5->10 |
| *Staphylococcus sciuri* (6) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 0 | 0 | >10 |
| *Streptococcus agalactiae* (5) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | >10 |
| *Streptococcus equi* ssp. *equi* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| *Streptococcus equi zooepidemicus* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Streptococcus intestinalis* (5) | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 4->10 |
| *Streptococcus milleri* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| *Streptococcus pneumoniae* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Streptococcus porcinus* (2) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | >10 |
| *Streptococcus pyogenes* (14) | 11 | 11 | 10 | 10 | 11 | 11 | 12 | 13 | 2 | 0 | 0.5->10 |
| *Streptococcus suis* (3) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 1 | >10 |
| *Yersinia enterocolitica* (1) | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 4->10 |
| Number (%) of strains resistant (n=128) | 99 (77.3) | 101 (78.9) | 99 (77.3) | 98 (76.6) | 101 (78.9) | 103 (80.5) | 104 (80.5) | 107 (83.6) | 32 (25) | 7 (5.5) | 0.5->10 |

GG, guggul oleogum incorporated in growth medium @10mg/ ml before autoclaving; GGE, methanolic extract discs of GG (5mg/ disc); CoT, 25 µg cotrimoxazole discs; G, 30 µg gentamicin discs; MIC, minimum inhibitory concentration of methanolic extract of GG (GGE) in µg/ ml

For testing the antibacterial activity of whole GGs, powdered gum was incorporated into MHA or BHI agar medium before autoclaving (1% w/v) and plates were used within three days. Bacterial strains were spot inoculated on GG plates with a loopful of fresh bacterial culture (0.1 $OD_{590}$). Fresh bacterial cultures having 0.1 $OD_{590}$ equalling to about 100-200 million bacteria/ ml was used for determining their MIC and sensitivity through disc diffusion assay. Incorporation of GG powder before autoclaving had one drawback that if anything heat labile may get destroyed during autoclaving. However, this method was found to be the only feasible method to incorporate GG in growth medium.

## Results

From 25 g of GG samples, 3.785, 4.22, 4.29 and 3.98 g of extract (GGE) were obtained from R1, R2, G1 and G2 GG samples, respectively. None of the gums or GGEs inhibited >23.4% of the 128 strains tested (Table1). Number of test strains inhibited to grow on medium incorporated with GG (10 mg/ ml, before autoclaving) was slightly more than those inhibited with GGE (5 mg) discs (Table 1). All the GGs and GGEs were significantly (p, <0.001) less antibacterial than gentamicin and cotrimoxazole. No significant difference was observed between GPBs and GNBs for their sensitivity to GGs/GGEs. Cotrimoxazole resistant strains had more probability of being resistant to GGs/ GGEs (p, 0.01). The minimum inhibitory concentration (MIC) of GGE for bacteria ranged between 0.5->10 mg/ml. MIC was minimum (0.5 mg/ ml) for *Staphylococcus intermedius* and *Streptococcus pyogenes* strains, but no specific trend of MIC for different groups of bacteria was evident (Table 1). Though sensitivity of bacteria to GGs or GGEs had no relation to sensitivity to gentamicin, cotrimoxazole resistant strains had more probability of being resistant to GGs/ GGEs (p, 0.01). No significant difference was evident among strains of 39 species of bacteria (Table 1) for their sensitivity to different GGs except the *E. coli* strains, more of which were sensitive to GGs/ GGEs than *Burkholderia* strains (p, 0.04-0.09). There was no significant difference in numerical results of antimicrobial activity recorded in disc diffusion assay (using discs containing 5 mg of GGE) and incorporation (10 mg/ ml) of GG in growth medium. However, a few more strains were inhibited on incorporation of GG in agar medium than tested with disc diffusion assay. The observation indicated that total antimicrobial activity of GG is not limited to its methanolic extract and better extraction methodology is needed to evaluate GGs for their antimicrobial activity.

## Discussion

Guggul gum extract (GGE) were obtained from different samples ranged from 15.14% to 17.16% (w/w) i.e., average recovery (16.29±0.925%) was comparatively less than reported earlier [4] probably due to variation in quality of different GG samples, evident from the study and earlier reports [6]. Guggul gums or GGEs inhibited only about 25% of the strains which were susceptible to gentamicin indicating that GGs or GGEs though antibacterial are inferiors than common antibiotics available. Less antimicrobial activity in GGS/ GGEs than in antibiotics is reported earlier [1, 4]. However, there is no significant difference in sensitivity of GPBs and GNBs for GG or GGE in our study, previous observations reported GPBs being more commonly sensitive than GNBs [1, 4]. This might be due to a limited number of strains of microbes (≤10) used in earlier studies [1, 4].

The minimum inhibitory concentration (MIC) of GGEs (0.5->10 mg/ml) observed in the study are in concurrence to earlier studies [4]. In a similar study on reference strains [1] a minimum MIC of GGE was observed for *S. aureus* (0.12 mg/ ml) and maximum for *B. subtilis* (2.048 mg/ ml), but *Escherichia coli* and *Salmonella* Typhi could not be inhibited even at 4.096 mg/ ml extract. In another study [3] MIC of guggul leaves'extract was reported between 0.125 mg/ml to 2 mg/ ml, minimum for *B. cereus* and maximum for *S. aureus*.

No significant difference in numerical results of antimicrobial activity of GGE (5 mg) discs and GG in growth medium (10 mg/ ml) indicated that for primary screening any of the two methods can be followed. For 5 mg of GGE about 30 mg of GG was needed. However activity of 10 mg of GG was almost equivalent to 5 mg of GGE (from ~30 mg of GG) indicating that all antimicrobial activity was not extracted through existing method or antimicrobial activity of GG was not only limited to its methanol soluble components thus there is possibility for

development of better extraction methodology for antimicrobial components of GG. Inhibition of growth of more number of test strains on medium incorporated with GG (10 mg/ ml, before autoclaving) than those inhibited with GGE (5 mg) discs (Table 1) indicated that antimicrobial activity of GG remained intact even after autoclaving and methanol may not solubilise all the antimicrobial components of the guggul gums. Therefore, 5(1-methyl, 1-amino ethyl)-5- methyl-2-octanone identified as major antibacterial compound [4] might just be one of the several antimicrobial compounds in GG.

## Conflict of Interest

We declare that we have no conflict of interest.

## Acknowledgements

Authors are thankful to Director, ICAR-IVRI, Izatnagar and ICAR- IINRG, Ranchi for permitting the undertaking of the study and Mr. HC Joshi and Laikurahman for consistent help in laboratory.

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