International Journal of Basic and Applied Biology
Print ISSN: 2394-5820, Online ISSN: 2349-2539, Volume 2, Number 6; July-September, 2015, pp. 399-403

http://www.krishisanskriti.org/ijbab.html

Phytochemical Screening and Antibacterial Analysis of *Launaea nudicaulis*

Savita Khatri[1] and Anil Kumar Chhillar[2]

[1,2]Centre for Biotechnology, Maharshi Dayanand University, Rohtak -124001, Haryana
E-mail: [1]savikhatri31@gmail.com, [2]anil.chhillar@gmail.com

Abstract—*Launaea nudicaulis was investigated for antibacterial activity against four bacterial strains namely Bacillus cereus (MTCC-10085), Staphyllococcus aureus (MTCC-3160), Salmonella enterica ser. typhi (MTCC-733) and Serratia marcescens (MTCC-9527). Five different plant extracts (petroleum ether, chloroform, acetone, methanol and water) were prepared from leaves by using soxhlet exraction method with % yield of 3.19 %, 35.22 %, 12.87 %, 30.96 % and 8.21 % respectively. Preliminary screening of various extracts for phytochemicals revealed the presence of many secondary metabolites including tannins, steroids, flavonoids, alkaloids, glycosides, terpenoids etc. Then, antibacterial activity was examined using Resazurin based Microbroth Dilution Assay (RMDA) and Disk Diffusion Assay (DDA). According to results of RMDA, acetone extract showed highest activity against S. aureus, S. enterica ser. typhi and S. marcescens i.e. MIC values 0.19 mg/ml, 0.39 mg/ml and 0.19 mg/ml respectively. In DDA, the acetone extract showed maximum zone of inhibition against S. aureus, S. enterica ser. typhi and S. marcescens i.e. 14.76 mm, 13.54 mm and 14.95 mm respectively. While petroleum ether, chloroform, methanol and water extracts showed less activity against tested bacteria. The mechanisms of antibiosis indicated that the acetone extract was highly bactericidal against S. aureus, S. enterica ser. typhi and S. marcescens. The antibacterial activities of L. nudicaulis against tested organisms provide the platform for its utilization in herbal industry.*

1. INTRODUCTION

Bacterial diseases are one of the major infectious diseases caused by pathogenic bacteria. Bacteria emit toxins in the body which damage cells and tissues that accordingly results in infection. Most frequently occurring pathogenic bacteria are *Neisseria meningitides, Streptococcus pneumoniae, Helicobacter pylori, Salmonella typhi* and *Staphylococcus aureus* which can cause meningitis, pneumonia, food poisoning, typhoid and skin infections respectively [1]. Multiple drug resistance has developed in many microorganisms against conventional antibiotics due to their indiscriminate use. In addition to these problems, antibiotics are also associated with adverse effects on the host including hypersensitivity and allergic reactions [2]. So, there is an essential need to discover alternative antimicrobial drugs from other sources. Medicinal plants with antimicrobial activities have gained more significance over conventional antibiotics [3]. Traditionally, herbal agents provide an unexplored source of potential drugs [4]. The pharmacological activities of medicinal plants are due to their secondary metabolites which are comparatively smaller molecules in contrast to the primary molecules such as proteins, carbohydrates and lipids. These natural products offer platform for the synthesis of new structural types of antimicrobial that are safer to mankind [5]. Natural products can be derived from any part of the plant like bark, leaves, flowers, seeds, etc.

Genus *Launaea* consists of about 40 species, belongs to family Asteraceae. Various *Launaea* species possesses many pharmacological activities i.e. insecticidal, antitumor and antioxidant activities. *L. nudicaulis* is a vital plant species of this genus and popularly used in folk medicine for the treatment of fever, toothache, eczema eruptions and rheumatism. It is one of the most fairly widespread plants, growing in dry, saline and sandy habitats. It is a perennial herb with sweet scent and yellow flowers of about 2 cm wide. Due to the wide applications in folk medicines including various potent biological activities such as insecticidal, antimicrobial and anti-inflammatory, this plant has been reported to be a source of various phytochemicals [6]. Considering the vast potentiality of *L. nudicaulis* as sources for antimicrobial drugs, a preliminary investigation was undertaken to examine its phytoconstituents and antibacterial activity against four bacterial strains.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaves of *L. nudicaulis* ware collected from campus of Maharshi Dayanand University, Rohtak, Haryana, India, in June 2012. The plant was identified from Department of Botany, Maharshi Dayanand University, Rohtak, Haryana (India).

