

Antimicrobial and antioxidant activity on Pakistan origin *Launaea nudicaulis* (L.) Hook f.: A promising halophyte

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Abstract: Facultative type of Halophytes is those which can easily grow on both saline and non-saline soil conditions *Launaea nudicaulis* (L.) Hook f. belongs to family compositae [Asteraceae] is facultative halophyte with great medicinal values. The main objective of this study is to explore anti-oxidant and anti-bacterial potential on different morphological parts of *Launaea nudicaulis* (L.) Hook f. The results of the antibacterial activity of methanolic extracts of leaves stem and roots of *Launaea nudicaulis* (L.) Hook f. showed that the potent antibacterial activity observed in all three morphological parts against *Staphylococcus aureus*. DPPH scavenging activity of extracts of root, stem, leaves against standard i.e., ascorbic acid at different concentrations of 20, 40, 60, 80 and 100 showed that root extract have more significant activity when compared to the ascorbic acid. Documented potentials of halophytes are elevating its prominence which motivates scientist to further explore with extensive research.

Keywords: Halophytes, salinization, antioxidant, antibacterial.

INTRODUCTION

With the rapid increase in population of world, increase in scarcity of the fresh water and increase in salinization of agricultural lands is already threatening the food supply (Lieth 1994). To find enough water to meet the world's escalating need of food is considered as one of the urgent global problems (Khan and Duke 2001). Increasing global problem of salinization is also associated with multibillion dollar losses. It is seen that very large areas in Pakistan, Australia, Central Asia, Egypt, India, Mexico, South America, and United States are facing salinization (Lieth and Hamdy, 1999).

Pakistan experiences diverse climate and physiography. Primarily Pakistan has arid and semi-arid land, except the just narrow belt in North, having low rainfall or variable rainfall. Annual Precipitation of the country starts from less than 100mm on western Balochistan coast to 1500 millimeters on southern step of Himalayas. Approximately 69% of country has rainfall just less than 250mm year long. In monsoons (June to September) Major rain falls here. However southwestern Balochistan with Mediterranean trend receives rain in winter and in some of the northwestern areas receives rain in both summer and winter (Khan and Qaisar 2006).

In freshwater scarcity areas halophytes play an important role as they are able to tolerate arid and harsh saline

conditions by the presence of some active constituents that are responsible to maximize their fitness, and from these halophytes many are source of potent medicines which are used for prevention and cure of wide range of diseases. Halophytes have some specific morphological and physiological characters which make them to tolerate salty conditions of soil without restriction in their growth (Hussain *et al.*, 2003). It's enticing to think that we might exploit these halophytic plants for saline water utilization and for rehabilitation of immensely saline soils (Khan and Duke 2001). Number of halophytes have already been proved as forage, vegetable and oilseed crops in the agronomic field trials, although other ones also already have showed remarkable potential to be develop as crop (Shabala and Mackay 2011). Halophytes has ability to seize NaCl in their cell vacuoles as prime plant osmoticum. Minimum functional Na⁺/H⁺ antiport system is required in tonoplast and some special membrane properties that avoid the leakage of Na⁺ from vacuole into the cytoplasm. To maintain the osmotic potential, they also accumulate some organic solutes in cytoplasm. Membrane lipids in halophytes also help in prevention of salt leakage which helps them to survive in salinated soils. These are remarkable plants which have the potential to fulfil almost every basic requirement of human like Fodder, Food, Fuel and also medicine (Glenn *et al.*, 1999; Hameed and Khan 2011).

Due to their adaptations to grow in harsh conditions these plants produce secondary metabolites which can be good source of many medicinally rich phytochemicals like

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Alkaloids, Flavonoids, Tannins and Terpenoids etc (Qasim *et al.*, 2014).

Facultative types of halophytes are those which can easily grow on both saline and non-saline soil conditions *Launaea nudicaulis* (L.) Hook f. belongs to family Compositae [Asteraceae] is facultative halophyte with great medicinal values. Its local name is jungli booti.

