Full Length Research Paper

Antimicrobial activity analysis of extracts of *Acacia* modesta, *Artimisia absinthium, Nigella sativa and* Saussurea lappa against Gram positive and Gram negative microorganisms

Anwar Khalid¹, Uzair-ur-Rehman¹, Ayesha Sethi², Sonia Khilji³, Urooj Fatima⁴, Muhammad Imran Khan⁴, Muhammad Khurram Waqas⁴, Qazi Najam-us-Saqib⁵, Muhammad Hassham Hassan Bin Asad⁵, Kalsoom Farzana⁶, Seema Mahmood⁷, Amir Waseem⁸, Tariq Ismail⁵ and Ghulam Murtaza*⁵

¹Department of Biochemistry, Hazara University Garden Campus, Mansehara, Pakistan.

²Department of Pharmacy, Government University, Faisalabad, Pakistan.

³Faculty of Pharmacy and alternative Medicines, The Isalamia University of Bahawalpur, Bahawalpur, Pakistan.

⁴School of Pharmacy, the University of Faisalabad, Faisalabad, Pakistan.

⁵Department of Pharmaceutical Sciences, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan.

⁶Faculty of Pharmacy, Bahauddin Zakariya University, Multan.

⁷Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

⁸Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan.

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Antimicrobial activity of extracts of certain herbs including Acacia modesta (leaf and stem), Artimisia absinthium (leaf and stem), Nigella sativa (seeds) and Saussurea lappa (root) was evaluated against three Gram positive and two Gram negative microorganisms. The Gram positive organisms included Bacillus subtalis (ATCC 6633), Entereococcus faecalis (ATCC 14506) and Staphylococcus aureus (ATCC 6538) and the Gram negative organisms included Pseudomonas aeruginosa (ATCC 27853) and Salmonella typhi (ATCC 14028). Methanolic, hot water and cold water extracts of these plants were taken for antibacterial assay through Discs agar diffusion technique using commercial filter paper discs applied on inoculated Muellar Hinton agar plates. The objective of this study was to explore the curative powers of these herbs that exist in nature as a tool to counter disease causing agents. The maximum zone of inhibition of 18 mm of methanolic extract of N. sativa was observed against B. subtalis and S. aureus. Similarly, the maximum zone of inhibition of 18 mm of cold water extract of N. sativa against S. aureus and methanolic extract of Saussurea lappa against S. aureus and Pseudomonas aeruginosa was also observed. The antibacterial action was compared with the effect of ceftriaxone, ceftriaxone sodium, cefuroxine, ciprofloxacin, gentamycine, levofloxacin, metronidazole and tranexamic acid that were used as standered drugs. Based on the results obtained in this study, it may be concluded that plant extracts of A. modesta, A. absinthium, N. sativa and Saussurea lappa have a stronger and broader spectrum of antimicrobial activity against a number of food borne bacteria.

Key words: Herbs, methanolic extract, *Acacia modesta, Bacillus subtalis*, gentamycine, gram negative, gram positive.

INTRODUCTION

Acacia modesta Wall (Phulai) belonging to the family of

Mimosaceae, is regularly used as miswak (tooth brush) for teeth cleaning in various parts of Pakistan (Asghar et al., 2003). Root extracts of *A. modesta* has been tested by Rashid and Hashmi (1999) for bacteriostatic activity against two Gram positive and two Gram negative strains.

^{*}Corresponding author. E-mail: gmdogar356@gmail.com. Tel: +92-314-2082826. Fax: +92-992-383441.

Asghar et al. (2003) checked antibacterial efficacy of twigs extract of *A. modesta* against dental pathogens Lactobacillus (Gram positive).

Artemisisa absinthium is a member of the family Compositae (Asteraceae) and is known by the common names wormwood (UK), absinthe (France) and wermut (Germany). The essential oils obtained by steam distillation from the aerial parts of A. absinthium has been analyzed by GC and GC-MS by Juteau et al. (2003). The oils of A. absinthium contain (Z)-epoxyocimene and chrysanthenyl acetate. Analysis of oils before and after anthesis showed some quantitative differences. As they contain no thujone, antimicrobial screening was performed on samples of French origin and showed that, A. absinthium oil inhibited the growth of both tested yeasts (Candida albicans and Saccharomyces cerevisiae var. chevalieri). The crude extracts obtained from the aerial parts of Artemisia annua Linn. (Asteraceae) by Gupta et al. (2009) have been tested against five Grampositive bacteria (Staphylococcus aureus, Bacillus subtilis. Bacillus pumilus, Bacillus cereus Micrococcus luteus) and three Gram-negative bacteria (Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa) by using agar well diffusion assays, in which the methanol extract showed the strongest activity against most bacteria used in this study. The most sensitive organism to the extracts was *M. luteus*.

