Research Article

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

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Background. Different parts of *Taraxacum officinale* (L.) were used in traditional medicine in various parts of the world for the treatment of health problems, and they possess significant biological activities. The present study aimed to estimate phytochemical and biological activities of *T. officinale* using different extraction solvents. Methods. Methanolic, acetone, and n-hexane extracts of selected species were prepared, and ten secondary metabolites were examined using standard protocols. The antioxidant activity was performed using three in vitro methods, namely, DPPH assay, total reducing power (TRP) assay, and total antioxidant capacity (TAC). Toxocolgical analysis was done using the brine shrimp cytotoxic assay and radish seed phytotoxic assay. Results. The *T. officinale* methanolic extract showed the highest phenolic (178.27 ± 17.17 mg/GAE/g) and flavonoid (18.50 ± 1.64 mg QE/g) contents. Similarly, the methanolic extract also revealed the highest DPPH activity (32.80 ± 9.66 IC50), reducing potential (0.53 ± 0.02 mg/g), and TAC (19.42 ± 1.64 mg QE/g) as compared to the acetone and n-hexane extracts. Pearson correlation analysis confirmed a strong positive correlation (r > 0.9) between total phenolic content (TPC), total flavonoid content (TFC), and all antioxidant assays. Furthermore, a heat map displayed the methanolic extract (red color) as a valuable source of phytochemicals and antioxidant agents. Moreover, the *T. officinale* methanolic extract also showed the highest (7.12 ppm) cytotoxic potential whereas both methanolic and acetone extracts were revealed as moderate phytotoxic agents when compared with the standard. Conclusion. The *T. officinale* methanolic extract exhibited comparatively notable phytochemicals that are actively involved in antioxidant activities and possess toxicological properties. This upholds the folkloric use of *T. officinale* as a possible source to develop natural plant-based drugs. Further investigations to isolate bioactive compounds and elements and on their safety need to be conducted.
1. Introduction

Azad Jammu and Kashmir (AJK) lies in the western part of the Himalayas, having an area of 13,269 km². Poonch is located in the northern mountainous region of AJK and is situated 1750–2500 m above the sea level between 33–36 north latitude and 73–75 east longitude [1]. Rawalakot is the capital city of the Poonch district and has a temperate climate with an average rainfall of 1600 mm, and the temperature ranges from 3 to 26°C [2]. Most of the area has sandy clay soil and retains a greater moisture content (95% humidity) which enables plant growth efficiently [3]. Hilly areas possess a spectacular diversity of plant species and are contemplated as a treasured grove of medicinal plants [4]. The Poonch valley exhibits diversity in terms of medicinal plants owing to the presence of unique climatic conditions. Almost 70 species of the 430 wild species of this region have been explored medicinally till now [3]. Medicinal plants contain numerous bioactive compounds that are involved in curing ailments [5]. Most of the diseases prevailing in these areas are cured by folklore medicine as proposed by various herbalists [2].

Taraxacum genus comprises wild medicinal plants with almost 2500 species reported worldwide [6]. Some of these species are invasive and grown worldwide such as *Taraxacum officinale* var. *erythrospermum*; however, few are scientifically investigated nowadays. In the beginning, these species were used owing to the popular knowledge and experience from our ancestors. The first evidence of its therapeutic use is in Arabic medicine during the 10th century to cure diseases of the liver and the spleen. Later on, Fuchs in 1593 described its use to treat diarrhea, blister, gout, and spleen and liver diseases [7]. Since the 12th century, many researchers have diverted their attention from traditional knowledge toward the scientific explanations related to the mode of action of *Taraxacum* against diseases. Exploring the chemical composition and the action mechanism of this species against diseases using multiple *in vitro* and *in vivo* assays could establish its potential as a commercial herb [8, 9].

Uncontrolled production of reactive oxygen species (ROS) in the body causes oxidative stress which ultimately leads to diseases such as diabetes, aging, and myocardial infarctions [10]. To counteract this condition, cells have multiplex enzymatic and nonenzymatic elements. The molecules of the nonenzymatic system have the ability to cause enzyme inhibition and chelation of trace elements that are involved in ROS production through other antioxidant defenses [11]. Besides these molecules, phenolic and flavonoid compounds play a vital role in scavenging ROS by neutralizing free radicals and thus act as natural antioxidants [12]. These compounds act as antioxidants as they are stable intermediates and possess the ability to donate hydrogen or electrons [13]. The antioxidant capacity of phenols present in plant extracts is effective at low concentrations and is involved in the prevention of cardiovascular and cancer diseases [14, 15].

