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In vitro Antibacterial Activity of *Aerva lanata* (L.) Juss.

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Abstract

The petroleum ether, chloroform and methanol extracts of *Aerva lanata* were screened against four gram-negative bacteria (*Escherichia coli* ATCC 69314, *Klebsiella pneumoniae* NCIM 2719, *Pseudomonas aeruginosa* NCIM 2200 and *Agrobacterium tumefaciens* NCIM 2943) and two gram-positive bacteria (*Staphylococcus aureus* NCIM 2080 and *Bacillus subtilis* MTCC 441). The *in vitro* antibacterial activity was performed by agar well diffusion method. All the three extracts showed promising inhibitory activity against both gram-negative and gram positive bacterial strains tested. The extracts exhibited high degree of sensitivity against gram-negative bacterial. Among the three extracts, methanol extract was highly active and it had a particular good activity against *Pseudomonas aureginosa, Agrobacterium tumefaciens, Staphylococcus aureus* and *Bacillus subtilis* and chloroform extract was highly active against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The most susceptible bacterium was *E. coli.* Our findings offer a scientific basis for the folkloric reputation of this plant.
Key words : Antibacterial activity, *Aerva lanata,* Plant extract, Agar well diffusion.

Nature has been a source of medicinal agents since time immemorial.The importance of plants in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Over the past few years, antibiotic resistance has become a global problem (1). To promote the proper use of herbal medicine and to determine their potentials sources for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified manner (2—4). Hence making antibacterial drug therapy effective, safe and affordable has been the focus of interest during recent years (5). There are several reports on antimicrobial activity of different herbal extracts (6—10). Plants are known to produce some chemicals that are naturally toxic to bacteria (11).Plant-based natural constituents can be derived from any part of the plant (12). Considering these aspects, an attempt was made to carry out the screening for preliminary antibacterial activity of *A. lanata* used in Indian folk medicine.

The plant *A. lanata* belongs to family Amaranthaceae, a branched herb, with the base hard as wood and the branches crect or creeping to the ground. Leaves alternate, wooly-tomentose, 1.3—2.5 cm long. Flower greenish-white, minute, borne in axillary panicles. Fruits greenish, roundish, compressed utricle ; seeds kidney shaped with shining black. It is found in open forests on mountain slopes, on waste and disturbed ground, deserted cultivation and coastal scrub and at altitudes from sea level to 900 meters. The plant finds its folkloric applications, is astringent, bitter, cooling emollient,vermifuge, suppurative, diuretic and lithontriptic. It is useful to treat boils, cephalalgia, cough, strangury, diabetes and lithiasis. Flowers are used for removal of kidney stone (13). The present study was undertaken to investigate the antibacterial activity of *A. lanata* (Amaranthaceae) against some enteric pathogens. The crude extracts of plant were tested for the potential antibacterial property and the selection of this plant was based on its traditional use.

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Methods

Plant Material

Fresh plant material was collected locally from Davangere region, Karnataka (India) in September 2005. Further the taxonomic identification was done by Dr M. Krishnappa, Professor, Department of Applied Botany, Kuvempu University, Shivamoga, Karnataka, India.

Extraction of Plant Constituents

The fresh plant was shade dried and powdered using mechanical grinder ; 200 g of powdered material was soaked in 100 ml of petroleum ether, chloroform and methanol separately for 48 hours. It was filtered by using Whatman no. 1 filter paper. The process was repeated twice. The solvent was distilled out completely from the filtrate under reduced pressure in Rota vapor to get the crude extract.

Test Organisms

The standard bacterial strains were obtained from the Department of Microbiology, PG Center, Kuvempu University, Tolahunase, Davangere, Karnataka, India. Four strains of gram-negative bacteria *Escherichia coli* ATCC 69314, *Klebsiella pneumoniae* NCIM 2719, *Pseudomonas aeruginosa* NCIM 2200 and *Agrobacterium tumefaciens* NCIM 2943 and two strains of gram-positive bacteria *Staphylococcus aureus* NCIM 2080 and *Bacillus subtilis* MTCC 441 were used. The organisms were maintained on nutrient agar slants at 4 C and subcultured in to nutrient broth by a picking off technique for 24 hours before use (14).

