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Comparative Antimicrobial Efficacy of Methanolic and Diethyl Ether Extracts of Rose (*Rosa Indica*) Hip Seeds Against Clinically Important Microbes

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Abstract

Rosehip seeds of different species of roses are known to contain many active ingredients including potentially antimicrobial ones, the present study compared the antimicrobial potential of *Rosa indica* hip seeds' methanolic (RME) and diethyl ether extracts (REE). From 150 g of rosehip seeds, 5.13 g of REE or 9.23 g of RME was obtained. Of the 162 strains of microbes tested, 24 and 159 strains were not inhibited by RME and REE, respectively at ≤50 mg mL^{-1} concentration. The minimum inhibitory concentration (MIC) of RME was for Gram-positive bacteria 13.99± 11.59 mg mL^{-1} followed by Gram-negative bacteria 32.85± 19.31 mg mL^{-1}, and *Candida* 43.75± 12.5 mg mL^{-1} strains. The minimum MIC (0.1 mg mL^{-1}) of RME was for one strain each of *Streptococcus anginosus, Bacillus brevis*, *Moraxella bovis* and *M. ovis*. Only one strain each of *M. ovis, Staphylococcus aureus,* and *Candida albicans* were susceptible to REE at ≤50 mg mL^{-1} concentration. Of the 162, 12 (7.41%) strains (of *Aeromonas trota, Staphylococcus aureus, S. xylosus, Proteus mirabilis, Flexibacter* spp. *Kocuria rosea, Streptococcus pyogenes, S. anginosus, M. bovis, Bacillus brevis*) were inhibited at ≤1.66 mg mL^{-1} concentration of RME. There was wide variability in the MIC of rosehip extracts for strains of the same species and different species of microbes. The MIC of RME was minimum (13.05± 15.34 mg mL^{-1}) for strains of buffalo origin and was maximum (40.63± 18.75 mg mL^{-1}) for strains from lions. The MIC of RME for *E. coli* of dog origin (38.64± 13.06 mg mL^{-1}) was almost similar to the MIC of RME (37.50± 16.14 mg mL^{-1}) for *Escherichia coli* strains from other sources. The study concluded that *Rosa indica* hip seeds had a wide spectrum of antimicrobial activity against 138 strains of 64 species of

microbes and methanol was a better solvent than diethyl ether for extraction of antimicrobial ingredients.

Keywords: Herbal antimicrobials, Bacteria, Fungi, *Candida*, *Moraxella, Aeromonas*

Introduction

Roses of many different species of genus *Rosa* are native to many countries/ regions and are used in day-to-day activities for several purposes. Rose petals are of high culinary, decorative, and essence value while rosehips often go waste despite their high therapeutic potential. Rosehip extract (from whole rose fruit, rosehip) and rosehip seed extract (from seeds of rose present in rosehips) are acclaimed for several health benefits specially to cure inflammatory acne and acne scars due to their richness in vitamin A, C, and E, and essential fatty acids like omega-6 [1-3]. Phenolic compounds and antioxidant activity of rosehip oil/ extracts and its ability to scavenge free radicals helps skin to maintain its tone and thus acting as an antiaging agent [4,5]. The important problems treated or predicted to be treated with rosehip oil (RHO), rosehip seed oil (RHSO) and other extracts include rheumatism and rheumatoid arthritis [1,6,7-11], cancer [4,12,13], osteoporosis [1], hyperlipidaemia [14], obesity [14,15], renal problems [16], hepatic problems [17], neurological damage [18-20], skin diseases [21-23], diarrhoea [1] and peptic ulcers [24]. Though the antimicrobial potential of RHO/ RHSO and extracts of seeds and flowers are reported in *Rosa canina* [25-27], *R. rugosa* [28-29], *R. damascene* [30], *R. multiflora* [31], *R. pisocarpa, R. nutkana* and *R. woodsii* [32] against strains of potentially pathogenic bacteria and fungi, little is understood about the antimicrobial potential of *Rosa indica* RHO/RHSO. The present study was undertaken to determine the antimicrobial potential of methanolic and ether extracts of rosehip seeds of *Rosa indica* against 158 strains of potentially pathogenic bacteria and 4 strains of yeasts.

