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Antimicrobial Activity of Ethanolic and Aqueous Extracts of Common Edible Gums against Pathogenic Bacteria of Animal and Human Health Significance

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Research Article

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ABSTRACT

The study was conducted to determine antimicrobial activity in ethanolic extracts and aqueous solution of 31 samples of edible gums from *Acacia nilotica* (17), *Buchanania lanzan* (7), *Sterculia* spp. (5), *Balanites aegyptiaca* (1) and *Prosopis juliflora* (1) plants.

Antimicrobial activity in ethanolic extracts (EE) was determined using disc (2 mg EE/disc) diffusion assay, while antimicrobial activity of aqueous solution (50 mg/ml) and minimum inhibitory concentration (MIC) of EE was determined through agar well dilution method against 101 bacterial strains including four reference strains (*Streptococcus milleri*, *Bacillus mycoides*, *E. coli*, *Salmonella Abortusequi*) and 97 clinical/environmental isolates (34 gram positive bacteria, GPBs and 63 gram negative bacteria, GNB). All the strains were also tested for sensitivity to ciprofloxacin disks (10 µg).

None of the 101 strain was sensitive to 4% (w/v) aqueous solution of gums but 50 (49.5%) strains were sensitive to one or other EE of two gum acacia (EEA) and 9 (8.9%) to EE of one gum chironji (EEC) samples. GPB strains were more commonly sensitive to EEA (p=0.0007) than GNB strains. However, no significant p=0.56 difference among GPBs and GNBs was evident for their sensitivity to EEC. MIC of EEA ranged between 80 µg to 2560 µg/ml for sensitive bacterial strains. MIC of EEC was lowest (160 µg) for *Streptococcus* equi but ranged between 640 µg to 2560 µg/ml for other sensitive strains. All resistant strains had MIC >2.56 mg/ml EEA or EEC.

Although antimicrobial activity of ethanolic extract of gum acacia and gum chironji had wide spectrum, it could be detected only in 2 of the 17 samples of gum acacia and one of the 7 samples of gum chironji. Though edible gums may have little utility as antimicrobials in therapeutics, might be containing some antibacterial component(s) which needs to be identified.

INTRODUCTION

Edible gums known by different names viz., Gond, Goond, Goond katira, Dinka, Gaund, Gondh are dried sap (exudates) of thorny trees and shrubs. Gums from trees and shrubs of Fabaceae family (*Acacia*, *Sterculia*, *Astragalus*, *Balanites*, *Buchanania*, *Anogeissus* species) are edible [1]. Edible gums are water soluble producing viscous gel like solution. They are used in food industry

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as thickening, gelling, emulsifying and stabilizing agents [1]. Due to their adhesives, binding, clarifying, encapsulating, flocculating, swelling and foam stabilizing properties gums have multiple applications in multiple industries [1,2]. Gums are ionic polymers (polyelectrolytes) of acidic glycopeptides containing several biologically active compounds and mineral salts [3]. Commonly available edible gums come from *Acacia* spp. trees and are known as gum acacia, chaar gund, char goond, or meska [1]. It is mostly obtained from trees of two *Acacia* species viz., *A. senegal* and *A. seyal*. Other edible gums available in market are; gum ghatti (from *Anogeissus* trees), gum tragacanth (from *Astragalus* shrubs, gond katira), karaya gum (from *Sterculia* trees), gum babul (from *Acacia nilotica*), gum chironji (from *Buchanania lanzan* trees), and gums from *Balanites aegyptiaca* and *Prosopis juliflora* are also edible [1,4].

