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Development and evaluation of dandelion (*Taraxacum officinale)* leaves nanogel for the treatment of bacterial skin infection

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Abstract
This study explores the antibacterial and pharmaceutical potential of *Taraxacum officinale* (dandelion) leaves, focusing on the development of a nanogel formulation for the treatment of bacterial skin infections. Leaves from dandelions were shade dried, powdered, then extracted using methanol, ethanol, and water through Soxhlet extraction. Flavonoids, alkaloids, tannins, saponins, and phenolic compounds were present as confirmed by the phytochemical screening. Solubility, partition coefficient and entrapment efficiency studies were performed to characterize the extract. The methanolic and ethanolic extracts exhibited excellent antibacterial activity against Staphylococcus aureus, having inhibition zones ranging between 12 - 20 mm and a MIC value was between 0.25 to 1.0 mg/mL. The findings suggest that dandelion leaves possess strong antibacterial properties and, when formulated into nanogels, could serve as a safe and natural alternative for managing bacterial skin infections.

Keywords: *Taraxacum officinale,* dandelion leaves, phytochemical analysis, nanogel formulation, antibacterial activity, Staphylococcus aureus

Introduction
Dandelion (*Taraxacum officinale*), a perennial herb of the Asteraceae family, comprises more than 300 species and has long been valued in both culinary and medicinal practices. The leaves have typically been eaten raw in salads and the roots have generally been roasted and used as a nifty coffee substitute [1]. Beyond its nutritional role, *Taraxacum officinale* has attracted considerable pharmacological interest due to its diverse bioactive phytochemicals, including phenolic compounds, flavonoids, sesquiterpene lactones, and triterpenes. These constituents contribute to the plant's well-documented antibacterial, antioxidant, anti-inflammatory activities [2]. The increasing prevalence of antibiotic resistance has heightened the demand for safer and more effective natural alternatives. In this regard, dandelion represents a promising candidate because of its broad spectrum of biological activities, particularly against bacterial pathogens such as Staphylococcus aureus, a common causative agent of skin infections [3]. The present study investigates the phytochemical profile, solubility characteristics, and *in vitro* antibacterial activity of *Taraxacum officinale* leaf extracts, with particular emphasis on their incorporation into a nanogel system for the treatment of S. aureus-induced skin infections [4].

Material and Method
Collection & Authentication of *Taraxacum officinale* leaves
Fresh leaves of *Taraxacum officinale* were collected from the Ganai area of Himachal Pradesh. and Authenticated by a qualified botanist Dr. Jagdeep Verma at the Department of Botany, in Sardar Patel University, Mandi, Himachal Pradesh, Ref: BOT/350.2 [5].

Preparation of Methanolic extract of *Taraxacum officinale* leaves
The powdered leaves that had been shade -dried were extracted using Soxhlet process, using methanol 500g of leaf were extracted with methanol for 72 hours to create the crude methanolic extract [10]. After extraction, the mixtures were allowed to cool and then solvent was removed by distillation and the prepared extract was stored in vacuum desiccator till further use [6].

Percentage Yield

The percentage yield of the extract indicates the efficiency of the solvent extraction process, representing the proportion of extract obtained from the leaves relative to the initial mass of plant material, expressed as a percentage [7].

The percentage yield was calculated using the following formula:

$$Percentage\ yield = \frac{weight of\ dried\ extract}{weight\ of\ dried\ plant\ material} \times 100$$

Phytochemical Screening

The methanolic extract of *Taraxacum officinale* leaves was subjected to standard phytochemical tests to evaluate its bioactive composition. The analysis revealed the presence of phenolics, flavonoids, coumarins, sesquiterpene lactones, triterpenes, alkaloids, tannins, saponins, and polysaccharides. these compounds are associated with significant therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory activities, underscoring the medicinal value of dandelion leaves [8].