2.2. Bacterial strains

Four standard bacterial strains i.e. *Bacillus cereus* (MTCC-10085), *Staphyllococcus aureus* (MTCC-3160), *Salmonella enterica* ser. *typhi* (MTCC-733) and *Serratia marcescens*

(MTCC-9527) were obtained from Institute of Microbial Technology (IMTECH), Chandigarh (India).

2.3. Chemicals and reagents

Petroleum ether, chloroform, acetone, methanol, dimethylsulfoxide (DMSO), Luria Broth (LB), Luria Agar (LA), Resazurin dye and standard antibiotic (Gentamicin) were purchased from Hi-Media.

2.4. Culture of bacterial strains

Bacterial strains were cultured in Luria broth (LB) under aseptic conditions. The final concentration was determined by spectrophotometric analysis. The OD_{600} = 0.5-1 corresponds to bacterial concentration of $5x10^6$ CFU/ml. The bacteria were inoculated and incubated at 37°C for 12-18 hrs in BOD incubator at 100 rpm. The CFU count was estimated by UV-Vis Spectrophotometer (Shimadzu) at 600 nm. A final concentration of $5x10^6$ CFU/ml of log phase culture of bacteria was adopted for the assay [7].

2.5. Sample preparation and extraction

The plant materials were shade dried for two weeks. The dried plant materials were grinded to powdered state. The powder was weighed before extract preparation. The powdered plant materials extracted successively with petroleum ether, chloroform, acetone, methanol and water [7]. The most common and popular method is the Soxhlet's extraction. In this method, each plant leaves were extracted separately in different solvents according to increasing polarity i.e. petroleum ether, chloroform, acetone, methanol and water. The extract was filtered and the filtrate was evaporated under reduced pressure to obtain crude extract of the plant. The percentage yields of crude extracts in various solvents were calculated by the following formulae:

Percentage yield =

$$\frac{\text{Weight of crude extract obtained in grams}}{\text{Total weight of dried plant material in grams}} \times 100$$

2.6. Phytochemical screening

The phytochemical analysis of five different solvent extracts was also performed by following the methods described by Harbone [8].

2.7. Anti-bacterial evaluation of various extracts

The antibacterial activity was studied by Resazurin based Microbroth Dilution Assay (RMDA) and Disc Diffusion Assay (DDA). The extracts were dissolved in 10% (v/v) DMSO to achieve a concentration of 25 mg/ml. Brief descriptions of these assays are given below:

2.7.1. Resazurin based Microbroth Dilution Assay. RMDA was performed in 96 well plates under aseptic conditions. A volume of 100 µl LB was added to the plates. Then, a volume of 100 µl of plant extract (25 mg/ml) was suspended and serially diluted in descending order of concentrations. 10 µl of bacterial suspension of concentration $5x10^6$ CFU/ml was added to each well. Finally, 10 µl of resazurin solution (5X, w/v) was added in each well. Each plate was wrapped with parafilm and placed in an incubator at 37°C for 18h at 100 rpm. The colour change was then observed visually. Any colour change from blue to pink was recorded as positive. The lowest concentration at which colour remained unchanged was taken as minimum inhibitory concentration (MIC) [7]. The minimum bactericidal concentration (MBC) was determined by sub-culturing 50 µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC. The mechanism of antibiosis of extracts was calculated using the ratio of MBC/MIC or MIC_{index} [9] to reveal whether the observed antibacterial effect was bactericidal or bacteriostatic. When the ratio of MBC/MIC was $\leq$2.0, the extract was considered bactericidal or otherwise bacteriostatic. If the ratio is $\geq$16.0, the extract was considered ineffective. Total activity (TA) is the volume at which test extract can be diluted with the ability to kill microorganisms. It is expressed in ml/g [10].