Materials and Methods

Preparation of rosehip seed methanolic (RME) and diethyl ether (REE) extracts

Ripened rosehips (orange-red in colour) were harvested in May 2020 from *Rosa indica* plants left unpruned after flowering in spring (due to lockdown implemented to contain COViD-19) at ICAR-IVRI, Izatnagar campus. Rosehip seeds were collected after cutting the rosehips. Seeds were dried at 60°C for 24 h and ground mechanically. A total of 300 g rosehip seed powder was divided into two equal parts into 1.0 L Borosil neutral glass screw-capped bottles and in one bottle 500 mL HPLC grade methanol (SD Fine Chemicals Ltd. India) and in other bottle, 500 mL of HPLC grade diethyl ether (SD Fine Chemicals Ltd. India) was added, and after tightening the caps both the bottles were kept at 30°C on shaking platform (60 rpm) for 24 h. Then contents of the bottles were filtered through Whatman filter paper No. 1 to collect the liquids separately and transferred to two glass bowls of 1 L capacity each, and incubated at 45°C for 48 h to evaporate methanol and diethyl ether, and have the concentrated extract free of solvents. Extracts were collected and stored at 4°C in amber-coloured glass bottles till tested for their antimicrobial potential.

Microbial strains used in the study

A total of 155 strains (Table 1) of bacteria (101 Gram-negative of 30 species of 21 genera, and 54 Gram-positive bacteria of 33 species belonging to 9 genera), 4 strains of *Candida* (*C. albicans* 2, *C. tropicalis* 1, *C. famata* 1) isolated from veterinary (buffaloes 14, cattle 16, dogs 55, birds 16, sheep 3, goat 1, lions 6, pig 1, pythons 3, sloth bears 2, spotted deer 1, tigers 10) and human [12] clinical cases, environmental (air 17, water 2) clinical samples (Table 2) and three reference strains (DH5α *Escherichia coli*, and *Staphylococcus aureus* ATCC 29312 and ATCC 43300) available in the repository of Division of Epidemiology, ICAR-IVRI, Izatnagar were revived and confirmed to identity through morphological, culture, staining and biochemical characterization [33,34]. Further, strains were confirmed by MALDI-ToF MS (MALDI Biotyper Sirius system, Bruker Daltonics). During the study, all strains were maintained in semisolid nutrient agar till tested for their susceptibility.

Table. 1: Minimum inhibitory concentration (MIC) of methanolic extract of rose (*Rosa indica*) hip seeds for microbes of different species

