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In-Vitro Antimicrobial Activities of Aqueous and Alcoholic Extracts from a Common Weed *Launaea nudicaulis* (Linn.) Hook.

F. K. PANDEY, SUNJEEV KUMAR, PURNIMA GOPINATHAN AND TRIPTI BHATNAGAR

Codon Institute of Biotechnology
Noida, Uttar Pradesh, India

Abstract

Antimicrobial properties of aqueous and alcoholic leaf extracts of *Launaea nudicaulis* (Linn.) Hook. were investigated against six bacteria *Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus* using the agar disc diffusion method and agar well diffusion method. The aqueous extract was found to be more effective against grampositive bacteria and alcoholic extract was reported to be comparatively more effective against gram-negative bacteria. Of all the microorganism tested the gram-positive were found to be slightly more susceptible to the extracts than the gram-negative bacteria.

Key words : *Launaea nudicaulis,* Antibacterial, Gram-negative, Gram-positive.

Antimicrobial substances are the substance that inhibits the growth and existence of microorganisms (1). These microorganisms could be pathogenic or non-pathogenic hence, antimicrobial substances are used in the treatment of various ailments. Quite a large number of antimicrobial substances exist and they are gotten from diverse sources such as microbes, plants, animals and chemicals (2). Plants have been an integral part of human civilization and their derived substance have recently become of great interest and importance owing to their versatile applications (3). The genus *Launaea* (family Asteraceae) comprises about 40 species. Its importance in folk medicine is illustrated in its use in bitter stomachic, skin diseases, anti tumor and insecticide activities (4). *Launaea nudicaulis* (Linn.) Hook. (Asteraceae) is a rosette herb which grows abundantly in parks, verges, ditches and in places with strong sunlight throughout the plains of Indian subcontinent.

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Methods

Collection of Plant Materials

Fresh plants of *Launaea nudicaulis* collected randomly from the outskirts of Noida and Greater Noida city. Leaves from fresh plants were taken and washed under running tap water, and rinsed with distilled water and air dried.

Extraction Procedures

For 20% aqueous extract preparation, 2 g of air dried plant material was crushed in 10 ml of sterile water in pestle and mortar. The extract was filtered using Whatman's filter paper No. 1. The filtrate was collected and stored at 4C in sterile tubes. For 20% alcohol extract, 2 g of air dried plant material was crushed in 10 ml of ethyl alcohol in pestle and mortar and incubated for 2—3 days for complete evaporation of ethyl alcohol and later dried in oven. Dried mixture was mixed with 10 ml of ethyl alcohol and filtered using Whatman's filter paper No. 1. The filtrate was collected and stored at 4C in sterile tubes.

Preparation of Test Organisms

Microbial cultures were obtained from CBPl, Noida. *Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus* were the bacteria used. Microorganisms were maintained at 4C on nutrient agar slant.

Antimicrobial Activity Testing

The antimicrobial assay was performed by two

Table 1. *In vitro* antimicrobial activity of aqueous and alcoholic extracts of *Launaea nudicaulis* (Linn.) Hook on test microorganisms Disc diffusion method.

Micro-organisms	Gram Stain + /-	*Launaea nudicaulis* (Linn.) Hook Aqueous extract	Alcoholic extract
		Zone of inhibition (mm)	
B. subtilis	+	16	13
E. aerogenes	-	09	11
E. coli	-	10	12
K. pneumoniae	-	13	14
P. aeruginosa	-	10	12
S. aureus	+	17	15

Table 2. *In vitro* antimicrobial activity of aqueous and alcoholic etracts of *Launaea nudicaulis* (Linn.) Hook on test microorganisms Well diffusion method.

Micro-organisms	Gram Stain + /-	*Launaea nudicaulis* (Linn.) Hook Aqueous extract	Alcoholic extract
		Zone of inhibition (mm)	
B. subtilis	+	18	15
E. aerogenes	-	10	12
E. coli	-	12	15
K. pneumoniae	-	16	18
P. aeruginosa	-	12	14
S. aureus	+	21	18

methods viz. agar disc diffusion method (5) and agar well diffusion method (6). Bacterial suspension was prepared and 1 ml of suspension was pippeted onto the surface of solidified agar plate, and gently rotated by hand so as to cover the entire surface with the microbial suspension. The plates were allowed to dry for at least 15 minutes. Sterile disc of 7 mm diameter was saturated with 100µl of the test compound (aqueous/alcohol), allowed to dry and was introduced on the upper layer of the seeded agar plate by means of sterile forceps. The control discs were also incorporated in the fourth quarter. The plates were incubated overnight at 37C in inverted position. For agar well diffusion method, a sterile cork borer No. 4 was used to make wells of 10 mm diameter in each plate for extracts. The bottoms of the wells were sealed with one drop of the sterile nutrient agar, to prevent diffusion of the extracts under the agar. In 3 of 4 wells 100µl of extract (aqueous/alcohol) was poured, the 4th well marked as control, filled with 100µl sterile water/alcohol. Then the plates were incubated overnight at 37C. The activity was evidenced by the presence of zone of inhibition surrounding the disc and well. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extract when compared to the controls.

Results and Discussion

The finding of this study reported in Tables 1 and 2. The results show that alcoholic extracts exhibited a considerably broader antimicrobial activity compared to aqueous extract except in both Gram-positive test organisms used. The agar well diffusion methods however, gave larger zones of inhibition compared to paper disc method (Tables 1 and 2). Agar well diffusion method allows better diffusion of the extract into the medium thus enhancing contact with the organisms (7). Paper discs may act as a barrier between the extract and the organisms thus, preventing total diffusion of active components absorbed by the discs into the medium and may be responsible for the observed differences.

The zone of inhibition of both aqueous and alcoholic extract in disc diffusion as well as in well diffusion method was found to be most effective for *Staphylococcus aureus* and least for *Enterobacter aerogenes*. The low antimicrobial effect of alcoholic plant extract in comparison to aqueous against gram-positive had also been reported earlier (8, 9).

Demonstration of antimicrobial activity of plant extract shows that successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (9, 10). Our finding showed that alcoholic extracts of Launaea nudicaulis (Linn.) Hook. was more effective mostly in gram-negative bacteria. This may be due to the better solubility of the active compounds in organic solvents (11).

Of all the microorganism tested the gram-positive were slightly more susceptible to the extracts than the gram-negative bacteria. This again is in agreement with previous reports that plant extracts are more effective against gram-positive bacteria than gram-negative bacteria (12,13).

Demonstration of antimicrobial activity of *Launaea nudicaulis* (Linn.) Hook . against these microorganism is an indication that there is possibility of discovering alternative antibiotic substance in this plant for the development of newer antimicrobial agents and carry out further pharmacological evaluation.

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