

Research Article

Exploring the Phytochemistry and Therapeutic Potential of Indigenously Grown *Taraxacum officinale* Leaves

Adil Jamal ¹, Amina Arif ², Akmal Zubair ³, Shumaila Kiran ⁴,
Muhammad Naveed Shahid ⁵ and Md. Belal Hossain ⁶

¹Department of Biochemistry and Biotechnology, Faculty of Sciences, The University of Faisalabad, Faisalabad 38000, Punjab, Pakistan

²Faculty of Science and Technology, University of Central Punjab, Lahore 54000, Pakistan

³Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan

⁴Department of Applied Chemistry, Government College University, Faisalabad 38000, Pakistan

⁵Department of Botany, Division of Science and Technology, University of Education, Lahore 54770, Pakistan

⁶Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka 1207, Bangladesh

Correspondence should be addressed to Md. Belal Hossain; dr.mbhossain@sau.edu.bd

Received 21 September 2024; Revised 6 November 2025; Accepted 14 November 2025

Guest Editor: Jayanta Kumar Patra

Copyright © 2025 Adil Jamal et al. Journal of Chemistry published by John Wiley & Sons Ltd. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Taraxacum officinale, popularly known as dandelion, is a well-known traditional medicinal plant with essential biological activities due to its biologically active substances and polyphenolic compounds. The current study aims to profile the bioactive components, phytochemicals, antioxidant, antibacterial, and hemolytic activity from dandelion leaves in different solvents. The bioactive compounds of dandelion were appraised using HPLC. Total phenolic and total flavonoids contents were documented in dandelion leaves. Antioxidants (SOD, POD, and CAT), protease, α -amylase, and hemolytic activities were measured using a spectrophotometer. Microbial (antibacterial and antifungal) inhibition assays were performed. The chemical compounds documented in leaves were quercetin, caffeic acid, gallic acid, vanillic acid, ferulic acid, coumaric acid, sinapinic acid, chlorogenic acid, cinnamic acid, and syringic acid. Additionally, phytochemical analysis of dandelion revealed the existence of alkaloids, flavonoids, saponins, flavonoids, terpenoids, and quinones in different solvents. The total phenolic (41 ± 0.021 mg GAE/g) and total flavonoids (17.5 ± 0.029 mg RUE/g) determined were maximum in leaves aqueous extract. Radical scavenging activity (DPPH) significantly varied in all solvents with maximum in ethanol (41%–48%). Protease ($40\% \pm 0.002\%$) and α -amylase ($37\% \pm 0.016\%$) showed activity in ethanol fraction. Peak zone of inhibition (ZOI mm) in *Escherichia coli* ($14 + 1.69$) and *Pseudomonas aeruginosa* ($14 + 1.55$) was noticeable in ethanol solvent, likewise *Staphylococcus aureus* ($13 + 1.24$) and *S. pneumonia* ($15 + 1.08$) showed maximum ZOI impact exerted by aqueous extract. Additionally, aqueous extract showed peak inhibitory effect against fungal pathogens *Aspergillus fumigatus* (15 ± 0.81), *Aspergillus flavus* (15 ± 0.75), and *Aspergillus niger* ($12 + 1.24$). Maximum hemolytic activity was documented by ethyl acetate ($0.857 + 0.002$), whereas minimum was exhibited by n-hexane ($0.30 + 0.008$) fraction. Our findings indicate that dandelion extracts and chemicals have the potential to be used for the alleviation of diseases caused by bacteria and fungal pathogens. Effective dandelion extracts could be used as a natural ingredient in the creation of future pharmaceutical products.

Keywords: antioxidants; dandelion; DPPH; hemolytic; phytochemicals

1. Introduction

According to the World Health Organization (WHO), most of global population accounting for 80% of global population utilize herbal medicines alongside conventional pharmaceuticals for basic healthcare purposes [1]. Herbal medicines have undergone rigorous testing and have been demonstrated to be effective as well as secure, with broad cultural acceptance and fewer adverse effects compared to contemporary pharmaceutical medications. The use of products derived from various herbs and plants are typically seen as safe owing to their multifunctional healing agents and bioactive substances. As per the Food and Agriculture Organization (FAO) investigation, in 2021, medicinal and aromatic plants were cultivated on 12.7 million hectares worldwide [2]. Biochemical approaches like HPLC previously has been employed for estimation of vitamin C (ascorbic acid), essential oil (eugenol), tannin (gallic acid), cinnamic acids (caffeic acid, syringic acid, *p*-coumaric acid, sinapic acid, and ferulic acid), and flavonoids (catechin, rutin, myricetin, quercetin, apigenin, and kaempferol) in different herbal plants [3, 4].

The genus *Taraxacum*, often known as dandelion, consists of more than three hundred species [5]. The *Taraxacum* genus is actively grown and abundantly distributed in various parts of Asia, including China, North Korea, Mongolia, and Russia [6]. Previous studies have reported antioxidant and antimicrobial properties of *T. officinale* from Europe, China, and North America. However, variations in phytochemical composition can arise due to geographical and climatic factors, meaning locally grown plants may differ significantly. Importantly, there is no comprehensive published study from Pakistan that integrates phytochemical profiling, antioxidant enzyme activities, hydrolase assays, radical scavenging, and hemolytic effects in one systematic analysis. Our work therefore addresses this knowledge gap and contributes region-specific insights.

The plant exhibits growth in temperate locations across the globe, encompassing various habitats such as lawns, roadsides, disturbed banks and coastlines of streams, as well as other regions characterized by damp soils. Dandelion (*Taraxacum* genus) has been extensively employed in traditional medicine, folk medicines, and substitution therapies across several cultures for the treatment of a wide range of diseases [7].

The literature highlights the traditional applications of dandelion, which include its utilization as a therapeutic intervention for various ailments such as kidney diseases, diabetes, bacterial infections, diuretics disorders, hepatic and renal dysfunctions, as well as its potential as an anti-inflammatory substance [8]. Polyphenols, specifically hydroxycinnamic acid derivatives (HCAs) and flavonoids (apigenin and luteolin derivatives), are present in the leaves and flowers of dandelion plants [9–11]. The aforementioned entities have robust antioxidant and hypocholesterolemic features. HCAs have been found to elicit antiradical and protective properties against oxidative processes [11]. On the other hand, flavonoids have been observed to impede the generation of reactive oxygen species (ROS) and nitrogen by

suppressing the production of NO synthase and COX-2 proteins [12, 13]. Dandelion roots also contain significant amounts of inulin [14, 15]. Inulin is a natural polysaccharide that belongs to the category of fructans, which are dietary fibers. Additionally, this particular plant serves as a significant reservoir of essential vitamins (A, C, E, K, and B) as well as minerals [15, 16]. The literature has revealed several major medicinal uses of *T. officinale*, including diuretic action and the treatment of hepatic problems [17], as antiviral [18], antibacterial [19], antioxidant [20], anti-inflammatory [21], antidiabetic [22], and anticancerous [23].

Prior studies validate the advantageous impact of a nutrient-rich diet and antioxidant compounds on human well-being. Nevertheless, various factors such as climate, and soil conditions, potentially affect biosynthesis and accumulation of plant secondary metabolites [24, 25]. The purpose of this work was to investigate the phytochemicals in indigenously grown dandelion (*T. officinale*) leaves in various solvent systems and to ascertain the bioactive compounds of *T. officinale*. Additionally, the objective of this study was to explore the antioxidants, hydrolases, radical scavenging (DPPH), antimicrobial (antibacterial, antifungal), and hemolytic activity present in various extracts of *T. officinale*. Our research will provide insights into phytochemical, bioactive components (polyphenolics) profile, and medicinal potential of the native *T. officinale* plant cultivated in Pakistan.

2. Materials and Methods

2.1. Chemicals. *n*-Hexane ($\geq 98\%$), chloroform ($\geq 99.8\%$), ethyl acetate ($\geq 99.5\%$), ethanol ($\geq 99.5\%$), methanol ($\geq 99.8\%$), acetone ($\geq 99.5\%$), acetonitrile ($\geq 99.9\%$), phosphoric acid ($\geq 99.0\%$), acetic acid ($\geq 99\%$), water, gallic acid (ACS reagent grade, $\geq 98.0\%$, powder), benzoic acid (ACS reagent grade, $\geq 99.5\%$, crystalline), rutin (90%), ascorbic acid ($\geq 99\%$, powder), Folin & Ciocalteu's phenol reagent, vanillin (99%), quercetin ($\geq 95\%$), silica gel (230–400 mesh, extent of labeling: $\sim 2.5\%$ loading, matrix active group, $\sim 8\%$ functionalized) and sea sand (quality level 200). All of the compounds listed above were analytical grade and purchased from Sigma Aldrich Ltd (Sigma Aldrich Chemie GmbH, Germany).

2.2. Sample Collection. Mature plants of indigenously grown *T. officinale* (G.H. Weber ex Wiggers) (Voucher PU.IAGS.806) were procured from herbarium, Institute of Agricultural Sciences (IAGS), University of the Punjab, Lahore Pakistan (N 31° 30' 4.3236", E 74° 18' 5.4684") and further botanical and morphological features were authenticated and confirmed by Botany Department, University of Education, Lahore under supervision of Prof. Dr. Muhammad Naveed Shahid. To ensure the removal of any dust particles or pollutants, the mature leaves of the harvested dandelion plants were subjected to a thorough rinsing process using running water, followed by several washings with distilled water. The washed leaves were stored and dried at 25°C–27°C for a duration of 10 days in the dark. During

this time, they were regularly monitored to prevent the growth of fungi or bacterial contamination. The leaves were subjected to air drying in a dark environment, followed by being subjected to a hot air oven (IG-HAO Series, iGene Labserve Pvt. Ltd, India) set at 40°C for a duration of 6–7 days, until their mass become constant. The constant weighted plants were ground into a fine powder using a pristine grinder. The powder was passed through a stainless-steel sieve (mesh size ≤ 1.0 mm), packed in sealed PA bags to ensure uniform particle size for extraction.

2.3. Preparation of Extract. The polyphenolics and flavonoids in leaves were identified by employing six polar solvents, including butanol, chloroform, ethanol, ethyl acetate, hexane, and aqueous (distilled) water. The choice of six solvents (butanol, chloroform, ethanol, ethyl acetate, hexane, aqueous) was based on their polarity index values, enabling extraction of a broad range of polar and nonpolar compounds. Preparation of extracts was done at room temperature.

In order to prepare the extracts, 30 g of powder was soaked in 270 mL of each solvent individually. The conical flasks were then incubated at a temperature of 37°C with shaking at 120 rpm for 60 min. The mixture was then left for 48 h. Subsequently, the extracts were filtered using Whatman N 1 filter paper, followed by condensation under decreased pressure using a rotatory evaporator (RAPIDVAP N2, Labconco, USA). Finally, the samples were stored at a temperature of –20°C until the subsequent analysis.

2.4. Chromatographic Analysis. The samples were analyzed using the RP HPLC technique using Shimadzu VP HPLC System (SpectraLab Scientific Inc.; USA). Chromatography conditions were as follows: detector: UV detector, detection wavelength: 280 nm, columns: Luna C18 columns (250 mm $\times$ 4.6 $\times$ 5 μ m), mobile phase: methanol:phosphate buffer = 25:75, velocity of flow: 0.7 mL/min, column temperature: 40°C, injection volume: 20 μ L, linear gradient mobile phase containing solvent A (solvents), solvent B (acetonitrile), and solvent C (5% formic acid in water, v/v).

2.5. Preparation of Standard and Sample Solutions. For HPLC analysis, a standard solution of gallic acid, quercetin, cinnamic acid, syringic acid, sinapinic acid, coumaric acid, ferulic acid, vanillic acid, and caffeic acid was prepared in aqueous (0.25 mg/mL). The sample solution for analysis was prepared by dissolving 1 mg of extract in 1 mL of aqueous. Both the standard and sample solutions were filtered through Whatman NYL 0.45 mm syringe filter. To ensure the purity and consistency of samples (fractions) and standards, the compounds were dissolved in aqueous and filtered through a 0.45 mm syringe filter. The responses were measured as peak areas (%) versus concentration (ppm).

2.6. HPLC Method Validation (Linearity, Repeatability, Reproducibility, and Accuracy). The HPLC method was validated through intraday and interday analysis of precision and accuracy according to the International Conference on

Harmonization (ICH) guidelines [26]. Linearity was obtained after receiving a peak of external standard at six concentrations (0.1, 1, 5, 10, 15, 20, and 25 g/mL), injected 20 times simultaneously at an automatic sample injection valve temperature of 19°C–20°C. Appropriate dilutions of these standard stock solutions were included, and the means of these received peak areas were averaged to obtain a plotted linear regression. To assess the repeatability and reproducibility of the developed method, precision was evaluated using intra- and interday variability measurements [26]. Intra- and interday precision values were expressed as the percentage of relative standard deviation (% RSD) of retention time and peak area. The precision evaluation was run at intermediate concentrations (0.1, 1, 5, 10, 15, 20, and 25 g/mL) for 10 consecutive runs.

To improve the accuracy of the method, three concentrations of 14 synergistic compounds identified in the extracts were prepared and added to dandelion extracts and incubated at room temperature (23°C–25°C) for 24 h. The extracts were then analyzed using the HPLC-DAD method as described, and each run was repeated 10 times. The extraction recovery value of each compound was calculated as a percentage of concentration of the compound spiked into a pulverized plant matrix obtained after extraction over that of an equivalent amount without extraction.

Phenolic acids and metabolites in samples were identified and quantified by comparison of retention time and UV spectra with that of authentic compounds. The procedure was optimized to resolve a mixture of the following phenolic acids: caffeic acid, cinnamic acid, phenolic acid, gallic acid, syringic acid, quercetin, vanillic acid, chlorogenic acid, ferulic acid, sinapinic acid, M-coumaric acid, P-coumaric acid, and benzoic acid.

2.7. Determination of Limit of Detection (LOD) and Limit of Quantification (LOQ) Values. LOD and LOQ are the two analytical performance parameters that were used to describe the smallest concentration of analyte that can be accurately measured by an analytical procedure. Three times the ratio of peak height (signal) to baseline noise (S/N) gives LOD value and 10 times of the same ratio gives LOQ value. The LOD and LOQ data obtained for 280–330 nm was calculated for identified volatile compounds and standards (gallic acid, quercetin, cinnamic acid, syringic acid, sinapinic acid, coumaric acid, ferulic acid, vanillic acid, and caffeic acid). To obtain accurate results, before calculations, the standards mixture was run multiple times in the HPLC system.

Area, concentration (ppm) and retention time (min) of identified compounds were imported into Microsoft Excel. Regression equation (slope and intercept), regression coefficient (R^2), ANOVA, standard error of intercept (SE), standard deviation (SD) of intercept, and LOD and LOQ of detected phenolic compounds in fractions of *T. officinale* were computed in Microsoft Excel (Ver 2019). Confidence interval for ANOVA was set at 95%. The LOD and LOQ were calculated from the calibration curve according to equations (1) and (2) [26].

$$\text{LOD} = 3.3 \times \frac{\sigma}{S(A)}, \quad (1)$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S(B)}, \quad (2)$$

where σ = SD of blank and S = angular coefficient in linear regression.