Nigella sativa is a member of the family Ranunculaceae and is known by the common names kalonji (Urdu), black seed or black cumin (English) and habat-baraka (Arabic). It is a spicy plant and is cultivated in various parts of the world (Ali and Blunden, 2003). N. sativa Linn. essential oil from N. sativa was studied by Salman et al. (2008) for antibacterial activity against various clinical isolates of bacteria. Among the Gram positive bacteria tested, S. aureus. S. epidermidis, other coagulase Staphylococci and Streptococcus pyogenes has been sensitive to the oil and Enterococcus faecalis, Streptococcus agalactiae has been resistant. Among the Gram negative bacteria tested, only P. aeruginosa has been sensitive to oil and the rest (Acinetobacter baumannii, Citrobacter freundii, Klebsiella pneumoniae, Proteus mirabilis, P. vulgaris and Vibrio cholerae) were insensitive. Masood (2008) studied antibacterial activity of aqueous infusions and aqueous decoctions of kalonji (N. sativa L., Ranu-nculaceae), against 188 bacterial isolates belonging to 11 different genera of Gram positive and Gram negative microorganisms isolated from oral cavity of apparently healthy individuals. The antimicrobial activity of N. sativa essential oil obtained by supercritical fluid extraction by carbon dioxide has been investigated by Nagi et al. (2008) against Gram Positive and Gram negative strains, isolated from clinical specimens.

Saussurea lappa is a member of family Compositae (Asteraceae) and is known by the common names Kuth, Minal (Urdu), Costus (English). S. lappa, Argyreia speciosa and Achyranthes aspera. S. lappa and A. speciosa were found by Gokhale et al. (2002) to

significantly inhibit paw edema induced by carrageenan and Freund's complete adjuvant and to prevent accumulation of inflammatory cells in carrageenan-induced peritonitis at doses of 50 to 200 mg/kg.

This study was performed to evaluate the antimicrobial activity of *A. modesta* (leaves and stem), *A. absinthium* (leaves and stem), *N. Sativa* (seeds) and *S. lappa* (root) being conventionally used as medicines.

MATERIALS AND METHODS

This study (BS339-ML-2009-HU) was carried out in the Microbiology Laboratory, Veterinary Research Institute, Abbottabad. Brief account of materials as well as procedures used is described further.

Chemicals and apparatus

Analytical grade methanol and n-hexane were purchased from Merck, Germany. Distilled water was prepared in the microbiology laboratory.

Centrifuge machine (5702, Eppendorf, Germany), rotary shaker (VRN-360, Gemmy Industrial Corporation, Pakistan), ready made blank antibiotic assay discs (Micropore, Pakistan), soxhlet apparatus (Pyrex, Germany) and water bath (Emmay, Pakistan) were used in this study.

Plant materials

Medicinal plants (registration no. X347B) like *A. modesta* (leaves and stem), *A. absinthium* (leaves and stem), *N. sativa* (seeds) and *S. lappa* (roots) were procured from Botanical Research Centre, Hazara University.

Preparation of extracts

In order to obtain the plants extract, plants were first dried at room temperature and 100 g of each dried plant (*A. modesta, A. absinthium, N. sativa* and *S. lappa*) were coarsely powdered with pestle and mortar and then ground into fine powdered form with the help of electric grinder. Extracts were prepared with solvents like water and methanol by soaking powdered plants for 24 h. The extracts were then stored in refrigerator at 4°C until used.

Cold water extraction

10 g of each powdered plants were soaked separately in 50 ml of distilled cold water and rotated on a rotary shaker at 150 rpm for 24 h. The extracts were filtered through Whatman filter paper no. 1 and then centrifuged at 5000 rpm for 5 min. The supernatants were taken and allowed to evaporate at room temperature until the volume became one fourth of the original volume and then stored in refrigerator at 4°C until used.

Hot water extraction

10 g of each powdered plants were soaked in 50 ml of distilled water for 1 h at 60° C. The extracts were filtered through Whatman filter paper no. 1 and then centrifuged at 5000 rpm for 5 min. The

Table 1. Test organisms.