Optimization of Nanoparticles:

To develop effective nanoparticles, four different formulations (F1-F4) were prepared using the solvent evaporation method. the optimization process involved varying the amounts of Chitosan and Tetracycline and adjusting their polymer-to-drug ratios, while keeping the solvent ratio of Dichloromethane to Chloroform constant (5:1). The optimized nanoparticles were subsequently subjected to characterization, including pH, appearance, odour, and other relevant physicochemical properties [9].

Formulation of Nanoparticles

Nanoparticles encapsulating amoxicillin were prepared using the solvent evaporation method. for each formulation (F1-F4), Chitosan and Tetracycline were weighed and dissolved in a mixture of dichloromethane and chloroform, with solvent ratios adjusted for each formulation [10]. separately, PVA solution was prepared as stabilizer. the organic phase was added dropwise into the aqueous phase under magnetic stirring to form an oil-in-water emulsion, which was sonicated in an ice bath. the mixture was stirred at room temperature for 3-4 hours to evaporate the solvents. nanoparticles were collected by centrifugation, washed, redispersed in water, and lyophilized with mannitol as cryoprotectant [11].

Optimization of Nanogel

The nanogel was optimized to achieve desirable physicochemical properties, stability, and therapeutic efficacy for topical application. formulations were prepared using carbomer 940 with methylparaben, glycerine and ammonia solution. the nanogels were evaluated for viscosity, spreadability, pH, homogeneity, and consistency, and the pH was adjusted using ammonia solution to maintain skin compatibility. nanoparticle characteristics, including particle size and zeta potential, were also considered to ensure stability and bioavailability [12].

Formulation of Nanogel

Nanogel was prepared for topical application using carbomer 940 gel base containing methylparaben as a preservative & glycerine. The ingredients were mixed under continuous stirring using a high-speed mechanical stirrer to achieve the required viscosity [13]. An aqueous dispersion was gradually incorporated into the mixture with continuous stirring, and the pH of the developed nanogel was neutralized using a few drops of ammonia solution. The prepared nanogels were selected for formulation and further experimental analysis [14].

Evaluation of Nanogel

Evaluation of herbal gel is the process of examining its physical, chemical, and functional properties to ensure stability, effectiveness, and suitability for topical application [15].

Organoleptic

The nanogel was observed for its colour, odour, and general appearance. These observations ensure that the gel is smooth, uniform, and acceptable [16].

pH

The pH of the gel was measured using a pH meter to determine its acidity or alkalinity. maintaining a near-neutral pH (around 6-7) is important for skin compatibility [17].

Viscosity

Viscosity indicates the thickness and flow of the gel, which affects ease of application and spreadability. it was measured using a viscometer to ensure proper consistency[18].

Spreadability

Spreadability determines how easily the gel spreads on the skin. it was calculated using the formula [19].

Formula S = m x l/t

Homogeneity

The gel was checked to ensure it is lump-free, smooth, and uniform throughout, indicating good [20].

Entrapment efficiency

Entrapment efficiency (EE) refers to the percentage of drug or active substance successfully incorporated within the nanoparticle matrix relative to the total amount used. It is an important parameter to evaluate the effectiveness of nanoparticle-based drug delivery systems. The EE% is determined by separating the free (unentrapped) drug from the formulation, usually by centrifugation, and quantifying it using spectrophotometric method [21].

Entrapment efficiency is calculated using the following formula:

$$EE\ (\%) = \frac{(Total\ drug - Free\ drug)}{Total\ drug} \times 100$$

Characterization of Nanoparticles

Particle size

Particle size refers to the average diameter of individual nanoparticles present within a nanogel formulation. It is a critical parameter that influences the stability, bioavailability, skin penetration, and release characteristics of the encapsulated bioactive compounds. Smaller and uniform particle sizes generally enhance therapeutic efficacy

and ensure consistent performance in topical applications [22].

Zeta potential

Zeta potential is the measure of the surface charge of nanoparticles or colloidal systems, expressed in millivolts (mV). It represents the electrical potential at the slipping plane of particles suspended in a medium. "Zeta potential measurements have been reported to provide important information on the electrostatic stability of nanoparticles, nanogels and all other colloidal formulations [23].