Moreover, phytochemicals also play a key role in plant adaptation under various growth conditions [16]. Plant growth could be reduced or adversely affected due to different weeds as they compete with them for available resources such as minerals, water, and space. Farmers use synthetic herbicides to control the growth of weeds which are harmful to human beings and also cause water and soil pollution [17]. Therefore, scientists have developed a great interest in exploring natural herbicides so that they could be utilized to enhance crop yield [18]. Moreover, plants also contain bioactive compounds which are toxic to organisms such as shrimps, and thus, the brine shrimp cytotoxicity test is usually recommended to determine the cytotoxic potential in plants [19].

Despite the longstanding vegetation growing in the Poonch valley, only a handful of species have been investigated scientifically. *Taraxacum* spp. (family Asteraceae) is commonly known as dandelion and is considered as a weed in several crops [20] while its leaves are rich in fibers, minerals, vitamins, and other bioactive compounds [21, 22]. Dandelion is a nontoxic herb that is exploited due to its diuretic, anti-inflammatory, and digestive stimulant properties [23]. It is extensively used for the treatment of eye diseases, cancer, osteoarthritis, and anemia [24]. In North American aboriginal medicine, decoctions and infusions of this herb are used for menstrual cramps, heartburn, dyspepsia, chest pain, and jaundice and to heal broken bones, bruises, swellings, and fractures [7]. In traditional Chinese medicine, dandelion is combined with other herbs to treat hepatitis and to increase immunity for upper respiratory tract infections, bronchitis, or pneumonia. *T. officinale* is also used to treat malaria in Venezuela, for toothache in Kosovo, and for hypertension in Ghana [8]. Glufraz et al. [25] revealed the CCl₄-induced hepatotoxicity potential of this plant in rats, and Khan et al. [26] determined the acaricidal potential of *T. officinale* against cattle tick *Rhipicephalus microplus* infestations. Moreover, Kenny et al. [27] reported the antimicrobial potential of methanolic root extracts of *T. officinale* against *Staphylococcus aureus* and *Bacillus cereus*.

Altogether, most of the previous studies have been focused on the ethnopharmacological potential of *T. officinale*, while few studies have been performed to confirm its biological potential. Hence, the present study was designed to explore the chemical composition, antioxidant, cytotoxic, and phytotoxic potential of *T. officinale* (L.) collected from the Poonch valley, by using multiple extraction solvents, to verify the rationale behind the use of this plant as a cure for various diseases.

2. Materials and Methods

2.1. Plant Collection and Extract Preparation. Fresh plant samples were collected from Rawalakot located in the Poonch district of Azad Kashmir. The plant sample was identified by Prof. Dr. Mir Ajab Khan (taxonomist), and a voucher number (QAU-AA-157) was assigned from the Herbarium of Pakistan, Quaid-i-Azam University (Islamabad). The plant material was washed, shade-dried, and powdered using an electric grinder. The plant material (20 gm) was extracted with 200 mL, each of methanol (polar), acetone (slightly polar), and n-hexane (nonpolar).
solvents for 48 hours. Subsequently, filtration was carried out using a Whatman filter paper (Schleicher & Schell Kent, England), and the whole process was repeated twice. Subsequently, the obtained filtrate was concentrated using a rotary evaporator (Scilogex Re100-Pro, Keyland Court, Bohemia, US) and crude extracts were stored at 4°C for experimental analysis.

2.2. Preliminary Phytochemical Tests. Qualitative tests were performed to detect the presence of various secondary metabolites using standard protocols, namely, alkaline detection assay for flavonoids, Mayer’s test for alkaloids, Salkowski test for glycosides, gelatin test for tannins, foam test for saponins, ferric chloride test for phenols, Libermann’s test for terpenoids and steroids, and sodium chloride (NaCl) was added to the extract to observe anthocyanins (bluish color) and coumarins (yellow color) [28, 29].

2.3. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). For TPC, each extract (20 μL) was added in the Folin–Ciocalteu reagent (90 μL) and then incubated for 5 minutes. Subsequently, 6% sodium carbonate (90 μL) was mixed and absorbance was measured at 630 nm. Gallic acid (standard) was used to obtain the calibration curve [30]. In the case of TFC, the aluminum chloride colorimetric method was followed and the calibration curve was prepared using different concentrations of quercetin, a flavonoid standard [31]. The plant extract (20 μL) was dissolved in 1 M potassium acetate (10 μL), 10% aluminum chloride (Al2Cl3) (10 μL), and distilled water (160 μL), followed by incubation (30 minutes), and then, absorbance (405 nm) was noted.