Alkaloids, flavonoids, phenolics, sesquiterpenes, diterpenoids, triterpenoids and sphingolipids have been reported in this plant (El-Newary *et al.*, 2021)

In folk medicines it was recommended as bitter tonic and wound healing accelerator. As per Ayurveda the potential wound healing property may be ascribed due to its capability to keep wounds free of infections through its antimicrobial effects or by enhancing wound healing through pharmacodynamics attributes (Kumar *et al.*, 2020)

It is used in treatment of bilious fever, eczema eruptions, cancers, cuts, itches, rheumatism and ulcers, it is also known to be effective against microbial pathogens. Leaves of the plant are used in children as anti-pyretic, and its latex is also used to relieve constipation. Further research proves it has anti-microbial, antioxidant insecticidal and cytotoxic activities. In Pakistan it is found self-grown in vacant lands, waste places and in cultivated fields (Maria *et al.*, 2017)

Traditional medicinal uses of this halophyte prompted us for further studies and research on this plant. In continuation of our main objective to explore this plant we conducted the research on anti-oxidant and antibacterial potential on different morphological parts of *Launaea nudicaulis* (L.) Hook f.

In natural metabolism of living organism including human being Reactive oxygen species (ROS) are regularly formed and play role in signal reduction, but they are also responsible for number of degenerative diseases like alzheimer's disease, atherosclerosis, cataract, diabetes, hypertension, malignant tumors, oxidative stress, parkinson's disease (Halliwell and Gutteridge 1984)

Naturally our body protects itself from such effects via antioxidant enzymatic system and there are also some non-enzymatic biomolecules and proteins are present in living organisms that performs as antioxidant, free radical scavengers. However, some food supplements having ascorbates, carotenoids, flavonoids, phenols and tocopherols plays quite significant role in dealing with Reactive oxygen species (Fritz *et al.*, 2003). The main reason for conducting anti-bacterial activity is need of discovering of new molecules that have capability to cope

with increasing antimicrobial resistance of already present antibiotics. Present need is to find best suitable molecule with broad spectrum antibiotic properties with good efficiency and significant efficacy.

MATERIALS AND METHODS

Collection of plant material

Mature whole fresh plants were collected from the premises of University of Karachi, Karachi and was identified by Prof. Dr. Rubina Dawar of Department of Botany, University of Karachi.

Preparation of plant extract

Firstly, collected plant was cleaned properly later roots, stems, leaves and flowers were separated. 1000g of each of leaves, Stems and roots were soaked separately in methanol and left for 20 days with random shaking. Whatmann filter paper No. 45. was used for filtration of each extract and Buchi Rota vapor R-700 used for solvent evaporation. All the four prepared extracts including leaves (Ln1l), Stems (Ln2s), and roots (Ln3r) were stored at 4°C for further experimentation.

Antibacterial activity

Antibacterial activity was performed using well diffusion method for each extract of root, stem and leaves separately. 100µl of each extract and antimicrobial agent introduced in each well plates were then allowed to incubate for 18-24 hrs. at 35°C - 37°C. Diameter of zone of inhibition was measured from inhibitory zones on plates. All extracts were tested for three times and their mean reading is taken (Mohamed and Ali, 2020).

Antioxidant activity

DPPH Radical scavenging activity

Free radical scavenging ability of extracts were determined by using DPPH. Solution of DPPH was prepared in solvent methanol (0.004% w/v). All four extracts (Ln1l), (Ln2s), and (Ln3r) were added in methanol 95% in order to prepare stock solutions of 10mg/100ml or 100µg/ml. Different volumes Like 2ml, 4ml, 6ml, 8ml and 10ml from stock solution taken and transfer in five different test tubes for serial dilution and make up volume of each up to 10ml. Now the freshly prepared solution of DPPH was then added in all 5 test tubes. After 10 minutes absorbance of each sample and standard was noted on spectrophotometer at 517nm. We used ascorbic acid as reference standard and prepared stock solution in distilled water of concentration 100µg/ml (Mohamed and Ali, 2020). Equation used to measure radical scavenging activity using absorbance of sample and control is as follows:

$$\text{Scavenging (\%)} = \frac{(\text{Absorbance}_{\{\text{control}\}} - \text{Absorbance}_{\{\text{sample}\}})}{\text{Absorbance}_{\{\text{control}\}}} \times 100$$

Table 1: Zone of inhibition (mm) of gram negative and gram-positive bacterial strains.