S/N	Test organism	Gram stain	Diseases caused
1	Bacillus subtalis	Positive	Bronchial disease, food poisoning.
2	Entereococcus faecalis	Positive	UTI, bacterimia, endocarditis, meningitis.
3	Pseudomonas aeruginosa	Negative	Wound infection in burnt patients, UTI.
4	Staphylococcus aureus	Positive	Atopic dermatitis, ritter' disease, endocarditis.
5	Salmonella typhi	Negative	Typhoid, paratyphoid, food born illness.

supernatants were taken and allowed to evaporate at room temperature until the volume became one fourth of the original volume.

Methanolic extraction

10 g of each powdered plants were soaked separately in 50 ml of methanol and rotated on a rotary shaker at 150 rpm for 24 h. The extracts were filtered through Whatman filter paper no. 1 and then centrifuged at 5000 rpm for 5 min. The supernatants were taken and allowed to evaporate at the room temperature until the volume became one fourth of the original volume and then stored in refrigerator at 4°C until used.

Oil extraction for N. sativa

Seeds of *N. sativa* were crushed and packed in paper bags and then extracted with n-hexane in soxhlet apparatus by heating at its boiling point until almost maximum oil had been extracted.

Determination of antibacterial activity

Test organisms

The antibacterial activity was determined against three Gram positive and two Gram negative microorganisms. The Gram positive organisms included *B. subtalis* (ATCC 6633), *E. faecalis* (ATCC 14506) and *S. aureus* (ATCC 6538) and the Gram negative organisms included *P. aeruginosa* (ATCC 27853) and *S. typhi* (ATCC 14028) (Table 1).

Preparation of media

Culturing media used for antibacterial assay was Mueller Hinton agar for the growth of respective bacteria. Mueller Hinton agar media (Table 2) was prepared in a conical flask by dissolving 38 g of powdered agar media in 1 L of distilled water. Flask was heated on open flame to dissolve the medium completely and then sterilized the medium in an autoclave at 121°C temperature for 15 min.

Antibiotic discs

Ready-made sterilized discs of size 6 mm were used, each having maximum capacity of 30 μ l. The plant extracts were taken in separate bottles and the discs were placed in these extracts such that each disc could absorb adequate quantity. Prepared discs were stored at 4°C in the refrigerator till use. The standard antibiotic discs contained ceftriaxone, ceftriaxone sodium, cefuroxine, cipro-

floxacin, gentamycine, levofloxacin, metronidazole and tranexamic acid in each disc. Methanol was used as a control.

Antibacterial assay

Antibacterial activity was determined by disc diffusion method. For this purpose, 10 ml of sterilized media was poured into sterilized 15 ml Petri dishes. Bacterial cultures were seeded on agar media in Petri plates by streaking method. Prepared antibiotic disks were applied on each plate and the plates were then incubated upside down at 37°C for 24 h. The inhibition zones were measured and recorded. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the disc.

RESULTS

In this study, antibacterial effects of *A. modesta* (leaves and stem), *A. absinthium* (leaves and stem), *N. sativa* (seeds) and *S. lappa* (root) against five microbial species (*B. subtalis, E. faecalis, S. aureus, P. aeruginosa* and *S. typhi*) were recorded. Standard antibiotic discs used for this purpose were ceftriaxone, ceftriaxone sodium, cefuroxine, ciprofloxacin, gentamycine, levofloxacin, metronidazole and tranexamic acid.

A. modesta (leaves)

Methanolic extracts showed maximum zone of inhibition of 8 mm against *E. faecalis*, *S. aureus* and *S. typhi* and no activity against *B. subtalis* and *P. aeruginosa* (Table 3). Cold water extracts showed maximum zone of inhibition of 10 mm against *S. aureus*, 9 mm against *E. faecalis* and *P. aeruginosa*, 8 mm against *B. subtalis* and no activity against *S. typhi* (Table 3).

Hot water extracts showed maximum zone of inhibition of 11 mm against *B. subtalis*, 10 mm against *S. aureus* and *P. aeruginosa*, 8 mm against *E. faecalis* and no activity against *S. typhi* (Table 3).

A. modesta (stem)

Methanolic extracts showed maximum zone of inhibition of 10 mm against *B. subtalis*, *E. faecalis* and *S. aureus*, 8 mm against *S. typhi* and no activity against *P. aeruginosa*

	Table 2. Com	position of	Mueller	Hinton agar.
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S/N	Ingredient	Amount (g/l)
1	Beef extract powder	2.0
2	Acid digest of casein	17.5
3	Starch	1.5
4	Agar	17.0

Table 3. Zone of inhibition (mm) of methanolic, cold water and hot water extracts of A. modesta leaves.