2.4. Antioxidant Assays

2.4.1. DPPH Scavenging Assay. The procedure described by Tepe et al. [32] was followed to observe the DPPH scavenging activity of selected extracts. In brief, each extract (10 μL) was added to 0.004% of DPPH solution (190 μL), and the final volume was made up to 200 μL. The reaction mixture was placed in the dark for 30 minutes, and then, absorbance was measured at 517 nm wavelength. Ascorbic acid was used as a standard, and the scavenging activity was calculated by using the following formula:

\[
\text{% scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100. \tag{1}
\]

2.4.2. Total Reducing Power (TRP) Assay. About 200 μL of each plant extract was added in potassium ferricyanide and phosphate buffer (500 μL each) and then incubated at 50°C for 20 minutes. Subsequently, 500 μL of trichloroacetic acid (TCA) was mixed followed by centrifugation (Model 2-6E, Sigma Laborzentrifugen, D-37520 Osterode am Harz, Germany) at 3000 rpm for 10 minutes. The supernatant was added in 0.1% ferric chloride (100 μL), and then, absorbance was measured at 630 nm. Ascorbic acid was used as a control, and results were expressed as μg AAE/mg of the extract [33].

2.4.3. Total Antioxidant Capacity (TAC) Assay. For the TAC assay, Prieto’s method [34] was followed with some modifications. In brief, the reaction mixture was prepared by adding the plant extract (50 μL) in 4 mM ammonium molybdate (0.25 g), 28 mM sodium phosphate (1.68 g), 1.63 mL of H2SO4, and 50 mL of distilled water, followed by incubation at 95°C for 90 minutes. Then, the samples were cooled at room temperature, and absorbance was measured at 630 nm.

2.5. Toxicological Analysis

2.5.1. Brine Shrimp Cytotoxicity Assay. For the cytotoxicity assessment, Sirajuddin et al.’s [35] method was used with some modifications. Three working solutions (50, 100, and 150 μg/mL) of each extract were prepared in dimethyl sulfoxide (DMSO) to ascertain the cytotoxicity potential of the selected extracts. Artemia salina eggs (Ocean Star, USA) were released in a bipartitioned tray filled with artificial saline water (3.8% sea salt in 1000 mL of distilled water; pH 7) and placed for incubation at 32°C. The lamp was used as a light source for the hatching of shrimps. After 24 hours, 10 shrimps were added to each vial along with different concentrations of the plant extract (5 μL) and left for incubation for the next 24 hours. Subsequently, the number of alive shrimps was counted with the help of the naked eye, and (%) mortality and lethal concentration 50% (LC50) values were evaluated.

2.5.2. Radish Seed Phytotoxicity Assay. For the radish seed phytotoxicity assay, the Arzu and Camper method [36] was followed with slight modifications. In this assay, the plant powder (50 mg) was dissolved in 5 mL of methanol, acetone, and n-hexane to prepare the test solution and water was used as a positive control. The solution was poured on the sterilized filter papers on the Petri plates inside the laminar hood (Model SC2-41 Changi South, Singapore) to avoid any contamination. After evaporation, 5 mL of distilled water was added, and then, 20 sterilized (using mercuric chloride) seeds were placed on each plate at a uniform distance. Petri plates were wrapped with parafilm and then incubated at 25°C in dim light. In the end, the percentage of seed germination and root length inhibition was determined using the following formula:

\[
\text{root length inhibition \%} = \frac{\text{root length in test sample}}{\text{root length in control}} \times 100. \tag{2}
\]

2.6. Statistical Analysis. All assays were performed in triplicate, and mean ± standard deviation was determined. Least significant difference (LSD) was measured using Statistix 8.1 by performing analysis of variance (ANOVA), and IC50
values were calculated using the GraphPad-Prism 5 software. Pearson correlation analysis was performed between phytochemicals and antioxidant assays using Microsoft Excel, whereas a heat map was prepared to observe variability among extracts in terms of antioxidant activities. Furthermore, LC50 values were determined to ascertain the toxicity potential of extracts by using Probit analysis software [37].