Total Activity = Total isolated extract (mg/g)/ MIC of extract (mg/ml)

2.7.2. Disc Diffusion Assay. The DDA was performed in sterilized petri plates of 10.0 cm diameter (Tarsons). The plant extracts at various concentrations were impregnated on the sterilized discs (6.0 mm in diameter) made up of Whatman filter paper no. 1. The discs were placed on the surface of the agar plates already inoculated with bacterial culture. The plates were incubated at 37°C and examined after 18- 24 hrs for zone of inhibition, if any, around the discs. The concentration, which developed the zone of inhibition of at least 7.0 mm diameter, was considered as MIC [7]. Zone of inhibition of extracts and similarly zone of inhibition of Antibiotic (Gentamicin) was measured to evaluate for activity index [11].

Activity Index = (Zone of inhibition of extract/Zone of inhibition of antibiotic)

3. RESULTS

3.1. Phytochemical screening

Various extracts of *L. nudicaulis* (leaves) examined for antibacterial activity. The percentage yield of extracts and phytochemicals present in five different extracts are shown in Fig. 1 and Table-1 respectively.

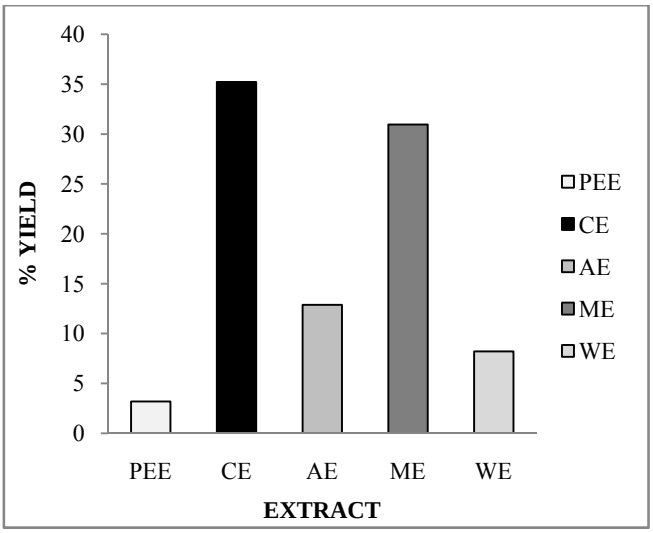

Fig. 1: Percentage yield of different plant extracts (PEE- Petroleum ether extract, CE- Chloroform extract, AE- Acetone extract, ME- Methanol extract, WE- Water extract).

Table 1: Various phytochemicals present in different extracts of *L. nudicaulis* (leaves).

Sr. No	Extract	Phytochemicals						
		A	T	S	G	SA	F	TE
1.	PEE	-	+	-	-	+	-	-
2.	CE	+	-	+	+	+	+	-
3.	AE	+	+	+	-	+	+	+
4.	ME	+	-	+	-	-	+	-
5.	WE	-	+	-	-	-	-	+

A- Alkaloids, T- Tannins, S- Steroids, G-Glycosides, SA- Saponins, F-Flavonoids, TE- Terpenoids, PEE- Petroleum ether extract, CE- Chloroform extract, AE- Acetone extract, ME- Methanol extract, WE- Water extract, - indicates absence and + indicates presence of phytochemicals.

3.2. Antibacterial activity

The MIC values from RMDA and zone of inhibition by DDA of Gentamicin (standard antibiotic) against four bacterial strains are given in Table-2.

3.2.1 Resazurin based Microbroth dilution assay. The various extracts of *L. nudicaulis* (leaves) were observed for antibacterial activity against *B. cereus*, *S. aureus*, *S. enterica ser. typhi*, *S. marcescens* at specific concentrations. The acetone extract showed highest activity against *S. aureus*, *S. enterica ser. typhi* and *S. marcescens* i.e. MIC values 0.19 mg/ml, 0.39 mg/ml and 0.19 mg/ml respectively. The chloroform extract exhibited highest activity against *B. cereus*, *S. enteric ser. typhi* and *S. marcescens* i.e. MIC values 0.78 mg/ml, 0.78 mg/ml and 0.39 mg/ml respectively. The petroleum ether extract showed less activity against all bacterial strains. The water extract also exhibited significant activity against *S. aureus* i.e. MIC value 0.78 mg/ml. The methanol extract also showed considerable activity towards *S. enterica ser. typhi* and *S. marcescens* i.e. MIC value 0.78 mg/ml. Acetone extract showed minimum bactericidal concentrations (MBCs) against *S. aureus*, *S. enterica ser. typhi* and *S. marcescens* i.e. MBC values 0.19 mg/ml, 1.56 mg/ml and 0.19 mg/ml respectively. Similarly, chloroform extract exhibited bactericidal activity against *B. cereus*, *S. enterica* and *S. marcescens* i.e. MBC values 1.56 mg/ml, 1.56 mg/ml and 0.39 mg/ml respectively. The methanol extract showed no activity against *B. cereus* up to tested concentration. The MIC and MBC values along with MIC_{index} of various extracts are shown in Table-3.

