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Research Article

Comparative Antimicrobial Potential of Green Synthesized Sliver-nano-particles Using Three Different Natural Gums on Potentially Pathogenic Microbes from Veterinary Clinical Cases and Associated Environment

Bhoj R Singh[1]*, Dharmendra K Sinha[1], Mahtab Z Siddiqui[2], Arnab Roy Chowdhury[3], Ravichnadran Karthikeyan[1], BS Pruthvishree[1], Akanksha Yadav[1], Varsha Jayakumar[1] and Himani Agri[1]

[1]Division of Epidemiology, ICAR-Indian Veterinary Research Institute, India

[2]Ex-Principal Scientist, ICAR-National Institute of Secondary Agriculture (formerly ICAR-Indian Institute of Natural Resins and Gums) Namkum, Ranchi, India

[3]Senior Scientist, Downstream Agro-Processing Division, ICAR-National Institute of Secondary Agriculture (formerly ICAR-Indian Institute of Natural Resins and Gums) Namkum, Ranchi, India

***Corresponding Author:** Bhoj R Singh, Division of Epidemiology, ICAR-Indian Veterinary Research Institute, India.

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Abstract

Metallic silver, its compounds and nano-particles (SNPs) are known for their wide-spectrum antimicrobial potential. However, little is understood about the best material for the green synthesis of SNPs. This study was conducted to find out the best of the three natural gums viz., piyar or chironji (*Buchanania lanzan*) gum, gum acacia (*Acacia nilotica*) and jhingan (*Lannea coromandelica*) gum used for green synthesis of SNPs and to evaluate the effect of aspirin, flunixin meglumine and pacetamol on *in vitro* antimicrobial activity of SNPs. The study was conducted on 376 Gram-negative (G-ve), 184 Gram-positive (G+ve) bacteria and 7 yeasts and moulds, belonging to 41 genera. The test strains were from clinical cases (392), environmental sources (120) and, reference culture (55) repositories. The MIC of silver-nano-particles for 567 isolates of bacteria and yeast and moulds was 1.6 ± 2.03, 2.13 ± 2.57, 1.46 ± 1.87, and 1.35 ± 1.85 µg of silver mL^{-1} in MHB when the source of silver in the medium was PGNP, AGNP, JGNP and silver nitrate, respectively. Strains of different origins had a wide difference in MIC of silver, 10 of the 12 strains having MIC of silver >4 µg mL^{-1} were detected from herbivores and one each was from a dog and a fish. Of the three SNPs, SNPs synthesized using gum acacia (AGNP) had the least antimicrobial activity ($p < 0.01$) while SNPs synthesized using jhingan gum (JGNPs) were the most effective among all the three SNPs. Significantly ($p \leq 0.05$) larger number of G-ve bacteria were resistant to SNPs than G+ve bacteria if susceptibility limit was set at 0.25 or 0.5 µg mL^{-1} but no such difference was evident at 1 or 2 µg mL^{-1} concentration of silver. Among strains of 17 different genera compared, *R. terrigena* isolates were significantly ($p < 0.05$) more often resistant than isolates of most of the other bacteria (*Acinetobacter, Aeromonas, Alcaligenes, Bacillus, Enterococcus, Escherichia, Klebsiella, Paenibacillus, Pasteurella, Salmonella, Staphylococcus, Streptococcus*) at both of the silver MIC levels i.e., at 0.25 µg mL^{-1} and 1.0 µg mL^{-1}. All isolates of *Alcaligenes* spp. were susceptible to silver at 1.0 µg mL-1. The MIC of flunixin meglumine for the test organisms was higher (5.58 ± 4.32 mg mL^{-1}) than the MIC of aspirin (2.75 ± 2.40 mg mL^{-1}) and paracetamol failed to inhibit any of the test microbial strains and MIC was always at higher side (≤5.12 mg mL^{-1}). Aspirin and flunixin meglumine in the test medium significantly ($p < 0.05$) reduced and paracetamol increased the MIC of AGNPs. The JGNPs had the best antimicrobial activity thus jhingan gum may be better option for green synthesis of SNPs. Two NASIDs, aspirin and flunixin meglumine, decreased while paracetamol increased the MIC of SNPs and finding may be useful in formulating creams/ ointments of SNPs for topical application.

Keywords: Silver Nano-particles (SNPs); Aspirin; Flunixin; Paracetamol; *Buchanania lanzan; Acacia nilotica; Lannea coromandelica*

Introduction

Silver and other heavy metals and their compounds are known for several health benefits including their antimicrobial activity. Silver often used as silver nitrate or silver sulfadiazine is an effective antimicrobial against wide spectrum of microbes; however, the colouring effect of silver nitrate and silver containing preparations on the area of application is always a problem [1,2]. Silver in form of nitrate or with sulfadiazine in creams is often used in dressings for wound specially the burns and ulcers [3]. Due to toxic nature of heavy metals, development of metallic nanoparticles (NPs) being safer and applicable in numerous fields, including biomedical sciences and engineering have gained impetus in last two decades

[2,4,5]. It resulted to development of numerous protocols for synthesis of nontoxic metal NPs specially the biosynthetic or green methods for this purpose [6,7]. For green synthesis, numbers of natural gums have been used including gum acacia, cashew gum, gum kondagogu, gum olibanum, gum karaya, gum tragacanth, gum ghatti, neem gum, gum acacia, piyar gum [8]. Silver nanoparticles (SNPs) have shown an excellent broad spectrum antimicrobial activity *in vitro* and *in vivo* against G+ve and G-ve bacteria causing infections [7-9]. Silver nano-particles trigger the oxidative stress, protein dysfunction, and membrane and DNA damage, leading to microbial cell damage [2,10].

Green fabricated silver-nanoparticles (SNPs) using different natural ingredients have shown a wide range of inhibitory activity varying significantly, the difference might be due to testing at different labs on different sets of a few microbial strains often from reference repositories [7-9,11-13]. Earlier studies have indicated that SNPs made through green synthesis process retain good antimicrobial activity; however, none of the earlier study compared the SNPs made of using different natural products to find out the best material to be used for preparing SNPs. Further, antimicrobials including antibiotics and others are often used in therapeutics along with anti-inflammatory drugs but no study revealed the possible effect of anti-inflammatory drugs on antimicrobial activity of SNPs. The present study was conducted to fill the gap in the knowledge with the objective to find out the best natural gum to be used for green synthesis of SNPs and also to evaluate the effect of most commonly used non-steroidal anti-inflammatory drugs (NSAIDs) on *in vitro* antimicrobial activity of SNPs. The present study was conducted on comparatively big number of microbial strains both of clinical origin and reference strains.

Materials and Methods

Silver nano-particles (SNPs)

Natural plant gums piyar (Chironji, *Buchanania lanzan*) (PGNP), acacia (*Acacia nilotica*) (AGNP) and jhingan (*Lannea coromandelica*) (JGNP) induced SNPs were synthesized adopting green synthetic methodology and well characterized using modern analytical techniques [8,9] have been used for their comparative antimicrobial potential on a wide array of pathogenic microbes from veterinary clinical cases and associated environment, for this study. All the SNPs were stored at 4°C till tested.

Microbial strains

A total of 567 microbial isolates (376 Gram-negative, 184 Gram-positive bacteria and 7 yeasts and moulds) belonging to 41 genera (Table 1) were revived from glycerol stocks of microbial isolates. Microbial isolates were from clinical cases (392), from environmental sources (120) isolated in past two years at the Clinical Epidemiology Laboratory, Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar, India. Besides, 55 reference strains (Table 2) were also included in the study. All the revived cultures were tested for purity and identity using standard protocols [14,15] and maintained on nutrient agar slant till the end of the study, at 4°C.