3. Results

3.1. Phytochemical Analysis. Preliminary phytochemical tests revealed that secondary metabolites were present more abundantly in their methanolic extracts as compared to the acetone and n-hexane extracts. Saponins and tannins were not detected in n-hexane extracts, while glycosides were absent in acetone extracts. However, all other compounds were present strongly to moderately in most of the selected extracts (Table 1).

Phenolic and flavonoid contents were determined quantitatively, and results showed that methanolic extracts exhibited the highest phenolic (178.273 ± 17.17 mg GAE/g) and flavonoid (18.5 ± 1.64 mg QE/g) contents as compared to the other extracts. Overall, phytochemicals were observed in decreasing order of methanolic extract > acetone extract > n-hexane extract, as shown in Figure 1.

3.2. Antioxidant Assays. The antioxidant activity of T. officinale extracts was assessed using three methods, and among these, the DPPH activity was evaluated at three different concentrations to observe the percentage inhibition and IC50 values. Results revealed that all extracts exhibited a concentration-dependent percentage inhibition of free radicals in the DPPH assay. Among all samples, the highest activity was recorded in the methanolic extract (IC50: 32.80 ± 9.66 µg/mL), followed by acetone (IC50: 42.63 ± 5.55 µg/mL) and n-hexane (IC50: 60.0 ± 8.37 µg/mL) extracts, respectively. The highest antioxidant potential of methanolic and acetone extracts was also found to be statistically significant when compared with that of the standard ascorbic acid (Figure 2(a)).

Similarly, different extracts of T. officinale were further examined using the TRP and TAC assay. Higher absorbance indicated higher reducing potential of the plant extract (Figure 2(b)). Moreover, the total antioxidant capacity was also observed in descending order of methanolic extract (19.42 ± 0.97 mg/g) > acetone extract (14.01 ± 2.51 mg/g) > n-hexane extract (11.70 ± 0.79 mg/g) (Figure 2(c)).

3.3. Pearson Correlation Analysis and Heat Map Visualization. The correlation of antioxidant assays with phenolic and flavonoid compounds was observed which showed a strong positive correlation (r > 0.9) in all three antioxidant assays (Figures 3(a)–3(c)). Furthermore, a heat map was prepared to perceive differences between different kinds of extracts based on phytochemical tests and antioxidant assays. A higher activity was displayed vividly in red color, while green color indicated the lowest activity. The obtained data revealed that the extract prepared in a polar solvent (i.e., methanol) possessed the highest TPC and TFC along with antioxidant potential as examined by different methods, followed by other extracts made in slightly polar (i.e., acetone) and nonpolar (i.e., n-hexane) solvents, respectively (Figure 3(d)).

3.4. Toxicological Assays. To determine the cytotoxic potential of the selected extracts, brine shrimps were tested using different concentrations of extracts and vincristine sulphate was used as a standard. Among all samples, the extract prepared in methanol showed a potent cytotoxic effect exhibiting 7.122 ppm LC50 value followed by the extract made in acetone (i.e., 10.32 ppm LC50 value). However, the n-hexane extract revealed the lowest cytotoxic potential as it showed 14.02 ppm LC50 value (Table 2).

In the phytotoxic assay, paraquat was used as a standard while methanol was taken as a negative control. Regarding root length inhibition, the acetone extract showed the highest inhibition (20.465 ± 1.54%), whereas in the case of seed germination, methanolic extract was found to be most potent showing 30% inhibition in the seed germination. In general, all extracts revealed a moderate phytotoxic potential

| Phytochemicals | Methanolic extract | Acetone extract | n-Hexane extract |
|----------------|--------------------|-----------------|------------------|
| Alkaloids      | ++                 | +               | +                |
| Flavonoids     | +++                | ++              | ++               |
| Phenols        | +++                | ++              | ++               |
| Terpenoids     | ++                 | ++              | +                |
| Steroids       | ++                 | +               | +                |
| Saponins       | ++                 | +               | –                |
| Tannins        | +++                | +               | –                |
| Anthocyanins   | +++                | ++              | +                |
| Coumarins      | +++                | ++              | +                |
| Glycosides     | ++                 | –               | +                |

Note. +++: abundantly present; ++: moderately present; +: weakly present; -: absent.

**Table 1: Qualitative phytochemical analysis of different extracts of T. officinale.**
**Figure 2:** Antioxidant activity observed in *T. officinale* using different extraction solvents. The number represents mean ± SD (3n) and each letter (A–C) shows a significant difference at *p* < 0.05 as determined by LSD. (a) DPPH activity of the selected extracts measured at different concentrations. (b) Total reducing power (TRP) and total antioxidant capacity (TAC) of the selected plant extracts.

