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Green Insights Into *Launaea Pinnatifida*: Extraction Techniques, Phytochemicals, Anti-Oxidant And Anti-Microbial Evaluation

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ABSTRACT
Aim: This study aimed to evaluate the extraction techniques, phytochemical composition, antioxidant potential, and antimicrobial properties of *Launaea pinnatifida* to explore its potential as a natural bioactive source.
Methods: The plant leaves were extracted using hydro alcoholic and petroleum ether solvents. Phytochemical screening identified the presence of alkaloids, flavonoids, tannins, and other secondary metabolites. Quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) was performed using spectrophotometric methods. Antioxidant activity was assessed using DPPH radical scavenging and reducing power assays, while antimicrobial activity was evaluated against *E. coli*, *S. aureus*, and *C. albicans* using the well diffusion method.
Results: The hydro alcoholic extract demonstrated a higher yield (5.30%) compared to petroleum ether (0.82%). The TPC and TFC were recorded as 41.90 mg GAE/g and 35.83 mg QE/g, respectively. The DPPH assay showed an IC50 value of 52.41 µg/mL, indicating moderate antioxidant potential. The hydro alcoholic extract exhibited significant antibacterial activity against *E. coli* and *S. aureus*, particularly in the nanosponge and nanosponge gel formulations, while no antifungal activity was observed against *C. albicans*.
Conclusion: The findings suggest that *Launaea pinnatifida* possesses promising antioxidant and antibacterial properties, supporting its potential use in pharmaceutical applications.

Keywords:*Launaea pinnatifida*, phytochemicals, antioxidant activity, antimicrobial evaluation, extraction techniques.

1.INTRODUCTION

The increasing interest in medicinal plants as sources of natural bioactive compounds has spurred extensive research into their chemical composition and therapeutic properties. Among these, *Launaea pinnatifida*, a member of the Asteraceae family, holds significant potential due to its diverse phytochemical profile and reported medicinal benefits. Native to various regions of Asia, Africa, and the Mediterranean, this halophytic plant has been traditionally utilized in folk medicine for its diuretic, anti-inflammatory, and hepatoprotective properties **(Kumar et al., 2013; Gupta & Jain, 2017).**The extraction of bioactive compounds from plants is a crucial step in phytochemical research, as it determines the yield, purity, and bioavailability of the desired constituents**(Sharma et al., 2015)**.Phytochemical screening of *Launaea pinnatifida* has revealed the presence of flavonoids, alkaloids, tannins, saponins, and phenolic compounds, all of which contribute to its therapeutic efficacy. Flavonoids and phenolic acids, in particular, are known for their strong antioxidant properties, which can mitigate oxidative stress and its associated cellular damage **(Das et al., 2016).** These bioactive compounds have attracted attention for their potential applications in pharmaceuticals, nutraceutical, and functional foods.Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, is implicated in the pathogenesis of various chronic diseases, including cancer, diabetes, and cardiovascular disorders **(Halliwell, 2007)**. Antioxidants derived from natural sources, such as *Launaea pinnatifida*, are increasingly preferred over synthetic antioxidants due to their safety, biocompatibility, and efficacy **(Singh et al., 2020)**.This study aims to provide a comprehensive analysis of *Launaea pinnatifida*, focusing on its extraction techniques, phytochemical composition, and antioxidant activity. By employing both conventional and extraction method, the research seeks to optimize the recovery of bioactive compounds while minimizing environmental impact. Furthermore, detailed phytochemical profiling and antioxidant evaluation will underscore the plant's potential as a natural source of therapeutic agents.