3.2.2. Disc Diffusion Assay. According to DDA, the acetone extract showed maximum zone of inhibition against *S. aureus*, *S. enterica ser. typhi* and *S. marcescens* i.e. 14.76 mm, 13.54 mm and 14.95 mm respectively. The chloroform extract exhibited maximum zone of inhibition against *B. cereus*, *S. enterica* and *S. marcescens* i.e. 12.72 mm, 12.56 mm and 13.27 mm respectively. The petroleum ether extract showed mild zone of inhibition against all bacterial strains. The water extract showed moderate zone of inhibition against *S. aureus* and *B. cereus* i.e. 12.37 mm and 11.65 mm respectively. The methanol extract also showed moderate zone of inhibition against *S. enterica ser. typhi* and *S. marcescens* i.e. 12.23 mm and 12.01 mm respectively. The zone of inhibition (ZI) in mm, activity index (AI) and total activity (TA) of various extracts against four tested bacterial strains is shown in Table-4.

Table 2: The MIC values (RMDA) and zone of inhibition (DDA) of Gentamicin (standard antibiotic) against four bacterial strains.

Sr. No.	Name of bacteria	Type	Gentamicin	
			MIC (µg/ml)	ZI (mm)
1	B. cereus	Gram positive	15.62	21
2.	S. aureus	Gram positive	15.62	20
3.	S. enterica ser. typhi	Gram negative	7.81	23
4.	S. marcescens	Gram negative	7.81	22

Table 3: The MIC and MBC values along with MIC_{index} of various extracts of *L. nudicaulis* against four tested bacterial strains.

Sr. No.	Bacterial Strains	Extract	MIC (mg/ml)	MBC (mg/ml)	MIC Index
1.	B. cereus	PEE	3.12	-	-
		CE	0.78	1.56	2
		AE	6.25	-	-
		ME	-	-	-
		WE	1.56	3.12	2
2.	S. aureus	PEE	6.25	-	-
		CE	3.12	-	-
		AE	0.19	0.19	1
		ME	12.50	-	-
		WE	0.78	1.56	2
3.	S. enterica ser. typhi	PEE	1.56	6.25	3
		CE	0.78	1.56	2

		AE	0.39	0.39	1
		ME	0.78	1.56	2
		WE	1.56	-	-
4.	S. marcescens	PEE	1.56	-	-
		CE	0.39	0.39	1
		AE	0.19	0.19	1
		ME	0.78	-	-
		WE	1.56	-	-

PEE- Petroleum ether extract, CE- Chloroform extract, AE- Acetone extract, ME- Methanol extract, WE- Water extract, - indicates no activity up to 25 mg/ml.

Table 4: ZI, AI and TA of various extracts of *L. nudicaulis* against four tested bacterial strains.

Sr. No.	Bacterial strains	Extract	ZI (mm)	AI	Total Activity (ml/g)
1.	B. cereus	PEE	10.59	0.50	10.22
		CE	12.72	0.61	451.54
		AE	9.78	0.46	20.59
		ME	-	-	-
		WE	11.65	0.55	52.63
2.	S. aureus	PEE	9.56	0.48	5.10
		CE	10.47	0.52	112.88
		AE	14.76	0.74	677.37
		ME	8.81	0.44	24.77
		WE	12.37	0.62	105.26
3.	S. enterica ser. typhi	PEE	11.34	0.49	20.45
		CE	12.56	0.55	451.54
		AE	13.54	0.59	330.00
		ME	12.23	0.53	396.92
		WE	11.02	0.48	52.63
4.	S. marcescens	PEE	11.29	0.51	20.45
		CE	13.27	0.60	903.08
		AE	14.95	0.68	677.37
		ME	12.01	0.55	396.92
		WE	11.21	0.51	52.63

PEE- Petroleum ether extract, CE- Chloroform extract, AE- Acetone extract, ME- Methanol extract, WE- Water extract, - indicates no activity up to 25 mg/ml.