Genus	Species of bacteria, number of strains tested	Strains tested	Average MIC of rosehip seed methanolic extract (mg/mL) ± STDV	Number of strains with MIC >50 mg/mL
Acinetobacter	*A. lwoffii* 2	2	25.00	0
Aerococcus	*A. christensenii* 1	1	3.12	0
Aeromonas	*A. bestiarum* 1, *A. eucranophila* 2, *A. popoffii* 1, *A. scubertii* 2, *A. trota* 1	7	26.84± 25.42	1
Alcaligenes	*A. faecalis* 2	2	15.63	0
Bacillus	*B. brevis* 1, *B. cereus* 1, *B. megaterium* 3, *B. mycoides* 1, *B. sphaericus* 1	7	15.12± 11.57	1
Burkholderia	*B. cepacia* 2	2	26.56	0
Candida	*C. albicans* 2, *C. famata* 1, *C. tropicalis* 1	4	43.75± 12.50	0
Edwardsiella	*E. tarda* 2	2	50.00	1
Enterobacter	*E. gregoviae* 1	1	50.00	0
Enterococcus	*E. asaccharolyticus* 1, *E. avium* 1, *E. faecalis* 3, *A. faecium* 3, *E. malodoratus* 1	9	19.53± 15.10	1
Erwinia	*E. cacticida* 1, *E. stewartii* 1	2	50.00	0
Escherichia	*E. coli* 24, *E. fergusonii* 1, *NDM E. coli* 3	27	39.88± 13.47	7
Flexibacter	*F. tractuolus* 1, *F. species* 11	12	17.59± 16.89	0
Geobacillus	*G. stearothermophilus* 3	3	13.54± 10.98	0
Haemophilus	*H. felis* 1	1	50.00	0
Hafnia	*H. alvei* 6	6	40.63± 8.75	2
Klebsiella	*K. pneumoniae* ssp. *pneumoniae* 8	8	50.00± 0.00	4
Kocuria	*K. rosea* 1	1	1.66	0
Micrococcus	*M. luteus* 1	1	6.25	0
Moelerella	*M. wisconsensis* 1	1	50.00	0
Moraxella	*M. bovis* 2, *M. ovis* 1	3	0.34± 0.42	0
Paenibacullus	*P. pantothenticus* 2	2	ND	2
Pantoea	*P. agglomerans* 3	3	16.67± 7.22	0
Pasteurella	*P. multocida* type B 1	1	6.25	0
Proteus	*M. mirabilis* 4, *M. vulgaris* 1	5	26.58± 23.10	0
Pseudomonas	*P. aeruginosa* 7, *P. paucimobilis* 1	8	45.83± 10.21	2
Raoultella	*R. terrigena* 2	2	ND	2
Salmonella	*S. enterica* ser Kentucky 1, *S. enterica* ser Naestved 1, *S. enterica* ser Typhimurium 4	6	43.75± 15.31	0
Serratia	*S. marcescens* 1	1	25.00	0
Staphylococcus	*S. aureus* 5, *S. capitis ssp. capitis* 3, *S. capitis* ssp. *urealyticus* 1, *S. caseolyticus* 1, *S. chromogenes* 2, *S. delphini* 1, *S. epidermidis* 2, *S. gallinarus* 1, *S. haemolyticus* 3, *S. hominis* 3, *S. lugduneresii* 1, *S. sciuri* 1, *S. xylosus* 1	23	14.22± 10.89	0
Streptococcus	*S. anginosus* 1, *E. equinus* 1, *S. milleri* 2, *S. phocae* 1, *S. pyogenes* 1	6	9.10± 10.11	1
Gram-positive bacteria	33 species of 9 genera	54	13.99± 11.59	5

Gram-negative bacteria	30 species of 21 genera	101	32.85± 19.31	19
Yeasts	3 species of *Candida*	4	43.75± 12.5	0
MIC for Reference strains				
ATCC43300	*Staphylococcus aureus*	0.83	0.83	0
ATCC29312	*Staphylococcus aureus*	12.5	12.5	0
DH5α	*Escherichia coli*	25	25	0
Total	**66 species of 31 genera**	**162**	**26.33 ± 19.15**	**24**

Testing of antimicrobial activity of RME and REE

To determine the minimum inhibitory concentration (MIC) of rosehip seed diethyl ether extract (REE) and methanolic extract (RME), both of the extracts were serially diluted starting from 1 g mL^{-1} up to 9th dilution (20 mg mL^{-1}) in 99.9% pure dimethyl sulfoxide (DMSO, Merck Ltd.) in sterile glass vials. All strains were tested using the agar-well diffusion assay described earlier for their MIC (35). All the strains were also tested for their susceptibility to DMSO alone, and gentamicin (Sigma Aldrich, USA) 3 mg mL^{-1} (150 µg in 50 µL) using a similar agar well diffusion protocol. In each of the 9 wells (6 mm diameter) of the Mueller Hinton agar (Difco, USA) plates seeded with 6 h old broth culture of the test microbial strain, 50 µL of the test solutions were added. For the first six hours, plates were incubated without inverting, and then for the next 18 hours after inverting, aerobically at 37°C. The zones of growth inhibition around wells were measured in mm and the well filled with the least concentration showing the measurable clear zone was considered to contain the quantity of the extract equal to the MIC. All tests were done in duplicate and in case of variation of the two readings tests were repeated a third time to take the final MIC reading.