**Figure 3:** Pearson correlation analysis and the heat map of TPC, TFC, and three antioxidant assays as detected in different plant extracts. (a) Correlation between the DPPH assay and phytochemicals. (b) Correlation between TRP and phytochemicals. (c) Correlation between TAC and phytochemicals. (d) The heat map showing the comparison of antioxidant assays and phytochemicals among different extracts by displaying the highest (red) to lowest (green) activity in colors.
when compared with the standard and presented significant statistical differences as shown in Figures 4(a) and 4(b).

### 4. Discussion

For ages, nature has proved and served as a sumptuous repository of medicinal plants owing to the existing bioactive constituents that contribute toward the isolation of natural drugs [38]. Till now, numerous drugs have been explored from natural resources including medicinal plants via exploiting multiple techniques and approaches. These herbal medicines are effectively used against various diseases, especially in rural areas of less developed countries due to their presumed safety compared to conventional medicine [39, 40]. Keeping in view the importance of natural flora, there is a growing interest in exploring novel species so that they could be utilized at the industrial level. Hence, this present study aimed at investigating the chemical composition along with relative bioefficacy and toxicity potential of the selected plant. Lł he number represents mean ± SD (3n), and each letter (A–C) shows a significant difference at *p* < 0.05 as determined by LSD. (a) Root length inhibition. (b) Seed germination inhibition.

![Figure 4: Phytotoxic potential of different extracts of *T. officinale*. The number represents mean ± SD (3n), and each letter (A–C) shows a significant difference at *p* < 0.05 as determined by LSD. (a) Root length inhibition. (b) Seed germination inhibition.](image-url)
significant antioxidant potential, and this was observed in the descending order of methanolic extract > acetone extract > n-hexane extract (Figure 2).

Our study correlates with the previous findings of Chon et al. [48] who documented a significant antioxidant potential in the methanolic extract of T. officinale collected from Korea. Similarly, our results are also coherent with the previous findings of Hu and Kitts [49] and Miłek et al. [50] who reported antioxidant activity in the water and ethyl acetate extracts of T. officinale leaves and flowers collected from Canada and Poland. Moreover, Kenny et al. [47] determined a DPPH activity of 227.72 ± 11.84 mg/g and a reducing potential of 463.06 ± 3.94 mg/g in the T. officinale root extract prepared in ethyl acetate. Thus, it can be proposed that T. officinale is equally effective against oxidative stress-related diseases regardless of geographical location and seasonal variations. These antioxidant assays provide a basis and rationale for using these extracts as antioxidant ingredients in various food and medicinal products. The higher phenolic content in the polar extract is also responsible for the higher antioxidant capacity of the selected plant extracts [19]. However, the decrease in antioxidant activity of slightly polar extracts can be due to the solubilization effect of polyphenols and their limited accessibility to DPPH radicals and other oxidized ions [49].

The obtained results revealed a good correlation between phytochemicals and antioxidant assays of the tested extracts (Figure 3) which suggests that phenolic and flavonoid compounds mainly affect the antiradical properties of the extracts. Herein, data indicated a strong positive (r > 0.9) correlation of phytochemicals with all antioxidant assays which is in parallel with the previous studies of Miłek et al. [50] who reported an r value above 0.8 between the antioxidant activities and TPC of T. officinale extracts. Therefore, it can be suggested that T. officinale collected from the Poonch valley also possesses a valuable reservoir of active compounds of pharmacological significance. In addition, differences between the three different kinds of T. officinale extracts were perceived by using a heat map which displayed high (red color) to low (green color) activities vividly (Figure 3(d)). Previously, Elhadef et al. [51] and Fernández-Poyatos et al. [52] also used a heat map to discriminate between different species and to observe the contribution of each extract to the biological activities.