2. MATERIAL AND METHOD

2.1 Pharmacognostical evaluation

2.1.1 Total Ash value

Approximately 5 g of powdered material was accurately weighed and placed in a pre-weighed and pre-ignited silica crucible. The powder was evenly spread in a thin layer at the bottom of the crucible and then gradually incinerated by increasing the temperature until it reached a dull red heat, ensuring the complete removal of carbon. After cooling, the crucible was weighed, and the process was repeated until a constant weight was achieved. The total ash content was then calculated as a percentage relative to the air-dried powder **(Gami et al., 2010).**

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$$\% \text{ Ash content} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Weight of crucible + sample - Weight of crucible}} \times 100$$

2.1.2 Water soluble ash-

The ash obtained following the total ash determination was boiled for five minutes with 25 mL of water. The insoluble residue was then collected on an ash less filter paper, rinsed with hot water, and transferred to a silica crucible. It was ignited for 15 minutes and weighed, with the process repeated until a constant weight was achieved. The weight of the insoluble residue was subtracted from the total ash weight, and the resulting difference was considered the water-soluble ash. The percentage of water-soluble ash was calculated based on the air-dried plant parts **(Gami et al., 2010).**

$$\% \text{ Water soluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} \times 100$$

2.1.3 Acid insoluble ash-

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug **(Gami *et al*., 2010).**

$$\% \text{ Acid insoluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} \times 100$$

2.1.4 Alcoholic extractive value-

A 5g sample of powdered material was placed into a 250mL stoppered conical flask containing 100mL of 90% ethanol, and the stopper was securely replaced. The flask was then agitated in a mechanical shaker for six hours before being left undisturbed for 18 hours. After this period, the mixture was filtered, and 20mL of the filtrate was transferred into a pre-weighed evaporating dish. The filtrate was evaporated to dryness, and the residue was dried in an oven at 105°C for approximately three minutes until a constant weight was obtained **(Egharevba et al., 2010).**The extractive value was calculated.

Alcohol soluble extractive value = Weight of reside /Weight of the drug ×100

2.1.5 Water extractive value-

The procedure was the same as above except that water used in place of 90% ethanol **(Egharevba and Kunle et al., 2010).**

Water soluble extractive value = Weight of residue /Weight of the drug × 100

2.1.6 Loss on drying

Place 2 to 6 g of the sample into a weighing bottle which has been accurately weighed, and weigh it accurately. Then, dry it at 105 o C for 5 - 6 hours and cool it in desiccators with silica gel. When the material is dried to a constant weight, the percent of loss on drying is determined**(Evans *et al.* 1997).**

$$\text{LOD \%} = \frac{\text{Wt. of petridish + crude drug - After drying Wt. of petridish + sample}}{\text{Weight of crude drug}} \times 100$$

2.2 Soxhlet extraction:

The dried and powdered leaves of *Launaea pinnatifida* were first defatted using petroleum ether and then placed in a Soxhlet apparatus. Extraction was performed using a hydro alcoholic solvent system at a temperature of 40-60°C on a heating mantle for 8-10 hours. Following the extraction process, the sample extract was filtered and concentrated until dry. The resulting extract was further evaporated using a rotary vacuum evaporator at 40°C. Finally, the extracts were stored in an airtight container **(Alara et al., 2019).**Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{ X } 100$$

2.3 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in hydroalcoholic extract of *Launaea Pinnatifida* using standard procedures **(Kokate *et al*., 2006).**

2.4 Quantitative Phytochemical estimation-

2.4.1 Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content (TPC) of *Launaea pinnatifida* hydro alcoholic extract was determined using the Folin-Ciocalteu assay. A 0.2 mL aliquot of the extract stock solution was mixed with 2.5 mL of Folin-Ciocalteu reagent. After 5 minutes, 10 mL of a 7.5% sodium carbonate (Na_2CO_3) solution was added, followed by 13 mL of deionized distilled water, and the mixture was thoroughly mixed. The reaction was incubated in the dark at 25°C for 90 minutes, and absorbance was measured at 760 nm. TPC was calculated based on a calibration curve prepared using gallic acid solutions (20–100 µg/mL). The analysis was performed in triplicate, and results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample **(Saeed et al., 2012).**