Results and Discussion

From 150 g each of the rosehip seed powder, 5.13 g of REE and 9.23 g of RME were recovered. All the 158 bacterial strains under study were inhibited by gentamicin (150 µg/ well) but none of the 4 *Candida* spp. strains were susceptible to gentamicin indicating that the test system worked well. None of the 162 strains under study was inhibited by DMSO indicating the suitability of DMSO as a diluent for making serial dilutions of REE and RME. A total of 24 microbial strains (5 G+ve and 19 G-ve bacteria) showed no zone of inhibition even around wells filled with 50 mg of RME (Tab. 1) indicating that MIC was >50 mg mL^{-1}. The rest of the138 strains had RME MIC ≤50 mg mL^{-1} and clear zones of growth inhibition were evident (Fig. 1). The average MIC of RME was minimum for G+ve bacteria (13.99± 11.59 mg mL^{-1}) followed by G-ve bacteria (32.85± 19.31 mg mL^{-1}), and *Candida* (43.75± 12.5 mg mL^{-1}) strains (Tab. 1). However, minimum MIC (0.1 mg mL^{-1}) of RME was determined for two strains each of G+ve (*Streptococcus anginosus* and *Bacillus brevis*) and G-ve (*Moraxella bovis* and *M. ovis*) bacteria, and it varied greatly even for strains of the same species (Tab. 1). The susceptibility of microbial strains to RME had no normal distribution (Fig. 2) and 92.6% of the test strains had MIC >1.66 mg mL^{-1}.