2.7.1. Cluster Heatmap Analysis. Hierarchical clustering was applied to compound concentrations and areas using R to identify groups of low- and high-abundance compounds. Clustering was performed using Euclidean distance and complete linkage. Heatmaps visualized compound abundance across replicates, highlighting reproducibility and relative abundance patterns.

2.7.2. Solvent-Compound Relationship Analysis. Circular chord diagrams were constructed in R (circlize package) to visualize the connectivity between solvents and compounds. Chord color gradients represented concentration levels, and chord thickness corresponded to chromatographic area, allowing assessment of solvent-specific enrichment of phenolic metabolites.

2.8. Phytochemical Analysis, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC). The Wagner's test was used to identify alkaloids in plant extracts [27]. The presence of flavonoids was determined using the alkaline reagent test [28]. Foaming test [29] was used to detect saponins. Terpenoids were tested by combining 3 mL of extract with 2 mL of CHCl_3 in a test tube [30]. Fehling solution was employed to determine the presence of glycosides [31]. Three drops of concentrated H_2SO_4 were added to 3 mL of extract; a red coloration indicates the presence of steroids. The tannins test was performed by adding 10% of freshly prepared KOH to 3 mL of the extract in $\text{Mn}(\text{OH})_2$ and observing for a dirty white precipitate, which confirmed the presence of tannins [32].

The TPC of the extracts was determined using the Folin Ciocalteu technique [33]. Gallic acid was utilized as the reference, and TPC was measured as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). The TFC of the extracts was determined using the aluminum chloride method reported by Quettier-Deleu et al. [34]. Rutin was utilized as the standard, and flavonoid contents were represented as milligram of rutin equivalents (RUE) per gram of extract (RUE/g of extract).

2.9. DPPH Radical Scavenging Activity. The DPPH (2,2-diphenyl-1-picrylhydrazyl) approach was employed to measure antioxidant capacity. The obtained DPPH stock solution with a concentration 0.5 mM or 200 $\mu\text{g/mL}$ was used. The free radical scavenging activity of DPPH was measured at 517 nm [35]. DPPH radical scavenging activity was measured for each extract type at concentrations of 50, 100, 150, and 200 $\mu\text{L/mL}$. Two-way ANOVA in R tested the

effects of extract type, concentration, and their interaction. Post hoc comparisons were performed using Tukey's and Fisher's LSD tests to identify significant differences among extract-concentration groups.

2.10. Antioxidants and Hydrolases. The activity of superoxide dismutase (SOD) was measured using the method published by Marklund and Marklund [36]. Catalase (CAT) activity was measured using the extract, as described by Aebi [37]. The activity of peroxidase (POD) was determined using a guaiacol reaction, which involved the oxidation of guaiacol extract [38]. Ascorbic acid was used as standard. The protease activity was determined using the approach given by Abd-El Khalek et al. [39]. The amount of tyrosine released from the filtrate was determined using a spectrophotometer at 280-nm wavelength. The α -amylase activity of *T. officinale* extracts following the methodology reported by Bhutkar and Bhise [40] at 530-nm wavelength. The experiments were conducted at least three times. Activities of SOD, POD, and CAT enzymes were measured at the same extract concentrations. Data visualization included bar plots with mean $\pm$ SD and error bars for replicate consistency.

2.11. Antibacterial Assay. The Gram-positive and Gram-negative bacterial strains in this study were procured from Microbial/Pathogen section, Microbiology Department, University of Central Punjab, Lahore. The test was performed on four common pathogenic bacteria: Gram-positive *S. aureus* (ATCC 25175), *S. pneumoniae* (ATCC 49619), Gram-negative *E. coli* (ATCC 2592), and Gram-negative *P. aeruginosa* ATCC (27853). The disc diffusion method [41] was used to assess antibacterial activity. A 0.2-mL volume of the test bacterial isolate's standard inoculums (10^7 CFU/mL) was dispersed on Mueller Hinton Agar (MHA) using a sterilized glass rod spreader and allowed to dry. Plant extracts (1200 $\mu\text{g/mL}$) were added to each well and left for 1 h at room temperature to diffuse into the medium before being incubated at 37°C for 24 h. Ciprofloxacin (1.2 mg/mL) was employed as a reference antibiotic (positive control) for all bacterial strains. DMSO was utilized as a negative control. The inhibition zone (ZOI) was assessed after 24 h and documented for all extracts and ciprofloxacin. The experiments were carried out in triplicate for each bacterial strain, and the final results were provided as the mean ZOI.

2.12. Antifungal Assay. The fungal spp in this study were obtained from Microbial/Pathogen section, Microbiology Department, University of Central Punjab, Lahore. Antifungal activity was tested on three common *Aspergillus* strains: *A. niger* (ATCC 16888), *A. flavus* (ATCC 16872), and *A. fumigatus* (ATCC 28282). Potato dextrose agar (PDA) medium (Oxoid) was prepared according to the manufacturer's instructions. The plant extracts' antifungal activity was determined using Surapuram et al. (2014) [42] method. The fungal sample was diluted to 10^8 CFU/mL. The antifungal activity values for each extract treatment were

expressed as the mean of the ZOI (mm) in triplicate. The diameters of ZOI were measured along fungicide fluconazole as positive control. Negative control (DMSO) plates deployed contained no plant extracts.

2.13. Hemolytic Assay. The hemolytic activity of plant extracts was determined in flat-bottom, 96-well dishes according to Lozano et al. [43]. The positive control solution (100 μ L) was used for hemolysis: 1% (v/v) Triton X-114

(Sigma-Aldrich Chemical Co., Saint Louis, MO, USA), 1% (v/v) saponin (Sigma Aldrich Chemical Co., Saint Louis, MO, USA), both in PBS solution. In all replicates, an equal quantity of DMSO-PBS was added; 200 μ L of plant extracts and compounds were prepared; and then 1:2 serial dilutions were made for 5.0 mg/mL. The optical density (OD) was quantified at 540-nm wavelength. The hemolytic activity for each sample was computed in triplicate.

$$\text{Hemolytic activity} = \text{OD sample} - \text{OD negative control} \\ \text{OD positive control} - \text{OD negative control.} \quad (3)$$

2.14. Statistical Analysis and Considerations. All experiments were done in triplicate. The statistical analysis was carried out using GraphPad Prism program. p values < 0.05 were considered statistically significant. The data were presented as mean and SD. Post hoc and Tukey tests were conducted in R program. Replicate measurements were used to ensure reproducibility. Significance thresholds were set at $p < 0.05$. Fold-changes and trends across concentrations were calculated to interpret dose-dependent responses.

3. Results

3.1. Phytochemical Analysis. Key phenolic compounds were identified in the different extracts of dandelion. Our study reported common bioactive compounds like gallic acid, quercetin (4.22, 2.91 min) (Figure 1(a)), (4.25, 2.94 min) (Figure 1(b)), (4.28, 2.90 min) (Figure 1(e)), (4.14, 2.86 min) (Figure 1(f)) in butanol, chloroform, aqueous and n-hexane, respectively (Table 1). Sinapinic acid (26.407 min) (Figure 1(c)), (26.66 min) (Figure 1(d)), (25.90 min) (Figure 1(e)), (25.76 min) (Figure 1(f)) was reported in ethanol, ethyl acetate, aqueous, and hexane fractions, respectively (Table 1). Likewise, syringic acid (16.52 min) (Figure 1(c)), (16.08 min) (Figure 1(d)), (16.11 min) (Figure 1(e)) was reported in ethanol, ethyl acetate, and aqueous extracts, respectively (Table 1). Butanol and chloroform showed the presence of cinnamic acid (24.79 min) (Figure 1(a)) (25.52 min) (Figure 1(b)), respectively. Butanol and ethanol revealed the incidence of caffeic acid (12.52 min) (Figure 1(a)), (12.66 min) (Figure 1(c)) (Table 1). Coumaric acid was reported in ethanol (20.38 min) (Figure 1(c)) and ethyl acetate (18.21 min) (Figure 1(d)).

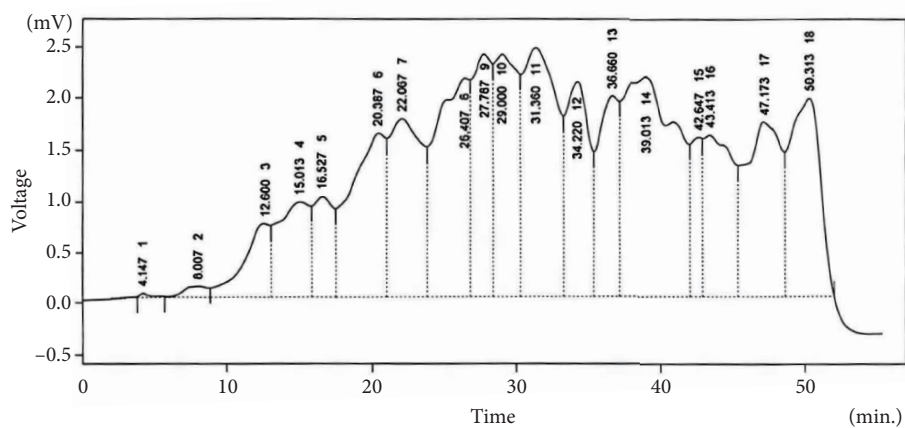
3.2. Abundance of Compounds Using Cluster Heatmap Analysis. Hierarchical clustering of the quantified compounds based on concentration and chromatographic area across three replicates revealed two distinct clusters (Figure 2). Cluster A (upper dendrogram) predominantly included low-abundance compounds, with concentration values ranging from 0.07 to 8.66 and area values from 0.1 to 8.63. Notable compounds in this cluster were Vanillic-Chloroform, Syringic-Ethylacetate, Syringic-Butanol,

Cinnamic-Chloroform, Sinapinic-Ethanol, Gallic-Hexane, Syringic-Aqueous, Coumaric-Ethylacetate, Gallic-Ethanol, Gallic-Butanol, Gallic-Ethylacetate, Quercetin-Hexane, Quercetin-Butanol, Quercetin-Aqueous, Gallic-Aqueous, Quercetin-Chloroform, Caffeic-Butanol, and Gallic-Chloroform. Cluster B (lower dendrogram) comprised higher-abundance compounds with concentrations from 2.25 to 22.28 and area values from 6.4 to 23.6, including Caffeic-Ethanol, Sinapinic-Ethylacetate, Coumaric-Ethanol, Syringic-Ethanol, Sinapinic-Aqueous, Cinnamic-Butanol, Chlorogenic-Ethanol, Sinapinic-Hexane, Phenolic-Butanol, Ferulic-Ethylacetate, Benzoic-Hexane, and Ferulic-Ethanol.

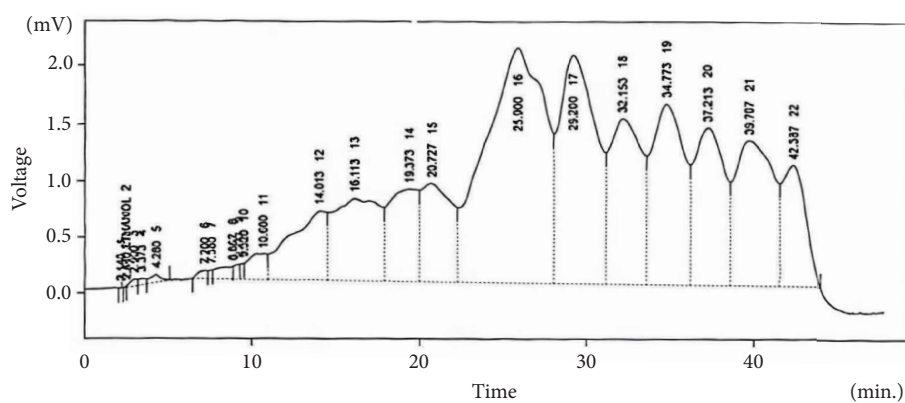
Replicate measurements demonstrated high reproducibility. Concentration replicates were highly consistent, for example, Vanillic-Chloroform (8.14–8.15) and Phenolic-Butanol (21.23–22.26). Area replicates also showed minimal variation, though some compounds displayed slightly higher variability, such as Gallic-Chloroform (1.1–1.4) and Caffeic-Ethanol (12.65–12.67). Among the compounds, Phenolic-Butanol (21.23–22.26), Ferulic-Ethanol (19.68–19.58), and Benzoic-Hexane (15.13–15.23) exhibited the highest concentrations, while Gallic-Ethanol (0.07), Quercetin-Hexane (0.18–0.19), and Gallic-Aqueous (0.11–0.13) showed the lowest. Similarly, Sinapinic-Aqueous (23.4–23.5), Phenolic-Butanol (14.1–15.5), and Chlorogenic-Ethanol (15.0–15.02) had the largest areas, whereas Gallic-Ethanol (4.14–4.15), Quercetin-Butanol (0.1–0.2), and Quercetin-Aqueous (0.1–0.2) had the smallest.

These results indicate that Cluster A compounds represent minor constituents, while Cluster B compounds are major contributors to the chemical profile. The hierarchical clustering effectively discriminates compounds based on combined concentration and area, and the low variability between replicates supports the robustness and precision of the analytical measurements.