2.4.2 Spectrophotometric Quantification of Total Flavonoid Content: -

The flavonoid content was analyzed using the Aluminium chloride method described by **Chang et al. (2002)**. In a 10 ml test tube, 0.5 ml of Launaea pinnatifida hydroalcoholic extract was combined with 3.4 ml of 30% methanol, 0.15 ml of 5% $NaNO_2$, and 0.15 ml of 10% $AlCl_3 \cdot 6H_2O$. After five minutes, 1 ml of 4% NaOH was added, and the mixture was thoroughly mixed. The absorbance was then recorded at 510 nm against a reagent blank. A standard curve for total flavonoids was prepared using rutin standard solutions (20–100 µg/ml) following the same procedure. The flavonoid content was expressed as milligrams of rutin equivalents per gram of dried fraction **(Senguttuvan et al., 2014).**

2.5 Activity (*In-vitro* Anti-oxidant Activity)

2.5.1 DPPH Radical Scavenging Activity

A 0.1mM DPPH solution was prepared in methanol. A fresh 1 mg/ml methanol solution of extracts or standard was also prepared. Varying volumes (20–100µl) of the extract or standard were taken from the stock solution, diluted with methanol to 1 ml, and mixed with 2 ml of 0.1mM DPPH solution. The mixture was incubated in the dark at room temperature for 30 minutes before measuring absorbance at 517 nm. For the control, 3 ml of 0.1mM DPPH solution was incubated under the same conditions, with absorbance recorded against methanol as a blank **(Sagar et al., 2011).**Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

2.5.2 Reducing power assay

A standard solution of ascorbic acid was prepared by dissolving 3 mg in 3 ml of distilled water. Serial dilutions were made to obtain concentrations of 20, 40, 60, 80, and 100 µg/ml. For extract preparation, stock solutions were prepared by dissolving 1 mg of dried extract in 1 ml of methanol, yielding a concentration of 1 mg/ml. These were further diluted to achieve sample concentrations of 20, 40, 60, 80, and 100 µg/ml. The reducing power assay was conducted by mixing 1.0 ml of different concentrations of ascorbic acid or extract with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, followed by cooling. Then, 2.5 ml of 10% trichloroacetic acid was added, and the solution was centrifuged at 3000 rpm for 10 minutes. The upper layer (2.5 ml) was combined with 2.5 ml of distilled water and 0.5 ml of freshly prepared 0.1% ferric chloride solution. Absorbance was measured at 700 nm using a UV spectrometer (Shimadzu-1700), with a blank prepared without the extract **(Quisumbing, 1978).**

2.6 Antimicrobial activity

2.6.1 Bacterial and fungal strains and culture conditions

The bacteria used in this study were *S. aureus* MTCC 10787, *E.coli*MTCC 42and*C. albicans*. *Bacteriaand fungus* were cultured in Nutrient agar and Saboured dextrose agar media. All strains were cultured at 37 °C for 24-48 h.

2.6.2 Antimicrobial activity by well diffusion assay

Antimicrobial assay of extracts of different plants was performed by agar well diffusion method in Nutrient agarmedia (NAM) andSaboured dextrose agar (SDA) plates. For the preparation of NAM Media, 28 g of Nutrient Media was dissolved in 1 liter of distilled water, and the pH was verified before autoclaving at 121°C and 15 lbs pressure for 15 minutes.For the preparation of SDAMedia suspend the 65gm of dehydrated SDA powder in distilled water, heat to boiling while stirring to completely dissolve it, adjust the pH to around 5.6, and then sterilize by autoclaving at 121°C for 15 minutes. Subsequently, the media was poured into plates and allowed to solidify in a laminar air flow. The test organisms were inoculated in broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5 × 108 CFU/ml. A well with a diameter of 5-6 mm was created on the agar plate using a cork borer. Next, 100µl of the inoculum was transferred to a fresh sterile solidified Agar Media Plate. The agar plate was inoculated by spreading the inoculum with a sterile spreader, and three wells of 6 mm were bored into the inoculated media. Each well was then filled with varying samples (Isolated compound, nanosponges and nanosponge gel). After 30 minutes of diffusion at room temperature, the plates were incubated at 27°C for 24 hours. The antimicrobial activity was determined by measuring the diameter (mm) of the clear zone of growth inhibition **(Manandhar et al., 2019).**