4. DISCUSSION

Medicinal herbs have played a crucial role in safeguarding various health ailments since antiquity [12]. As a result of increasing demand of herbal medicines for treatment of several diseases, many plants from ayurvedic system are being explored globally [7]. The leaves extracts of *L. nudicaulis* in five different solvents i.e. petroleum ether, chloroform, acetone, methanol and water were evaluated for antibacterial activity against standard strains of *B. cereus*, *S. aureus*, *S. enterica ser. typhi* and *S. marcescens*. The test bacterial strains used in this work are associated with diverse forms of human infections. From a clinical point of view, *S. marcescens* is now acknowledged as an opportunistic pathogen causing major outbreaks of nosocomial infections, such as urinary tract infections, respiratory tract infections, conjunctivitis, endocarditis, meningitis and wound infections [13]. Infections caused by *B. cereus* and *S. typhi* are serious public health problems associated with food industry in developing countries [14, 15]. *S aureus* causes superficial skin lesions (boils, styes), osteomyelitis, endocarditis and other skin infections [16]. The investigation of antibacterial activity against both gram-negative and gram-positive bacteria is an indication that the plant can be a source of active compounds with broad spectrum functions. Different solvents have diverse degrees of solubility for various phytochemicals and assessed with different range of antimicrobial effect [17]. The ethanol extract and methanol extract of *L. nudicaulis* (Arial parts) has reported for good antibacterial activity against *S. aureus* and *Escherichia coli* [18]. In the present study, we found that acetone extract of *L. nudicaulis* leaves showed highest antibacterial activity against *S. aureus*, *S. enterica ser. typhi* and *S. marcescens* and chloroform extract exhibited highest activity against *B. cereus, S. enterica* and *S. marcescens*. The mechanisms of antibiosis indicated that the acetone extract and chloroform extract are highly bactericidal against tested bacterial strains. As per observations in this study, the plant extracts showed the broad range activity maybe due to presence of multiple phytoconstituents or their synergic effects. This supports the improvement of efficient herbal products derived from active extracts.

5. CONCLUSION

Due to the development of multi drug resistant in various bacterial strains, it is necessary to isolate the potential lead antibacterial compounds from other sources. Medicinal plants provide leads to uncover therapeutically active compounds, thus more efforts should be made towards isolation and characterization of these compound by high throughput screening. The present study disclose that highest antibacterial potential was observed with acetone extract and chloroform extract of *L. nudicaulis* leaves against tested bacterial strains. Hence, this study clearly indicates the antibacterial effect of *L. nudicaulis* leaves thereby provide the possibility to exploring potent bioactive agents possessing antibacterial potential.

6. ACKNOWLEDGEMENTS

The authors wish to thank University Grants Commission, Special Assistance Programme (SAP), India and Maharshi Dayanand University, Rohtak for the necessary support.

REFERENCES

[1] Tiwari, P., Jain, R., Kumar, K., Mishra, R., and Chandy, A., "Antibacterial activity and physicochemical evaluation of roots of *Butea monosperma*", *Asian Pacific Journal of Tropical Biomedicine*, 2, 2, February 2012, pp. S881–S883.

[2] Djeussi, D. E., Noumedem, J. A. K., Seukep, J. A., Fankam, A. G., Voukeng, I. K., Tankeo, S. B., Kuete, A. H. L. N., and Kuete, V., "Antibacterial activities of selected edible plants extracts against multidrug resistant Gram-negative bacteria", *BMC*

Complementary and Alternative Medicine, 13, 164, July 2013, pp. 1-8.

[3] Saranraj, P., and Sivasakthi, S., "Medicinal plants and its antimicrobial properties: A review", *Global Journal of Pharmacology*, 8, 3, March 2014, pp. 316-327.