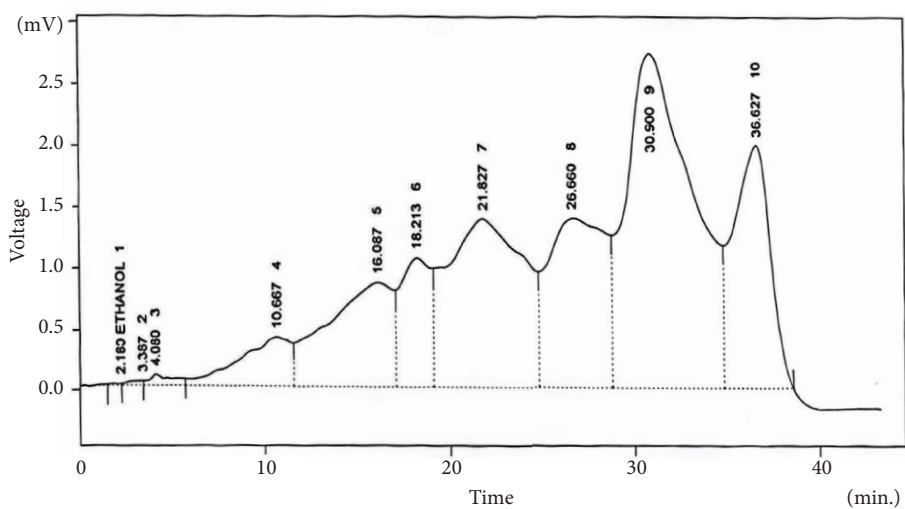
3.3. Multivariate Analysis of Solvent-Compound Relationships. The circular chord diagram (Figure 3) depicts the connectivity between solvent fractions (left half) and phenolic/secondary metabolites (right half) based on concentration (Conc_mean) and chromatographic area (Area_mean). Each solvent or compound is represented as



(a)

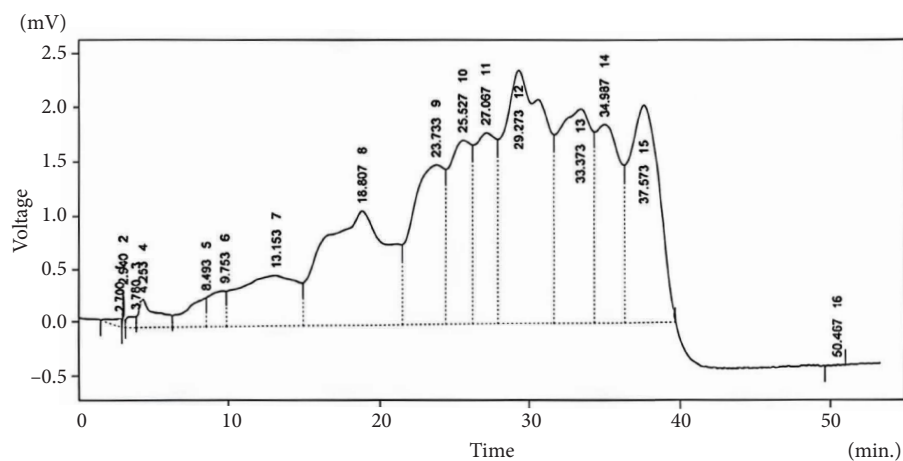


(b)

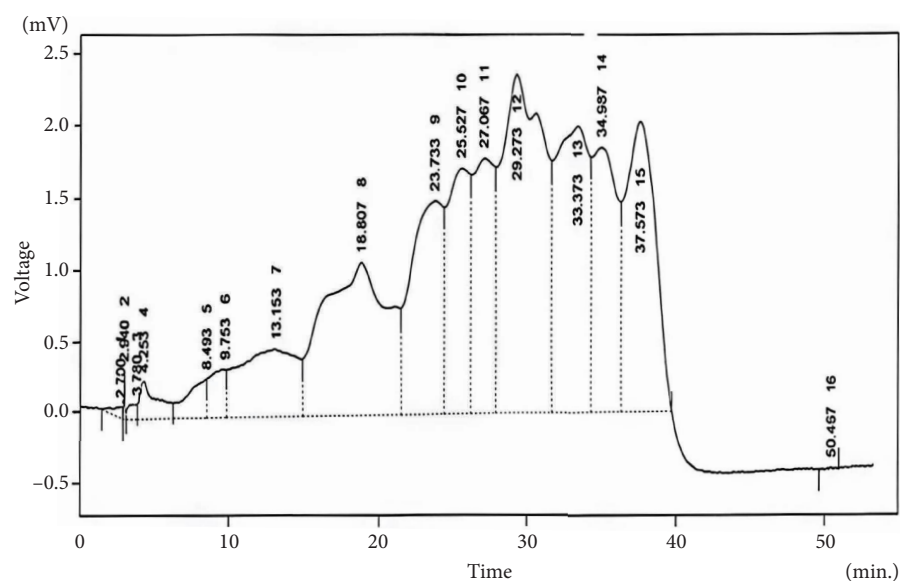


(c)

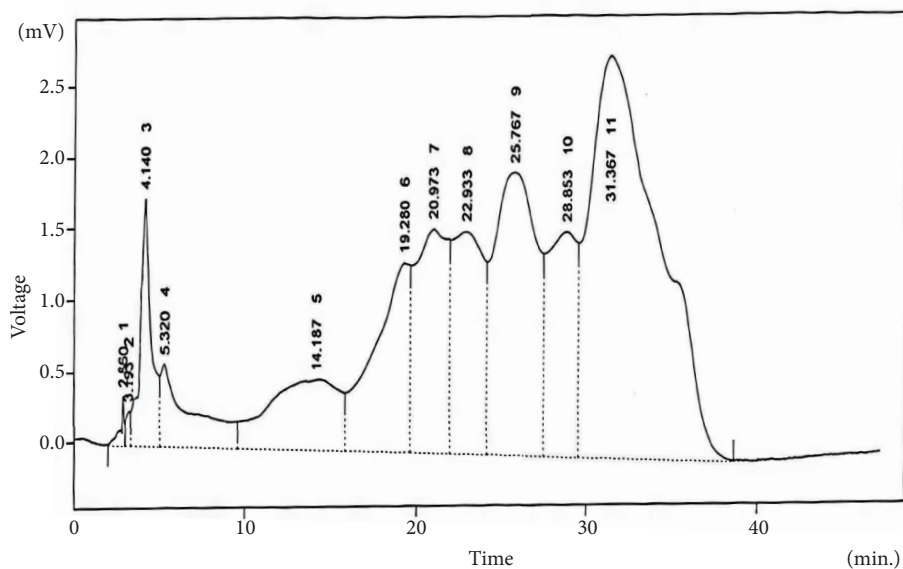
FIGURE 1: Continued.



(d)



(e)



(f)

FIGURE 1: (a) HPLC analysis of butanol fraction of *T. officinale* leaves, (b) HPLC analysis of chloroform fraction of *T. officinale* leaves, (c) HPLC analysis of ethanol fraction of *T. officinale* leaves, (d) HPLC analysis of ethyl acetate fraction of *T. officinale* leaves, (e) HPLC analysis of aqueous fraction of *T. officinale* leaves, and (f) HPLC analysis of *n*-hexane fraction of *T. officinale* leaves.

TABLE 1: HPLC analysis of *n*-butanol, chloroform, ethanol, ethyl acetate, aqueous, and *n*-hexane fraction from *T. officinale*.

Fractions/compound name	Molecular formula	Retention time (min)	Area (%)	Concentration (ppm)
<i>n</i> -Butanol				
Caffeic acid	C ₉ H ₈ O ₄	12.52	0.9	0.7848
Cinnamic acid	C ₉ H ₈ O ₂	24.79	18.9	13.1515
Phenolic acid	C ₆ H ₅ OH	21.97	15.5	22.2565
Gallic acid	C ₇ H ₆ O ₅	4.22	2	1.4024
Syringic acid	C ₉ H ₁₀ O ₅	16.95	11.7	5.8369
Quercetin	C ₁₅ H ₁₀ O ₇	2.91	0.2	0.1788
<i>Chloroform</i>				
Cinnamic acid	C ₉ H ₈ O ₂	25.52	7.4	6.1451
Gallic acid	C ₇ H ₆ O ₅	4.25	1	0.8306
Quercetin	C ₁₅ H ₁₀ O ₇	2.94	1	0.1141
Vanillic acid	C ₈ H ₈ O ₄	13.15	5.6	8.1508
<i>Ethanol</i>				
Caffeic acid	C ₉ H ₈ O ₄	12.66	12.6	4.2845
Chlorogenic acid	C ₁₆ H ₁₈ O ₉	15.01	15.0	11.1036
Gallic acid	C ₇ H ₆ O ₅	4.14	4.1	0.0653
Ferulic acid	C ₁₀ H ₁₀ O ₄	22.06	6.9	19.5768
Sinapinic acid	C ₁₁ H ₁₂ O ₅	26.40	8.6	4.4049
M-Coumaric acid	C ₉ H ₈ O ₃	20.38	20.3	3.2164
Syringic acid	C ₉ H ₁₀ O ₅	16.52	16.5	2.3440
<i>Ethyl acetate</i>				
Gallic acid	C ₇ H ₆ O ₅	4.08	0.4	0.2796
Syringic acid	C ₉ H ₁₀ O ₅	16.08	10.2	5.2386
P-Coumaric acid	C ₉ H ₈ O ₃	18.21	5.8	1.5409
Ferulic acid	C ₁₀ H ₁₀ O ₄	21.82	19.2	21.2156
Sinapinic acid	C ₁₁ H ₁₂ O ₅	26.66	14.4	3.8483
<i>Aqueous</i>				
Quercetin	C ₁₅ H ₁₀ O ₇	2.90	0.1	0.1014
Gallic acid	C ₇ H ₆ O ₅	4.28	0.1	0.1022
Syringic acid	C ₉ H ₁₀ O ₅	16.11	6.3	3.4441
Sinapinic acid	C ₁₁ H ₁₂ O ₅	25.90	23.6	6.6591
<i>n</i> -Hexane				
Quercetin	C ₁₅ H ₁₀ O ₇	2.86	0.3	0.3071
Gallic acid	C ₇ H ₆ O ₅	4.14	3.5	2.8296
Benzoic acid	C ₇ H ₆ O ₂	14.18	6.5	15.2324
Sinapinic acid	C ₁₁ H ₁₂ O ₅	25.76	15.6	12.1291

a segment on the circle, with chords linking solvents to the compounds they solubilized. The color gradient of chords represents concentration categories (yellow=low, purple=high), while the thickness of chords reflects chromatographic area (thin=low area, thick=high area). Polar solvents such as ethanol, butanol, and chloroform display the highest connectivity, forming thick, dark chords with compounds including syringic acid, caffeic acid, coumaric acid, and quercetin, indicating both high concentration and high area abundance. Ethanol, in particular, shows broad associations, suggesting its efficiency in extracting diverse high-abundance phenolics. In contrast, aqueous and hexane fractions exhibit fewer and thinner linkages, primarily with gallic acid, quercetin, and cinnamic acid, which correspond to low-abundance compounds. Ethyl acetate shows an intermediate pattern, capturing both moderate and high-abundance phenolics.

Notably, compounds such as gallic acid, quercetin, and vanillic acid demonstrate multiple solvent linkages but with thin, lighter chords, reinforcing their classification as minor

constituents. Conversely, ferulic acid, caffeic acid, and sinapinic acid are predominantly enriched in ethanol and butanol, marked by thick, dark chords, confirming their status as major contributors to the phytochemical profile. The structural layout reveals distinct solvent-compound specificity, highlighting solvent polarity as a determinant of metabolite recovery. Overall, this chord diagram complements the hierarchical clustering results by visually mapping how each solvent fraction selectively enriches specific phenolics, emphasizing ethanol and butanol as the most effective for extracting high-abundance bioactive compounds, whereas aqueous and hexane extracts primarily recover trace-level metabolites.

3.4. Phenolic Acid Standards. Thirteen analyzed major phenolic acids, caffeic acid, cinnamic acid, phenolic acid, gallic acid, syringic acid, quercetin, vanillic acid, chlorogenic acid, ferulic acid, sinapinic acid, M-coumaric acid, P-coumaric acid, and benzoic acid, were resolved under the

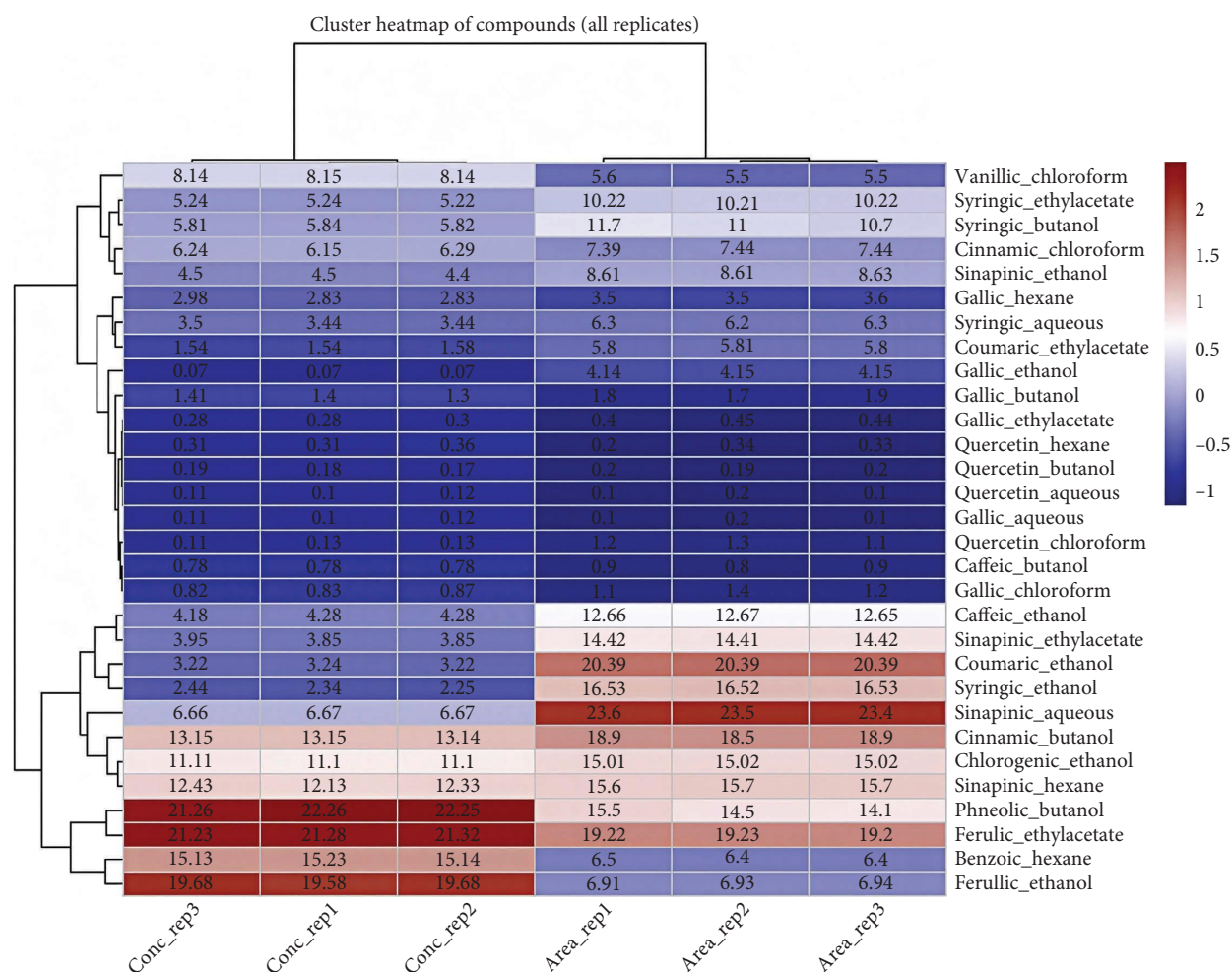


FIGURE 2: Hierarchical clustering of the quantified compounds based on concentration and chromatographic area of fractions of *T. officinale* leaves.

chromatographic conditions described (Figure 4). Calibration curves of all analytes routinely yielded regression coefficients 0.75 or better except sinapinic acid (Table 2).

3.5. Phytochemical Profile. Table 3 provides an overview of the phytochemical features of the *T. officinale* being examined. The secondary metabolites in dandelion were found to be more in the aqueous, ethyl acetate, ethanol, and chloroform extracts compared to the butanol and hexane extracts (Table 3). All fractions of *T. officinale* exhibited the presence of alkaloids. All fractions illustrated the absence of coumarins and tannins. The phytochemical profiles of chloroform, ethanol, and ethyl acetate exhibited echoes. The hexane and butanol fractions did not include the majority of phytochemicals that were reported in our investigation (Table 3).

The TPC (mg GAE/g) in aqueous extract was maximum (41 ± 0.021) followed by ethanol (32 ± 0.013), ethyl acetate (30 ± 0.032), butanol (28 ± 0.007), chloroform (23 ± 0.010), and hexane (18 ± 0.014). Significant differences were reported among TPC ($p < 0.05$). TFC (mg RUE/g) were reported maximum in aqueous extract (17.5 ± 0.029) followed by butanol (9.2 ± 0.018), ethanol (8.5 ± 0.022), ethyl acetate (8.0 ± 0.040), chloroform (6.1 ± 0.019), and hexane (2.7 ± 0.024) (Table 4).