Antimicrobial activity of gums is always being a controversial issue due to several contrasting reports [1]. Alcoholic extract and aqueous extracts of gum acacia are reported to inhibit growth of *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Pseudomonas aeroginosa*, *Proteus merabilis*, *Acinetobacter*, *Enterobacter*, *Klebsiella pneumoniae*, *Serratia* spp., *E. coli*, *Salmonella typhi*, *Cerospora pongamae*, *Candida albicans* and *Aspergillus niger* [5,6]. Though gum chironji is reported beneficial in intercostals' pain and diarrhoea, reports are scant on its antimicrobial activity [1]. Antibacterial and antimycotic activity has been reported in alcoholic extracts of *Balanites aegyptiaca* leaves, bark and fruit mesocarps [7-11] inhibiting of metallo-B-lactmase producing *E. coli*, *Klebsiella* spp. and *Citrobacter* spp. [11] Though hot and cold aqueous extracts of *Prosopis juliflora* (Vilayati kikar) leaves inhibited *Bacillus subtilis*, *E. coli*, *Enterobacter faecalis*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *S. pyogenes*, *S. typhi* and *S. typhimurium* [12], its gum has rarely been reported to have antimicrobial activity. Gum Karaya from *Sterculia urens* tree has been used as an adhesive for dental fixtures and osteomyo- equipment, and as a base for salicylic acid patches [13,14], and is known to reduce bacterial adhesion by 98% when applied as protective coating to dentures [14] but its antibacterial activity is not reported yet. Antimicrobial activity of oleoresins (inedible gums) also similar to edible gums in texture is often reported [15], information on antimicrobial activity of edible gums is scanty and mostly based on tests using reference laboratory strains [1]. The scant information on antimicrobial activity that too not on clinically important pathogens, limits the understanding of utility of edible gums as natural antimicrobials. This study aimed at determining antimicrobial activity of ethanolic extracts and aqueous solutions of commonly available edible gums in India on clinically important bacteria.

MATERIALS AND METHODS

Gums

A total of 31 samples (duly authenticated and catalogued at ICAR-Indian Institute of Natural Resins and Gums (IINRG), Namkum, Ranchi) of *Acacia nilotica* (Babul) gum (17), *Buchanania lanzan* (Chironji) gum (7), *Sterculia* (Karaya) gum (5), *Balanites aegyptiaca* gum (1) and *Prosopis juliflora* gum (1) were collected/procured from fresh collections (gathered from different parts of the country) available at IINRG, Ranchi **(Table 1).** All the gum samples after manual cleaning and sorting were converted into fine powder and passed through 0.4 mm mesh sieve and packed in air tight containers for further analysis.

Table 1. Source wise distribution of gum samples tested for antimicrobial activity in the study.

Gum type	Place of collection	Serial numbers of samples collected
Gum Acacia	Sitapur, Uttar Pradesh	1
	Hissar, Haryana	2
	Karnal, Haryan	3
	Rohtak, Haryana	4
	Amritsar, Punjab	5
	Bundi, Rajasthan	6
	Bilaspur, Chattis Garh	7
	Balaghat, Madhya Pradesh	8
	Jabalpur, Madhya Pradesh	9
	Gondia-I, Maharashtra	10
	Mayurbhanj, Orrisa	11
	Anand, Gujarat	12
	Jodhpur, Rajasthan	13, 14
	Gondia-II, Maharashtra	15
	Merk, India	25
	Hi-Media, Mumbai	24
Gum Chironji	Bilaspur, Chattisgarh	16
	Simdega, Ranchi, Jharkhand	17
	ICAR-IINRG Farm, Ranchi, Jharkhand	18a
	Dindori , Madhya Pradesh	18b
	Umaria, Madhya Pradesh	19, 20
	Sitapur, Uttar Pradesh	21

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Gum *Balanites aegyptiaca*	Jodhpur, Rajasthan	22
Gum *Prosopis juliflora*,	Jodhpur, Rajasthan	23
Gum Karaya	Girijan Cooperative Corporation Ltd., Visakhaptnam, Andhra Pradesh	26-30

