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ESSENTIAL OIL COMPOSITION AND ANTIMICROBIAL SCREENING OF *LAUNAEA NUDICAULIS* GROWN IN OMAN

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ABSTRACT

The essential oil of the aerial parts of *Launaea nudicaulis* was isolated using Clevenger's apparatus and analyzed by GC/MS instrument. Different extracts of the aerial parts of *Launaea nudicaulis* were tested for their activity against *Staphylococcus aureus* and *Escherichia coli*. Seventeen constituents were identified in the essential oil. The major constituents in the essential oil are limonene, Z-citral and E-citral. The three polar extracts (hydro alcoholic, Ethyl acetate and butanol extracts) showed good activity against Gram +ve and Gram-ve bacteria. On the other hand, non-polar extracts (petroleum ether extract and chloroform extract) did not show any antibacterial activity.

INTRODUCTION: *Launaea nudicaulis* is an herb which belongs to the family Compositae (*Asteraceae)*. It's known in Pakistan as Jangli booti and its Arabic name is Al-Hewa [1, 2]. The genus *Launaea* possesses phytochemical features, such as terpenoids, phenolics, flavones and coumarins [3-13].

Many of the plants belonging to the genus *Launaea* are used in folk medicine for skin disease, as anti-tumors, anthelmintics, insecticidal and for renal disorders [14-17].

In Pakistan, *Launaea nudicaulis* has many uses in folk medicine; its milky material is taken during constipation. Leaves are used to relieve fever in children, in treatment of itches of skin, cuts, ulcers, swelling, bilious fever, eczema, eruption and rheumatism. Its roots are used in toothache [1].

In Omani traditional medicine, *Launaea nudicaulis* is used to accelerate healing of wounds and for the treatment of abscesses due to its antimicrobial activity [2]. In this study, we aimed to isolate essential oil from the aerial parts of *Launaea nudicaulis* and analyze it by GC/MS technique to identify its constituents in comparison with the constituents of essential oil isolated from the aerial parts of the same plant grown in Saudi-Arabia [18].

Alcoholic extracts of *Launaea nudicaulis* grown in Pakistan were tested for their antimicrobial activity [1]. Our second rationale was to obtain different extracts of the aerial parts of *Launaea nudicaulis* grown in Sultanate of Oman to be tested for their activity against Gram +ve and Gram-ve bacteria as compared to the results of the Pakistani study.

MATERIALS AND METHODS:

Materials: Clevenger's apparatus (1 Liter), Normkam Company Instrument GC "Perkin Elmer Model # Clarus

600: equipped with MS "Perkin Elmer Model # Clarus 600C". The oven temperature was programmed from 60-280◦C (hold for 2 minutes) at a rate of 3◦C/min. Helium was used as carrier gas with flow rate of 1ml/min. Mass spectra were continuously recorded from 40-500 m/z. The MS operating parameters were: ionization voltage of 70ev, scan rate of 500amu/s. Each compound was identified by comparison of their spectral data with reference spectra in the data bases (Wiley AccessPak V7, May 2003 and NIST2005 version 2.1.0)

Absolute ethanol, butanol, chloroform, ethyl acetate, and petroleum ether were obtained from Sigma chemical company.

Gram negative bacteria, *Escherichia coli* and Staphylococcus *aureus* were obtained from Nizwa Hospital, Nizwa, Sultanate of Oman.

Filter paper discs of diameter 6 mm, Whatmman Company, Catalogue number: 8174900, Sharlau Chemie Company Nutrient agar and plastic petri dishes. Incubator: Gen Lab, Model: MINO/75F, Serial number: Y5K041 Volt: 240A.C., 1 PH, 50 Hz, Load 1 K.W, 4 MPS.

Plant collection: The plant samples were collected from Sultanate of Oman, Al-Mudhaibi region, Ba'ad Village, 90 Km from Sahwa Tower, 18Km on the left, in November 2009. They were identified by Dr. Sulaiman Said Al-Khinjari, at the research center, Nizwa University and the identity was confirmed in the Department of Botany, Sultan Qaboos University. A voucher specimen was deposited at the herbarium of Sultan Qaboos University, Muscat.

Isolation of Essential Oil: The aerial parts of *Launaea nudicaulis* were separated from fresh plant material (2.8Kg) and were subjected to steam distillation using Clevenger's apparatus (1L) to isolate the essential oil. About 1ml of light oil was obtained and analyzed by GC/MS instrument. The antibacterial activity of the isolated oil was tested against two organisms, *E-coli*, and *S. aureu*s on nutrient agar plates using disc diffusion method [5]. The plates were placed in an incubator for 24 hours at 37^{O}C and then observed the diameters of inhibition zones.

Extraction: After collection and identification of the plant, the aerial parts were separated, washed and dried in the shed for one week. 140g of the dried plant material were macerated in 2L of 99.9% ethanol for one week. The ethanolic extract was filtered, dried and weighed. The total weight of the ethanolic extract was 9.5g. The ethanolic extract was re-dissolved in 400ml ethanol: distilled water mixture in 1:1 ratio and transferred to a separating funnel. The hydroalcoholic extract was successively shaken with petroleum ether 750ml, chloroform 500ml, ethyl acetate 250ml and butanol 250ml.