[4] Malik, V., "Antifungal activity of plant extracts of *Alstonia scholaris*, *Argemone maxicana* and *Datura alba* to Control *Candida albicans*", *Biological Forum – An International Journal*, 7, 1, February 2015, pp. 241-243.

[5] Kalimuthu, S., Rajesh, P., Kannan, V. R., Balamurugan, B., and Chandrasekar, T. M., "Antiulcer activity of methanolic extract of *Acalypha indica Linn.* (Euphorbiaceae) by pylorous ligature and swim stress induced ulceration", *Journal of pharmacy research*, 3, 11, November 2010, pp. 2779-2783.

[6] Alkhathlan, H. Z., Khan, M., Abdullah, M. M. S., Abdullah, M., Mousa, A. A., and Mohammad, Z. A. A., "*Launaea nudicaulis* as a source of new and efficient green corrosion inhibitor for mild steel in acidic medium: A comparative study of two solvent extracts", *International Journal of Electrochemical Science*, 9, December 2013, pp. 870 -889.

[7] Dhankar, S., Dhankar, S., Kumar, M., Ruhil, S., Balhara, M., and Chhillar, A. K., "Analysis towards innovative herbal antibacterial and antifungal drugs", *Recent Patents on Anti-infective Drug Discovery*, 7, December 2012, pp. 242-248.

[8] Harborne, J. B., *Phytochemical methods*. 2nd ed. London: Chapman and Hall; 1991.

[9] Shanmughapriya, S. A., Manilal, A., Sujith, S., Selvin, J., Kiran, G. S., and Natarajaseenivasan, K., "Antimicrobial activity of seaweeds extracts against multiresistant pathogens", *Annals of Microbiology*, 58, 3, September 2008, pp. 535–541.

[10] Sharma, B., and Kumar, P., "Extraction and pharmacological evaluation of some extracts of *Tridax procumbens* and *Capparis decidua*", *International Journal of Applied Research in Natural Products*, 1, 14, August 2009, pp. 5-12.

[11] Ranjan, S., Dasgupta, N., Saha, P., Rakshit, M., and Ramalingam, C., "Comparative study of antibacterial activity of garlic and cinnamon at different temperature and its application on preservation of fish", *Advances in Applied Science Research*, 3, 1, February 2012, pp. 495-501.

[12] Mahima, Rahal, A., Deb, R., Latheef, S. K., Samad, H. A., Tiwari, R., Verma, A. K., Kumar, A., and Dhama, K., "Immunomodulatory and therapeutic potentials of herbal, traditional/indigenous and ethnoveterinary medicines", *Pakistan Journal of Biological Sciences*, 15, 16, August 2012, pp. 754-774.

[13] Su, L. H., Ou, J. T., Leu, H. S., Chiang, P. C., Chiu,Y. Pi., Chia, J. H., Kuo, A. J., Chiu, C. H., Chu, C., Wu, T. L., Sun, C. F., Riley, T. V., and Chang, B. J., "Extended epidemic of nosocomial urinary tract infections caused by *Serratia marcescens*", *Journal Of Clinical Microbiology*, 41, 10, October 2003, pp. 4726-4732.

[14] Mastroeni, P., "Immunity to systemic *Salmonella* infections", *Current Molecular Medicine*, 2, 4, June 2002, pp. 393-406.

[15] Bottone, E. J., "*Bacillus Cereus*, a volatile human pathogen", *Clinical Microbiology Reviews*, 23, 2, April 2010, pp. 382-398.

[16] Stryjewski, M. E., and Chambers, H. F., "Skin and soft-tissue infections caused by community-acquired methicillin-resistant *Staphylococcus aureus*", *Clinical Infectious Diseases*, 46, 5, June 2008, pp. S368–S377.

[17] Cowan, M. M., "Plant products as antimicrobial agents", *Clinical Microbiology Reviews*, 12, 4, October 1999, pp. 564-582.

[18] Al-Mahrezi, J. A., Al-Sabahi, J. N., Akhtar, M. S., Selim, Dina., and Weli, A. M., "Essential oil composition and antimicrobial screening of *Launaea nudicaulis* grown in oman", *International Journal Of Pharmaceutical Science And Research*, 2, 12, November 2011, pp. 3166-3169.