Ethanolic extracts of gums

To make Ethanolic extract (EE), 250 g of powdered gum was mixed in a 1 L conical flask with 500 ml of 99.9% pure ethanol (SD Fine Chem Ltd, Mumbai). Flasks were kept overnight at 25°C, over a rotary (30 rpm) platform. Thereafter, all the contents of flasks were filtered through glass wool filter to recover the filtrate. Filtrate was transferred to sterile shallow bowls and ethanol was allowed to evaporate at 50°C in an oven with exhaust overnight. Next morning all the dried material available in bowls was collected, weighed and kept in airtight screw capped vials at 4°C till tested within a month. To test the antimicrobial activity, discs of EE were prepared by dissolving 300 mg of EE in to 3 ml of ethanol and then adsorbing the solution on to 6 mm sterile discs (20 µl each), discs were allowed to dry at room temperature in desiccators and then stored in dry and sterile vials at 4°C till used within a month.

Aqueous extract (solution) of gums

To make gum solution in water (4% w/v), 2 gm of gum was added to 50 ml of sterile water in a sterilized conical flask and solubilized through keeping flasks in warm (50°C) shaking (50 rpm) water bath for 30 min. All gum solutions were tested on the same day for antimicrobial activity using agar well diffusion assay [16].

Bacterial strains

Four reference strains, sensitive to all commonly used antimicrobials (*Streptococcus milleri* SM-22; *Bacillus mycoides* B29-19-1; *E. coli* E-382; *Salmonella abortusequi* E-155), available in Epidemiology Laboratory of Indian Veterinary Research Institute, were revived from glycerol stocks and tested for purity and identity and maintained on nutrient agar slants without further sub-culturing throughout the study [17]. Besides, 97 isolates from clinical and environmental sources **(Table 2)** maintained in glycerol stocks and isolated during last three months at Clinical Epidemiology Laboratory of the Division, belonging to 24 species of 6 genera of Gram positive (34) bacteria (GPB) and to 27 species of 19 genera of Gram negative (63) bacteria (GNB) were included in the study to screen the antibacterial activity of EE and aqueous solutions of gums.

Table 2. Antimicrobial activity and minimum inhibitory concentration of alcoholic extracts of gum Acacia (*Acacia nilotica*) and gum Chironji (*Buchanania lanzan*) against bacterial strains of public health concern.

Bacteria tested (no. of strains tested)	Number of strains sensitive				MIC of EE in µg/ml		
	GA9	GA4	GC19	Cip10	GA9	GA4	GC
Acinetobacter baumannii (1)	1	1	1	1	1280	1280	2560
Actinobacillus actinomycetencomitans (1)	1	1	0	1	2560	2560	>2560
Aerococcus sanguinicola (1)	1	1	0	1	320	320	>2560
Aerococcus urinae (1)	1	1	0	1	320	320	>2560
Aeromonas bestiarum (6)	3	3	0	6	80->2560	80->2560	1280->2560
Aeromonas schubertii (1)	1	1	0	1	160	320	>2560
Aeromonas veronii (2)	2	2	2	2	160-640	160-640	1280-2560
Bacillus alvei (1)	1	1	0	1	160	160	>2560
Bacillus cereus (1)	1	1	0	1	80	80	>2560
Bacillus firmus (1)	1	1	0	1	320	320	>2560
Bacillus mycoides (4)	4	4	2	4	80-640	80-640	1280->2560
Bacillus sphaericus (1)	1	1	0	1	320	320	>2560
Bordetella bronchiseptica (1)	1	1	0	1	160	160	>2560
Brucella abortus (6)	6	6	1	5	160-640	160-640	640->2560
Citrobacter freundii (2)	1	1	0	2	1280->2560	1280->2560	>2560
Edwardsiella tarda (1)	0	0	0	1	>2560	>2560	>2560
Enterobacter agglomerans (3)	0	0	0	2	>2560	>2560	>2560
Enterococcus avium (1)	0	0	0	1	>2560	>2560	>2560
Enterococcus durans (1)	0	0	0	1	>2560	>2560	>2560