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
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http://www.veterinaria.org
Article Received: Revised: Accepted:

3. RESULTS

3.1 Pharmacognostical evaluation

Table 1: Pharmacognostical evaluation of *Launaea Pinnatifida* (Leaves) plant sample

Parameters	**Value in percentage (%)**
	***Launaea Pinnatifida* (Leaves)**
Total ash value	1.96
Water soluble ash	0.85
Acid insoluble ash	0.76
Water extractive value	1.56
Alcoholic extractive value	2.89
Loss on drying	1.97

3.2 Percentage yield

Table 2: Percentage yield of extracts

S. No.	**Plant name**	**Solvent**	**Color of extract**	**Theoretical weight (gm)**	**Yield (gm)**	**% Yield**
1.	*Launaea Pinnatifida*	Petroleum Ether	Yellow to Brownish	100	0.821	0.82
2.	*Launaea Pinnatifida*	Hydro alcoholic	Dark Brown	99.00	5.248	5.30

3.4 Solubility determination of Hydro alcoholic extractof *Launaea Pinnatifida* -

Table 3: Solubility Determination of Hydro alcoholic extract of *Launaea Pinnatifida*

S. No.	**Solvent**	**Result**
1.	Water	Partially Soluble
2.	Ethanol	Soluble
3.	Ethyl Acetate	Sparingly Soluble
4.	DMSO	Soluble
5.	Petroleum Ether	Insoluble
6	Methanol	Soluble
7	Chloroform	Soluble
8	Acetone	Partially Soluble

3.5 Qualitative Phytochemical Analysis of Hydro alcoholic extractof *Launaea Pinnatifida*

Table 4: Phytochemical analysis of Hydro alcoholic extract of Launaea Pinnatifida

S. No.	**Experiment**	**Result**	
		Petroleum ether	**Hydroalcoholic**
Test for Carbohydrates			
1.	Molisch's Test	+	+
2.	Fehling's Test	-	+
3.	Benedict's Test	+	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	+	+
2.	Hager's Test	-	-
3.	Wagner's Test	-	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	-	-
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	+	+
2.	Lead Acetate Test	-	+
3.	Gelatine Test	-	+
Test for Saponins			

1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	-
2.	Biuret's Test	-	+
Test for Glycosides			
1.	Legal's Test	-	+
2.	Keller Killani Test	-	+
3.	Borntrager's Test	-	+

3.6 Quantitative Phytochemical analysis of Hydro alcoholic extractof *Launaea Pinnatifida* -

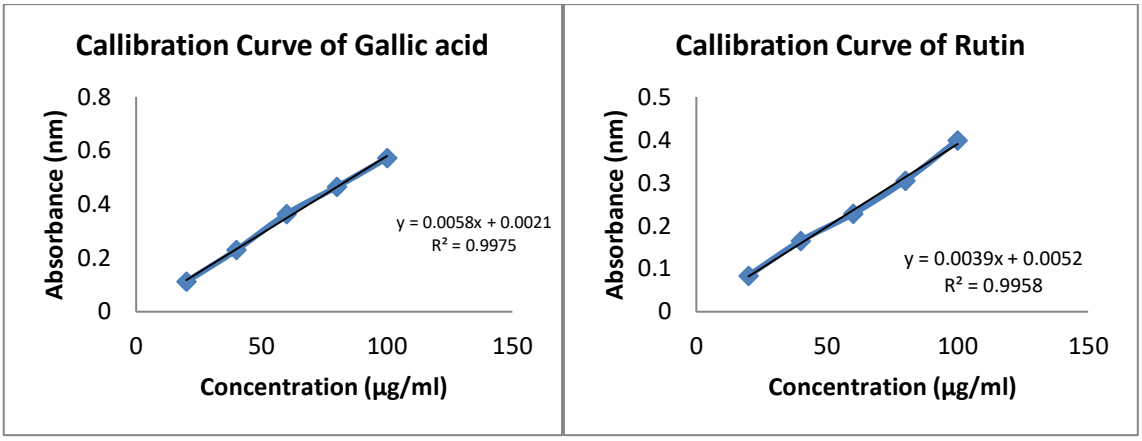
3.6.1 Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) Estimation