In the current study, the brine shrimp assay was adopted to appraise the cytotoxicity potential of the selected extracts. The chemical or drug that kills nauplii is contemplated as a cytotoxic agent, and lethal concentration (LC50) values are needed to express their cytotoxic level, whereby low values represent high cytotoxicity. According to Meyer’s criteria, plant extracts with LC50 > 1000 μg/mL are nontoxic, whereas plants with LC50 < 1000 μg/mL are toxic [53]. Likewise, as per Clarkson’s cytotoxicity criteria, the extract can be classified as nontoxic when LC50 > 1000 μg/mL, slightly toxic when LC50 500 to 999 μg/mL, moderately toxic when LC50 99 to 499 μg/mL, and highly toxic when LC50 0 to 100 μg/mL [54]. Consequently, based on Meyer’s and Clarkson’s criteria, all extracts of T. officinale were highly toxic as they had LC50 values less than 100 μg/mL (Table 2). The plant extracts with LC50 values < 20 μg/mL exhibit more chances of producing anticancer compounds [55]. Hence, it can also be suggested that T. officinale can be used as a cytotoxic agent in particular conditions. Yet further toxicological studies could be conducted to establish the toxicity and safety profile of the selected extracts.

Moreover, weeds are the most important factors responsible for the reduction in crop yield. To counteract unwanted weeds, synthetic chemicals are used which are more or less associated with pollution, carcinogenesis, and high cost and thus their use is restricted [4, 18]. Accordingly, the search for alternative natural herbicides which are safe and cost-effective is recommended. In our study, both methanolic and acetone extracts were revealed as the most potent phytotoxic agents as they showed the highest root length inhibition (17.36 ± 2.48% and 20.46 ± 1.44%) and seed germination inhibition (30 ± 1.5% and 20 ± 1.0%) (Figure 4). So far, this is the first study on the brine shrimp cytotoxicity and radish seed phytotoxicity potential of T. officinale collected from the Poonch valley. It can be concluded from the present study that T. officinale can serve as a significant source of natural herbicides for weed control sustainably to enhance per acre yield, which warrants detailed investigations.

5. Conclusion

From this study, we came across a judgement that biological characteristics of plants are greatly influenced by the solvents used for extraction. As the solvent polarity reduces, the extraction process is hindered which eventually alters the chemical reactions inside the plants. The methanolic and acetone extract of T. officinale showed promising bioactive compounds and antioxidant activities which support its traditional use in industries. In contrast, the n-hexane (nonpolar) extract of T. officinale exhibits less antioxidant potential and is moderately toxic. Thus, polar extracts of the selected species can effectively serve as a natural source to formulate antioxidant and toxicological agents; however, these results cannot be applied directly to humans.

6. Recommendations

This research explored that the extracts of T. officinale prepared in polar solvents have higher medicinal value than the extracts prepared in other nonpolar solvents; however, further empirical investigations using in vivo models are needed. Besides this, isolation of pure compounds and their characterization are required to analyze their mode of action against various diseases. All-inclusive, the selected plant exhibits multiple properties and thus could be utilized by humans and animals in their dietary items and in preparing pharmaceutical products.

Data Availability

The data used to support the findings of this study are included within the article. Any additional data will be delivered by the authors upon reasonable request.
Conflicts of Interest
The authors declare that they have no conflicts of interest.

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References

[1] S. Rashid, M. Ahmad, M. Zafar et al., "Ethnobotanical survey of medicinally important shrubs and trees of Himalayan region of Azad Jammu and Kashmir, Pakistan," *Journal of Ethnopharmacology*, vol. 166, pp. 340–351, 2015.

[2] M. A. Khan, M. A. Khan, and M. Hussain, "Medicinal plants used in folk recipes by the inhabitants of Himalayan region Poonch Valley Azad Kashmir (Pakistan)," *Journal of Basic and Applied Sciences*, vol. 8, pp. 35–45, 2012.

[3] M. A. Khan, S. A. Khan, M. A. Qureshi et al., "Ethnobotany of some useful plants of Poonch Valley Azad Kashmir," *Journal of Medicinal Plants Research*, vol. 5, no. 26, pp. 6140–6151, 2011.

[4] F. Kiran, M. A. Khan, R. Batool, S. Kanwal, S. L. Shah, and T. Mahmood, "Biological evaluation of some medicinal plants from Poonch valley, Azad Kashmir, Pakistan," *Journal of Traditional Chinese Medicine*, vol. 39, no. 6, pp. 753–763, 2019.

[5] I. Fatima, W. Akhtar, N. K. Bangash et al., "Volatile profiling, elemental composition and biological activities of aerial parts of seven Poaceae species," *Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology*, vol. 162, pp. 1–18, 2021.

[6] J. Kirschner and J. Štepánek, "Clonality as a part of the evolution process in *Taraxacum*," *Folia Geobotanica et Phytotaxonomica, Czech Republic*, vol. 29, no. 2, pp. 265–275, 1994.