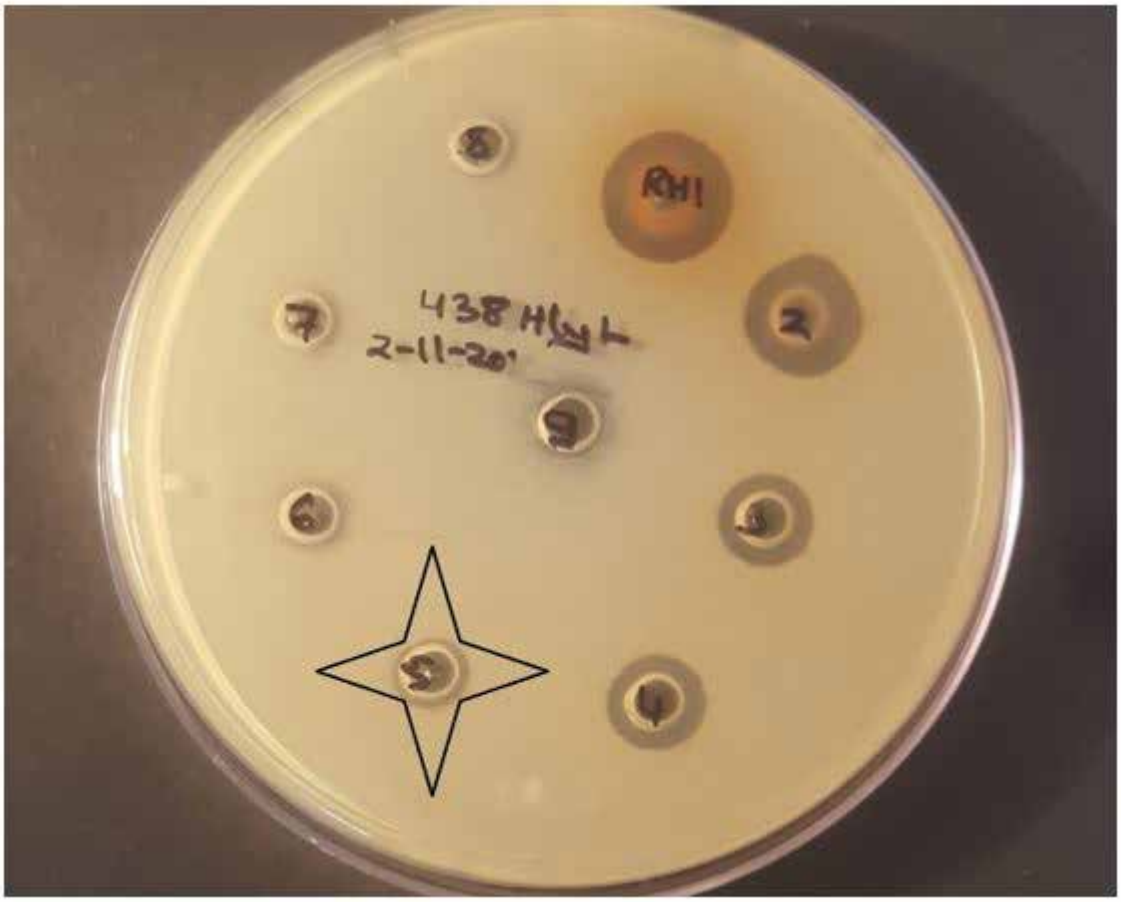

Figure 1: Photograph showing minimum inhibitory concentration (MIC= 1.56 mg mL^{-1}) of methanolic extract of rose (*Rosa indica*) hip seeds for *Bacillus mycoides* 438 HlyL

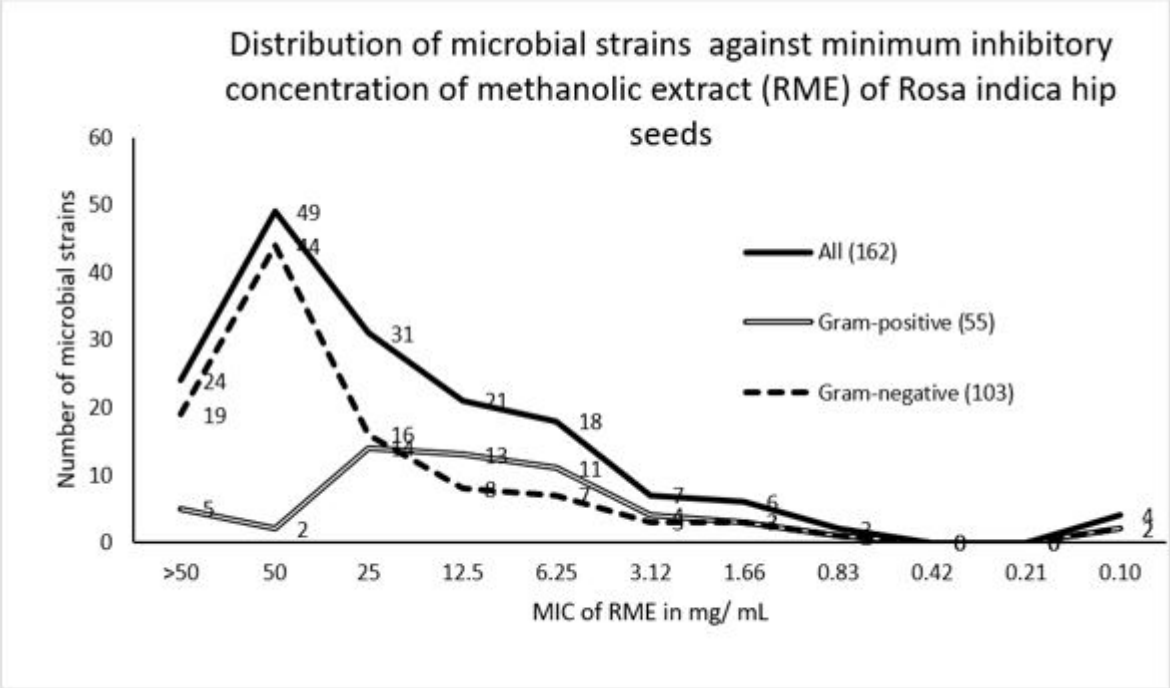

Figure 2: Minimum inhibitory concentration of methanolic extract (RME) of *Rosa indica* hip seeds for microbial strains (162) tested

Except for one strain each of *Moraxella ovis, Staphylococcus aureus* (ATCC 43300), and *Candida albicans* none of the strains tested was susceptible to REE, and MIC of REE MIC was equal to 25.0, 12.5, and 25.0 mg mL^{-1}, respectively. The observation indicated that diethyl ether as a solvent for extraction of rosehip seeds antimicrobial ingredients was not a better option than methanol.