Genus of bacteria, yeast and moulds	Species, number of strains tested	Total strains tested	Average MIC in µg mL^{-1} (standard deviation)			
			PGNP	AGNP	JGNP	$AgNO_3$
Acinetobacter	*A. calcoaceticus* 5, *A. ewoffli* 1, *A. haemoticus* 6, *A. lwoffii* 4, *A. scindleri* 2	18	1.07 (0.66)	1.31 (0.84)	0.96 (0.64)	0.87 (0.67)
Aerococcus	*Aerococcus christensenii* 2	2	0.31 (0.27)	0.31 (0.27)	0.31 (0.27)	0.31 (0.31)
Aeromonas	*A. bestiarum* 6, *A. caviae* 1, *A. eucranophila* 2, *A. hydrophila* 3, *A. jandaei* 3, *A. miedia* 3, *A. popoffii* 3, *A. salmonicida* 3, *A. schubertii* 8, *A. trota* 2	34	1.74 (2.66)	2.44 (3.18)	1.77 (2.66)	1.59 (2.66)
Alcaligenes	*A. denitrificans* 1, *A. faecalis* 10	11	0.35 (0.280	0.60 (0.56)	0.32 (0.31)	0.29 (0.30)
Arsenophonus	*A. nasoniae* 1	1	1.00	2.00	1.00	1.00
Aspergillus	*A. flavus* 1	1	0.06	0.06	0.06	0.06
Bacillus	*B. badius* 1, *B. cereus* 5, *B. coaggulans* 1, *B. licheniformis* 1, *B. megaterium* 2, *B. mycoides* 2, *B. subtilis* 1, *B. thurigiensis* 1	14	1.37 (1.05)	1.71 (1.170`	1.28 (1.05)	1.08 (1.03)
Brucella	*B. abortus* 2	2	1.00	2.00	1.00	1.00
Burkholderia	*B. cepacia*	1	1.00	1.00	1.00	1.00
Candida	*Candia spp.* 4, *C. albicans* 2	6	2.42 (2.80)	2.33 (1.37)	1.13 (0.74)	0.96 (0.60)
Citrobacter	*C. freundii* 2	2	1 (0.0)	1.5 (0.71)	1 (0.0)	2 (0.0)
Edwardsiella	*E. hoshniae* 1, *E. tarda* 3	4	2.25 (1.26)	3.00 (1.15)	2.25 (1.260	2.25 (1.261
Enterobacter	*E. gregoviae* 1	1	2.00	2.00	2.00	2.00
Enterococcus	*E. durans* 2, *E. faecalis* 8, *E, faecium* 11, *E. mundtii* 1, *E. solitarus* 4	26	1.53 (1.45)	1.71 (0.71)	1.24 (0.62)	1.16 (0.68)

Erwinia	*E. amylovora* 2, *E. aphidicola* 1, *E. caeotovora* 1, *E. mallotivora* 1, *E. nimipresuuralis* 1, *E. stewartii* 1, *E. tasmaniensis* 1	8	1.05 (0.69)	1.19 (0.74)	0.92 (0.57)	0.84 (0.62)
Escherichia	*E. coli* 90, *E. fergusonii* 4, *E. hermanii* 1	95	1.76 (2.49)	2.12 (2.950	1.60 (2.10)	1.42 (1.98)
Falvimonas	*F. oryzihabitans* 2	2	0.53 (0.66)	1.25 (1.06)	0.31 (0.270	0.31 (0.27)
Flexibacter	*Flexibacter spp.*	2	0.75 (0.35)	0.75 (0.35)	0.75 (0.35)	0.63 (0.53)
Gallibacterium	*G. anatis* 4	4	0.47 (0.39)	0.78 (0.83)	0.47 (0.39)	0.42 (0.43)
Gardnerella	*Gardnerella spp.* 1	1	1.00	1.00	1.00	1.00
Geobacillus	*G. stearothermophilus* 7	7	0.79 (0.62)	0.82 (0.59)	0.79 (0.62)	0.69 (0.69)
Hafnia	*H. alvei*	12	1.64 (1.32)	1.73 (1.29)	1.64 (1.32)	1.61 (1.35)
Klebsiella	*K. oxytoca* 3, *K. ozaenae* 1, *K. neumoniae* 27	31	2.23 (2.84)	2.75 (3.71)	2.07 (2.81)	1.92 (2.85)
Lysinibacillus	*L. sphaericus* 2	2	0.19 (0.09)	0.16 (0.13)	0.25 (0.0)	0.09 (0.4)
Micrococcus	*M. luteus* 1	1	0.13	0.25	0.13	0.06
Moelerella	*M. wisconsensis* 1	1	2.00	2.00	2.00	2.00
Moraxella	*M. bovis,* 1, *M. canis* 1, *M. osloensis* 1, *M. phenyl-pyruvica* 1	4	0.56 (0.31)	0.56 (0.31)	0.50 (0.35)	0.44 (0.41)
Paenibacillus	*P. amylolyticus* 1, *P. pantothenticus* 13	14	1.17 (1.35)	1.17 (1.35)	1.17 (1.35)	1.09 (1.40)
Pantoea	*P. agglomerans* 16	16	0.75 (0.67)	0.97 (0.66)	0.71 (0.70)	0.65 (0.73)
Pasteurella	*P. aerogenes* 1, *P. canis* 12, *P. multocida* 2	15	2.34 (3.21)	2.79 (3.08)	2.33 (3.21)	2.30 (3.23)
Proteus	*P. mirabilis* 10, *P. penneri* 2, *P. vulgaris* 2	14	2.02 (1.48)	2.59 (1.18)	1.98 (1.52)	1.82 (1.58)
Providencia	*P. stuartii* 3	3	0.52 (0.47)	1.02 (0.97)	0.52 (0.47)	0.52 (0.47)
Pseudomonas	*P. aeruginosa* 11, *P. alcaligenes* 1, *P. diminuta* 1, *P. pseudomobilis* 1	15	1.48 (1.01)	1.58 (0.93)	1.34 (1.00)	1.30 (1.03)
Raoultella	*Raoultella terrigena*	16	4.28 (4.910	4.25 (4.71)	3.88 (4.89)	3.75 (4.92)
Roseomonas	*Roseomonas spp.*	1	0.25	0.25	0.25	0.13
Salmonella	*S.* 6,7:- 1, *S. abortusequi* 1, *S. Enteritidis* 1, *S. Gallinarum* 4, *S. Illinois* 1, *S. indica* 1, *S. Infantis* 1, *S. Kentcky* 3, *S. Miyazaki* 1, *S. Naestved* 1, *S. Paratyphi A* 1, *S. Pullorum* 2, *S. Typhi* 1, *S. Typhimurium* 16, *S. Virchow* 11	46	1.81 (1.25)	4.00 (3.33)	1.45 (0.90)	1.34 (0.81)
Serratia	*S. fonticola* 1, *S. grimesii* 1, *S. marcescens* 2, *S. odorifera* 7	11	2.59 (2.73)	3.77 (4.49)	2.05 (2.05)	2.02 (2.08)
Staphylococcus	*S. arlettae* 1, *S. aureus* 11, *S. auricularis* 1, *S. capitis* 5, *S. capriae* 1, *S. carnosus* 1, *S. caseo-lyticus* 1, *S. chromogenes* 4, *S. delphini* 5, *S. epidermidis* 15, *S. equorum* 3, *S. gallinarum* 3, *S. haemolyticus* 13, *S. hominis* 7, *S. hyicus* 2, *S. intermedius* 4, *S. lentus* 5,*S. lugdunerisii* 6, *S. schleiferi* 2, *S. sciuri* 2, *S. simulans* 1, *S. xylosus* 2	95	1.29 91.01)	1.62 (1.37)	1.22 (1.00)	1.12 (1.01)
Stenotrophomonas	*S. maltophilia* 1	1	0.25	0.50	0.25	0.25
Streptococcus	*S. milleri* 14, *S. phocae* 1, *S. pneumoniae* 4, *S. porcinus* 1, *S. pyogenes* 1, *S. salivaris* 2	23	1.47 (1.75)	2.63 (2.85)	1.44 (1.86)	1.29 (1.78)
Xenorhabdus	*X. bovienni* 3, *X. luminescens* 1	4	0.78 (0.44)	0.77 (0.47)	0.81 (0.38)	0.77 (0.47)
Gram negative bacteria		376	1.74 (2.31)	2.35 (2.93)	1.60 (2.15)	1.48 (2.12)
Gram positive bacteria		184	1.30 (1.20)	1.67 (1.58)	1.21 (1.12)	1.10 (1.11)
Yeast and moulds		7	2.08 (2.71)	2.01 (1.51)	0.97 (0.78)	0.83 (0.64)
All microbes tested		567	1.60 (2.03)	2.13 (2.57)	1.46 (1.87)	1.35 (1.85)