[7] K. Schütz, R. Carle, and A. Schieber, "*Taraxacum* - a review on its phytochemical and pharmacological profile," *Journal of Ethnopharmacology*, vol. 107, no. 3, pp. 313–323, 2006.

[8] M. Martinez, P. Poirrier, R. Chamy et al., "*Taraxacum officinale* and related species - an ethnopharmacological review and its potential as a commercial medicinal plant," *Journal of Ethnopharmacology*, vol. 169, pp. 244–262, 2015.

[9] S. Rasool and B. Sharma, "*Taraxacum officinale*: a high value less known medicinal plant," *Annals of Plant Sciences*, vol. 3, no. 12, pp. 908–915, 2014.

[10] C. A. Juan, J. M. Pérez de la Lastra, F. J. Plou, and E. Pérez-Lebeña, "The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies," *International Journal of Molecular Sciences*, vol. 22, no. 9, p. 4642, 2021.

[11] C. C. Barua, S. Sen, A. S. Das et al., "Comparative study of the in vitro antioxidant property of different extracts of *Acorus calamus* Linn.," *Journal of Natural Product and Plant Resources*, vol. 4, pp. 8–18, 2014.

[12] Y. Pang, S. Ahmed, Y. Xu et al., "Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice," *Food Chemistry*, vol. 240, pp. 212–221, 2018.

[13] N. Nićiforović, V. Mihailović, P. Mašković, S. Solujić, A. Stojković, and D. P. Muratspahić, “Antioxidant activity of selected plant species: potential new sources of natural antioxidants,” *Food and Chemical Toxicology*, vol. 48, pp. 3125–3130, 2010.

[14] A.-N. Li, S. Li, Y.-J. Zhang, X.-R. Xu, Y.-M. Chen, and H.-B. Li, “Resources and biological activities of natural polyphenols,” *Nutrients*, vol. 6, no. 12, pp. 6020–6047, 2014.

[15] I. Balmus, A. Ciobica, A. Trifan, and C. Stanciu, "The implications of oxidative stress and antioxidant therapies in inflammatory bowel disease: clinical aspects and animal models," *Saudi Journal of Gastroenterology*, vol. 22, no. 1, pp. 3–17, 2016.

[16] M.-M. Oh, H. N. Trick, and C. B. Rajashkekar, "Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce," *Journal of Plant Physiology*, vol. 166, no. 2, pp. 180–191, 2009.

[17] B. Ahmad, I. Khan, S. Bashir, S. Azam, and F. Hussain, “Screening of *Ziziphus jujuba* for antibacterial, phytotoxic and haemagglutination activities,” *African Journal of Biotechnology*, vol. 10, no. 13, pp. 2514–2519, 2011.

[18] M. Ayaz, M. Junaid, F. Subhan et al., "Heavy metals analysis, phytochemical, phytotoxic and anthelmintic investigations of crude methanolic extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L.,” *BMC Complementary and Alternative Medicine*, vol. 14, no. 1, p. 465, 2014.

[19] I. Fatima, S. Kanwal, and T. Mahmood, "Evaluation of biological potential of selected species of family Poaceae from Bahawalpur, Pakistan," *BMC Complementary and Alternative Medicine*, vol. 18, no. 1, pp. 27–13, 2018.

[20] M. González-Castejón, F. Visioli, and A. Rodriguez-Casado, "Diverse biological activities of dandelion," *Nutrition Reviews*, vol. 70, no. 9, pp. 534–547, 2012.

[21] G. Clyton, "Botanical feed additives," *BioFeed International*, vol. 21, no. 4, pp. 271–277, 2000.

[22] Y. Xue, S. Zhang, M. Du, and M.-J. Zhu, "Dandelion extract suppresses reactive oxidative species and inflammatory response in intestinal epithelial cells," *Journal of Functional Foods*, vol. 29, pp. 10–18, 2017.

[23] E. Dermesonlugu, K. Fileri, A. Orfanoudaki, M. Tsevdou, T. Tsironi, and P. Taoukis, "Modelling the microbial spoilage and quality decay of pre-packed dandelion leaves as a function of temperature," *Journal of Food Engineering*, vol. 184, pp. 21–30, 2016.

[24] 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*, vol. 19, pp. 190–197, 2015.

[25] M. Gulfráz, D. Ahammad, M. S. Ahmad et al., "Effect of leaf extracts of *Taraxacum officinale* on CCl4 induced hepatotoxicity in rats, in vivo study," *Pakistan Journal of Pharmaceutical Sciences*, vol. 27, no. 4, pp. 825–829, 2014.