Antibacterial Assay

In-vitro antibacterial activity of the crude extract was studied by the agar well plate method (15). Nutrient agar (Hi Media, India) was used as the bacteriological medium. The extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 100 mg/ml. Pure DMSO was taken as the negative control and ciprofloxacin 50 mg/ml as the positive control.

One hundred µl of inoculums was aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculums. Wells were prepared in the agar plates using a sterile cork borer of 6.0 mm diameter ; 100 µl of test and control compounds were introduced in the well. The same procedure was used for all the strains. The plates were incubated aerobically at 35 C and examined after 24 hours. The diameter of zone of inhibition produced by each agent was measured with a ruler and compared to those produced by the commercial antibiotic ciprofloxacin. The results of antibacterial activity of *A. lanata* against the bacterial strains were recorded.

Results and Discussion

The antibacterial activity of the crude extract of *A. lanata* was determined against six microorganisms which include gram-negative and gram-positive bacteria. The presence of antibacterial substances in the plants is well established (16). The results revealed that the leaf extract showed antibacterial activity with various magnitude. Both gram-positive and gram-negative bacteria were sensitive to the plant extracts. However, the results were more promising in gram-negative than gram-positive bacteria tested contradicting the earlier findings that most medicinal plants are more effective against gram-positive than gram-negative bacteria (16, 17). The differences in susceptibility may be explained by differences in cell wall composition and genetic content of plasmids that can be easily transferred among bacterial strains (18).

In vitro studies in this work showed that the plant extracts inhibited bacterial growth. The present results offer a scientific basis for the therapeutic potency of *A. lanata* used in traditional medicine. The observation may be attributed to two reasons ; firstly, due to the nature of biologically active compounds (alkaloids, flavonoids, sterols, tannins, quinine) which may be enhanced in the presence of a solvent (methanol) and secondly, alkaloids, flavonoids and tannins are plants metabolites well known for their antimicrobial activity (19). However, further studies about the safety and toxicity of the extract and isolation of compounds are needed to evaluate possible clinical application in therapy of infectious diseases.

Hence the compounds could be bacteriostatic in nature. These findings indicate the presence of

Table 1. Antibacterial activity of *Aerva lanata* extracts determined by the Well plate method. (a) Petroleum ether, chloroform and methanol. (b) Diameter of inhibition zone in mm. (c) 100 µl of plant extract. (d) Positive control 100 µl of ciprofloxacin per disc for bacteria.

Type of microorganisms	Microorganisms	Type of extract[a]	Zone of inhibition in mm[b] Test[c]	Control[d]
	Agrobacterium tumefaciens		12.1	21.8
	Klebsiella pneumoniae		6.2	8.0
Gram negative bacteria	*Pseudomonas aeruginosa*		7.1	25.0
	Escherichia coli	Methanol	5.9	25.8
Gram positive	*Staphylococcus aureus*		8.2	27.8
bacteria	*Bacillus subtilis*		10.8	20.9
Gram negative	*Agrobacterium tumefaciens*	Chloroform	1.9	20.1
bacteria	*Klebsiella pneumoniae*		6.0	8.0
	Pseudomonas aeruginosa		11.8	24.7
	Escherichia coli		3.5	14.5
Gram positive	*Staphylococcus aureus*		3.1	19.2
bacteria	*Bacillus subtilis*		2.8	24.8
	Agrobacterium tumefaciens		7.1	9.5
Gram negative	*Klebsiella pneumoniae*		6.5	16
bacteria	*Pseudomonas aeruginosa*	Petroleum Ether	6.1	30
	Escherichia coli		7.2	9.1
Gram positive	*Staphylococcus aureus*		5.2	27
bacteria	*Bacillus subtilis*		4.3	25

antibacterial principles in the plant of *A. lanata.* In conclusion, all of the plant extracts tested in this study had potential antibacterial activity against the gram negative bacteria and particularly methanol extract was quite active against *Pseudomonas aeruginosa, Agrobacterium tumefaciens, Staphylococcus aureus* and *Bacillus subtilis* and chloroform extract was active against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Table 1). These results support the use of the plants as traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search of new drugs. The antimicrobial activity could be enhanced if the active components are purified and adequate dosage determined for administration.