Though rosehip oils/ extracts from *R. rugosa* [28-29], *R. damascene* [30], *R. multiflora* [31], *R. pisocarpa, R. nutkana* and *R. woodsii* [32] are reported to inhibit several bacterial and fungal strains including *Bacillus subtilis* [28, 31], *B. cereus* [30], *E. coli* [25, 28-31], *Salmonella enterica* ser Typhimurium [30,31], *Staphylococcus aureus* [28,30-32], *S. epidermidis, Enterococcus faecalis* [28,31,32], *Klebsiella pneumoniae* [28], *Micrococcus luteus* [28], *Proteus mirabilis* [28], *Pseudomonas aeruginosa* [28, 30], *Aspergillus niger* [30] and *Candida* strains [28,30, 32] , no study yet reported antimicrobial potential of *R. indica* hips on any of the microbes. However, only few studies determined MIC of the test preparations from *Rosa* species extracts against two strains of *E. coli* [25, 28] and one strain each of *S. aureus, S. epidermidis, B. subtilis, M. luteus, K. pneumoniae, P. aeruginosa, P. mirabilis* [28] in range of 0.1-1.25 mg mL^{-1} for *E. coli* and 1.25 mg mL^{-1} for rest of the strains. In the present study, 12 strains belonging to *Aeromonas trota, S. aureus, S. xylosus, P. mirabilis, Flexibacter* spp. *Kocuria rosea, Streptococcus pyogenes, S. anginosus, M. bovis, B. brevis* (Tab. 1) were inhibited at ≤1.66 mg mL^{-1} concentration of RME and observations are in concurrence to earlier observations for some of the microbial strains [28]. In the present study, none of the *Candida* strains had MIC <25 mg mL^{-1} and it is not in agreement with earlier observations reporting MIC of *Rosa rugosa* hip extract equal to 0.166 mg mL^{-1}. The variability among the two studies for MIC of rosehip extract might be due to the use of extracts from the different rose species, different strains of *Candida* tested and different procedures used for extraction. The study revealed a wide range of variability in MIC of rosehip extracts for strains of the same species and different species of microbes and this revelation was probably possible due to the use of large number of strains of different species of microbes instead of a few select strains.

Microbial strains isolated from different origins had a difference in MIC values of RME; minimum MIC (13.05± 15.34 mg mL^{-1}) was for strains isolated from buffaloes and maximum MIC (40.63± 18.75 mg mL^{-1}) was for isolates from lions (Tab. 2). The variation in the MIC of RME for strains might be due to differences in species and origin of strains included in the study. For assessing the real impact of the source of microbes on the MIC of RME sizeable and equitable number of strains of different species need to be compared. To some extent, it was possible to assess the impact of the source of strains on the MIC of RME for *E. coli*. Fourteen *E. coli* strains of dog origin [38.64± 13.06 mg mL^{-1}] had an almost similar MIC of RME (37.50± 16.14 mg mL^{-1}) observed for 10 *E. coli* strains from other sources. Though 25 staphylococci belonged to different species, MICs of RME were 23.96± 15.01 mg mL^{-1}, 12.12± 8.42 mg mL^{-1}, and 9.49± 5.18 mg mL^{-1} for 6 staphylococci strains from cattle and buffalo, 12 from dogs and 7 from other sources, respectively. The observations indicated the need for more targeted studies to assess the impact of species and source of isolation of the microbial strains on the MIC of RME.

Table. 2. Minimum inhibitory concentration (MIC) of methanolic extract of rose (*Rosa indica*) hip seeds for microbes of different origin