Table 1: Average (standard deviation) minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of silver nitrate ($AgNO_3$) for different categories of microbes.

Source of microbes (number of strains) tested	Silver preparation	Number of strains with MIC µg mL^{-1} of silver (standard deviation)					
		≤0.25	0.5	1	2	4	>4
Birds (70)	PGNP	13	30	48	68	70	0
	AGNP	10	26	45	68	70	0
	JGNP	15	33	51	68	70	0
	$AgNO_3$	27	33	51	68	70	0
Buffalo (62)	PGNP	9	19	30	51	54	8
	AGNP	3	8	21	36	50	12
	JGNP	9	25	38	54	57	5
	$AgNO_3$	11	29	39	55	57	5
Carnivores in zoos and wildlife sanctuaries (41)	PGNP	3	7	17	35	41	0
	AGNP	1	7	17	35	41	0
	JGNP	3	7	17	35	41	0
	$AgNO_3$	4	7	22	35	41	0
Cattle (38)	PGNP	12	19	27	34	37	1
	AGNP	7	14	23	32	36	2
	JGNP	16	22	27	34	37	1
	$AgNO_3$	17	22	30	34	37	1
Dog (77)	PGNP	7	14	42	69	76	1
	AGNP	4	10	28	67	76	1
	JGNP	8	16	42	69	76	1
	$AgNO_3$	12	16	52	71	76	1
Environment (120)	PGNP	18	36	79	113	120	0
	AGNP	15	34	77	108	116	4
	JGNP	15	29	92	112	119	1
	$AgNO_3$	21	43	94	115	120	0
Fish (4)	PGNP	0	0	2	0	3	1
	AGNP	0	0	1	2	3	1
	JGNP	0	1	1	2	3	1
	$AgNO_3$	0	1	1	2	3	1
Sheep and Goats (8)	PGNP	1	3	3	6	7	1
	AGNP	0	3	3	6	7	1
	JGNP	1	3	3	7	7	1
	$AgNO_3$	1	3	3	7	7	1
Herbivores in zoos and wild-life sanctuaries (19)	PGNP	8	11	13	14	16	3
	AGNP	7	8	11	14	16	3
	JGNP	8	10	13	14	16	3
	$AgNO_3$	8	11	13	14	16	3
Human (64)	PGNP	20	30	44	57	64	0
	AGNP	15	28	33	53	63	1
	JGNP	20	30	44	57	64	0
	$AgNO_3$	23	30	45	57	64	0
Lab animals (2)	PGNP	1	1	2	2	2	0
	AGNP	1	1	2	2	2	0
	JGNP	1	1	2	2	2	0
	$AgNO_3$	1	1	2	2	2	0

Pig (7)	PGNP	2	2	3	7	7	0
	AGNP	0	2	3	7	7	0
	JGNP	2	2	3	7	7	0
	$AgNO_3$	2	2	3	7	7	0
Reference (55): *Brucella abortus* 2, *Candida albicans* 2, *Escherichia coli* 8, *Pasteurella multocida* 1, *Salmonella enterica* ssp. e*nterica* 38, *Staphylococcus aureus* 3, *Staphylococcus epidermidis* 1	PGNP	1	5	29	52	53	2
	AGNP	0	4	10	26	48	7
	JGNP	2	10	38	54	55	0
	$AgNO_3$	2	10	41	55	55	0
Herbivores (127)	PGNP	30	52	73	105	113	14
	AGNP	17	33	58	88	109	18
	JGNP	34	60	81	109	117	10
	$AgNO_3$	37	65	85	110	117	10
Carnivores (118)	PGNP	10	21	59	104	117	1
	AGNP	5	17	45	102	117	1
	JGNP	11	23	59	104	117	1
	$AgNO_3$	16	23	74	106	117	1
Omnivores (147)	PGNP	36	63	99	136	146	1
	AGNP	26	57	84	132	145	2
	JGNP	38	67	101	136	146	1
	$AgNO_3$	53	67	102	136	146	1
All (567)	PGNP	95	177	339	510	549	18
	AGNP	63	145	274	456	535	32
	JGNP	100	189	371	515	554	13
	$AgNO_3$	129	208	396	522	555	12

Table 2: The minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP), Jhingan (*Lannea coromandelica*) gum (JGNP) and silver nitrate ($AgNO_3$) for from different sources.

Source of reference strains: *Brucella abortus* from National *Brucella* Centre; *Candida albicans,* from Mycology laboratory, all *Salmonella* and *Escherichia coli* from National *Salmonella* (Vet) Centre, *Staphylococcus aureus* ATCC700699, ATCC29312, ATCC43300, available in the Epidemiology Laboratory. *Pasteurella multocida* from National *Pasteurella* Reference, ICAR-Indian Veterinary Research Institute, Izatnagar-243 122, India, and *E. coli* MTCC723 and *Staphylococcus epidermidis* MTCC1425, from Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

Antimicrobial assays for determining minimum inhibitory concentration (MIC)

The micro-dilution assay in 96-well plates was used to estimate the antimicrobial activity of the green synthesized silver nano-particles (SNPs). *Staphylococcus aureus* (ATCC43300, MIC 1 µg of silver mL^{-1}), and *Escherichia coli* (MTCC723, MIC 1 µg of silver mL^{-1}), strains available in the laboratory were used as control test strains for Gram-positive and Gram-negative bacteria, respectively. The test microbes were grown from six colonies of pure culture to avoid any culturing error [16] in Mueller Hinton broth (MHB) and adjusted to the turbidity of 0.1 at 590 nm (OD_{590}). Serial dilutions of silver-nano-particles and silver-nitrate were made in 100 µL of MHB in each well of 96 well tissue culture plates to contain 16, 8, 4, 2, 1, 0.5, 025, 0.125, 0.0625, 0.031, 0.016 and 0.00 µg of silver mL^{-1}. Thereafter, each well was inoculated with 2 µL of test culture adjusted to 0.1 OD_{590}. Each microbe was tested in duplicate and in each plate appropriate control culture was included and one row was kept as un-inoculated control. After 24 h of incubation at 37°C plates were read for visible turbidity and any turbidity was considered no inhibition of growth. The highest dilution of silver-nano-particles able to inhibit the growth of the test microbe was recorded as the MIC of SNPs.