Plant extract	B. subtalis ATCC ® 6633	E. faecalis ATCC ® 14506	S.aureus ATCC ® 6538	P. aeruginosaATCC ®27853	S. typhi ATCC ® 14028
Methanolic	Nil	8	8	Nil	8
Cold water	8	9	10	9	Nil
Hot water	11	8	10	10	Nil

table (Table 4).

Cold water extracts showed maximum zone of inhibition of 10 mm against *E. faecalis* and *S. aureus*, 9 mm against *S. typhi* and no activity against *B. subtalis* and *P. aeruginosa* (Table 4).

Hot water extracts showed maximum zone of inhibition of 10 mm against *S. typhi*, 9 mm against *B. subtalis* and *S. aureus* and 8 mm against *E. faecalis* and *P. aeruginosa* (Table 4).

A. absinthium (leaves)

Methanolic extracts showed maximum zone of inhibition of 16 mm against *S. aureus*, 10 mm against *B. subtalis*, 9 mm against *E. faecalis*, 8 mm against *P.aeruginosa* and no activity against *S. typhi* (Table 5).

Cold water extracts showed maximum zone of inhibition of 10 mm against *P. aeruginosa*, 9 mm against *B. subtalis*, 8 mm against *S. aureus* and *S. typhi* and no activity against *E. faecalis* (Table 5).

Hot water extracts showed maximum zone of inhibition of 11 mm against *B. subtalis*, 10 mm against *S. typhi*, 8 mm against *S. aureus* and *P. aeruginosa* and no activity against *E. faecalis* (Table 5).

A. absinthium (stem)

Methanolic extracts showed maximum zone of inhibition of 12 mm against *S. aureus*, 10 mm against *B. subtalis*, 9 mm against *P. aeruginosa* and *S. typhi* and 8 mm against *E. faecalis* (Table 6).

Cold water extracts showed maximum zone of inhibition of 9 mm against *E. faecalis*, 8 mm against *B. subtalis* and *S. aureus* and no activity against *P. aeruginosa* and *S. typhi* (Table 6).

Hot water extracts showed maximum zone of inhibition

of 9 mm against *B. subtalis*, *P. aeruginosa* and *S. typhi*, 8 mm against *S. aureus* and no activity against *E. faecalis* (Table 6).

N. sativa (seeds)

Methanolic extracts showed maximum zone of inhibition of 18 mm against *B. subtalis* and *S. aureus*, 12 mm against *P. aeruginosa* and no activity against *E. faecalis* and *S. typhi* (Table 7).

Cold water extracts showed maximum zone of inhibition of 18 mm against *S. aureus*, 8 mm against *B. subtalis* and *S. typhi* and no activity against *E. faecalis* and *P. aeruginosa* (Table 7).

Hot water extracts showed maximum zone of inhibition of 12 mm against *S. aureus*, 10 mm against *B. subtalis* and *S. typhi* and 8 mm against *E. faecalis* and *P. aeruginosa* (Table 7).

Oil extracts showed maximum zone of inhibition of 16 mm against *B. subtalis*, *S. aureus*, *P. aeruginosa* and *S. typhi* and 8mm against *E. faecalis* (Table 7).

S. lappa (roots)

Methanolic extracts showed maximum zone of inhibition of 18 mm against *S. aureus* and *P. aeruginosa,* 16 mm against *B. subtalis,* 14 mm against *S. typhi* and 10 mm against *E. faecalis* (Table 8).

Cold water extracts showed maximum zone of inhibition of 13 mm against *P. aeruginosa*, 10 mm against *B. subtalis* and *S. aureus*, 9 mm against *S. typhi* and no activity against *E. faecalis* (Table 8).

Hot water extracts showed maximum zone of inhibition of 12 mm against *S. aureus*, 11 mm against *P aeruginosa* and *S. typhi*, 9 mm against *B. subtalis* and 8 mm against *E. faecalis* (Table 8).

Table 4. Zone of inhibition (mm) of methanolic, cold water and hot water extracts of A. modesta stem.