Scanning Electron Microscope

Scanning Electron Microscope is an advanced microscopy technique that uses a focused beam of electrons to produce highly magnified images of the surface of a sample, giving detailed information about its morphology and surface structure [24].

In Vitro Antibacterial Assay

The antibacterial activity of the prepared nanogel was evaluated against *Staphylococcus aureus* following the ATCC100 method with minor modifications. The bacterial strain was first cultured in Mueller-Hinton broth, and a standardized suspension (2×10^5 CFU/mL) was prepared. Different concentrations of the nanogel (1250, 2500, and 5000 µg/mL) were obtained by serial dilution of the stock solution in Mueller-Hinton broth. A 2 mL aliquot of each dilution was inoculated with the bacterial suspension in sterile Petri plates and incubated at 37 °C for 24 h. After incubation, 10 µL of supernatant from each plate was spread onto Mueller-Hinton agar and further incubated (37 °C, 24 h). Colony-forming units (CFUs) were counted, and bacterial growth (%) was calculated using the formula: Bacterial growth (%) = (CFU of sample / CFU of control) × 100.

Simultaneously, the Minimum Inhibitory Concentration (MIC) of the nanogel was determined by the broth dilution method according to CLSI guidelines. Two-fold serial dilutions of the nanogel were inoculated with the standardized bacterial suspension and incubated under the same conditions. The MIC was defined as the lowest concentration of nanogel that inhibited visible bacterial growth (absence of turbidity) compared with the control. Mueller-Hinton broth with bacterial suspension served as the positive control, while broth containing nanogel without inoculation was used as the negative control.

Results

Percentage yield

Sr.no.	Plant name	Solvent	Theoretical Weight (kg)	Yield (g)	% Yield
1.	*Taraxacum officinale*	Methanol	1000	46	4.6%

The percentage yield was calculated as follows

$$Percentage\ yield = \frac{weightof\ dried\ extract}{weight\ of\ dried\ plant\ material} \times 100$$

$$\frac{46}{1000} \times 100 = 0.046$$

$$0.046 \times 100 = 4.6\%$$

The percentage yield is 4.6%

Phytochemical Tests

Table 1: Phytochemical Screening of Dandelion leaves Methanol extract

Sr. No.	Phytochemical	Phytochemical Test	Result
1.	Phenols	Feeric chloride test	+
2.	Carbohydrates	Molish test	-
		Benedict test	+
		Fehling's test	-
3.	Alkaloids	Mayer's reagent	+
		Dragendroff test	-
		Hager's test	+
4.	Glycosides	Anthraquinone glycosides	+
		Borntrager's test	+
5.	Proteins	Million test	+
		Biuret test	-
6.	Amino Acid	Ninhydrin test	-
7.	Flavonoids	Shinoda test	+
		Alkali test	+
8.	Tannis	Lead acetate test	-
		Ferric chloride	+
9.	Saponins	Foam test	-

Table 2: Optimization of Nanogel

Formulation no.	Texture	Homogeneity	Viscosity	pH
F3	Soft, Smooth	Uniform, no lumps	Moderated	6.8

Table 3: Evaluation of Nanogel

Parameters	Results
Appearance	Good And Clear
Homogeneity	Uniform
pH	6.5±0.2
Viscosity	6505 ± 110
Spreadability	7.1±0.1

Entrapment efficiency

The entrapment efficiency (EE) was calculated as follows:

$$EE\ \% = \frac{(Total\ drug - Free\ drug)}{Total\ drug} \times 100$$

Total drug added = 12.00 mg
Measured free drug in supernatant = 7.00 mg

$$EE\% = \frac{12.00 - 7.00}{12.00} \times 100$$

$$= \frac{5.00}{100} \times 100$$

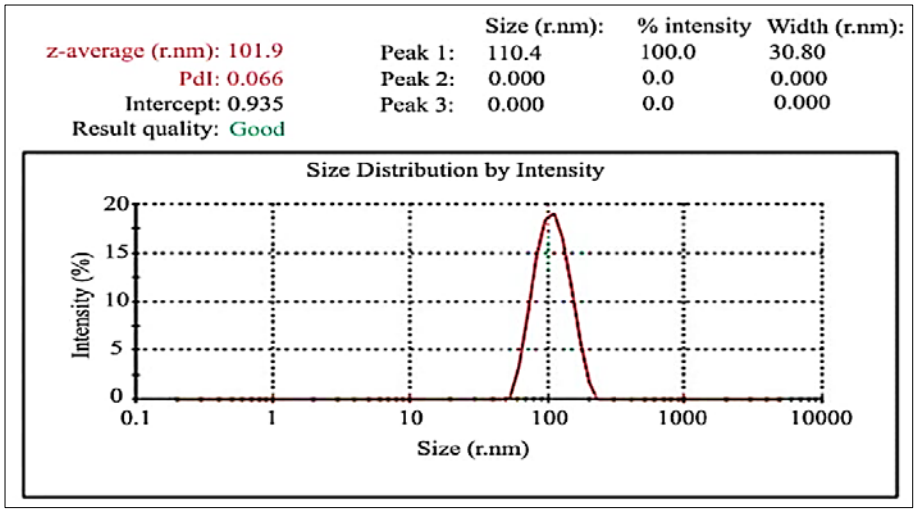
$$= 41.67\%$$

= 41.67% If measured in triplicate, show mean ± SD (eg. EE=19.5±21.0%, n=3)

Characterization of Nanoparticles

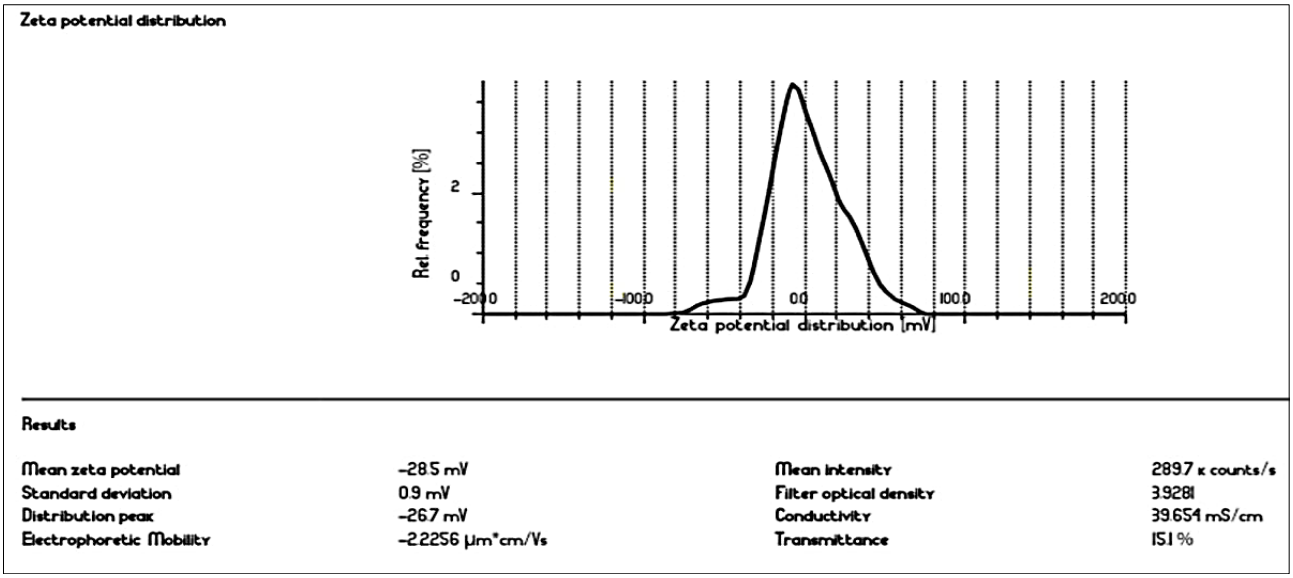
Particle size

The particle size of the nanogel was determined using the dynamic light scattering (DLS) technique. For analysis, the formulation was appropriately diluted with distilled water and filtered to remove dust or aggregates. The diluted sample was then analysed, and the average particle size along with the size distribution was recorded.