Table 5: Standard table for Gallic acid

Concentration (µg/ml)	Absorbance (nm)
20	0.112
40	0.23
60	0.364
80	0.465
100	0.572

Table 6 : Standard table for Rutin

Concentration (µg/ml)	Absorbance (nm)
20	0.084
40	0.165
60	0.228
80	0.305
100	0.399



Graph 1: Graph represent standard curve of Gallic acid and Rutin

Table 2 : TPC and TFC in *Launaea Pinnatifida* Hydro alcoholic Extract-

Extracts	Total Phenolic content (mg/gm equivalent to Gallic acid)	Total Flavonoid content (mg/gm equivalent to Rutin)
Absorbance Mean±SD	0.2115±0.003	0.1125±0.002
TPC	41.90	35.83

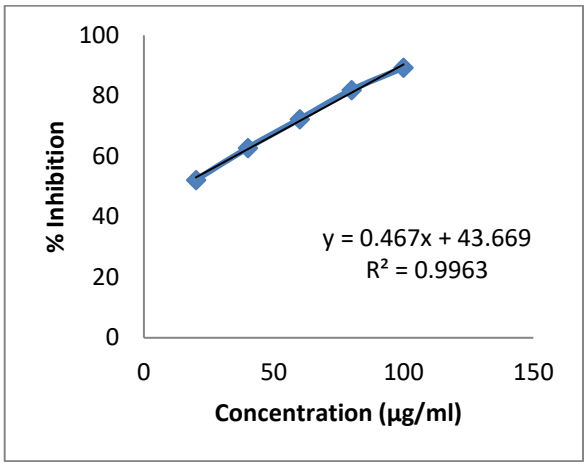
REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

3.7 Anti-oxidant activity

3.7.1 DPPH Assay

Table 8: DPPH radical scavenging activity of Ascorbic acid

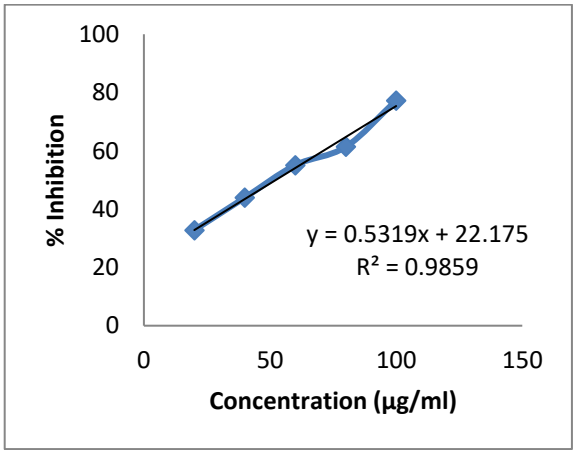
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.442	52.164
40	0.344	62.770
60	0.256	72.294
80	0.167	81.926
100	0.099	89.285
Control	0.924	
IC50		**13.576**



Graph 2: Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

Table 9: DPPH radical scavenging activity of Hydroalcoholic extract of *Launaea pinnatifida*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.621	32.792
40	0.518	43.939
60	0.415	55.086
80	0.357	61.363
100	0.21	77.272
Control	0.924	
IC50		**52.410**