For determining MIC of asprin (acetylsalicylic acid), paracetamol (acetaminophen) and flunixin meglumine (Merc India) similar procedure described earlier [17] and as was adopted as for SNPs except that the starting concentration was kept at 10.24 mg mL^{-1} (for flunixin), 5.12 mg mL^{-1} (for aspirin and paracetamol), and serially diluted to achieve concentration of 2.56, 1.28, 0.64, 0.32, 0.16, 0.08, 0.04, 0.02, 0.01 and 0.0 mg mL^{-1}.

To assess the effect of asprin, paracetamol and flunixin meglumine on MIC of silver-nano-particles synthesized through green synthesis using acacia gum (AGNP), only those microbes having

≥ 1.28 mg mL^{-1} MIC of aspirin, paracetamol and flunixin were included to test the interaction. The MIC of AGNP was determined for selected microbial strains in absence (0.0 mg mL^{-1}) and presence of varying concentration of aspirin, paracetamol and flunixin (1.28-0.1 mg mL^{-1}) as described above in 96 well microplates.

Statistical analysis

The MIC data of microbial strains was entered in an Excel sheet and analyzed using appropriate statistical tools.

Results

The MIC of silver-nano-particles for 567 clinical isolates of bacteria and yeast and moulds was 1.6 ± 2.03, 2.13 ± 2.57, 1.46 ± 1.87, and 1.35 ± 1.85 µg of silver mL^{-1} in MHB when source of silver in the medium was PGNP, AGNP, JGNP and silver nitrate, respectively. However, MIC varied for different group of microbes (Table 1). The MIC of silver in all SNPs and silver nitrate was minimum (0.016 µg mL^{-1}) for a strain of *Staphylococcus hominis* isolated from heart blood of a bird died of septicaemia and was maximum (16 µg mL^{-1}) for one strain each of *Aeromonas trota* (from necrotic lesion of a fish), *Escherichia coli* (from diarrheic faeces of a goat kid), *Klebsiella pneumoniae* ssp. *pneumoniae* (from mastitis in buffalo) and two strain of *Raoultella terrigena* causing mastitis in buffaloes. Strains of different origin (Table 2) had a wide difference in MIC of silver, 10 of the 12 strains having MIC of silver (from silver nitrate) >4 µg mL^{-1} were detected from herbivores including isolates from buffaloes 5, cattle 1, goat 1, spotted deer 3 and rest two, one each from a dog and a fish.

Of the three SNPs, SNPs synthesized using gum acacia (AGNP) had the least antimicrobial activity ($p < 0.01$) at both of the concentration i.e., 0.25 µg mL^{-1} and 1.0 µg mL^{-1} inhibiting only 11.11% and 48.32% of the microbial strains, respectively. The SNPs synthesized using jhingan gum (JGNPs) were the most effective among all the three SNPs as antimicrobials inhibiting 17.64% and 64.43% of the tested microbes at level of 0.25 µg mL^{-1} and 1% µg mL^{-1}co-centration of silver, respectively. The SNPs made using piyar gum (PGNPs) stood in between of the two, the least active AGNPs and the most active JGNPs, inhibiting 16.75% and 59.79% of the microbial strains tested at 0.25 µg mL^{-1} and 1.0 µg mL^{-1}concentrations, respectively. At 0.25 µg mL^{-1} level of silver concentration all the three SNPs were significantly ($p< 0.04$) less inhibitory to microbes than silver in nitrate form but at 1.0 µg mL^{-1} level of silver, JGNPs though having a bit less activity, not differed significantly ($p >0.05$) in antimicrobial activity from $AgNO_3$. Though at 0.25 µg silver mL^{-1} level antimicrobial activity of PGNPs and JGNPs not differed significantly ($p>0.05$), at 1.0 level JGNPs had better antimicrobial activity than of PGNPs ($p < 0.05$) against tested microbial strains.

Of the 12 isolates having MIC of silver (from silver nitrate) >4 µg mL^{-1}, 11 were G-ve bacteria; 1 *A. trota* from fish, 3 *E. coli* one each from diarrhoeic goat, dog and cattle, 1 *K. pneumoniae* ssp. *pneumoniae* from buffalo with mastitis, 3 *Pasteurella canis* from septicemic spotted deer, 2 *R. terrigena* from buffalo with mastitis and 1 *Serratia odorifera* causing mastitis in buffalo and only one was an isolate of *Streptococcus milleri* from a case of buffalo mastitis.

Significantly ($p \leq 0.05$) larger number of Gram -ve bacteria were resistant to all types of SNPs and silver nitrate than G+ve bacteria if the criteria of susceptibility was set either 0.25 or 0.5 µg mL^{-1} but no such difference was evident if the susceptibility limit was set at 1 or 2 µg per mL. Also, no such difference was evident between bacteria and yeast and moulds' strains (Table 3).

Type of Microbes (number of strains)	Type of silver preparation	Percent of strains susceptible at MIC equivalent to µg of silver mL^{-1} of growth medium										
		16	8	4	2	1	0.5	0.25	0.125	0.063	0.031	0.016
Gram-negative bacteria (376)	PGNP	100.00	98.40	96.01	88.30	58.78	28.72	13.83	6.65	3.99	0.00	0.00
Gram-positive bacteria (184)	PGNP	100.00	100.00	98.91	93.48	61.96	36.41	22.83	11.41	7.07	1.09	0.54
Candida and *Aspergillus* (7)	PGNP	100.00	100.00	85.71	85.71	57.14	28.57	14.29	14.29	14.29	0.00	0.00
Gram-negative bacteria (376)	AGNP	100.00	97.07	92.82	78.46	46.28	21.81	8.78	3.46	2.66	0.00	0.00
Gram-positive bacteria (184)	AGNP	100.00	100.00	97.28	84.78	52.72	33.70	15.76	7.07	5.43	1.09	0.54
Candida and *Aspergillus* (7)	AGNP	100.00	100.00	100.00	71.43	42.86	14.29	14.29	14.29	14.29	0.00	0.00
Gram-negative bacteria (376)	JGNP	100.00	98.67	96.81	89.36	64.63	30.32	14.36	8.24	4.79	0.00	0.00
Gram-positive bacteria (184)	JGNP	100.00	100.00	99.46	93.48	66.85	39.13	23.91	13.04	8.70	1.09	0.54
Candida and *Aspergillus* (7)	JGNP	100.00	100.00	100.00	100.00	71.43	42.86	28.57	14.29	14.29	0.00	0.00
Gram-negative bacteria (376)	AgNO3	100.00	98.67	97.07	90.96	68.62	33.78	18.09	13.30	7.18	0.00	0.00
Gram-positive bacteria (184)	$AgNO_3$	100.00	100.00	99.46	94.02	71.74	42.39	32.07	20.65	12.50	1.09	0.54
Candida and *Aspergillus* (7)	$AgNO_3$	100.00	100.00	100.00	100.00	85.71	42.86	28.57	14.29	14.29	0.00	0.00

Table 3: Per cent susceptibility of different groups of microbes at different minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) and silver nitrate ($AgNO_3$).