Plant extract	B. subtalis ATCC ® 6633	E. faecalis ATCC ® 14506	S.aureus ATCC ® 6538	P. aeruginosa ATCC ®27853	S. typhi ATCC ® 14028
Methanolic	10	10	10	Nil	8
Cold water	Nil	10	10	Nil	9
Hot water	9	8	9	8	10

Table 5. Zone of inhibition (mm) of methanolic, cold water and hot water extracts of A. absinthium leaves.

Plant extract	B. subtalis ATCC ® 6633	E. faecalis ATCC ® 14506	S. aureus ATCC ® 6538	P. aeruginosa ATCC ®27853	S. typhi ATCC ® 14028
Methanolic	10	9	16	8	Nil
Cold water	9	Nil	8	10	8
Hot water	11	Nil	8	8	10

Table 6. Zone of inhibition (mm) of methanolic, cold water and hot water extracts of *A. absinthium* stem.

Plant extract	B. subtalis ATCC ® 6633	E. faecalis ATCC ® 14506	S.aureus ATCC ® 6538	P. aeruginosa ATCC ®27853	S. typhi ATCC ® 14028
Methanolic	10	8	12	9	9
Cold water	8	9	8	Nil	Nil
Hot water	9	Nil	8	9	9

Table 7. Zone of inhibition (mm) of methanolic, cold water, hot water and oil extracts of N. sativa seeds.

Plant extract	B. subtalis ATCC ® 6633	E. faecalis ATCC ® 14506	S.aureus ATCC ® 6538	P. aeruginosa ATCC ®27853	S. typhi ATCC ® 14028
Methanolic	18	Nil	18	12	Nil
Cold water	8	Nil	18	Nil	8
Hot water	10	8	12	8	10
Oil	16	8	16	16	16

Table 8. Zone of inhibition (mm) of methanolic, cold water and hot water extracts of S. lappa roots.

Plant extract	B. subtalis ATCC ® 6633	E. faecalis ATCC ® 14506	S.aureus ATCC ® 6538	P. aeruginosa ATCC ®27853	S. typhi ATCC ® 14028
Methanolic	16	10	18	18	14
Cold water	10	Nil	10	13	9
Hot water	9	8	12	11	11

Ceftriaxone

The maximum zone of inhibition of 31 mm against *E. faecalis* and *S. typhi*, 30 mm against *B. subtalis*, 26 mm against *S. aureus* and 25 mm against *P. aeruginosa* were recorded (Table 9).

Ceftriaxone sodium

The maximum zone of inhibition of 34 mm against *S. typhi*, 32 mm against *E. faecalis*, 29 mm against *P. aeruginosa* and 27 mm against *B. subtalis* and *S. aureus* were recorded (Table 9).

Table 9. Zone of inhibition (mm) of standard antibiotics.

Antibiotic	B. subtalis ATCC ® 6633	E. faecalis ATCC ® 14506	S.aureus ATCC ® 6538	P. aeruginosa ATCC ®27853	S. typhi ATCC ® 14028
Ceftriaxone	30	31	26	25	31
Ceftriaxone sodium	27	32	27	29	34
Cefuroxine	21	27	10	27	26
Ciprofloxacin	28	27	26	25	26
Gentamycine	22	29	18	30	22
Levofloxacin	28	28	23	29	27
Metronidazole	17	13	10	16	18
Tranexamic acid	19	21	10	10	17
Methanol(control)	Nil	Nil	Nil	Nil	Nil

Cefuroxine

The maximum zone of inhibition of 27 mm against *E. faecalis* and *P. aeruginosa*, 26 mm against *S. typhi*, 21 mm against *B. subtalis* and 10 mm against *S.aureus* were recorded (Table 9).

Ciprofloxacin

The maximum zone of inhibition of 28 mm against *B. subtalis*, 27 mm against *E. faecalis*, 26 mm against *S. aureus* and *S. typhi* and 25 mm against *P. aeruginosa* were recorded (Table 9).

Gentamycine

The maximum zone of inhibition of 30 mm against *P. aeruginosa*, 29 mm against *E. faecalis*, 22 mm against *B. subtalis* and *S. typhi* and 18 mm against *S. aureus* were recorded (Table 9).

Levofloxacin

The maximum zone of inhibition of 29 mm against *P. aeruginosa*, 28 mm against *B. subtalis* and *E. faecalis*, 27 mm against *S. typhi* and 23 mm against *S. aureus* were recorded (Table 9).

Metronidazole

The maximum zone of inhibition of 18 mm against *S. typhi,* 17 mm against *B. subtalis,* 16 mm against *P. aeruginosa,* 13 against *E. faecalis* and 10 mm against *S. aureus* were recorded (Table 9).