3.6. DPPH Radical Scavenging Activity

3.6.1. DPPH Radical Scavenging Activity Comparison.

The antioxidant capacity of dandelion was assessed by employing the radical scavenging, which evaluates its ability to scavenge free radicals. Aqueous, n-hexane, and ethyl acetate fractions inhibited radical scavenging by 21%–25%, 14%–17%, and 20%–24%, respectively, at varying doses from 50 to 200 $\mu\text{g/mL}$ (Table 5). Chloroform, ethanol, and n-butanol reported DPPH inhibition activity of 18%–19%, 41%–48%, and 19%–20%, respectively, at varying extract concentration (Table 5). SD values for experiments performed for all fractions in triplicates each ranged from 0.001 to 0.005. All the fractions in our study showed varying inhibition activity in different fractions. n-Butanol, chloroform, and hexane showed no similar differences among varying concentrations of extracts ($p < 0.05$). Ethanol and ethyl acetate depicted higher radical scavenging activity at low concentrations and vice versa, while aqueous, hexane, chloroform, and butanol displayed peak radical scavenging activity at maximum concentration (Figure 5).

The two-way ANOVA revealed highly significant effects of extract type, concentration, and their interaction on DPPH radical scavenging activity as results of all analysis are



FIGURE 3: Circular chord diagram showing the connectivity between solvent fractions (left half) and phenolic/secondary metabolites (right half) based on concentration (Conc_mean) and chromatographic area (Area_mean).

mentioned in Supporting File 1. Specifically, there was a highly significant main effect of extract type [$F(6, 56) = 774046.770$, $p < 0.0001$], indicating that the different fractions exhibited distinct levels of antioxidant activity. Concentration also exerted a significant influence [$F(3, 56) = 24210.702$, $p < 0.0001$], suggesting that radical scavenging activity varied with increasing concentration. Moreover, the interaction effect between extract type and concentration was highly significant [$F(18, 56) = 33464.582$, $p < 0.0001$], implying that the effect of concentration differed across the various extracts. Together, these findings establish that both extract composition and dosage play crucial roles in determining antioxidant efficiency.

3.6.2. Tukey's HSD Post Hoc Test Interpretation. Post hoc comparisons using Tukey's HSD demonstrated multiple significant differences among the extract-concentration groups. For instance, the aqueous fraction at 100 $\mu\text{L/mL}$ differed significantly from its own values at 150 $\mu\text{L/mL}$ (mean difference = 1.00, $p < 0.001$) and 200 $\mu\text{L/mL}$ (mean

difference = 3.00, $p < 0.001$). Strong differences were also observed between aqueous and chloroform extracts at the same concentrations, as well as between aqueous and the control (ascorbic acid). Furthermore, ethanol fractions at all concentrations were significantly higher in radical scavenging activity compared with aqueous, chloroform, ethyl acetate, and *n*-hexane fractions ($p < 0.001$). The control (ascorbic acid) consistently exhibited significantly greater scavenging activity compared to most plant fractions, except for ethanol, which approached the control values at higher concentrations.

3.6.3. Fisher's LSD Test Interpretation. The Fisher's LSD test confirmed and further highlighted the magnitude of differences among extracts. The most striking contrasts were observed between ethanol fractions and control (ascorbic acid) at different concentrations. For example, ethanol at 50 $\mu\text{L/mL}$ showed significantly lower scavenging activity than the control at 200 $\mu\text{L/mL}$ (difference = -2.00, $t = -496.83$, $p < 0.0001$), while ethanol at 200 $\mu\text{L/mL}$ was also

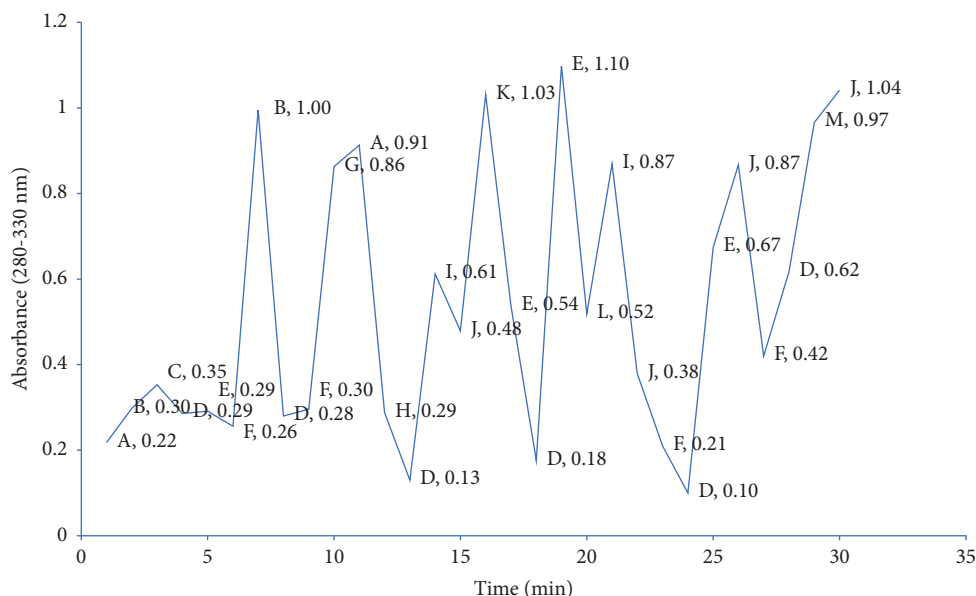


FIGURE 4: HPLC chromatogram of phenolic acids standards of *T. officinale*. Alphabets and numbers above each peak correspond to respective phenolic acids and absorbance values, respectively: (A) caffeic acid, (B) cinnamic acid, (C) phenolic acid, (D) gallic acid, (E) syringic acid, (F) quercetin, (G) vanillic acid, (H) chlorogenic acid, (I) ferulic acid, (J) sinapinic acid, (K) M-coumaric acid, (L) P-coumaric acid, and (M) benzoic acid.

significantly lower than the control at 200 $\mu\text{L/mL}$ (difference = -9.00 , $t = -2238.73$, $p < 0.0001$). Within ethanol fractions themselves, large significant differences were observed between lower and higher concentrations, such as ethanol 200 $\mu\text{L/mL}$ versus ethanol 50 $\mu\text{L/mL}$ (difference = -7.00 , $p < 0.0001$). These findings indicate that ethanol fractions exhibit strong, concentration-dependent antioxidant potential but still remain below the potency of the standard ascorbic acid.

3.7. Antioxidants and Hydrolases. Our study results elucidated that SOD activity (%) measured was minimum ($20 + 0.5$) at 50- μL quantity in aqueous extract as compared to increased aqueous extract quantity which exhibited increased ($25 + 0.5$ to $35 + 0.5$) SOD activity (Figure 6, Supporting file 2). The peak POD activity ($33 + 0.6$) was measured in maximum aqueous extract quantity (200 μL), while POD activity declines successively with reduced dose of aqueous extract. Peak ($35 + 0.7$) CAT activity was measured in 200 μL , while CAT activity declines with increased aqueous extract quantity (Figure 6). Significant differences were computed for protease and α -amylase activity ($p < 0.05$) among different extracts. Maximum protease (%) and α -amylase (%) activity was reported in ethanol ($40 + 0.002$, $37 + 0.016$) followed by n-butanol ($35 + 0.001$, $35 + 0.001$), chloroform ($34 + 0.001$, $32 + 0.012$), aqueous ($33 + 0.005$, $31 + 0.009$), ethyl acetate ($30 + 0.001$, $29 + 0.001$), and hexane ($28 + 0.002$, $25 + 0.002$) (Figure 7).

3.8. Antimicrobial Activity (Antibacterial and Antifungal)

3.8.1. Antimicrobial Activity (Antibacterial and Antifungal Means Comparison). The present findings indicate that

extracts derived from dandelion leaves exhibit inhibitory effects on the growth of both Gram-positive and Gram-negative bacteria. The inhibitory activity (ZOI, mm) of chloroform and aqueous solutions turned out to be comparable across Gram-negative bacteria. However, ethanol exhibited the most inhibition activity ($14 + 1.69$) and hexane displayed the minimal inhibition activity ($10 + 2.49$ – $11 + 0.81$) on the tested *E. coli* strain. The inhibitory effect of chloroform ($12 + 1.24$) and ethyl acetate ($12 + 1.24$) fractions on *P. aeruginosa* has been found to be similar (Figure 8). Our study found that aqueous ($14 + 0.80$) had the strongest inhibitory impact against gram-positive bacteria *S. pneumonia* and *S. aureus*, while hexane extract demonstrated minimal inhibition in both *S. pneumonia* and *S. aureus* (Figure 8). Aqueous extract exhibited most inhibitory (mm) impact on *A. fumigatus*, *A. flavus* ($15 + 0.81$), as well as *A. niger* ($12 + 1.24$), whereas chloroform fraction had the least inhibitory action in *A. fumigatus* ($6 + 0.81$), *A. flavus* ($3 + 0.81$), and *A. niger* ($2 + 0.81$) (Figure 9). In *A. fumigatus*, butanol and ethyl acetate extracts unveiled comparable inhibition effects with no noticeable differences, whereas ethanol and ethyl acetate fractions showed same inhibition effects in *A. niger* (Figure 9).

3.8.2. Antimicrobial Dose-Response Relationship (Antibacterial and Antifungal)

3.8.2.1. Antibacterial Activity. The antibacterial dose-response evaluation of *T. officinale* fractions demonstrated significant differences in inhibitory potential across tested strains (ANOVA, $p < 0.05$). The aqueous fraction exhibited the strongest inhibition, producing a zone of inhibition (ZOI) of 15 mm against *S. pneumoniae*, followed by ethanol

TABLE 2: Regression equation, regression coefficient (R^2), intercept (standard error, standard deviation), limit of detection (LOD), and limit of quantification (LOQ) of detected phenolic compounds in different fractions of *T. officinale*.

Fractions/compound name	Regression equation	Regression coefficient (R^2)	Intercept S.E	Intercept S.D	λ (nm)	LOD (ppm)	LOQ (ppm)
<i>n-Butanol</i>							
Caffeic acid	$y = 0.0028x + 0.7822$	0.8555	0.001	0.0026	330	3.116	9.444
Cinnamic acid	$y = 0.0253x + 12.674$	0.9999	0.039	0.0553	280	7.225	21.895
Phenolic acid	$y = 0.5779x + 13.427$	0.5223	3.640	5.1478	280	29.396	89.079
Galic acid	$y = 0.555x + 0.3825$	0.8176	0.140	0.1989	280	25.948	78.631
Syringic acid	$y = 0.0291x + 5.4971$	0.9824	0.283	0.4015	280	45.533	137.980
Quercetin	$y = 1.5x - 0.1161$	0.7500	0.051	0.0721	330	0.158	0.480
<i>Chloroform</i>							
Cinnamic acid	$y = 2.4526x - 11.98$	0.9064	0.739	1.0459	270	1.407	4.2657
Galic acid	$y = 0.15x + 0.6557$	0.7500	0.228	0.3225	280	7.097	21.506
Quercetin	$y = 0.12x - 0.0178$	0.7500	0.086	0.1218	330	3.349	10.150
Vanillic acid	$y = 0.1x + 7.5908$	0.9999	0.381	0.5394	280	17.803	53.949
<i>Ethanol</i>							
Caffeic acid	$y = 5.0001x - 59.051$	0.7500	0.368	0.5206	330	4.666	14.1421
Chlorogenic acid	$y = 1.4769x - 11.072$	0.8531	2.661	3.7643	330	8.411	25.488
Galic acid	$y = 0.0272x - 0.0475$	0.8858	0.001	0.0029	280	0.353	1.071
Ferulic acid	$y = 3.595x - 5.2583$	0.8939	5.722	8.0924	280	7.428	22.510
Sinapinic acid	$y = 2.4995x - 17.066$	0.2500	0.774	1.0955	330	1.446	4.383
M-Coumaric acid	$y = 9.75x - 195.55$	0.7462	8.891	12.573	280	4.255	12.896
Syringic acid	$y = 97.15x - 1603.2$	0.9997	2.920	4.1299	280	4.326	13.110
<i>Ethyl acetate</i>							
Galic acid	$y = 0.2858x + 0.1634$	0.4286	0.075	0.1071	280	1.237	3.749
Syringic acid	$y = 2.0005x - 15.207$	0.9999	0.736	1.0422	280	1.719	5.209
P-Coumaric acid	$y = 3.9999x - 21.658$	0.9999	3.239	4.5817	280	3.780	11.454
Ferulic acid	$y = 2.9321x - 35.074$	0.9857	4.582	6.4805	320	7.293	22.102
Sinapinic acid	$y = 4.99x - 68.058$	0.2508	3.261	4.6117	330	3.049	9.241
<i>Aqueous</i>							
Quercetin	$y = 0.1503x + 0.0914$	0.7512	0.032	0.0454	330	0.997	3.022
Galic acid	$y = 0.1499x + 0.0922$	0.7498	0.065	0.0919	280	2.023	6.132
Syringic acid	$y = 0.2825x + 1.6926$	0.2595	0.347	0.4921	280	5.748	17.419
Sinapinic acid	$y = 0.05x + 5.4908$	0.7497	0.024	0.0419	330	2.765	8.380
<i>n-Hexane</i>							
Quercetin	$y = 2.5021x - 0.5018$	0.7506	0.056	0.0794	330	0.104	0.317
Galic acid	$y = 1.4997x - 2.4192$	0.9999	0.519	0.7345	280	1.617	4.900
Benzoic acid	$y = 0.9497x + 9.0593$	0.9918	1.441	2.0388	280	7.084	21.468
Sinapinic acid	$y = 2.5001x - 26.872$	0.8929	3.033	4.2898	330	5.662	17.158

Abbreviations: LOD = Limit of detection, LOQ = Limit of quantification, S.D = Standard deviation, S.E = Standard error.

TABLE 3: Phytochemical analysis of different extracts of *T. officinale*.

Phytochemical tests	Phytochemicals in <i>T. officinale</i>					
	<i>n</i> -butanol	Chloroform	Ethanol	Ethyl acetate	<i>n</i> -Hexane	Aqueous
Alkaloids	+	+	+	+	+	+
Coumarins	–	–	–	–	–	–
Flavonoids	–	+	+	+	+	+
Tannins	–	–	–	–	–	–
Terpenoids	+	+	+	+	–	+
Saponins	–	+	+	+	–	+
Quinones	–	+	+	+	+	+

Note: + = present, – = not present.

TABLE 4: Total phenolic and total flavonoids contents of different extracts of *T. officinale*.

Fractions	Total phenolic (mg GAE/g) (Mean $\pm$ SD)	Total flavonoids (mg RUE/g) (Mean $\pm$ SD)
<i>n</i> -Butanol	28 $\pm$ 0.007	9.2 $\pm$ 0.018
Chloroform	23 $\pm$ 0.010	6.1 $\pm$ 0.019
Ethanol	32 $\pm$ 0.013	8.5 $\pm$ 0.022
Ethyl acetate	30 $\pm$ 0.032	8.0 $\pm$ 0.040
Aqueous	41 $\pm$ 0.021	17.5 $\pm$ 0.029
<i>n</i> -Hexane	18 $\pm$ 0.014	2.7 $\pm$ 0.024

TABLE 5: DPPH radical scavenging activity in fractions of *T. officinale* leaf fractions.