[26] A. Khan, N. Nasreen, S. Niaz et al., "Acaridical efficacy of *Calotropis procera* (Asclepiadaceae) and *Taraxacum officinale* (Asteraceae) against *Rhipicephalus microplus* from Mardan, Pakistan," *Experimental & Applied Acarology*, vol. 78, no. 4, pp. 595–608, 2019.

[27] O. Kenny, N. P. Brunton, D. Walsh, C. M. Hewage, P. McLoughlin, and T. J. Smyth, "Characterisation of antimicrobial extracts from dandelion root (*Taraxacum officinale*)
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using LC-SPE-NMR,” Phytotherapy Research, vol. 29, no. 4, pp. 526–532, 2015.
[28] G. Trease and W. Evans, “Pharmacognosy,” BrailiarTiridel Can, Macmillan Publishers Ltd, New York, NY, USA, 11th edition, 1989.
[29] J. B. Harborne, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK, 1973.
[30] G. Clarke, K. Ting, C. Wiart, and J. Fry, “High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest,” Antioxidants, vol. 2, no. 1, pp. 1–10, 2013.
[31] I. Hussain, R. Ullah, R. Ullah et al., “Phytochemical analysis of selected medicinal plants,” African Journal of Biotechnology, vol. 10, pp. 7487–7492, 2013.
[32] B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, and J. B. Harborne, G. Trease and W. Evans, “Pharmacognosy,” 11th edition, 1989, pp. 243–2447, 2018.
[33] B. Singh, J. P. Singh, A. Kaur, and N. Singh, “Insights into the phenolic compounds present in Jambolan (Syzygium cumini) along with their health-promoting effects,” International Journal of Food Science & Technology, vol. 53, no. 11, pp. 2431–2447, 2018.
[34] B. Singh, J. P. Singh, A. Kaur, and N. Singh, “Phenolic composition, antioxidant potential and health benefits of citrus peel,” Food Research International, vol. 132, Article ID 109114, 2020.
[35] A. S. Khan, K. Arif, B. Munir et al., “Estimating total phenolics in Taraxacum officinale (L.) extracts,” Polish Journal of Environmental Studies, vol. 28, no. 1, p. 497, 2019.
[36] 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, vol. 4, no. 1, pp. 55–61, 2014.
[37] S.-U. Chon, C.-H. Bae, and S.-C. Lee, “Antioxidant and cytotoxic potentials of methanol extracts from Taraxacum officinale F. H. Wigg at different plant parts,” Korean Journal of Polar Research, vol. 25, no. 2, pp. 232–239, 2012.
[38] C. Hu and D. D. Kitts, “Antioxidant, prooxidant, and cytotoxic activities of solvent-fractionated dandelion (Taraxacum officinale) flower extracts in vitro,” Journal of Agricultural and Food Chemistry, vol. 51, no. 1, pp. 301–310, 2003.
[39] M. Milek, D. Marcincakova, and J. Legath, “Polyphenols content, antioxidant activity, and cytotoxicity assessment of Taraxacum officinale extracts prepared through the micelle-mediated extraction method,” Molecules, vol. 24, no. 6, p. 1025, 2019.
[40] K. Elhafed, S. Akermi, H. B. Hlima et al., “Tunisian pistachio hull extracts: phytochemical content, antioxidant activity, and foodeborne pathogen inhibition,” Journal of Food Quality, vol. 2021, Article ID 9953545, 18 pages, 2021.
[41] M. D. P. Fernández-Poyatos, E. J. Llorent-Martínez, and A. Ruiz-Medina, “Phytochemical composition and antioxidant activity of Portulaca oleracea: influence of the steaming cooking process,” Foods, vol. 10, no. 1, p. 94, 2021.
[42] B. Meyer, N. Ferrigni, J. Putnam, L. Jacobsen, D. Nichols, and J. McLaughlin, “Brine shrimp: a convenient general bioassay for active plant constituents,” Planta Medica, vol. 45, no. 5, pp. 31–34, 1982.
[43] Clarkson, V. J. Maharaj, N. R. Crouch et al., “In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa,” Journal of Ethnopharmacology, vol. 92, no. 2–3, pp. 177–191, 2004.
[44] A. Ali, M. S. Haider, S. Hanif, and N. Akhtar, “Assessment of the antibacterial activity of Cuscuta pedicellata Lede,” African Journal of Biotechnology, vol. 13, pp. 430–433, 2014.