S No.	Bacterial Strains	Bacteria Type	Ln1l (mm)	Ln2s (mm)	Ln3r (mm)	Ofloxacin (mm)
1.	<i>Staphylococcus aureus</i>	Gram Positive	25	23	15	25
2.	<i>Bacillus subtilis</i>	Gram Positive	15	13	11	26
3.	<i>Escherichia coli</i>	Gram Negative	16	14	12	18
4.	<i>Pseudomonas aeruginosa</i>	Gram Negative	13	12	10	10
5.	<i>Salmonella Typhi</i>	Gram Negative	12	10	10	15
6.	<i>Klebsiella pneumonia</i>	Gram Negative	19	16	14	24

Table 2: DPPH Radical scavenging activity of leaves, stems, roots of *Launaea nudicaulis* (L.) Hook f.

Concentration (µg/ml)	DPPH (Radical Scavenging %)			
	Ln1l	Ln2s	Ln3r	Ascorbic Acid
20	31.54	34.50	36.76	66.83
40	32.39	37.25	40.63	76.76
60	32.46	39.36	42.25	79.57
80	32.74	39.50	46.76	84.50
100	33.45	42.25	49.29	91.54

STATISTICAL ANALYSIS

All the analyses were performed in triplicate in order to minimize the incidence of error. MSTATC statistical computer package were used for analysis.

RESULTS

Antibacterial activity

The results of the antibacterial activity of methanolic extracts of leaves, stem, and roots of *Launaea nudicaulis* (L.) Hook f. was tabulated in table 1. *Staphylococcus aureus* shows most significant antibacterial activity in all three morphological parts. *Klebsiella pneumonia*, *Escherichia coli* and *Bacillus subtilis* also gives antibacterial activity while *Pseudomonas aeruginosa* and *Salmonella Typhi* shows lesser activity.

Antioxidant activity

DPPH Radical scavenging activity is commonly used method for evaluation of antioxidant activity of extracts of plants. Scavenging activity of extracts of roots, stems, leaves against standard i.e., Ascorbic acid at concentrations of 20, 40, 60, 80 and 100 were showed in table 2.

DISCUSSION

Medicinal plants usage in the treatment of diseases is growing its worth not only in developed countries but also in developing areas (Astutik *et al.*, 2019). Scientific research is now our major goal to screen the medicinally important plants especially in salt affected areas (Partricia *et al.*, 2005). Cultivation of salt tolerant crops is now gaining interest for utilization of saline land, conserving freshwater reservoirs and due to economic feasibility

(Lieth 1994). Halophytes chiefly being used as (1) food (2) fodder (3) forage (4) medicine (5) ornament (6) source of phytochemicals, wood including timber and fibers (Arya *et al.*, 2019).

Out of total 410 approximately 272 halophytes are found in Pakistan from which 51 could be source of medicines, 38 are used as food, 47 as fodder, 48 can be used as forage, 34 as ornament and remaining are source of fiber, timber, wood and various chemical (Khan and Qaiser 2006). Proved potentials of halophytes raising its importance and urges researchers to continue its exploration through extensive research. They could revolutionize the future. Previous researches suggest that *Launaea nudicaulis* (L.) Hook f. is proved to be economically feasible, easily accessible and environment friendly plant that has potential to be the important aid to humanity.

There is growing need of antibacterial studies from natural sources to meet the future requirement of antimicrobials for the treatment of infections. Increasing resistance in presently available antibiotics threatening us and demanding researchers for availability of more antibiotics. All three parts of *Launaea nudicaulis* (L.) Hook f. as compared to standard show good activity against *Pseudomonas aeruginosa*. Results showed that leaves possess potential antibacterial activity as compared to other two extracts. The data we found can definitely help in future in choosing better research strategy.

DPPH is itself a stable type of free radical that has ability to accept an electron or hydrogen to change into stable diamagnetic molecule. Freshly prepared solution of DPPH is of deep purple color, and it disappears when an antioxidant is added in the solution. Results obtained from

DDPH assay for antioxidant activity shows activity in leaves while roots and stems have no significant activities. For further studies we can check antioxidant activity on higher concentrations.

CONCLUSION

Proved potentials of halophytes raising its importance and urges researchers to continue its exploration through extensive research. They could revolutionize the future. Previous research suggests that *Launaea nudicaulis* (L.) Hook f. is proved to be economically feasible, easily accessible and environment friendly plant that has potential to be the important aid to humanity. Results show that leaves possess potential antibacterial activity as compared to other two extracts. The outcome of the research we found can help in future in choosing better research strategy.

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