References

1. Westh H., C. S. Zinn, Rosdahl and V. T. Sarisa. 2004. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microbial Drug Resist. 10 : 169—176.
2. Roja G. and P. S. Rao. 2000. Anticancer compounds from tissue cultures of medicinal plant. J. Herbs, Spices and Medicinal Pl. 7 : 71—102.
3. Awadh Ali N. A., W. D. Juelich, C. Kusnick and U. Lindequist. 2001. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol. 74 : 173—179.
4. Nitta T., T. Arai, H. Takamatsu, Y. Inatomi, H. Murata, M. Linuma, T. Tanaka, T. Ito, F. Asif, I. Ibrahim, T. Nakanishi and K. Watanabe. 2002. Antibacterial activity of extracts preparated from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus.* J. Hlth. Sci. 48 : 273—276.
5. Sharma K. K., H. Sangraula and P. K. Mediratta. 2002. Some new concepts in antibacterial drug therapy. Indian J. Pharmacol. 34 : 390.
6. Adelakun E. A., E. A. Finbar, S. E. Agina and A. A. Makinde. 2001. Antimicrobial activity of *Boswellia dalziellii* stems bark. Fitoterapia 72 : 822.
7. Camporese A., M. J. Balik, R. Arvigo, R. G. Esposito, N. Morsellino, F. De Simone and A. Tubaro. 2003. Screening of antibacterial activity of medicinal plants from Belice. J. Ethnopharmacol. 87 : 103.
8. Bonjar S. 2004. Evaluation of antibacterial properties of some medicinal plants used in Iran. J. Ethanopharmacol. 94 : 301.
9. De Boer H. J., A. Kool, A. Broberg, W. R. Mziray, I. Hedberg and J. J. Levenfors. 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol. 96 : 461.
10. Nair R., T. Kalariya and S. Chanda. 2005. Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol. 29 : 41—47.
11. Basile A., S. Giordano, J. A. Lopez-Saez and R. C.

Cobianchi. 1999. Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry 52 : 1479—1482.

12. Gordon M. C. and J. N. David. 2001. Natural product drug discovery in the next millennium. Pharmaceutical Biol. 39 : 8—17.
13. Narayan Das P., S. S. Purohit, A. K. Sharma and T. Kumar. 2003. A hand book of medicinal plants of India, volume 1. Agrobios India Publ., Jodhpur, India.
14. Aneja K. R. 2003. Experiments in microbiology, plant pathology and biotechnology, 4th edition. New Age International Ltd., New Delhi, India.
15. Perez C., M. Paul and P. Bezique. 1990. An antibiotic assay by the agar well diffusion method. Acta Bio. Med. Exp. 15 : 113—115.
16. Srinivasan D., S. Nathan, T. Suresh and O. Perumalsamy. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharmacol. 74 : 217—220.
17. Lin J., A. R. Opoku, M. Geheeb-Keller, A. D. Hutchings, S. E. Terblanche, A. K. Jager and J. Van Staden. 1999. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J. Ethnopharmacol. 68 : 267—274.
18. Karaman I., F. Sahin, M. Gulluce, H. Ogutcu, M. Sngul and A. Adiguzel. 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol. 85 : 231—235.
19. Tschesche R. 1971. Advances in the chemistry of antibiotics substances from higher plants : Pharmacognosy and phytochemistry. Pages 274—289 *in* H. Wagner and L. Horharmmer, editors. Proc. 1st Int. Cong., Murich, 1970. Springer-Verlag, Berlin.