Extracts	Concentrations (μ L/mL)	Radical scavenging effect (%) (Mean $\pm$ S.D)
<i>n</i> -Butanol	200	20 $\pm$ 0.005
	150	20 $\pm$ 0.002
	100	19 $\pm$ 0.002
	50	19 $\pm$ 0.001
Chloroform	200	19 $\pm$ 0.002
	150	19 $\pm$ 0.002
	100	19 $\pm$ 0.001
	50	18 $\pm$ 0.001
Ethanol	200	41 $\pm$ 0.003
	150	43 $\pm$ 0.002
	100	46 $\pm$ 0.002
	50	48 $\pm$ 0.001
Ethyl acetate	200	20 $\pm$ 0.002
	150	21 $\pm$ 0.002
	100	22 $\pm$ 0.005
	50	24 $\pm$ 0.001
<i>n</i> -Hexane	200	17 $\pm$ 0.005
	150	17 $\pm$ 0.002
	100	16 $\pm$ 0.002
	50	14 $\pm$ 0.23
Aqueous	200	25 $\pm$ 0.002
	150	23 $\pm$ 0.002
	100	22 $\pm$ 0.001
	50	21 $\pm$ 0.002
Control (Ascorbic acid)	200	49 $\pm$ 0.002
	150	45 $\pm$ 0.001
	100	34 $\pm$ 0.002
	50	24 $\pm$ 0.003

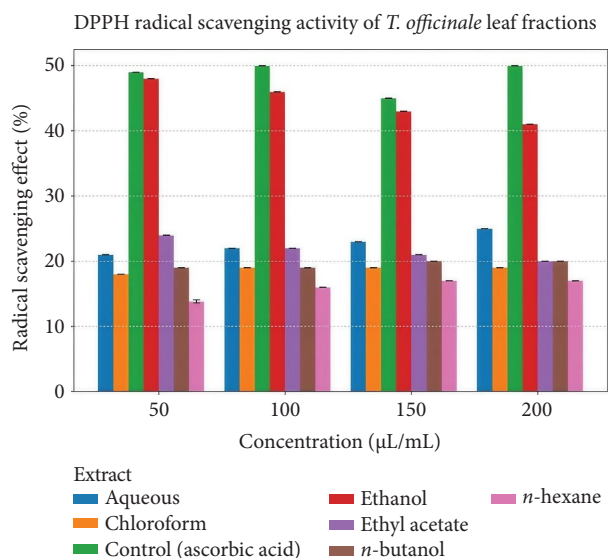


FIGURE 5: Radical scavenging activity (DPPH) of *T. officinale* leaf fractions.

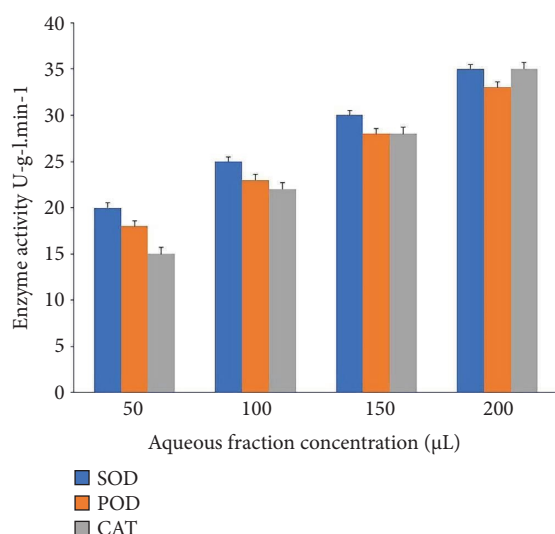


FIGURE 6: Antioxidants concentration of *T. officinale* in aqueous extract. Bars in the graph pooled from three independent replicates ($n=3$) and showing the statistical difference ($p < 0.05$), SOD = superoxide dismutase; CAT = catalase; POD = peroxidase.

with ZOI of 14 mm against both *E. coli* and *S. pneumoniae*. These responses were statistically higher ($p < 0.05$) compared to *n*-butanol, chloroform, ethyl acetate, and *n*-hexane, which showed moderate activity in the range of 10–13 mm. As expected, the positive control ciprofloxacin displayed the highest antibacterial effect across all strains (20–25 mm; $p < 0.001$). The dose–response curves with SE bars (Figure 10) confirmed reproducibility and highlighted the superior activity of aqueous and ethanol fractions compared to other solvent extracts.

3.8.2.2. Antifungal Activity. The antifungal dose–response analysis also revealed significant variability among fractions

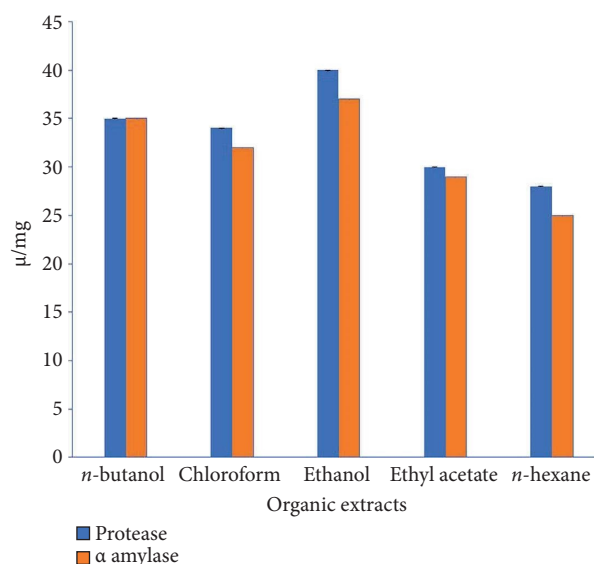


FIGURE 7: Hydrolases enzymes in aqueous extract of *T. officinale*.

(ANOVA, $p < 0.05$). The aqueous and ethanol fractions showed the strongest inhibitory effects, particularly against *A. fumigatus* and *A. flavus* (up to 15 mm and 13 mm, respectively), with effects that were statistically higher than those of chloroform and *n*-hexane fractions ($p < 0.01$). Ethyl acetate and *n*-butanol demonstrated intermediate activity (8–12 mm), while chloroform exhibited the weakest antifungal response, with inhibition zones as low as 2 mm against *A. niger*. The standard control fluconazole consistently produced the highest antifungal activity (15–20 mm; $p < 0.001$). The plotted dose–response curves with error bars (Figure 11) further validated the statistical reliability of the results, confirming aqueous and ethanol fractions as the most promising antifungal candidates.

3.9. Hemolytic Activity. Our results revealed significant ($p < 0.05$) differences across extraction systems. The hemolytic activity of dandelion leaf extracts ranged between 0.30 and 0.85 (30%–85%). Hemolytic activity of various extraction systems, including *n*-butanol, hexane, chloroform, ethyl acetate, and aqueous was 0.44 ± 0.003 (44%), 0.30 ± 0.011 (30%), 0.75 ± 0.01 (75%), 0.85 ± 0.002 (85%), and 0.60 ± 0.001 (60%), respectively (Figure 12). In contrast to other dandelion leaf extracts, ethyl acetate (85%) demonstrated the most hemolytic activity, while hexane ($30 \pm 0.011\%$) showcased the least activity (Figure 12).

4. Discussion

The curative benefits of traditional medicinal plants are contingent upon the specific compounds present inside them [44]. Over the past several decades, investigators have adeptly isolated the bioactive compounds in dandelion due to its excellent pharmacological effects. According to Fan et al. [45], the biological activity of the substance is impacted by a multitude of complex chemical constituents, mostly including phenolic compounds, flavonoids, sphingolipids,

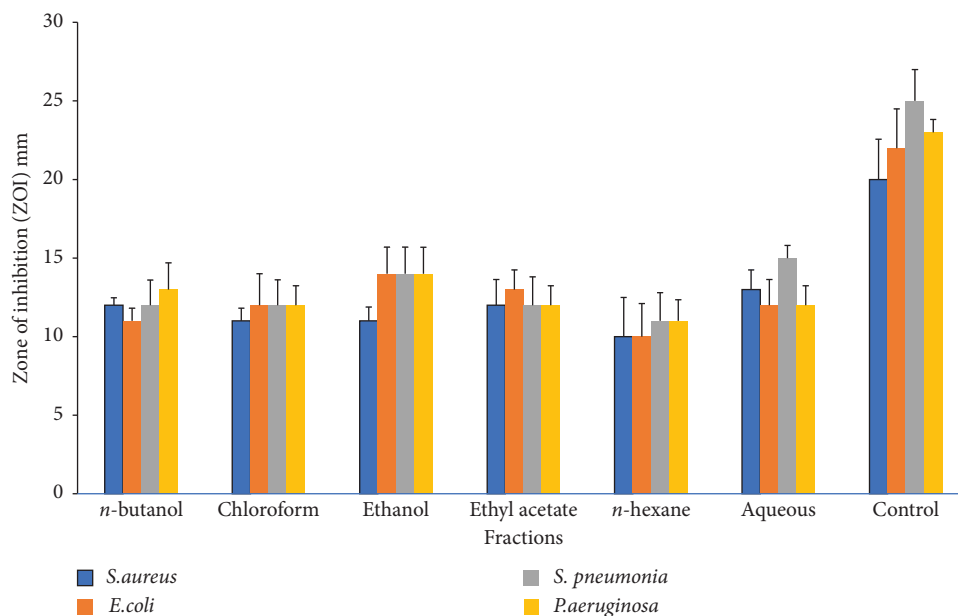


FIGURE 8: Inhibition zones (ZOI) in Gram-negative and Gram-positive bacterial strains formed by *T. officinale* extracts with control (ciprofloxacin), bars in the graph pooled from three independent replicates ($n = 3$) and show the statistical difference ($p < 0.05$).

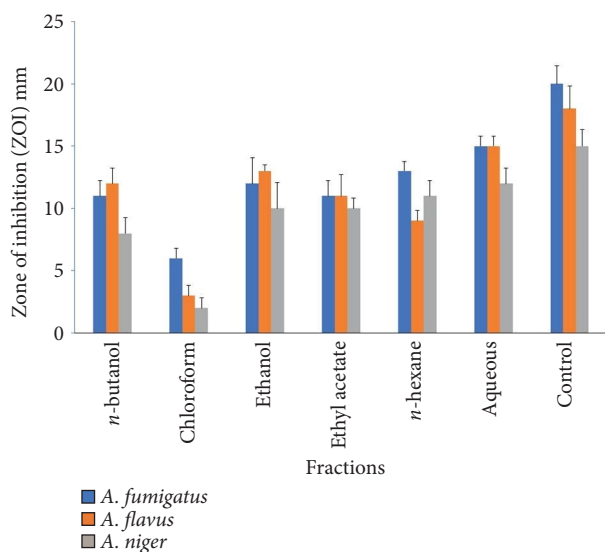


FIGURE 9: Inhibition zones in fungal strains formed by *T. officinale*. Different fractions with control, values are means and standard deviations of diameter zone of inhibition (mm) of triplicate determinations. Values showed a significant difference among treatments ($p < 0.05$).

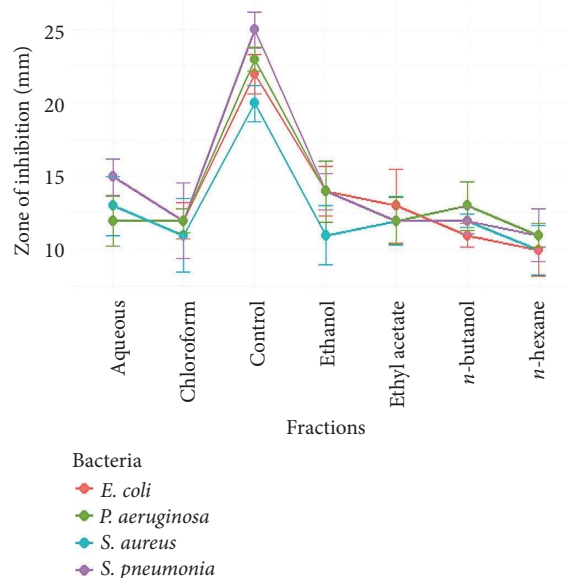


FIGURE 10: Dose-response relationship curve (bacterial strains and fractions).

triterpenoids, sterols, coumarins, and others. Polyphenolic compounds are ubiquitous in several plant species.

Numerous phenolic compounds with potential activity have been extracted from various species within the *Taraxacum* genus [10, 46]. Quercetin, gallic acid, caffeic acid, syringic acid, vanillic acid, chlorogenic acid, coumaric acid, ferulic acid, sinapinic acid, benzoic acid, and cinnamic acid were effectively determined through several extraction techniques, as reported in our study [45]. These compounds have been uncovered for antioxidative and

immunostimulant activities. Such phenolics have been documented earlier [10, 46, 47]. The present investigation determined comparable profiles of secondary metabolites in ethyl acetate [48], aqueous [49], chloroform and hexane [31], and ethanol [50] extracts, which align substantially with previous investigations. Certain substances found in plant extracts can be ascribed to physiological and biosynthetic responses, as well as the environmental factors [51]. Furthermore, the absence of compounds in the n-hexane extract of *T. officinale* in our study could be attributed to the solvent's low polarity, which hampered extraction [52, 53]. As a result, it is possible to deduce that the observed variances

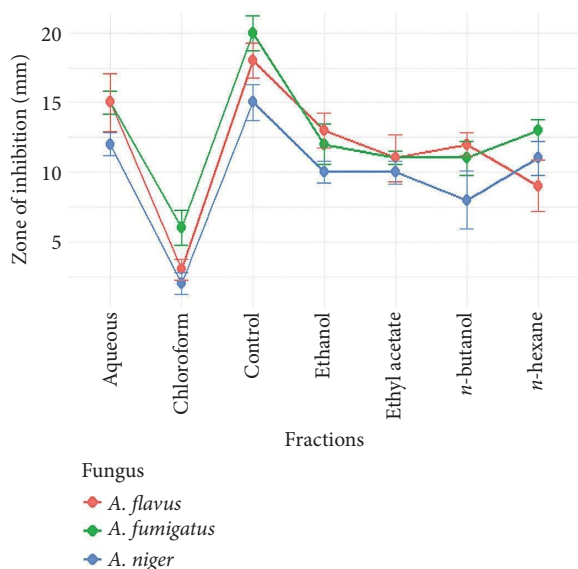


FIGURE 11: Dose-response relationship curve (fungal strains and fractions).