Tranexamic acid

The maximum zone of inhibition of 21 mm against E.

faecalis, 19 mm against *B. subtalis,* 17 mm against *S. typhi* and 10 mm against *S. aureus* and *P. aeruginosa* were recorded (Table 9).

Methanol (control)

Methanol was used as a control in this study and no inhibitory results were found against any bacterial strain (Table 9).

DISCUSSION

Antimicrobial activities of different plant parts of A. modesta, A. absinthium, N. sativa and S. lappa were checked against B. subtalis, E. faecalis, S. aureus, P. aeruginosa and S. typhi and standard antibiotics used for the study were ceftriaxone, ceftriaxone sodium, cefuroxine, ciprofloxacin, gentamycine, levofloxacin, metronidazole and tranexamic acid. Antimicrobial activity of stem and leaves of A. modesta was checked. The end results of stem extracts of A. modesta with five bacterial strains showed that these specific parts of the plant have good antibacterial activity and same results were obtained by Rashid and Hashmi (1999) and Asghar et al. (2003). Similarly, their leaves extract also gives good antibacterial activity against these specified microbes. This study revealed that the methanolic and hot water extracts of A. modesta stem and leaves can be used as an alternate of cefuroxine, metronidazole in the treatment of S. aureus infections and tranexamic acid in the treatment of S. aureus and P. aeruginosa infections.

In this study, positive results of leaves and stem extracts of *A. absinthium* with five specified bacterial strains were obtained. Juteau et al. (2003) obtained oil extract from leaves of *A. absinthium* and found positive inhibitory effect against *C. albicans* and *S. cerevisiae* only. We also observed positive results for both leaves and stem extracts. Methanolic extracts of *A. absinthium* leaves and stem can be used as an alternate for cefuroxine, metronidazole and tranexamic acid in the

treatment of *S. aureus* infections, while cold water extracts can be used as an alternate of tranexamic acid in the treatment of *P. aeruginosa* infections.

Antibacterial activity of seeds of N. sativa was checked against B. subtalis, E. faecalis, S. aureus, P. aeruginosa and S. typhi. The end results of all the extracts with five bacterial strains gives excellent antibacterial activity. Alhaj et al. (2008), Salman et al., (2008) and Masood (2008) tested the antibacterial activity of seed extracts of N. sativa against number of bacterial strains and found excellent antibacterial activity. Methanolic extracts, hot and cold water extracts of N. sativa can be used in medicines instead of cefuroxine and gentamycine in the treatment of S. aureus infections, metronidazole in the treatment of B. subtalis and S. aureus infections and tranexamic acid in the treatment of S. aureus infection and P. aeruginosa infections. Oil extracts can be used as alternate of cefuroxine in the treatment of S. aureus infections, metronidazole in the treatment of B. subtalis, S. aureus and P. aeruginosa infections and tranexamic acid in the treatment of S. aureus, P. aeruginosa and S. typhi infections.

Antibacterial activity of roots extracts of Saussurea lappa was tested against B. subtalis, E. faecalis, S. aureus, P. aeruginosa and S. typhi. End result of root extracts of S. lappa against five bacterial strains showed excellent antibacterial activity and the results were confirmed by Patil et al. (2009). Methanolic extracts of S. lappa can be preferred upon cefuroxine and gentamycin in the treatment of S. aureus infections, metronidazole in the treatment of B. subtalis, S. aureus and P. aeruginosa infections and tranexamic acid in the treatment of S. aureus and P. aeruginosa infections. Cold water extracts can be preferred upon cefuroxine and metronidazole in the treatment of S. aureus infections and tranexamic acid in the treatment of S. aureus and P. aeruginosa infections. Hot water extracts can be preferred upon cefuroxine, metronidazole and tranexamic acid in the treatment of S. aureus infections.

Our results showed that the extracts of *S. lappa* (root) and *N. sativa* (seeds) possessed highest antibacterial activity among these plants.

Conclusion

Based on the results obtained in this study, it may be concluded that plant extracts of *A. modesta*, *A. absinthium*, *N. sativa and S. lappa* have a stronger and broader spectrum of antimicrobial activity against a number of food borne bacteria and the extracts may be used to discover bioactive natural products that may serve as basic source for the development of new antimicrobial compounds to overcome the problem of increasing resistance to known traditional antibiotics.

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