The distribution of bacterial strains of different genera according to MIC of silver nitrate was a little skewed. For G-ve (Figure 1), G+ve (Figure 2) bacteria, *Aeromonas* ssp. (Figure 3), *Salmonella enterica* (Figure 4) and *Staphylococcus* spp. (Figure 5) isolates median value for MIC of silver using $AgNO_3$ and JGNP showed a shift to right (lower MIC). The right shift in median was noted for AGNP and PGNP silver MIC among isolates of *Enterococcus* spp. (Figure 6) while the right shift was evident among *Klebsiella* spp. isolates (Figure 7) for PGNP and $AgNO_3$ silver, but for *Escherichia* spp. isolates (Figure 8) the median was at the same level and distribution looked quite normal.

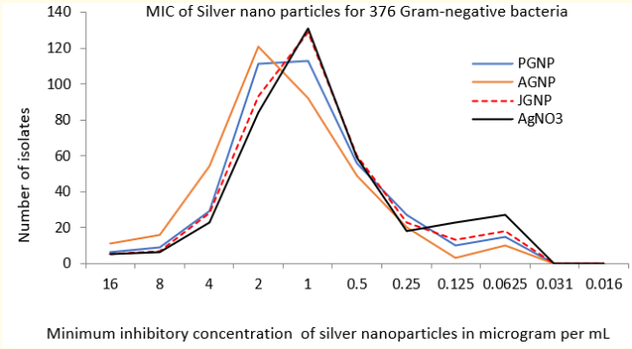

Figure 1: Distribution of strains of Gram-negative bacteria according to average minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of silver nitrate ($AgNO_3$).

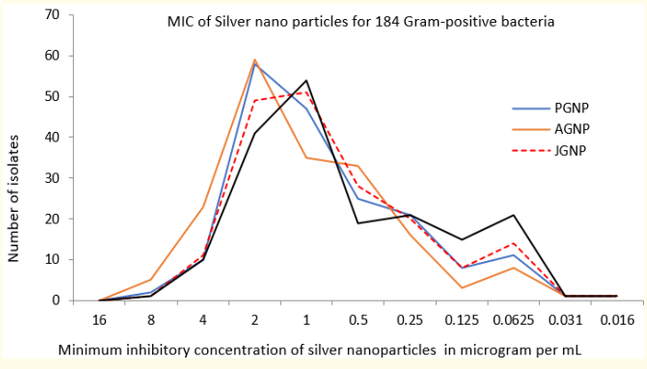

Figure 2: Distribution of strains of Gram-positive bacteria according to average minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of silver nitrate ($AgNO_3$).

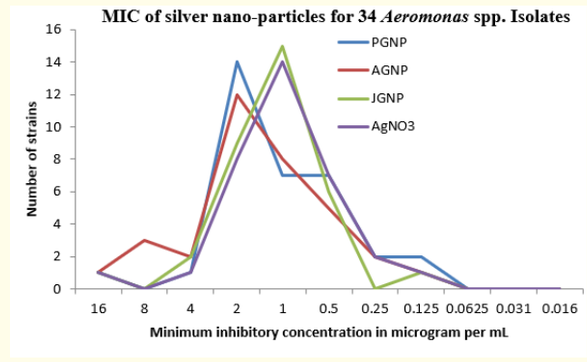

Figure 3: Distribution of strains of 34 *Aeromonas* spp. isolates according to average minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of silver nitrate ($AgNO_3$).

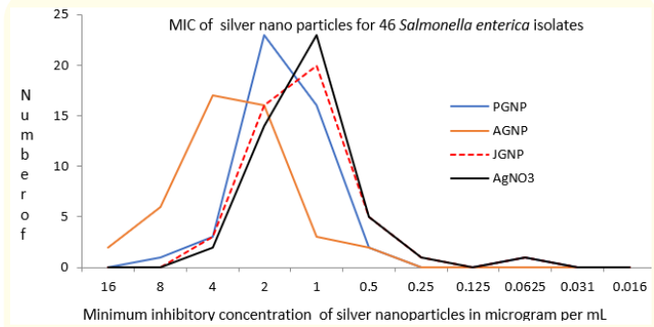

Figure 4: Distribution of strains of 46 *Salmonella* enterica isolates according to average minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of Silver nitrate ($AgNO_3$).

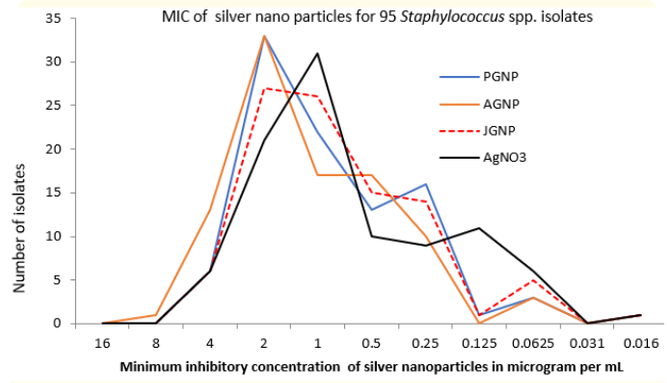

Figure 5: Distribution of strains of 95 *Staphylococcus* spp. isolates according to average minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of silver nitrate ($AgNO_3$).

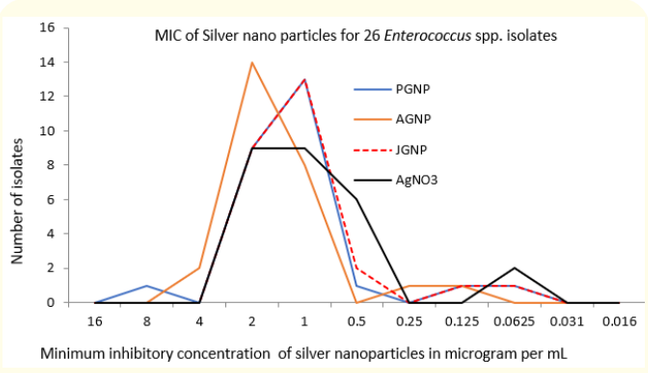

Figure 6: Distribution of strains of 26 *Enterococcus* isolates according to average minimum inhibitory concentration (MIC) of Silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of Silver nitrate ($AgNO_3$).

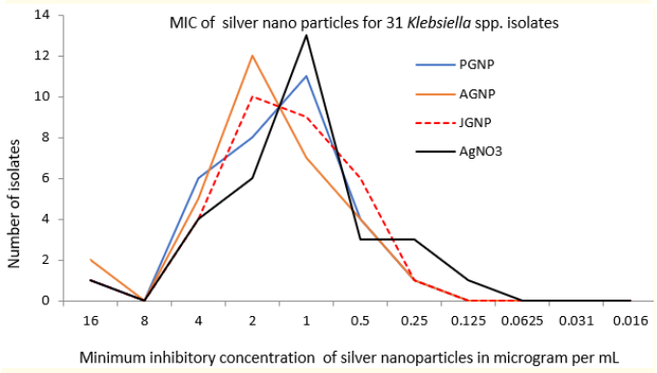

Figure 7: Distribution of strains of 31 *Klebsiella* spp. isolates according to average minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of silver nitrate ($AgNO_3$).