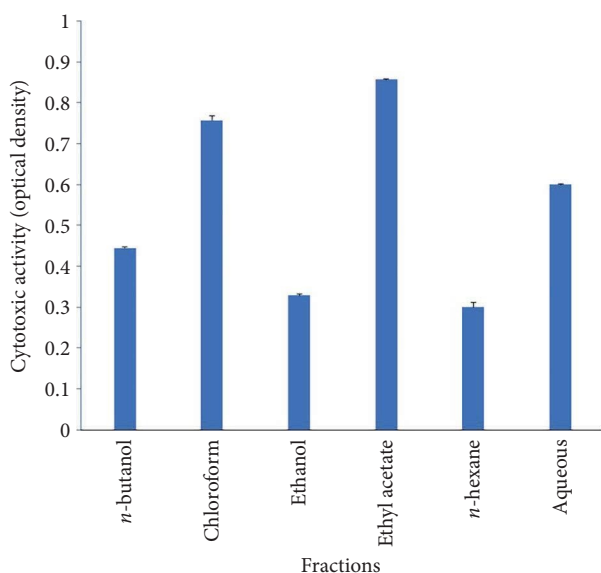


FIGURE 12: Hemolytic activity of different extracts of *T. officinale*. Values are means and standard deviations of triplicate determinations. Values showed a significant difference among different solvents ($p < 0.05$).

were mostly caused by the solvents' incompatible polarity indices. Consistent with previous studies [54–56], our study indicated that ascorbic acid, gallic acid, cinnamic acids (caffeic acid, syringic acid, *p*-coumaric acid, sinapic acid, and ferulic acid), and flavonoids (catechin, rutin, quercetin) and their derivatives have hydroxyl group substitutions that are associated with their protective potential. Flavonoids, for instance, had been demonstrated to increase antihemolytic protection due to their chemical structure and well-known liposolubility increased antioxidant capacity [57].

Dandelion leaves and flowers are rich in polyphenols, particularly HCAs and flavonoids [10, 11]. They are known

to possess excellent antioxidant and hypocholesterolemic characteristics. HCAs have antioxidative and curative effects [10], whereas flavonoids limit the production of ROS and nitrogen by blocking NO synthase and COX-2 protein expression [13]. Polyphenols identified in our study encompassing caffeic acid, gallic acid, ferulic acid, and syringic acid acts as antioxidative properties [58, 59], cinnamic acid as antidiabetic [60], quercetin as antioxidative [45], chlorogenic acid as antioxidant, anti-inflammatory, antibacterial, antiviral, hypoglycemic, lipid-lowering, antiscavenger, antimutagenic, anticancer, immunomodulatory [61], sinapic acid as antidepressant [62], vanillic acid as antioxidative [59] and immunostimulant capabilities [45], *p*-coumaric acid as anti-inflammatory, bacteriostatic [45], *p*-coumaric as anti-inflammatory, and antioxidative qualities [63].

Our results showed that water had the highest TPC, followed by ethyl acetate and ethanol. Previous investigations found that ethanol has the highest TPC, followed by aqueous [63]. While our aqueous extract showed the highest TPC, other studies have reported ethanol as superior. This discrepancy may arise due to soil composition and climatic variation in Pakistan, which can alter phenolic biosynthesis. Thus, our findings highlight the influence of geographical origin on phytochemistry.

It also implies that water and ethyl acetate are the primary conduits of phenolic-based compounds, as previously reported [58], which may potentially account for their comparatively strong antioxidant activity. The aqueous extract entailed twofold more TFC than the ethyl acetate extract. Previous research cited the highest TFC in the aqueous extract of dandelion [64]. The quantity of TFC in dandelion ethyl acetate extract assessed by Ghaima et al. [65] was closely comparable to that reported in our investigations. The shift in TPC and TFC concentrations could be explained by solvent polarity. As the solvent polarity decreases, the extraction process impedes, eventually influencing the chemical processes [51] thereby affecting the concentration. Table 4 shows that the ethanol extracts of dandelion leaves have more formidable antioxidant activity (radical scavenging) values than previous results [66]. Previous investigations uncovered increased radical scavenging activity in ethyl acetate extracts of dandelion leaves [58]. There have been no studies conducted on radical scavenging activity in dandelion in concentration-specific manner. This shows that ethyl acetate contains considerable amounts of phenolic compounds, which could account for its relatively high antioxidant activity [55]. Our study's aqueous extract stated less radical scavenging activity in leaves than ethanol, which is consistent with previous research [67]. Radical scavenging activity differed based on many factors, including the plant part, the solvent deployed, and the duration of extraction.

Plants exhibit antioxidant protection enzyme systems, such as SOD, CAT, and POD [68]. The present investigation determined a statistically significant increase ($p < 0.05$) in the aqueous dandelion leaf extract. There is a lack of research detailing the AOX capabilities of dandelion enzymes in both polar and nonpolar solvents. The presence of one or more

phytochemical components of dandelion has been accountable for the observed antioxidant activity in aqueous extract [69]. Ethanol extract followed by n-butanol, showcased the most pertinent inhibitory effect against protease and β -amylase. This elevated α -amylase activity seen in dandelion has been credited to the containment of phenolic substances, dietary polyphenols [70], and flavonoids [71].

Research on antibacterial and antifungal aspects in both developing and developed countries primarily focuses on alleviating the need for affordable new pharmaceuticals or products derived from traditional medicine. The ethanol extract exhibited a significant ZOI followed by ethyl acetate fraction against both Gram-negative (*E. coli*, *P. aeruginosa*) bacteria. This finding aligns with prior research conducted by Qiao and Sun [72]. This antibacterial activity could be explained by the considerable amount of flavonoids and phenolic acids present in these fractions [72]. The dandelion stem, leaves, and root extracts contain polyphenolics including chlorogenic acid and caffeic acid, which demonstrated bactericidal activity owing to its cellular membrane-breaking efficacy [73, 74]. Among the Gram-positive (*S. aureus*, *S. pneumonia*) detected, aqueous extract has been shown to be more effective in thwarting the growth of bacteria, followed by ethyl acetate extract. According to Ionescu et al. [75], polar chemicals have an increased capacity for inhibiting microbial growth than nonpolar compounds. As a result, the findings of the relevant study, which uncovered that polar plant extracts had a stronger antibacterial impact than nonpolar solvent extracts, are unanimously agreed with earlier reports [76]. Some researchers have speculated that the antibacterial action implied by plant extracts may be due to secondary metabolites [77, 78]. Studies on the antibacterial mechanism of natural alkaloids show that they can disrupt the bacterial cell membrane [79], affect the DNA function [80], and inhibit protein synthesis [81].

Our research focused on the *Aspergillus* species, which are among the most frequent fungal ailments worldwide. *Aspergillus* infection arises by *Aspergillus* spp. and has been believed to cause human infections. *Aspergillus fumigatus* is the primary species inducing infection in humans and the leading cause mortality among infected people. Nevertheless, it has been documented that nonfumigatus species of *Aspergillus*, such as *A. flavus* and *A. niger*, can also induce aspergillosis. The preliminary antifungal activity analysis results indicate that the aqueous extract demonstrated the highest ZOI activity across all *Aspergillus* species examined in our study. Conversely, the chloroform extract exhibited the lowest activity. There is lack of information about the antifungal inhibitory activity of dandelion leaves extract in *Aspergillus* species.

In this study, we found that dandelion extracts include polyphenols (active compounds) such as coumaric acid, ferulic acid, quercetin, gallic acid, cinnamic acid, syringic acid, and caffeic acid. In our work, dandelion extracts inhibited mycelial growth and exhibited fungistatic action. Dandelion active compounds are capable of destroying fungal cell membranes, elevating cell membrane permeability, macromolecule leakage, and, eventually, disrupting

cell metabolism. This shows that dandelion active components or polyphenolics may suppress fungal growth by disrupting the cell wall and cell membranes, resulting in mycelial growth inhibition [82, 83]. Polyphenols may disrupt the glycosidic link of (1,3)- β -d-glucan in fungal cell walls, slowing growth or causing cell death. The inhibiting impact of certain plant species on fungi and bacteria is primarily attributable to flavonoids as reported earlier [84]. The antimicrobial effects observed likely result from synergistic actions of quercetin, gallic acid, and caffeic acid, which are known to disrupt microbial membranes, inhibit nucleic acid synthesis, and chelate essential metal ions. Flavonoids may also interfere with bacterial quorum sensing, while polyphenols disrupt fungal cell walls by targeting β -glucans.

A highly polar solvent like aqueous may extract numerous polyphenol chemicals from plants and vice versa, which may explain the apparent link between polyphenolics and antifungal activity. The antifungal activity observed in the chemical analysis may be attributed, in part, to the presence of polyphenol chemicals, which are typically the primary antifungal agents found in most plant extracts. In summary, current research indicates that the antifungal activity mechanism is quite intricate, necessitating further in-depth investigations to fully understand this mechanism. Currently, as far as we know, there have been no detailed research conducted regarding the antifungal properties of polyphenolics against fungal species.

The hemolytic activity assay is a frequently utilized for assessing the detrimental effects of xenobiotics and environmental influences on red blood cells (RBCs). The hemolytic activity was studied to figure out if the damage to human erythrocytes might impede the usage of solvents (extracts) and chemicals derived from dandelion, regardless of the biological attribute being assessed. To date, there have been no documented investigations on hemolytic activity of dandelion extracts. The ethyl acetate, chloroform, and aqueous fractions exhibited significant hemolytic activity, which aligned with previous findings in other plant species [43]. Furthermore, it has been demonstrated that the ethanol extract derived from dandelion possesses the ability to prevent damage and improve the integrity of the erythrocyte membrane. This effect is likely attributed to the elevated levels of phenolic compounds found in many plant species [85–87]. While our investigations have stipulated us with a basic grasp of the nontoxic properties of dandelion extracts, it is not feasible to draw a conclusive assessment of their safety. Different leaf extracts from medicinal plants prevent production of nitric oxide radicals and superoxide anion [3, 4]. Additional investigations are necessary to ascertain the potential toxicity of dandelion. The available data on the hemolytic activity of dandelion is currently scant. Hence, this study presents the initial documentation of the antibacterial and hemolytic properties exhibited by leaf extracts derived from this particular species.

The antimicrobial activities observed in different fractions of *T. officinale* can be attributed to the presence of phenolic acids and flavonoids, which are known to interfere with microbial physiology through multiple mechanisms.

Phenolic compounds such as caffeic acid, gallic acid, and syringic acid can integrate into microbial cell membranes, disrupting their integrity and altering permeability, which leads to leakage of intracellular contents. Flavonoids such as quercetin exert additional effects by binding to bacterial enzymes like DNA gyrase and topoisomerases, thereby inhibiting nucleic acid synthesis. Furthermore, several phytochemicals can chelate essential metal ions required for microbial growth or induce oxidative stress by promoting ROS generation, leading to cellular damage. In fungi, these compounds may additionally interfere with ergosterol biosynthesis, compromising membrane stability. Collectively, these mechanisms may underlie the dose-response antimicrobial effects observed in this study, and future work will focus on direct mechanistic validation using targeted assays such as membrane permeability, ROS generation, and enzyme inhibition analyses.

More investigation must be undertaken to explore the activities of *T. officinale*-derived polyphenolics and flavonoids. This study provides the first integrated analysis of phytochemistry, antioxidant enzymes, hydrolases, radical scavenging, antimicrobial, and hemolytic activities of *T. officinale* grown in Pakistan. Standard controls (e.g., ciprofloxacin, fluconazole, ascorbic acid) were used for comparative purposes. Although the absence of dose-response data and mechanistic validation is a limitation, the results provide novel insights into the bioactive potential of region-specific dandelion extracts and lay groundwork for future pharmacological and mechanistic studies.

5. Conclusion

In conclusion, we demonstrated the bioactive components, phytochemical screening, TPC and TFC, radical scavenging, antioxidants, antimicrobial, and hemolytic activity of various dandelion leaf extracts. HPLC examination of dandelion leaf fractions revealed approximately 12 bioactive compounds. Secondary metabolites of dandelion extracts from leaves included alkaloids, flavonoids, saponins, quinones, and terpenoids in organic fractions. None of the fractions reported coumarins or tannins. The aqueous fraction had the most concentrations of total flavonoids and total phenolics. In comparison to other extracts, the ethanol extract exhibited superior radical scavenging and hydrolase activity. Intriguing antibacterial activity against Gram-negative and Gram-positive pathogenic clinical bacteria is observed in ethanol and aqueous extracts, respectively. Our findings revealed that the water-soluble extract of dandelion had high antifungal activity against *Aspergillus* spp. The current study unambiguously suggests antioxidants, hydrolases, and antibacterial action due to the broad repertoire of flavonoids and polyphenolic compounds encountered in dandelion leaves.

Dandelion, a well-known complementary and alternative medicine, has an array of bioactive components such as terpenes, flavonoids, polyphenolic compounds, phytosterols, etc. However, previous research centered mainly on extracting and identifying active component compounds from several types of dandelion. To be more specific,

T. officinale is the most widely employed species in complementary and alternative medicine. Furthermore, dandelion extracts (G.H. Weber ex Wiggers) possess potent antimicrobial, antioxidant, and cytotoxic properties. Furthermore, the extracts from leaf tissues endured good aforementioned actions, providing solid evidence about the utilization of the traditional medicinal herb as an antibacterial and antifungal medicine.

5.1. Study Limitations and Challenges. The study's limitations include an emphasis on a single geographic region (Central Punjab, Pakistan) for the collecting of *T. officinale* leaves, which may limit the results' applicability to other regions. *Taraxacum officinale* species, environmental conditions (soil, cultivation tactics, climate, harvesting, storage, processing, handling), extraction (analytical) procedures, solvents, and other characteristics all have an impact on the findings, and hence these variables cannot be reliably standardized. Antimicrobial experiments with more bacterial and fungal species need to be conducted to evaluate against *T. officinale* fractions. Other in-depth research employing other solvents and different plant tissues is needed to fully grasp its potential medicinal benefits. We also highly recommend the structural explication of *T. officinale* bioactive compounds and their in silico studies against different diseases.

6. Future Perspectives

The current study provides an overview of the phytochemical composition and bioactivities of indigenously grown *Taraxacum officinale* leaves. However, the current study serves as a preliminary basis for the plant's medicinal potential. In the future, compound-level validation should be a priority using LC-MS/MS, GC-MS, FTIR, and NMR spectroscopy. These methods would help identify and quantify the active metabolites responsible for biological functions. Also, bioactivity-guided fractionation strategies should be utilized to isolate individual phenolic and flavonoid constituents and correlate their molecular structures with their biological activities.

To unravel the mechanistic underpinnings of *T. officinale* bioactivity, future studies should integrate in vitro cell-based assays targeting oxidative stress pathways, inflammatory mediators, and microbial virulence factors. Investigations using enzyme inhibition kinetics, receptor-ligand binding assays, and pathway-specific molecular markers (e.g., NF- κ B, COX-2, Nrf2, and MAPK signaling cascades) can provide mechanistic clarity regarding antioxidant and antimicrobial modes of action. In addition, by using computational methods, whether in the Wet Lab or in silico through molecular docking, molecular dynamics (MD) simulations, and quantitative structure-activity relationship (QSAR) studies, the possible dynamics of interaction with the targeted protein can be identified.

Equally critical are in vivo evaluations, which remain indispensable for confirming the pharmacological relevance

and safety of *T. officinale*-derived bioactives. Research on the pharmacokinetics, bioavailability, dose-response relations, systemic toxicity, and toxicokinetics in animal models will inform the therapeutic windows and toxicity. Chemical and disease tests can provide extra information about the safety of specific fractions. Moving from the lab to real-world use requires following strict rules to ensure its safe and works. This will be necessary for any new treatment.