Source of sample	N	Species of microbes and number of isolates	MIC mg mL^{-1} ± STDV
Buffalo	14	*Aeromonas eucranophila* 2, *Bacillus megaterium* 2, *Burkholderia cepacia* 1, *Enterococcus avium* 1, *E. faecium* 1, *Escherichia coli* 2, *Moraxella bovis* 2, *Staphylococcus aureus* 1, *Atreptococcus anginosus* 1, *S. phocae* 1	13.05± 15.34
Cattle	16	*Aeromonas schubertii* 1, *Candida albicans* 2, *Erwinia stewartii* 1, *E. coli* 1, *Geobacillus stearothermophilus* 1, *H. alvei* 1, *Klebsiella pneumoniae* 1, *Proteus mirabilis* 1, *Salmonella Typhimurium* 1, *Staphylococcus capitis* 1, *S. hominis* 1, *S. epidermidis* 2, *S. sciurii* 1, *Streptococcus milleri* 1	35.42± 16.98
Dog	55	*Acinetobacter lwoffii* 2, *Aerococcus christensenii* 1, *Bacillus brevis* 1, *Edwardsiella tarda* 1, *Enterococcus asachrolyticus* 1, *E. faecalis* 1, *E. faecium* 1, *E. malodoratus* 1, *E. coli* 16, *E. fergusonii* 1, *G. stearothermophilus* 2, *Haemophillus felis* 1, *K. pneumoniae* 2, *Paenibacillus pantothenticus* 2, *Pantoea agglomerans* 1, *Proteus mirabilis* 3, *P. vulgaris* 1, *Pseudomonas aeruginosa* 2, *Raoultella terrigena* 1, *S. aureus* 1, *S. capitis* 2, *S. caseolyticus* 1, *S. chromogenes* 1, *S. delphini* 1, *S. gallinarum* 1, *S. haemolyticus* 2, *S. hominis* 1, *S. lugduneresii* 1, *S. xylosus* 1, *Streptococcus equinus* 1, *S. pyogenes* 1	24.55± 18.51
Birds	16	*Aeromonas popoffii* 1, *Alcaligenes faecalis* 1, *Enterococcus gregoviae* 1, *Hafnia alvei* 3, *Micrococcus luteus* 1, *P. agglomerans* 1, *P. aeruginosa* 2, *R. terrigena* 1, *Salmonella Kentucky* 1, *S. Naestved* 1, *S. Typhimurium* 3,	38.42± 18.94
Environmental samples	19	*Alcaligenes* 1, *Bacillus cereus* 1, *B. mycoides* 1, *B. sphaericus* 1, *Flexibacter tractuolus* 1, *Flexibacter spp.* 11, *Kocuria rosae* 1, *Serratia marscescens* 1, *Staphylococcus capitis* 1	16.85± 14.91
Sheep & Goat s	4	*A. scubertt* 1, *B. megaterium* 1, *E. coli* 1, *Moraxella ovis* 1	31.46± 23.58
Human	12	*Aeromonas trota* 1, *Candia tropicalis* 1, *Erwinia cacticida* 1, *E. coli* 1, *H. alvei* 1, *K. pneumoniae* 2, *Moelerella wisonsensis* 1, *P. agglomerans* 1, *S. aureus* 1, *S. chromogenes* 1, *Streptococcus milleri* 1	29.35± 20.44
Lions	6	*Aeromonas bestiarum* 1, *E. coli* 2, *H. alvei* 1, *K. pneumoniae* 2	40.63± 18.75
Other animals	7	*Burkholderia cepacia* 1, *E. faecalis* 1, *E. coli* 1, *Pasteurella multocida* 1, *P. aeruginosa* 2, *Staphylococcus haemolyticus* 1	31.25± 18.75
Tiger	10	*Candia famata* 1, *Edwardsiella tarda* 1, *E. faecalis* 1, *E. faecium* 1, *E. coli* 2, *K. pneumoniae* 1, *Pseudomonas paucimobilis* 1, *P. aeruginosa* 1, *Staphylococcus hominis* 1	33.20± 23.20
Reference	3	*S.* aureus (ATCC29312, ATCC43300) 2, *E. coli* DH5α 1	12.78± 12.09

Conclusion

The study concluded that *Rosa indica* hip seeds had antimicrobial activity against 138 strains of 64 species of microbes. On one hand none of the two strains tested each of *R. terrigena* and *P. pantothenticus* species was inhibited by rosehip extract even at 50 mg mL^{-1} while strains *S. anginosus, B. brevis*, *M. bovis* and *M. ovis* were susceptible even at 0.1 mg mL^{-1} concentration of RME. To extract the antimicrobial active ingredient of rosehips methanol proved as a better solvent than diethyl ether. The study indicated that for further studies for purification and identification of the active antimicrobial compounds (s) in rosehip methanolic extracts of rosehip seeds may be an option.

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Conflict of Interests

None to declare

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