The five extracts were collected and dried to obtain five dried extracts: petroleum ether extract 3.02g, chloroform extract 1.00g, ethyl acetate extract 0.360g, butanol extract 0.96g and hydroalcoholic extract 2.53g.

Antimicrobial screening: The extracts were tested for their antimicrobial activity using two microorganisms, grown on nutrient agar plates using disc diffusion technique. Four concentrations were prepared for each extract 1000µg/mL, 500µg/ml, 250µg/ml and 125µg/ml, using dimethylsulphoxide as a solvent and as a control.

Each of the previously prepared concentrations of different extracts of the ariel parts was tested for its antimicrobial activity against Gram +ve bacteria *Staphylococcus aureus* and against one Gram-ve bacteria *Escherichia coli* on nutrient agar plates using disc diffusion method [5].

RESULTS: The composition of the essential oil is presented in **Table 1**, where compounds are listed in increasing order of retention time (Tr).

DISCUSSION: Seventeen components were identified in the essential oil of *L. nudicaulis* by matching their spectra with reference spectra in the data base. The major constituents in the essential oil are Z-citral, E-citral., and limonene (**Table 1**).

In previous work which is published in J. Saudi Chemical. Soc. [4], only 12 constituents have been reported from essential oil of *L. nudicaulis* which are mostly long chain hydrocarbons of no medicinal value. In this study, a total of 17 constituents have been identified which are mostly of triterpenoid nature

indicating the probability of having the biological activities. Further, biological investigations are to be done in the future.

TABLE 1: GC/MS DATA OF THE MAJOR CONSTITUENTS OF THE ESSENTIAL OIL OF *L. NUDICAULIS*

Peak #	Compound Name	KI	%
1	DL-Limonene	1035.77	18.7
2	Beta-ocimene Y	1051.82	1.5
3	L-Linalool	1106.34	1.7
4	Citronellal	1159.86	1.2
5	Z-Citral	1251.82	22.2
6	E-Citral	1281.02	30.0
7	Citronellyl acetate	1362.595	2.5
8	Neryl acetate	1374.045	1.9
9	Geranyl acetate	1392.366	8.4
10	beta-Elemene	1408.13	1.0
11	trans-Caryophyllene	1441.46	6.7
12	(Z,E)-alpha-Farnesene	1452.03	0.6
13	cis-alpha-bisabolene	1476.42	0.9
14	Germacrene D	1504.27	0.3
15	(E,E)alpha-Farnesene	1521.37	0.7
16	Beta-Bisabolene	1525.64	0.6
17	Germacrene B	1584.61	1.0

It's obviously noticed that only polar extracts (hydro alcoholic extract, ethyl acetate extract and butanol extract) showed activity against gram positive and gram negative bacteria. On the other hand, non-polar extracts (petroleum ether extract and chloroform extract) did not show any activity against gram positive or gram negative bacteria.

For the three polar extracts, activity of each extract against *E. coli* (gram negative) and *S. aureus* (gram positive) was almost equivalent. The three extracts showed close activities against each of the tested organisms. These activities were shown to be comparable to the activities of standard antibiotic, augmentin against the tested organisms (**Table 2**).

TABLE 2: ANTIBACTERIAL ACTIVITY OF FIVE EXTRACTS AGAINST *E. COLI* AND *S. AUREUS*

Name of extract/ standard antibiotic & concentration	Diameter of inhibition (Zone in mm) *(E-coli)*	Diameter of inhibition (Zone in mm) *(S. aureus)*
Hydro alcoholic extract		
1000 µg/ml	**20**	13
500 µg/ml	18	10
250 µg/ml	10	8
125 µg/ml	9	No activity
Butanol extract		
1000 µ g/ml	13	13
500 µg/ml	11	10
250 µg/ml	6	9
125 µ g/ml	5	7
Ethyl acetate extract		
1000 µg/ml	14	11
500 µg/ml	12	9
250 µg/ml	10	9
125 µg/ml	9	7
Chloroform extract 1000 µg/ml 500 µg/ml 250 µg/ml 125 µg/ml	No activity	No activity
Petroleum ether 1000 µg/ml 500 µg/ml 250 µg/ml 125 µg/ml	No activity	No activity
Control (Dimethyl sulfoxide)	No activity	No activity
Augmentin 30 µg/ml	24	50
Isolated essential oil	No activity	No activity

One of the papers has been published in Pakistan Journal of Biological Sciences [1] on *Launaea nudicaulis* (Roxb.) where the polar extracts like ethanolic and methanolic extracts showed good antibacterial activity against Gram +ve and Gram-ve organisms. As compared to our recent finding about antimicrobial activity of *Launaea nudicaulis*, it is obvious that the Omani *Launaea nudicaulis* has better antimicrobial

activity than the Pakistani species. The hydroalcoholic extract showed more inhibition as compared to any other extract of *Launaea nudicaulis*.

CONCLUSION: The present study reveals some useful information about the antibacterial activity of the polar extracts of *Launaea nudicaulis against* Gram +ve and Gram-ve bacteria. Seventeen compounds were identified in the essential oil of the plant and the major constituents are Z-citral, E-citral and limonene,

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