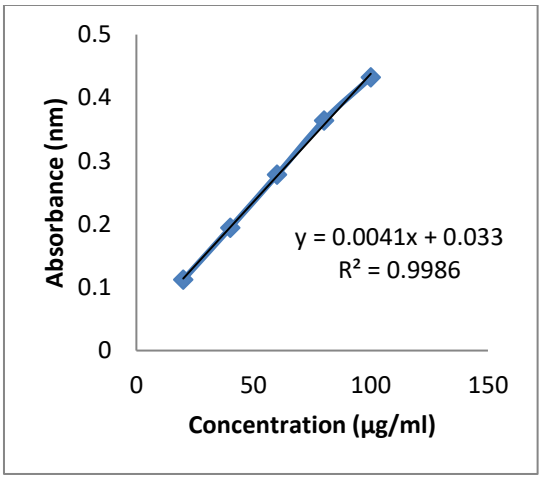
Graph 3: Graph represents the Percentage Inhibition *Vs* Concentration of Hydroalcoholic extract of *Launaea pinnatifida*

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

3.7.2 Reducing power scavenging activity

Table 10: Reducing power scavenging activity of Ascorbic acid

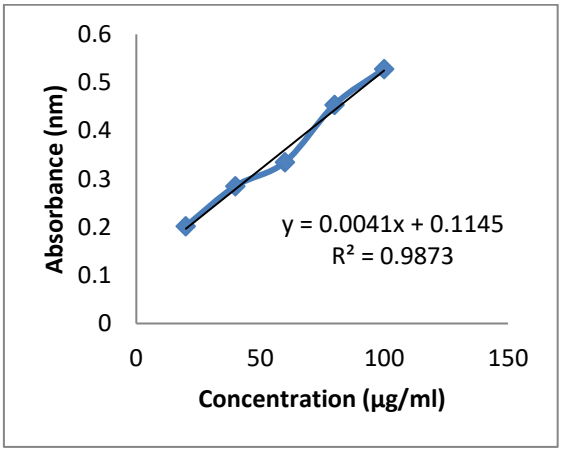
Concentration (µg/ml)	Absorbance
20	0.112
40	0.194
60	0.278
80	0.364
100	0.432



Graph 4: Graph represents the Absorbance *Vs* Concentration of Ascorbic acid

Table 31 :Reducing power scavenging activity of Hydroalcoholic extract of *Launaea Pinnatifida*

Concentration (µg/ml)	Absorbance
20	0.202
40	0.285
60	0.335
80	0.454
100	0.528



Graph 5: Graph represents the Absorbance *Vs* Concentration of Hydroalcoholic extract of *Launaea Pinnatifida*

3.8 Antimicrobial activity of samples

Table 12 Anti-bacterial activity of samples against *E. coli*

Sample name	Plate 1	Plate 2	Plate 3	Mean±SD
Isolated compound	7	10	8	8.333±1.527
Nanosponges	12	12	11	11.666±0.577
Nanosponges gel	16	15	17	16±1

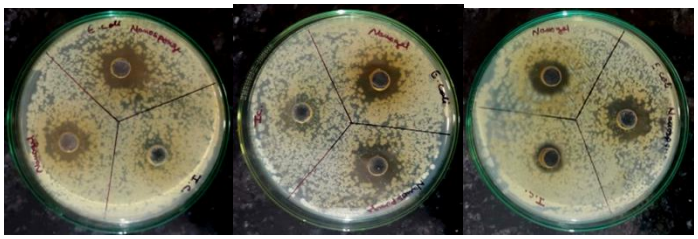


Table 14Anti-bacterial activity of samples against *S. aureus*

Sample name	Plate 1	Plate 2	Plate 3	Mean±SD
Isolated compound	9	10	9	9.333±0.577
Nanosponges	11	11	12	11.333±0.577
Nanosponges gel	14	15	18	15.666±2.081