Furthermore, multiomics integration (metabolomics, transcriptomics, and proteomics) should be incorporated to map the molecular networks influenced by dandelion phytochemicals, enabling a systems-level understanding of their biological impact. By using these omics approaches in combination with network pharmacology, the synergistic interactions of bioactive compounds and their multitarget therapeutic effects will be predicted. Examining the phytochemicals of plants grown in different areas and using different methods may help us understand which factors influence their growth.

Ultimately, integrating in silico, in vitro, and in vivo methodologies, along with compound-level validation and mechanistic assays, will enable a holistic elucidation of *Taraxacum officinale* therapeutic potential. Such comprehensive approaches will facilitate the translation of dandelion-derived bioactives into standardized, evidence-based phytopharmaceuticals and nutraceutical formulations. These efforts will not only reinforce the ethnomedicinal value of *T. officinale* but also contribute to the discovery of novel, naturally derived therapeutic agents with validated pharmacological efficacy and safety for clinical use.

Data Availability Statement

The data used to support the findings of this study are included within the article.

Ethics Statement

There were no ethical considerations to be considered in this research.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Adil Jamal, Amina Arif, Md. Belal Hossain: conceptualization, methodology, project administration, supervision, data curation, resources, formal analysis, writing—original draft, writing—review and final editing, and validation. Akmal Zubair: investigation and writing—review and editing. Muhammad Naveed Shahid: methodology, investigation, and data analysis.

Funding

This study did not receive any sort of funding from any government, semigovernment, and private organization.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*)

All the required Supporting information (Supporting tables) have been provided.

References

- [1] R. Hoenders, R. Ghelman, C. Portella, et al., “A Review of the WHO Strategy on Traditional, Complementary, and Integrative Medicine From the Perspective of Academic Consortia for Integrative Medicine and Health,” *Frontiers of Medicine* 11, no. 11 (2024): 1395698, <https://doi.org/10.3389/fmed.2024.1395698>.
- [2] (2021), <https://www.fao.org/faostat/en/#data/TCL>.
- [3] R. A. P. Purba and P. Paengkoum, “Exploring the Phytochemical Profiles and Antioxidant, Antidiabetic, and Anti-hemolytic Properties of *Sauropus androgynus* Dried Leaf Extracts for Ruminant Health and Production,” *Molecules* 27, no. 23 (2022): 8580, <https://doi.org/10.3390/molecules27238580>.
- [4] P. Rap and P. F. Paengkoum, “(*Psidium guajava* L.) Dried Leaf Extracts: Phytochemical Profiles, Antioxidant, Anti-Diabetic, and Anti-Hemolytic Properties for Ruminant Health and Production,” *Molecules* 16, no. 24 (2022): 8987, <https://doi.org/10.3390/molecules27248987>.
- [5] Y. Li, Y. Chen, and D. Sun-Waterhouse, “The Potential of Dandelion in the Fight Against Gastrointestinal Diseases: A Review,” *Journal of Ethnopharmacology* 293 (2022): 115272, <https://doi.org/10.1016/j.jep.2022.115272>.
- [6] S. Zhang, C. Li, W. Gu, et al., “Metabolomics Analysis of Dandelions From Different Geographical Regions in China,” *Phytochemical Analysis* 32, no. 6 (2021): 899–906, <https://doi.org/10.1002/pca.3033>.
- [7] C. Li, Y. Tian, C. Zhao, et al., “Application of Fingerprint Combined With Quantitative Analysis and Multivariate Chemometric Methods in Quality Evaluation of Dandelion (*Taraxacum mongolicum*),” *Royal Society Open Science* 8, no. 10 (2021): 210614, <https://doi.org/10.1098/rsos.210614>.
- [8] D. Esiyok, S. Otles, and E. Akcicek, “Herbs as a Food Source in Turkey,” *Asian Pacific Journal of Cancer Prevention* 5, no. 3 (2004): 334–339.
- [9] C. A. Williams, F. Goldstone, and J. Greenham, “Flavonoids, Cinnamic Acids and Coumarins From the Different Tissues and Medicinal Preparations of *Taraxacum officinale*,” *Phytochemistry* 42, no. 1 (1996): 121–127, [https://doi.org/10.1016/0031-9422\(95\)00865-9](https://doi.org/10.1016/0031-9422(95)00865-9).
- [10] K. Schütz, D. R. Kammerer, R. Carle, and A. Schieber, “Characterization of Phenolic Acids and Flavonoids in Dandelion (*Taraxacum officinale* WEB. Ex WIGG.) Root and Herb by High-Performance Liquid Chromatography/Electrospray Ionization Mass Spectrometry,” *Rapid Communications in Mass Spectrometry* 19, no. 2 (2005): 179–186, <https://doi.org/10.1002/rcm.1767>.
- [11] D. Jędrejek, B. Kontek, B. Lis, A. Stochmal, and B. Olas, “Evaluation of Antioxidant Activity of Phenolic Fractions From the Leaves and Petals of Dandelion in Human Plasma Treated With H₂O₂ and H₂O₂/Fe,” *Chemico-Biological Interactions* 262 (2017): 29–37, <https://doi.org/10.1016/j.cbi.2016.12.003>.
- [12] C. Hu and D. D. Kitts, “Luteolin and luteolin-7-O-glucoside From Dandelion Flower Suppress iNOS and COX-2 in

- RAW264.7 Cells,” *Molecular and Cellular Biochemistry* 265, no. 1-2 (2004): 107–113, <https://doi.org/10.1023/b:mcbi.0000044364.73144.fe>.
- [13] K. Tsai, C. Kao, C. Hung, Y. Cheng, H. Lin, and P. Chu, “Chicoric Acid is a Potent Anti-atherosclerotic Ingredient by Anti-Oxidant Action and Anti-Inflammation Capacity,” *Oncotarget* 8, no. 18 (2017): 29600–29612, <https://doi.org/10.18632/oncotarget.16768>.
 - [14] D. Zolotova, R. Teterovska, D. Bandere, L. Lauberte, and S. Niedra, “Antidiabetic Properties of the Root Extracts of Dandelion (*Taraxacum officinale*) and Burdock (*Arctium lappa*),” *Plants* 13, no. 7 (2024): 1021, <https://doi.org/10.3390/plants13071021>.
 - [15] C. Jalili, M. Taghadosi, M. Pazhouhi, et al., “An Overview of Therapeutic Potentials of *Taraxacum officinale* (Dandelion): A Traditionally Valuable Herb With a Reach Historical Background,” *World Cancer Res. J.* 7 (2020): e1679, https://doi.org/10.32113/wcrj_20209_1679.
 - [16] B. Olas, “New Perspectives on the Effect of Dandelion, its Food Products and Other Preparations on the Cardiovascular System and Its Diseases,” *Nutrients* 14, no. 7 (2022): 1350, <https://doi.org/10.3390/nu14071350>.
 - [17] I. O. Pfingstgraf, M. Taulescu, R. M. Pop, et al., “Protective Effects of *Taraxacum officinale* L. (dandelion) Root Extract in Experimental Acute on Chronic Liver Failure,” *Antioxidants* 10, no. 4 (2021): 504, <https://doi.org/10.3390/antiox10040504>.
 - [18] Y. Yang, G. Ying, S. Wu, F. Wu, and Z. Chen, “In Vitro Inhibition Effects of Hepatitis B Virus by Dandelion and Taraxasterol,” *Infectious Agents and Cancer* 15, no. 1 (2020): 44–10, <https://doi.org/10.1186/s13027-020-00309-4>.
 - [19] H. Goyal, R. C. Gupta, S. K. Pradhan, and R. Goel, “Anti-microbial and Hepatoprotective Activity of Different Cytotypes of *Taraxacum officinale* From the Indian Himalayas,” *Journal of Herbs, Spices, & Medicinal Plants* 30, no. 1 (2023): 12–25, <https://doi.org/10.1080/10496475.2023.2248040>.
 - [20] A. Epure, A. E. Părvu, L. Vlase, et al., “New Approaches on the Anti-Inflammatory and Cardioprotective Properties of *Taraxacum officinale* Tincture,” *Pharmaceuticals* 16, no. 3 (2023): 358, <https://doi.org/10.3390/ph16030358>.
 - [21] B. Ge, R. Sang, W. Wang, et al., “Protection of Taraxasterol Against Acetaminophen-Induced Liver Injury Elucidated Through Network Pharmacology and *in Vitro* and *in Vivo* Experiments,” *Phytomedicine* 116 (2023): 154872, <https://doi.org/10.1016/j.phymed.2023.154872>.
 - [22] A. Ikram, R. Khan, S. Kauser, A. A. Khan, M. T. Arshad, and M. Ahmad, “*Taraxacum officinale* (Dandelion),” in *Edible Flowers*, ed. A. K. Gupta, V. Kumar, B. Naik, and P. Mishra (Uttarakhand, India: Elsevier: Amsterdam, The Netherlands; Academic Press, 2024), 281–300.
 - [23] H. Gui and X. Fan, “Anti-Tumor Effect of Dandelion Flavone on Multiple Myeloma Cells and Its Mechanism,” *Discover Oncology* 15, no. 1 (2024): 215, <https://doi.org/10.1007/s12672-024-01076-z>.
 - [24] E. U. Alum, “Climate Change and Its Impact on the Bioactive Compound Profile of Medicinal Plants: Implications for Global Health,” *Plant Signaling & Behavior* 19, no. 1 (2024): 2419683, <https://doi.org/10.1080/15592324.2024.2419683>.
 - [25] K. Yeshi, D. Crayn, E. Ritmejeriyte, and P. Wangchuk, “Plant Secondary Metabolites Produced in Response to Abiotic Stresses Has Potential Application in Pharmaceutical Product Development,” *Molecules* 27, no. 1 (2022): 313, <https://doi.org/10.3390/molecules27010313>.
 - [26] ICH harmonised Tripartite Guidelines, “Validation of Analytical Procedures: Text and Methodology Q2(R1),” in *International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Current Step 4 Version* (October 1994).
 - [27] J. Senguttuvan, S. Paulsamy, and K. Karthika, “Phytochemical Analysis and Evaluation of Leaf and Root Parts of the Medicinal Herb, *Hypochaeris radicata* L. for *in Vitro* Antioxidant Activities,” *Asian Pacific Journal of Tropical Biomedicine* 4 (2014): 359–367, <https://doi.org/10.12980/apjtb.4.2014c1030>.
 - [28] J. Zhishen, T. Mengcheng, and W. Jianming, “The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals,” *Food Chemistry* 64, no. 4 (1999): 555–559, [https://doi.org/10.1016/s0308-8146\(98\)00102-2](https://doi.org/10.1016/s0308-8146(98)00102-2).
 - [29] H. P. Makkar, P. Siddhuraju, and K. Becker, *Methods in Molecular Biology: Plant Secondary Metabolites* (Totowa: Human Press, 2007).
 - [30] I. S. Akande, T. A. Samuel, U. Agbazue, and B. L. Olowolagba, “Comparative Proximate Analysis of Ethanolic and Water Extracts of Cymbopogon Citrates (Lemongrass) and Four Tea Brands,” *Journal of Pharmaceutical and Biomedical Sciences* 3 (2012): 29–35.
 - [31] O. S. Edori, A. C. Marcus, and M. C. Maduagu, “Phytochemical and Anti-Microbial Screening of *Phyllanthus Fratenus* and *Taraxacum Officinale* Leaves,” *Biochemistry & Analytical Biochemistry* 8, no. 1 (2019): 376.
 - [32] C. M. Ejikeme, C. S. Ezeonu, and A. N. Eboatu, “Determination of Physical and Phytochemical Constituents of Some Tropical Timbers Indigenous to Niger Delta area of Nigeria,” *European Scientific Journal* 10, no. 18 (2014): 247–270.
 - [33] P. C. Wootton-Beard, A. Moran, and L. Ryan, “Stability of the Total Antioxidant Capacity and Total Polyphenol Content of 23 Commercially Available Vegetable Juices Before and After *in Vitro* Digestion Measured by FRAP, DPPH, ABTS and Folin-Ciocalteu Methods,” *Food Research International* 44, no. 1 (2011): 217–224, <https://doi.org/10.1016/j.foodres.2010.10.033>.
 - [34] C. Quettier-Deleu, B. Gressier, J. Vasseur, et al., “Phenolic Compounds and Antioxidant Activities of Buckwheat (*Fagopyrum esculentum* Moench) Hulls and Flour,” *Journal of Ethnopharmacology* 72, no. 1-2 (2000): 35–42, [https://doi.org/10.1016/s0378-8741\(00\)00196-3](https://doi.org/10.1016/s0378-8741(00)00196-3).
 - [35] J. He, B. Huang, X. Ban, J. Tian, L. Zhu, and Y. Wang, “*In Vitro* and *in Vivo* Antioxidant Activity of the Ethanolic Extract From *Meconopsis quintuplinervia*,” *Journal of Ethnopharmacology* 141, no. 1 (2012): 104–110, <https://doi.org/10.1016/j.jep.2012.02.006>.
 - [36] S. Marklund and G. Marklund, “Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase,” *European Journal of Biochemistry* 47, no. 3 (1974): 469–474, <https://doi.org/10.1111/j.1432-1033.1974.tb03714.x>.
 - [37] A. C. Maehly and M. Chance, “Assay of Catalases and Peroxidases,” *Method Enzyme* 2 (1954): 764–817.
 - [38] D. R. Doerge, R. L. Divi, and M. I. Churchwell, “Identification of the Colored Guaiacol Oxidation Product Produced by Peroxidases,” *Analytical Biochemistry* 250, no. 1 (1997): 10–17, <https://doi.org/10.1006/abio.1997.2191>.
 - [39] M. Abd-El Khalek Aya, D. M. Seoudi, O. A. Ibrahim, N. S. Abd-Rabou, and E. M. Abd ElAzeem, “Extraction, Partial Purification, Characteristics, and Antimicrobial Activity of Plant Protease From *Moringa oleifera* Leaves,” *Journal of Applied Biotechnology Reports* 7, no. 4 (2020): 243–250.