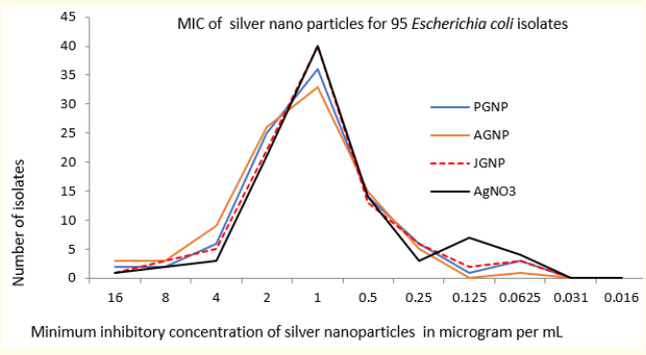

Figure 8: Distribution of strains of 95 *Escherichia coli* isolates according to average minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Piyar (Chironji, *Buchanania lanzan*) gum (PGNP), Acacia (*Acacia nilotica*) gum (AGNP) and Jhingan (*Lannea coromandelica*) gum (JGNP) in comparison of silver nitrate ($AgNO_3$).

The susceptibility of isolates of bacteria of 17 genera (having tested >10 isolates) was compared, and difference was assessed through odds ratio analysis. Among isolates of 17 different genera, *R. terrigena* isolates were significantly ($p < 0.05$) more often resistant than isolates of most of the other bacteria (*Acinetobacter, Aeromonas, Alcaligenes, Bacillus, Enterococcus, Escherichia, Klebsiella, Paenibacillus, Pasteurella, Salmonella, Staphylococcus, Streptococcus*) at both of the silver MIC levels i.e, at 0.25 µg mL^{-1} (Table 4) and 1.0 µg mL^{-1} (Table 5). All isolates of *Alcaligenes* spp. were susceptible to silver at 1.0 µg mL^{-1}, and were significantly ($p < 0.05$) more susceptible than isolates belonging to other 16 genera compared (Table 5).

Bacteria	Bacteria with no significant ($p > 0.05$) difference in susceptibility	Significantly ($p < 0.05$) more susceptible bacteria	Significantly ($p < 0.05$) less susceptible bacteria
Acinetobacter	*Aeromonas, Bacillus, Enterococcus, Escherichia, Hafnia, Klebsiella Proteus, Pseudomonas, Raoultella, Salmonella, Serratia, Staphylococcus*	*Alcaligenes, Paenibacillus Pasteurella Streptococcus*	
Aeromonas	*Acinetobacter, Alcaligenes, Enterococcus, Escherichia, Hafnia, Klebsiella, Paenibacillus, Proteus, Pseudomonas, Raoultella, Salmonella, Serratia,*	*Bacillus Pasteurella Staphylococcus Streptococcus*	
Alcaligenes/ Paenibacillus	*Bacillus, Hafnia, Pasteurella, Proteus, Streptococcus*		*Acinetobacter, Aeromonas, Enterococcus, Escherichia, Klebsiella, Pseudomonas, Raoultella, Salmonella Serratia, Staphylococcus*
Bacillus/Hafnia/ Proteus	*Acinetobacter, Aeromonas, Alcaligenes, Enterococcus, Escherichia, Klebsiella, Paenibacillus, Pasteurella, Pseudomonas, Serratia, Staphylococcus, Streptococcus*		*Raoultella, Salmonella*
Enterococcus/ Escherichia/ Klebsiella	*Acinetobacter, Aeromonas, Bacillus, Hafnia, Proteus, Pseudomonas, Raoultella, Salmonella, Serratia*	*Alcaligenes, Paenibacillus, Pasteurella, Staphylococcus, Streptococcus*	

Pasteurella	*Alcaligenes, Bacillus, Hafnia, Paenibacillus, Proteus, Serratia, Staphylococcus, Streptococcus*		*Acinetobacter, Aeromonas, Enterococcus, Escherichia, Klebsiella, Pseudomonas, Raoultella, Salmonella*
Pseudomonas	*Acinetobacter, Aeromonas, Bacillus, Enterococcus, Escherichia, Hafnia, Klebsiella, Proteus, Raoultella, Salmonella, Serratia, Staphylococcus, Streptococcus*	*Alcaligenes, Paenibacillus, Pasteurella*	
Raoultella/ Salmonella	*Acinetobacter, Aeromonas* *Enterococcus, Escherichia, Klebsiella* *Pseudomonas, Serratia*	*Alcaligenes, Bacillus, Hania, Paenibacillus, Pasteurella, Proteus, Staphylococcus, Streptococcus*	
Serratia	*Acinetobacter, Aeromonas, Bacillus, Enterococcus, Escherichia, Hafnia, Klebsiella, Pasteurella, Proteus, Pseudomonas, Raoultella, Salmonella, Staphylococcus*	*Alcaligenes, Paenibacillus, Streptococcus*	
Staphylococcus	*Acinetobacter, Bacillus, Hafnia, Klebsiella, Pasteurella, Proteus, Pseudomonas, Serratia, Streptococcus*	*Alcaligenes,* *Paenibacillus*	*Aeromonas, Enterococcus, Escherichia,* *Raoultella, Salmonella*
Streptococcus	*Alcaligenes,* *Bacillus, Hafnia, Paenibacillus Pasteurella, Proteus, Streptococcus*		*Acinetobacter, Aeromonas, Enterococcus, Escherichia, Klebsiella,Pseudomonas, Raoultella, Salmonella, Serratia*

Table 4: Difference in susceptibility of different group of microbes when limit of susceptibility was set as MICof silver ≤0.25 µg mL^{-1}.

Bacterial genera	Significantly ($p < 0.05$) more susceptible bacteria	Significantly ($p < 0.05$) less susceptible bacteria
Alcaligenes	No	All other 16 genera
Raoultella	*Acinetobacter, Aeromonas, Alcaligenes, Bacillus, Enterococcus, Escherichia, Klebsiella, Paenibacillus, Pasteurella, Salmonella, Staphylococcus, Streptococcus*	
Hafnia	*Alcaligenes*	No
Proteus	*Alcaligenes*	No
Pseudomonas	*Alcaligenes*	No
Serratia	*Alcaligenes*	No
Acinetobacter	*Alcaligenes*	*Raoultella*
Aeromonas	*Alcaligenes*	*Raoultella*
Enterococcus	*Alcaligenes*	*Raoultella*
Escherichia	*Alcaligenes*	*Raoultella*
Klebsiella	*Alcaligenes*	*Raoultella*
Paenibacillus	*Alcaligenes*	*Raoultella*
Pasteurella	*Alcaligenes*	*Raoultella*
Salmonella	*Alcaligenes*	*Raoultella*
Staphylococcus	*Alcaligenes*	*Raoultella*
Streptococcus	*Alcaligenes*	*Raoultella*

Table 5: Difference in susceptibility of different group of microbes when limit of susceptibility was set as MIC of silver ≤1.0 µg mL^{-1}.

The MIC of aspirin tested for 71 isolates ranged between 1.28 mg/mL to 10.24 mg mL^{-1} (Table 6), was relatively less for G+ve bacteria (2.41 ± 2.22 mg mL^{-1}) than for G-ve bacteria (2.78 ± 2.31 mg mL^{-1}) and yeasts (4.69 ± 4.85 mg mL^{-1}). The MIC of flunixin meglumine for 24 of bacteria and three yeasts isolates tested (Table 7) was higher (5.58 ± 4.32 mg mL^{-1}) than the MIC of aspirin (2.75 ± 2.40 mg mL^{-1}). However, none of the 29 bacteria and three strains yeast tested was inhibited (Table 8) by paracetamol at concentration of ≤5.12 mg mL^{-1}.