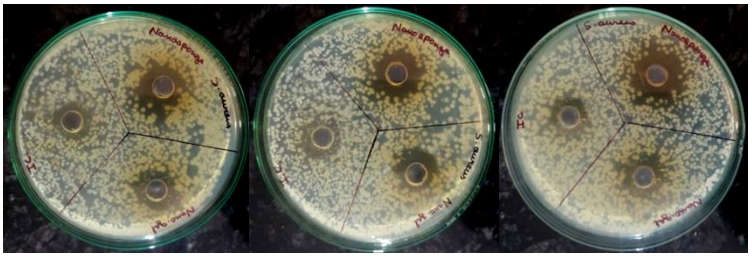


Table 5 Anti bacterial activity of samples against *C. albican*

Sample name	Plate 1	Plate 2	Plate 3	Mean±SD
Isolated compound	0	0	0	0±0
Nanosponges	0	0	0	0±0
Nanosponges gel	0	0	0	0±0

4. DISCUSSION

The Pharmacognostical evaluation of *Launaea pinnatifida* leaves revealed significant findings regarding its physicochemical properties. The total ash value was 1.96%, with water-soluble and acid-insoluble ash values of 0.85% and 0.76%, respectively. The extractive values indicated that the plant contained more alcohol-soluble components (2.89%) than water-soluble ones (1.56%), and the loss on drying was recorded at 1.97%. The percentage yield of extracts showed that the hydro alcoholic extract had a significantly higher yield (5.30%) compared to the petroleum ether extract (0.82%). Solubility tests of the hydro alcoholic extract indicated solubility in ethanol, methanol, chloroform, and DMSO, while it was partially soluble in water and acetone but insoluble in petroleum ether and ethyl acetate. Phytochemical analysis confirmed the presence of various bioactive compounds, including carbohydrates, alkaloids, terpenoids, flavonoids, tannins, saponins, glycosides, and proteins. However, certain tests, such as Hager's test for alkaloids and protein detection via the Ninhydrin test, yielded negative results. The quantitative estimation of phytochemicals showed a total phenolic content (TPC) of 41.90 mg/g (equivalent to gallic acid) and a total flavonoid content (TFC) of 35.83 mg/g (equivalent to rutin), confirming the plant's rich antioxidant potential. Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by Reactive Oxygen Species. Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process. The antioxidant activity of plant extracts was determined by different in vitro methods such as the DPPH and Reducing power assay. The antioxidant activity of *Launaea pinnatifida* was assessed through DPPH and reducing power assays. The hydro alcoholic extract exhibited dose-dependent free radical scavenging activity, though its IC50 value (52.410 µg/ml) was significantly higher than that of ascorbic acid (13.576 µg/ml), indicating moderate antioxidant

potency.Ascorbic acid was used as a reference compound. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. In the reducing power assay, the extract demonstrated a concentration-dependent increase in absorbance, suggesting electron-donating capacity similar to ascorbic acid. Antimicrobial activity was evaluated against *E. coli*, *S. aureus*, and *C. albicans*. The nanosponges gel formulation exhibited the highest antibacterial activity, with inhibition zones of 16±1 mm against *E. coli* and 15.666±2.081 mm against *S. aureus*, outperforming both nanosponges and the isolated compound. However, none of the tested formulations showed any activity against *C. albicans*, indicating the extract's lack of antifungal efficacy. These findings suggest that *Launaea pinnatifida* possesses promising antibacterial and antioxidant properties, warranting further investigation for potential pharmaceutical applications.

5. CONCLUSION

The study on *Launaea pinnatifida* provides valuable green insights into its extraction techniques, phytochemical composition, antioxidant, and antimicrobial potential. The hydro alcoholic extract exhibited a higher yield and a rich presence of bioactive compounds, including flavonoids, phenolics, alkaloids, and saponins. The antioxidant assays confirmed significant free radical scavenging activity, highlighting its potential as a natural antioxidant. Antimicrobial evaluation demonstrated strong antibacterial activity against *E. coli* and *S. aureus*, particularly in nanosponges and nanosponges gel formulations, while showing no effect against *C. albicans*. These findings suggest that *Launaea pinnatifida* holds promise for pharmaceutical and nutraceutical applications, supporting its traditional medicinal use and encouraging further research into its therapeutic potential.

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