- [40] M. A. Bhutkar and S. B. Bhise, "In Vitro Assay of Alpha Amylase Inhibitory Activity of Some Indigenous Plants," *International Journal of Chemical Sciences* 10, no. 1 (2012): 457–462.
- [41] P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover, *Manual of Clinical Microbiology*, 6th ed. (Washington, DC, USA: ASM, 1995).
- [42] V. Surapuram, W. N. Setzer, R. L. McFeeters, and H. McFeeters, "Antifungal Activity of Plant Extracts Against *Aspergillus Niger* and *Rhizopus stolonifer*," *Natural Product Communications* 9, no. 11 (2014): 1603–1605.
- [43] L. Lozano, C. Ramirez, J. M. Lozano, et al., "Antibacterial and Hemolytic Activity of Extracts and Compounds Obtained From Epicarps and Seeds of *Garcinia madruno* (Kunth) Hammel. Bol. Latinoam. Caribe," *Medicinal and Aromatic Plants Research Journals* 21, no. 3 (2022): 309–322.
- [44] R. Hänsel, M. Kartarahardja, J.-T. Huang, and F. Bohlmann, "Sesquiterpenlacton- β -d-glucopyranoside Sowie Ein Neues Eudesmanolid Aus *Taraxacum Officinale*," *Phytochemistry* 19, no. 5 (1980): S857–s861.
- [45] M. Fan, X. Zhang, H. Song, and Y. Zhang, "Dandelion (*Taraxacum* Genus): A Review of Chemical Constituents and Pharmacological Effects," *Molecules* 28, no. 13 (2023): 5022, <https://doi.org/10.3390/molecules28135022>.
- [46] H. J. Chen, B. S. Inbaraj, and B. H. Chen, "Determination of Phenolic Acids and Flavonoids in *Taraxacum formosanum* Kitam by Liquid chromatography-tandem Mass Spectrometry Coupled With a Post-Column Derivatization Technique," *International Journal of Molecular Sciences* 13, no. 1 (2011): 260–285, <https://doi.org/10.3390/ijms13010260>.
- [47] E. J. Mo, J. H. Ahn, Y. H. Jo, S. B. Kim, B. Y. Hwang, and M. K. Lee, "Inositol Derivatives and Phenolic Compounds From the Roots of *Taraxacum coreanum*," *Molecules* 22, no. 8 (2017): 1349, <https://doi.org/10.3390/molecules22081349>.
- [48] K. K. Ghaima, N. M. Hashim, and S. A. Ali, "Antibacterial and Antioxidant Activities of Ethyl Acetate Extract of Nettle (*Urtica dioica*) and Dandelion (*Taraxacum officinale*)," *Journal of Applied Pharmaceutical Science* 3, no. 5 (2013): 96.
- [49] M. A. Mir, S. S. Sawhney, and M. M. S. Jassal, "Qualitative and Quantitative Analysis of Phytochemicals of *Taraxacum officinale*. Wudpecker," *Journal of Pharmacy and Pharmacology* 2, no. 1 (2013): 001–005.
- [50] P. Sasikala, S. Ganesan, T. Jayaseelan, et al., "Phytochemicals and GC-MS Analysis of Bioactive Compounds Present in Ethanolic Leaves Extract of *Taraxacum officinale* (L)," *Journal of Drug Delivery and Therapeutics* 9, no. 1 (2019): 90–94, <https://doi.org/10.22270/jddt.v9i1.2175>.
- [51] W. Akhtar, G. Ali, N. Ashraf, et al., "Efficiency of Multiple Extraction Solvents on Antioxidant, Cytotoxic, and Phytotoxic Potential of *Taraxacum officinale* (L.) Weber Ex F.H. Wigg. From Poonch Valley, Azad Kashmir, Pakistan," *Evidence-Based Complementary and Alternative Medicine* 2022 (2022): 1–9, <https://doi.org/10.1155/2022/5118553>.
- [52] T. Dhanani, S. Shah, N. A. Gajbhiye, and S. Kumar, "Effect of Extraction Methods on Yield, Phytochemical Constituents and Antioxidant Activity of *Withania somnifera*," *Arabian Journal of Chemistry* 10 (2017): S1193–S1199, <https://doi.org/10.1016/j.arabjc.2013.02.015>.
- [53] S. K. Mokua, J. M. Mbaria, T. E. Maitho, and G. A. Moriasi, "Ethnobotanical Documentation, Phytochemical Screening, and Cytotoxicity Evaluation of Medicinal Plants Used to Manage Snakebite Envenomation in Mwingi West Subcounty, Kenya," *Evidence-based Complementary and Alternative Medicine* 2021 (2021): 1–12, <https://doi.org/10.1155/2021/4167296>.
- [54] D. Zhang, N. M. A. Ivane, S. A. Haruna, et al., "Recent Trends in the Micro-Encapsulation of Plant-Derived Compounds and Their Specific Application in Meat as Antioxidants and Antimicrobials," *Meat Science* 191 (2022): 108842, <https://doi.org/10.1016/j.meatsci.2022.108842>.
- [55] J. González-Romero, E. J. Guerra-Hernández, and C. Rodríguez-Pérez, "Chapter 19- Bioactive Compounds From *Moringa oleifera* as Promising Protectors of in Vivo Inflammation and Oxidative Stress Processes," in *Current Advances for Development of Functional Foods Modulating Inflammation and Oxidative Stress*, ed. B. Hernández-Ledesma and C. Martínez-Villaluenga (Cambridge, MA, USA: Academic Press, 2022), 379–399.
- [56] R. A. P. Purba, S. Paengkoum, C. Yuangklang, and P. Paengkoum, "Flavonoids and Their Aromatic Derivatives in *Piper betle* Powder Promote in Vitro Methane Mitigation in a Variety of Diets," *Ciencia E Agrotecnologia* 44 (2020): E012420, <https://doi.org/10.1590/1413-7054202044012420>.
- [57] A. Ullah, S. Munir, S. L. Badshah, et al., "Important Flavonoids and Their Role as a Therapeutic Agent," *Molecules* 25, no. 22 (2020): 5243, <https://doi.org/10.3390/molecules25225243>.
- [58] O. Kenny, T. J. Smyth, C. M. Hewage, and N. P. Brunton, "Antioxidant Properties and Quantitative UPLC-MS/MS Analysis of Phenolic Compounds in Dandelion (*Taraxacum officinale*) Root Extracts," *Free Radicals and Antioxidants* 4, no. 1 (2014): 55–61, <https://doi.org/10.5530/fra.2014.1.9>.
- [59] O. Kenny, T. J. Smyth, C. M. Hewage, and N. P. Brunton, "Quantitative UPLC-MS/MS Analysis of Chlorogenic Acid Derivatives in Antioxidant Fractionates From Dandelion (*Taraxacum officinale*) Root," *International Journal of Food Science and Technology* 50, no. 3 (2015): 766–773, <https://doi.org/10.1111/ijfs.12668>.
- [60] A. Savych, S. Marchyshyn, M. Kyryliv, and I. Bekus, "Cinnamic Acid and Its Derivatives in the Herbal Mixtures and Their Antidiabetic Activity," *Farmacia* 69, no. 3 (2021): 595–601, <https://doi.org/10.31925/farmacia.2021.3.23>.
- [61] M. Miao and L. Xiang, "Pharmacological Action and Potential Targets of Chlorogenic Acid," in *Advances in Pharmacology*, ed. D. Guanhu, 87 (London, OH, USA: Academic Press, 2020), 71–88, <https://doi.org/10.1016/bs.apha.2019.12.002>.
- [62] C. Gao, S. Kong, B. Guo, X. Liang, H. Duan, and D. Li, "Antidepressive Effects of *Taraxacum officinale* in a Mouse Model of Depression are due to Inhibition of Corticosterone Levels and Modulation of Mitogen-Activated Protein Kinase phosphatase-1 (Mkp-1) and Brain-Derived Neurotrophic Factor (Bdnf) Expression," *Medical Science Monitor* 25 (2019): 389–394, <https://doi.org/10.12659/msm.912922>.
- [63] A. S. Khan, K. Arif, B. Munir, et al., "Estimating Total Phenolics in *Taraxacum officinale* (L.) Extracts," *Polish Journal of Environmental Studies* 28, no. 1 (2018): 497–501, <https://doi.org/10.15244/pjoes/78435>.
- [64] N. Petkova, I. Ivanov, S. Topchieva, P. Denev, and A. Pavlov, "Biologically Active Substances and in Vitro Antioxidant Activity of Different Extracts From Dandelion (*Taraxacum officinale*) Roots," *Scientific Bulletin Series F. Biotechnologies* (2015): 190–198.
- [65] K. K. Ghaima, N. M. Hashim, and S. A. Ali, "Antibacterial and Antioxidant Activities of Ethyl Acetate Extract of Nettle (*Urtica dioica*) and Dandelion (*Taraxacum officinale*)," *Journal of Applied Pharmaceutical Science* 3, no. 5 (2013): 96.

- [66] D. Ivanova, D. Gerova, T. Chervenkov, and T. Yankova, "Polyphenols and Antioxidant Capacity of Bulgarian Medicinal Plants," *Journal of Ethnopharmacology* 96, no. 1-2 (2005): 145–150, <https://doi.org/10.1016/j.jep.2004.08.033>.
- [67] A. Nowak, W. Duchnik, J. Zielonka-Brzezicka, et al., "The Antioxidant Activity of Ethanolic and Aqueous Extracts of Dandelion (*Taraxacum officinale* L.)," *Pomeranian Journal of Life Sciences* 64, no. 4 (2019): 83–88, <https://doi.org/10.21164/pomjlifesci.639>.
- [68] E. F. Santos, J. M. Kondo Santini, A. P. Paixão, et al., "Physiological Highlights of Manganese Toxicity Symptoms in Soybean Plants: Mn Toxicity Responses," *Plant Physiology and Biochemistry* 113 (2017): 6–19, <https://doi.org/10.1016/j.plaphy.2017.01.022>.
- [69] S. Dedić, A. Džafirović, and H. Jukić, "Chemical Composition and Antioxidant Activity of water-ethanol Extracts of Dandelion (*Taraxacum officinale*)," *Journal of Functional Foods in Health and Disease* 11, no. 1 (2022): 8–14.
- [70] Y. Huang, P. Wu, and X. D. Chen, "Mechanistic Insights into the Influence of Flavonoids From Dandelion on Physicochemical Properties and in Vitro Digestibility of Cooked Potato Starch," *Food Hydrocolloids* 130, no. 2022 (2022): 107714, <https://doi.org/10.1016/j.foodhyd.2022.107714>.
- [71] Y. Huang, P. Wu, J. Ying, Z. Dong, and X. D. Chen, "Mechanistic Study on Inhibition of Porcine Pancreatic α -amylase Using the Flavonoids From Dandelion," *Food Chemistry* 344, no. 2021 (2021): 128610, <https://doi.org/10.1016/j.foodchem.2020.128610>.
- [72] H. Qiao and T. J. Sun, "Antibacterial Activity of Ethanol Extract and Fractions Obtained From *Taraxacum mongolicum* Flower," *Res J Pharmacog* 1, no. 4 (2014): 35–39.
- [73] P. Xu, X. B. Xu, A. Khan, T. Fotina, and S. H. Wang, "Antibiofilm Activity Against *Staphylococcus aureus* and Content Analysis of *Taraxacum officinale* Phenolic Extract," *Polish Journal of Veterinary Sciences* 24, no. 2 (2021): 243–251, <https://doi.org/10.24425/pjvs.2021.137659>.
- [74] P. J. Xie, L. X. Huang, C. H. Zhang, S. S. Ding, Y. J. Deng, and X. J. Wang, "Skin-Care Effects of Dandelion Leaf Extract and Stem Extract: Antioxidant Properties, Tyrosinase Inhibitory and Molecular Docking Simulations," *Industrial Crops and Products* 111 (2018): 238–246, <https://doi.org/10.1016/j.indcrop.2017.10.017>.
- [75] D. Ionescu, G. Predan, and D. Rizea, "Antimicrobial Activity of Some Hydroalcoholic Extracts of Artichoke (*Cynara Scolymus*), Burdock (*Atrium Lappa*) and Dandelion (*T. Officinale*)," *Bulletin of the Transilvania University of Brasov* 6 (2013): 114–120.
- [76] B. Chi, D. Jo, S. Cha, M. J. Chi, H. W. Jeong, and K. H. Kang, "The Effect on Growth Inhibition of *S. mutans* by Lotus Leaf and Dandelion Extracts. J Korea Academia-Indus," *Journal of the Korea Academia-Industrial cooperation Society* 12 (2011): 5773–5778, <https://doi.org/10.5762/kais.2011.12.12.5773>.
- [77] I. Z. Sohail, M. Afzal, A. Afzal, et al., "In Vitro Antibacterial Study of *Taraxacum officinale* Leaves Extracts Against Different Bacterial Pathogenic Strains," *Journal of Pharmacognosy and Phytochemistry* 3, no. 2 (2014): 15–17.
- [78] S. Roshanak, B. A. Behbahani, F. Shahidi, F. T. Yazdi, A. R. Vasiee, and N. Norouzi, "Evaluation of Chlorophyll Content, Antioxidant Activity and Antimicrobial Effect of Dandelion Leaves Extract," *Iranian Food Science and Technology Research Journal* 17, no. 3 (2021): 63–72.
- [79] N. Li, S. N. Tan, J. Cui, et al., "PA-1, a Novel Synthesized Pyrrolizidine Alkaloid, Inhibits the Growth of *Escherichia coli* and *Staphylococcus aureus* by Damaging the Cell Membrane," *Journal of Antibiotics* 67, no. 10 (2014): 689–696, <https://doi.org/10.1038/ja.2014.49>.
- [80] E. Larghi, A. Bracca, A. A. Arroyo Aguilar, et al., "Neocryptolepine: A Promising Indolisoquinoline Alkaloid With Interesting Biological Activity. Evaluation of the Drug and Its Most Relevant Analogs," *Current Topics in Medicinal Chemistry* 15, no. 17 (2015): 1683–1707, <https://doi.org/10.2174/1568026615666150427113937>.
- [81] C. Kelley, S. Lu, A. Parhi, M. Kaul, D. S. Pilch, and E. J. Lavoie, "Antimicrobial Activity of Various 4- and 5-Substituted 1-Phenylphthalenes," *European Journal of Medicinal Chemistry* 60 (2013): 395–409, <https://doi.org/10.1016/j.ejmech.2012.12.027>.
- [82] D. Rongai, P. Pulcini, B. Pesce, and F. Milano, "Antifungal Activity of Some Botanical Extracts on *Fusarium oxysporum*," *Open Life Sciences* 10, no. 1 (2015): 409–416, <https://doi.org/10.1515/biol-2015-0040>.
- [83] Y. Liang, H. Duan, P. Zhang, et al., "Extraction and Isolation of the Active Ingredients of Dandelion and Its Antifungal Activity Against *Candida albicans*," *Molecular Medicine Reports* 21, no. 1 (2020): 229–239, <https://doi.org/10.3892/mmr.2019.10797>.
- [84] L. F. Tan, V. L. Yap, M. Rajagopal, et al., "Plant as an Alternative Source of Antifungals Against Aspergillus Infections: A Review," *Plants* 11, no. 22 (2022): 3009, <https://doi.org/10.3390/plants11223009>.
- [85] S. Luqman, S. Kaushik, S. Srivastava, et al., "Protective Effect of Medicinal Plant Extracts on Biomarkers of Oxidative Stress in Erythrocytes," *Pharmaceutical Biology* 47, no. 6 (2009): 483–490, <https://doi.org/10.1080/13880200902832900>.
- [86] P. Saroni Arwa, M. L. Zeraik, V. F. Ximenes, L. M. da Fonseca, V. d. S. Bolzani, and D. H. Siqueira-Silva, "Redox-Active Biflavonoids From *Garcinia brasiliensis* as Inhibitors of Neutrophil Oxidative Burst and Human Erythrocyte Membrane Damage," *Journal of Ethnopharmacology* 174 (2015): 410–418, <https://doi.org/10.1016/j.jep.2015.08.041>.
- [87] A. Ghosh, S. Banik, M. N. Amin, and J. Ahmed, "Evaluation of Antinociceptive, Antihyperglycemic, and Membrane Stabilizing Activities of *Garcinia lancifolia* Roxb," *Journal of Traditional and Complementary Medicine* 8, no. 2 (2018): 303–307, <https://doi.org/10.1016/j.jtcme.2017.04.009>.