The presence of aspirin (Table 6) and flunixin meglumine (Table 7) in the test medium significantly ($p < 0.05$) reduced the MIC of AGNP. However, the effect was concentration dependent. The reduction in MIC of AGNP was evident in presence of ≥0.08 mg mL^{-1} of aspirin while reduction in AGNP MIC was evident in presence of ≥0.02 mg mL^{-1} of flunixin meglumine. The presence of paracetamol at concentration ≥0.02 mg mL^{-1} adversely affected antimicrobial activity of AGNP (Table 8).

Species, number of strains tested	MIC of aspirin mg mL^{-1}	Average MIC of AGNP (µg mL^{-1}) in presence of different concentrations of aspirin in mg mL^{-1}								
		1.28	**0.64**	**0.32**	**0.16**	**0.08**	**0.04**	**0.02**	**0.01**	**0**
Aeromonas popoffii 2, *A. schubertii* 2	2.24	0.75	3.25	3.25	3.50	3.50	3.50	3.50	1.78	0.01
Bacillus mycoides 1	10.24	0.25	0.50	0.50	0.50	1.00	1.00	2.00	2.00	2.00
Candia albicans 1, *C. famata* 2	4.69	1.00	1.17	1.33	1.33	1.67	1.67	1.67	1.67	1.33
Enterococcus faecalis 2. *E. faecium* 2, *E. malodoratus* 1	2.82	0.62	0.91	1.41	1.41	1.41	1.61	1.61	1.61	0.40
Eewinia stewartii 1	2.56	1.00	4.00	4.00	4.00	4.00	4.00	4.00	0.03	0.01
Escherichia coli 14	2.29	1.21	1.66	1.77	1.85	1.99	2.15	2.33	2.12	0.74
Geobacillus stearo-thermophilus 1	1.28	0.13	0.13	0.13	0.13	0.25	0.25	0.50	0.50	0.13
Hafnia alvei	2.13	1.08	2.83	3.00	3.00	3.00	3.00	4.00	4.00	0.42
Klebsiella pneu-moniae 7	4.39	0.95	1.59	2.02	2.02	2.07	2.07	2.07	2.07	0.25
Moraxella ovis 2	1.28	0.53	0.56	0.56	0.56	0.56	0.63	0.75	0.75	0.25
Pantoea agglomer-ans 1	1.28	0.50	0.50	0.50	0.50	0.50	1.00	2.00	2.00	0.25
Pproteus mirabilis 6	2.56	2.79	3.08	3.83	3.83	3.83	3.83	4.17	3.50	1.54
Pseudomonas aeru-ginosa 1, *P. pseudoal-caligenes* 2	4.69	0.19	1.33	1.67	1.67	1.67	1.67	1.67	1.67	0.34
Raoultella terrigena 1	2.56	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	0.25
Salmonella enterica ssp. enterica ser Naestved	5.12	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	0.50
Serratia marcescens 1, *S. plymuthica* 1, *S. rubidiae* 1	2.13	0.71	2.00	2.00	2.33	2.33	2.33	3.00	3.00	0.17
Staphylococcus aureus 2, *S. capitis* 2, *S. chromogenes* 1, *S. epidermidis* 3, *S. hae-molyticus* 2, *S. hyicus* 1, *S. intermedius* 1, *S. lentus* 1, *S. sciuri* 1	1.87	0.39	1.41	1.36	1.36	1.39	1.39	1.52	1.52	0.15
Streptococcus sali-varis 1	1.28	0.01	0.01	0.01	0.01	0.03	0.03	0.03	0.03	0.03
G-positive Bacteria, 22	2.41 (2.22)	0.28 (0.51)	0.40 (0.37)	1.13 (1.14)	1.21 (1.14)	1.21 (1.14)	1.27 (1.10)	1.31 (1.11)	1.45 (1.08)	1.45 (1.09)
G-negative Bacteria, 46	2.78 (2.31)	0.56 (1.41)	1.19 (1.65)	2.04 (1.84)	2.27 (1.99)	2.34 (1.96)	2.39 (1.97)	2.45 (1.93)	2.69 (1.92)	2.30 (1.80)
Candida species, 3	4.69 (4.85)	1.33 (0.58)	1.00 (0.87)	1.17 (0.76)	1.33 (0.58)	1.33 (0.58)	1.67 (0.58)	1.67 (0.58)	1.67 (0.58)	1.67 (0.58)
Total, 71	2.75 (2.40)	0.51 (1.18)	0.94 (1.40)	1.72 (1.67)	1.90 (1.79)	1.95 (1.78)	2.01 (1.77)	2.07 (1.75)	2.26 (1.76)	2.01 (1.61)

Table 6: Effect of aspirin on minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Acacia (*Acacia nilotica*) gum (AGNP) for different microbes (values in brackets indicates standard deviation).

Species, number of strains tested	MIC of flunixin mg mL^{-1}	Average MIC of AGNP (µg mL^{-1}) in presence of different concentrations of flunixin in mg mL^{-1}								
		1.28	0.64	0.32	0.16	0.08	0.04	0.02	0.01	0
Aeromonas popoffii 2, *A. schubert* 2	4.32	0.01	0.51	0.57	0.66	1.38	2.13	2.13	1.78	1.78
Bacillus mycoides 1	10.24	0.01	0.01	0.25	0.25	0.50	1.00	2.00	2.00	2.00
Candida albicans 1, *C. famata* 2	7.04	0.01	0.01	0.75	0.75	1.00	1.33	1.67	1.67	1.67
Enterococcus faecium 3	7.25	0.01	0.01	0.34	0.83	1.33	2.00	2.00	2.00	2.00
Escherichia coli 9	7.43	0.01	0.12	0.78	0.92	1.31	1.33	1.39	2.78	2.78
Hafnia alvei 1	5.12	0.01	0.01	0.25	0.50	1.00	1.00	1.00	4.00	4.00
Klebsiella pneumoniae 2	6.40	0.01	0.01	1.06	1.06	2.06	2.06	2.25	2.25	2.25
Pantoea agglomerans 1	2.56	0.01	0.01	0.25	0.50	1.00	1.00	1.00	2.00	2.00
Proteus mirabilis 4	7.04	0.51	0.51	1.81	1.88	2.50	2.50	3.75	3.75	4.25
Pseudomonas aeruginosa 1, *P. pseudoalcaligenes* 1	10.24	0.01	0.01	0.25	0.75	1.25	1.50	1.50	1.50	1.50
Staphylococcus aureus 1, *S. capitis* 1, *S. epidermidis* 2, *S. haemolyticus* 1, *S. intermedius* 1, *S. lentus* 1	2.27	0.01	0.01	0.36	0.29	0.36	0.38	0.52	1.68	1.68
Gram-positive bacteria 11	4.00 (4.66)	0.01 (0.00)	0.01 (0.00)	0.33 (0.42)	0.41 (0.39)	0.59 (0.56)	0.81 (1.07)	0.98 (1.10)	1.68 (1.27)	1.69 (1.26)
Gram-negative bacteria 23	6.16 (4.00)	0.09 (0.40)	0.21 (0.57)	0.79 (0.96)	0.92 (0.95)	1.44 (1.43)	1.59 (1.38)	1.83 (1.79)	2.45 (2.13)	2.53 (2.15)
Candida species 3	7.04 (5.54)	0.01 (0.00)	0.01 (0.00)	0.75 (1.09)	0.75 (1.09)	1.00 (0.87)	1.33 (0.58)	1.67 (0.58)	1.67 (0.58)	1.67 (0.58)
Total 32	5.58 (4.32)	0.06 (0.31)	0.13 (0.46)	0.65 (0.85)	0.76 (0.84)	1.15 (1.24)	1.34 (1.28)	1.56 (1.57)	2.16 (1.84)	2.21 (1.87)

Table 7. Effect of flunixin meglumine on minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using acacia (*Acacia nilotica*) gum (AGNP) for different microbes (values in brackets indicates standard deviation).