Enterococcus faecalis (1)	1	1	0	0	1280	1280	>2560
Erwinia caratovora (1)	1	1	0	1	640	640	>2560
Erwinia herbicola (1)	1	1	1	1	640	640	640
Escherichia coli (14)	1	0	0	12	1280->2560	>2560	>2560
Escherichia fergusonii (2)	0	0	0	1	>2560	>2560	>2560
Klebsiella oxytoca (1)	0	0	0	1	>2560	>2560	>2560
Klebsiella pneumoniae (4)	0	0	0	4	>2560	>2560	>2560
Micrococcus varians (3)	3	3	0	2	160	160	>2560
Moraxella osloensis (1)	1	1	0	1	80	80	>2560
Pasteurella canis (1)	1	1	0	1	80	80	>2560
Pragia fontium (1)	0	0	0	1	>2560	>2560	>2560
Proteus mirabilis (2)	1	1	0	1	320	640	>2560
Proteus vulgaris (1)	0	0	0	2	>2560	>2560	>2560
Providencia stuartii (1)	1	1	0	1	640	640	>2560
Pseudomonas aeruginosa (2)	0	0	0	2	>2560	>2560	>2560
Pseudomonas fluorescens (3)	0	0	0	3	>2560	>2560	>2560
Pseudomonas testosteronii (1)	1	1	0	1	1280	1280	>2560
Raoultella terrigena (3)	0	0	0	3	>2560	>2560	>2560
Salmonella abortusequi (1)	0	0	0	1	>2560	>2560	>2560
Salmonella typhimurium (1)	0	0	0	1	>2560	>2560	>2560
Staphylococcus aureus ssp. (1)	0	0	0	1	>2560	>2560	>2560
Staph. capitis ssp. *urealyticus* (1)	0	0	0	0	>2560	>2560	>2560
Staph. caseolyticus (1)	1	1	0	0	160	160	>2560
Staph. chromogenes (2)	2	2	0	1	160	160	>2560
Staph. felis (1)	1	1	0	1	160	160	>2560
Staph. haemolyticus (2)	1	1	0	1	160	160	>2560
Staph. intermedius (2)	1	1	0	1	160	160	>2560
Staph. hominis (1)	1	1	0	0	320	640	>2560
Streptococcus bovis (2)	1	1	0	2	160	160	>2560
Strept. dysgalactiae (1)	0	0	0	1	>2560	>2560	>2560
Strept. milleri (4)	2	2	1	4	1280->2560	1280->2560	>2560
Strept. equi ssp. *equisimilis* (1)	1	1	1	1	80	80	160
Strept. pyogenes (1)	1	1	0	1	160	160	>2560
Total (101) sensitive strains (%)	50 (49.5)	49 (48.5)	9 (8.9)	88 (87.1)	80 to >2560	80 to >2560	160 to >2560

EE, ethanolic extract (EE); GA9, 2 mg discs of gum Acacia EE sample number 9; GA4, 2 mg discs of gum Acacia EE sample number 4; GC19, 2 mg discs of gum Chronji EE sample number 19, Cip10, ciprofloxacin discs (10 µg).

Testing antimicrobial activity

Disc diffusion assay using EE discs was performed as described earlier [16,18] for different bacteria in triplicate on Mueller Hinton agar (MHA, BD BBL and Difco) plates, swab inoculated with overnight broth culture (0.1 OD590). All strains were tested for sensitivity on MHA but *Moraxella, Streptococcus, Brucella, Bordetella* and *Pasteurella* (slow growing, fastidious) strains were tested on Brain heart infusion (BHI) agar (BD BBL and Difco) instead of MHA [18]. All strains were tested at 37 °C aerobically except *Brucella*, which were incubated at 37 °C in 5% CO_2 enriched environment. Ciprofloxacin (10 µg) discs (BD BBL and Difco), and

blank discs (first soaked in ethanol and dried in similar way as for EE discs) were used as standard antimicrobial and negative control discs, respectively.