Zeta Potential

Zeta potential measurements have been reported to provide important information on the electrostatic stability of nanoparticles, nanogels and all other colloidal formulations.



Scanning Electron Microscope

Scanning Electron Microscope is an advanced microscopy technique that uses a focused beam of electrons to produce highly magnified images of the surface of a sample, giving detailed information about its morphology and surface structure.

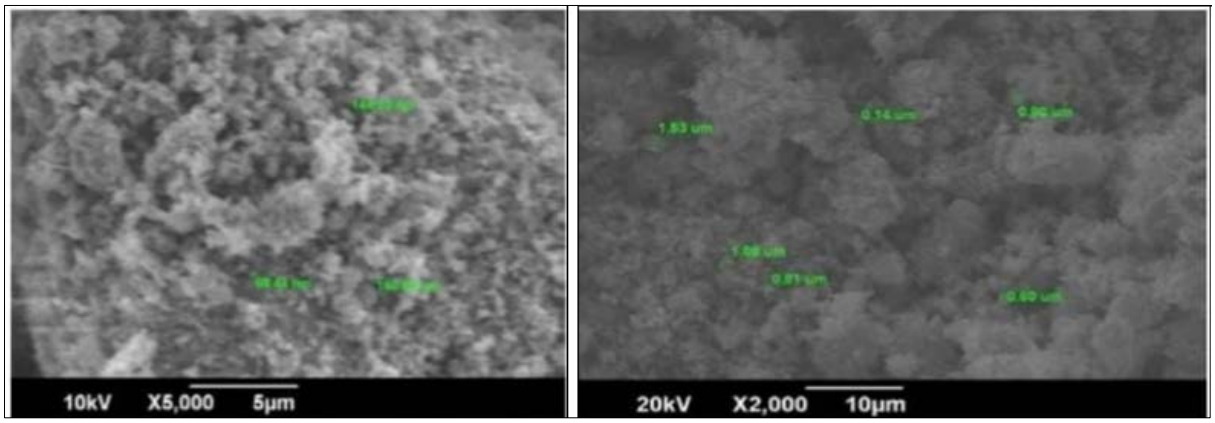


Fig 1: SEM Image of Nanoparticles

Antibacterial Activity

Antibacterial activity of formulation was determined against Staphylococcus. The results of the present study shoes that most of the organic solvent extracts of Dandelion leaf showed significant activity against the tested bacterial strains.

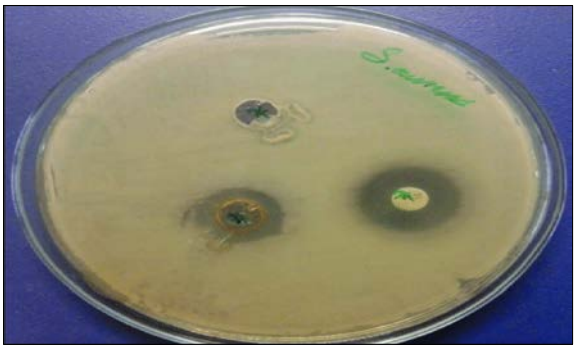


Fig2: Zone of inhibition of control and herbal formulation against Staphylococcus aureus

Test Microorganism	Zone of Inhibition (20mm)	
Staphylococcus aureus	Control	Herbal Formulation
	13 ± 0.3 mm	8.0 ± 2 mm

Conclusion

The in-vitro findings of this study revealed that dandelion (*Taraxacum officinale*) leaves nanogel possesses significant antibacterial activity against selected skin pathogens. The activity can be attributed to its phytoconstituents, mainly flavonoids, phenolics, and terpenoids, while the nanogel system enhanced solubility, stability, and potential skin applicability. These results suggest that dandelion nanogel is a promising natural alternative for the treatment of bacterial skin infections; however, further in-vivo studies and clinical trials are required to establish its therapeutic efficacy and safety.

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