Species of microbes (number of strains tested)	MIC of Paracetamol mg mL^{-1}	Average MIC of AGNP (µg mL^{-1}) in presence of different concentrations of paracetamol mg mL^{-1}								
		1.28	0.64	0.32	0.16	0.08	0.04	0.02	0.01	0.00
Aeromonas popoffii (2)	>5.12	12.00	6.00	6.00	6.00	6.00	4.00	4.00	4.00	4.00
Aeromonas schubertii (2)	>5.12	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Candida albicans (2)	>5.12	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32
Candida famata (1)	>5.12	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Enterococcus faecalis (1)	>5.12	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Enterococcus faecium (2)	>5.12	2.50	2.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Erwinia stewartii (1)	>5.12	8.00	8.00	8.00	4.00	4.00	4.00	4.00	4.00	4.00
Escherichia coli (8)	>5.12	10.75	3.63	3.63	3.13	3.13	3.13	3.13	2.63	2.63
Klebsiella pneumoniae (2)	>5.12	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Moraxella ovis (1)	>5.12	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Proteus mirabilis (3)	>5.12	8.67	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Pseudomonas pseudoalcaligenes (1)	>5.12	4.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Staphylococcus aureus (1)	>5.12	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Staphylococcus haemolyticus (2)	>5.12	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Staphylococcus intermedius (1)	>5.12	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Staphylococcus lentus (1)	>5.12	1.28	0.64	0.32	0.16	0.08	0.04	0.02	0.01	0.01
Streptococcus salivaris (1)	>5.12	0.64	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Gram-negative bacteria (20)	>5.12	8.40 (6.11)	5.05 (3.63)	4.45 (2.56)	4.05 (2.26)	4.05 (2.26)	3.85 (2.06)	3.85 (2.06)	3.65 (2.13)	3.65 (2.13)
Gram-positive bacteria (9)	>5.12	1.99 (1.24)	1.74 (1.12)	2.60 (2.46)	2.58 (2.48)	2.57 (2.49)	2.56 (2.50)	2.56 (2.50)	2.56 (2.50)	2.56 (2.50)
Candida spp. (3)	>5.12	2.21 (1.69)	2.21 (1.69)	2.21 (1.69)	2.21 (1.69)	2.21 (1.69)	2.21 (1.69)	2.21 (1.69)	2.21 (1.69)	2.21 (1.69)
Total (32)	>5.12	6.02 (5.76)	3.85 (3.33)	3.72 (2.59)	3.46 (2.35)	3.46 (2.35)	3.34 (2.20)	3.33 (2.20)	3.21 (2.22)	3.21 (2.22)

Table 8: Effect of paracetamol on minimum inhibitory concentration (MIC) of silver-nano-particles synthesized through green synthesis using Acacia (*Acacia nilotica*) gum (AGNP) for different microbes (values in brackets indicates standard deviation).

Discussion

Silver-nanoparticles (SNPs) made through three different gums in the study had wide range of inhibitory activity as shown in earlier studies [7,8,11-13].

The jhingan gum SNPs being the most active *in vitro* inhibitors of microbes at almost all concentrations used in the study suggesting it a better gum than piyar gum and gum acacia for synthesis of SNPs for antimicrobial activity. Though there are no earlier studies comparing the SNPs using different gums, several gums including gum acacia, cashew gum, gum kondagogu, gum olibanum, gum karaya, gum tragacanth, gum ghatti, neem gum, gum acacia, piyar gum, gum tragacanth [7,8,11] have been tried to produce antimicrobial SNPs indicating variations in their antimicrobial potential.

In the present study, all SNPs and silver nitrate have shown to be more active antimicrobial against G+ve bacteria than on G-ve bacteria and observations corroborate with earlier reports on SNPs using gum tragacanth (*Astragalus gummifer*) showing better antimicrobial activity against *S. aureus* than G-ve bacteria including *E. coli* and *P. aeruginosa* [11]. Similarly, piyar gum based SNPs tested against 8 G-ve and 9 G+ve bacterial strains had lower MIC for G+ve bacteria than for G-ve bacteria [8]. The SNPs made with use of *Carduus crispus* extract also showed better antibacterial activity against G+ve bacteria than on G-ve bacteria [13].

As a group, the most susceptible strains for SNPs were *S. hominis* strains among all types of microbes and their MIC ranged between 0.25 to 1.00 µg of silver mL^{-1}. Whereas, *Alcaligenes* species strains were the most susceptible and *R. terrigena* strains the most resistant and all other bacteria including all G+ve strains those stood in between of these two groups of bacteria.

In earlier studies on MIC of SNPs wide variations have been reported [8,12]. In the present study MIC of SNPs for pseudomonads was 1.48 ± 1.01, 1.58 ± 0.93 and 1.34 ± 1.00 for PGNPs, AGNPs and JGNPs, respectively and ranged between 0.13-4.00 µg mL^{-1}. The observations are in concurrence to the earlier observations on SNPs synthesized using branched cyclodextrin having MIC ranging from 1.406–5.625 µg mL^{-1} against a few selected strains of *P. aeruginosa* [12].

In the present study PNGs had MIC ranging from 0.02 to 16 µg mL^{-1} and corroborate with an earlier study of PGNPs reporting MIC ranging from 0.52 µg mL^{-1} to >8.5 µg mL^{-1} for 17 strains of bacteria [8].

The MIC of aspirin for 71 bacteria ranged between 1.28 - 10.24 mg µg mL^{-1} and for 0.64 - 10.24 mg µg mL^{-1} G-ve and G+ve bacteria, respectively. The MIC was much higher for flunixin meglumine (5.58 ± 4.32 mg mL^{-1}) and paracetamol (≤5.12 mg mL^{-1}). The observations are in concurrence to earlier findings [17,18] and revealed no therapeutic utility of NSAIDs as antimicrobials for internal therapeutic use.

The study has shown that aspirin and flunixin meglumine may enhance the antimicrobial activity of SNPs depending on concentrations of the two NSAIDs. In contrast, another NSAID, paracetamol, had adverse effect on MIC of SNPs and increased MIC in most instances. However, the concentrations of NSAIDs at which they may affect the MIC of SNPs *in vitro* (≥0.02 mg mL^{-1}) is unachievable therapeutically *in vivo* unless toxic doses of NSAIDs are administered [18]. Therefore, interaction between NDAIDs and SNPs has little or no significant role in internal therapeutic use of SNPs unless SNPs are used in preparation of ointments or creams

for topical applications. Similar, type of interactions of NSAIDs have been reported with some antibiotics too [18,19]

Conclusion

The study concluded that SNPs possess wide spectrum antimicrobial activity but their antimicrobial potential differs significantly with the material used for their green synthesis. Among all the three gums used for green synthesis of SNPs, SNPs made using jhingan gum had the best antimicrobial activity. Use of NSAIDs may certainly impact the MIC (decreased with aspirin and flunixin meglumine, increased with paracetamol) but not at therapeutically achievable serum concentrations; however, it may be important finding in formulating creams/ointments of SNPs for topical applications.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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