Determination of MIC of EE of gum samples

It was performed using agar well diffusion assay using suitable agar plates (MHA/ BHIA) having 9 wells bored in each plate [16]. Plates were swab inoculated with test strain as described for disc diffusion assay and then 50 µl of EE solution in dimethyl sulphoxide (DMSO, Merck India) was poured into each well. In first well DMSO without EE was used as negative control and in 2nd to 9th well 50 µl DMSO containing 20, 40, 80, 160, 320, 640, 1280 µg and 2560 µg of gum EE, respectively was poured in. Plates were incubated without inverting for 3 hr. so that contents of the well get diffused into the surrounding medium and then incubated as for disc diffusion assay. After 24 hr. of incubation plates were observed for inhibition of growth around the wells. The inhibition of growth around the wells with minimum EE was considered MIC of the EE of the gum. If no inhibition of bacteria was observed in a plate then MIC was recorded as >2560 µg/ml.

Statistical analysis

To determine correlation between diameter of zone of inhibition (in mm) of bacteria around EE discs and MIC, correlation coefficient was calculated using MS Office Excel-7. To estimate association between sensitivity of bacteria to EE of gums and species of bacteria, χ^2 test was performed in MS Office Excel-2007. The statistical comparison was done for only those genera of bacteria where number (n) of strains tested of the genus was ≥ 10.

RESULTS

None of the 101 strains including 4 reference (*Streptococcus milleri* SM-22; *Bacillus mycoides* B29-19-1; *E. coli* E-382; *Salmonella abortusequi* E-155) strains was inhibited by aqueous solution of any of the 31 gum samples. Discs of EE of only three gum samples (no. 4 and 9 of gum acacia and no. 19 of gum chironji) induced variable zone of growth inhibition **(Table 2).** All the three EEs inhibiting growth of bacteria failed to inhibit growth of both of the two reference GNB strains while both of the reference GPB strains were inhibited by all the three EEs having antimicrobial activity. Of the 101 strains, 50 strains (49.5%) were sensitive to EEA-9, 49 (48.5%) to EEA-4 and 9 (8.9%) to EEC-19. There was near to perfect (r=0.87) correlation between inhibition zones caused by EEA-4 and EEA-9 discs for different bacteria.

Including 4 reference strains, a total of 88 (87.1%) strains were sensitive to ciprofloxacin disks. Of the 36 GPBs tested, 26 were sensitive to EEA-4 and EEA-9, 4 to EEC-19 and 28 to ciprofloxacin disks. Of the 65 GNB strains tested, 60, 24, 23 and 5 were sensitive to ciprofloxacin, EEA-9, EEA-4, and EEC-19 disks, respectively.

Sensitivity to EEA was more common among GPBs (p=0.0007) than GNBs but no significant difference was evident for EEC (p=0.56). Ciprofloxacin resistance was more (p=0.04) common in GPB than in GNB strains.

Minimum inhibitory concentration (MIC) for EEA from gum acacia sample no. 4 and 9 for sensitive strains ranged between 80 µg to 1280 µg/ml for GNBs and 80 µg to 640 µg for GPBs **(Table 2).** For sensitive strains MIC was minimum (80 µg/ml) for *Aeromonas bestiarum*, *Moraxella osloensis*, *P. canis* and *Streptococcus equi* and was maximum (2560 µg/ml) for *Actinobacillus actinomycetencomitans* strains. MIC of EEC-19 was the lowest (160 µg) for *Strept.* equi and maximum (2560 µg/ml) for *Acinetobacter baumanii* strains, and ranged between 640 µg to 1280 µg/ml for other sensitive strains. For all the strains resistant to EEA and EEC MIC was >2.56 mg/ml **(Table 2).**

DISCUSSION

Natural edible gums though reported to be antimicrobial in some reports [5,6], are generally considered devoid of antimicrobial activity [1]. In presented study three of the 31 gum samples had detectable antibacterial activity in concurrence of earlier observations [1]. Variations reported in composition of different gum samples [1,3,4] might be responsible for antimicrobial activity in EE of three gum samples and not in other gum samples. Antibacterial activity only in alcoholic extracts but not in aqueous solution of gums was in agreement to earlier studies [5,6,11,19-22]. The study also revealed that EE of acacia gum sample no. 9 (from Jabalpur) was a little better in inhibiting growth of 50 strains of bacteria than EE of acacia gum sample no. 4 from Rohtak (inhibiting only 49), but the difference was statistically insignificant and MIC studies also indicated the similar MIC of both the EEAs for different bacteria. The difference in antimicrobial activity of gum acacia samples cannot be explained on the basis of source (geographical region) as in the same region samples without antimicrobial activity were available. The difference might be attributed to individual plant source but it needs further studies for verification and elucidation of exact reason of variation.

Wide spectrum of activity of EE of gum acacia against 50 strains of bacteria belonging to 35 species of GPBs and GNBs both indicated presence of a promising antimicrobial compound in gum acacia. In earlier studies gum acacia extracts are reported to inhibit growth of 12 reference strains of *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *P. aeroginosa*, *Proteus merabilis*, *Acinetobacter*, *Enterobacter*, *Klebsiella pneumoniae*, *Serratia* spp., *E. coli*, *Salmonella typhi*, *Candida albicans* [6] and reference strains of *Bacillus cereus*, *E. coli*, *Aspergillus niger* and *Cercospora pongamiae* [23] indicating wide spectrum of its antimicrobial activity. However, this study seems to be first to report the antimicrobial activity of gum acacia extracts on clinically important bacteria. Moreover,

from earlier studies [6,23] variation in sensitivity of different strains of the same bacteria was not apparent, this study elucidated that only small fraction of *E. coli*, *Proteus*, *Aeromonas*, *Staphylococcus* and *Streptococcus* strains was susceptible to antimicrobial action of gum acacia. The present study suggested that conclusion based on studies using only a few reference strains may not be always practically useful. Further, detection of sensitivity in a few strains of a bacteria or resistance in a few strains of other bacteria indicated that there might be some mechanism for emergence of resistance against herbal antimicrobials as proposed earlier [16,24-26].

Antimicrobial activity in gum chironji or its extract has not been reported earlier [1,19]. In the present study one of the 7 gum samples had some antimicrobial potential that too against a few strains of *Aeromonas veronii*, *B. mycoides*, *Brucella abortus* and *S. milleri* indicating its weak antimicrobial potential.

Lack of detection of any antimicrobial activity in gum samples of *Sterculia* (Karaya), *Balanites aegyptiaca* and *Prosopis juliflora* is in concurrence of the earlier studies [1,11,12,27-29]. It might be either due to the absence of antimicrobial potential in those edible gums or due to less number of samples analyzed.

All the three gum samples having antimicrobial activity in their alcoholic extract were from three different regions **(Table 1)** but from the same or nearby regions other samples of the same gum were negative for antimicrobial potential. For the observation no reason could be assigned on the basis of present study and it needs to be explored further.

Though edible gums may not be considered useful antimicrobials for therapeutic use [1], utility of gums in green synthesis of nanoparticles, nano-fibers for antimicrobial scaffolds useful in dressing and nano-capsules for drug delivery or membranes of packaging is emerging fast [27,30-34]. Moreover, further studies may lead to identification of active antimicrobial component of gum which might be a potential one.

CONCLUSION

Although, antimicrobial activity in acacia gum has wide spectrum, was detected in only two of the 17 samples. Similarly, only one of the 7 gum chironji samples possessed a little antimicrobial activity. To elucidate the reasons behind antimicrobial potential only in a few gum samples and for identification of active antimicrobial component in edible gum samples need further studies. Moreover, identification of the active antimicrobial substance in alcoholic extracts of gum acacia and gum chironji might be important area of further research.

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