Antioxidant and Antidiabetic Activities of Dandelion (*Taraxacum officinale*)

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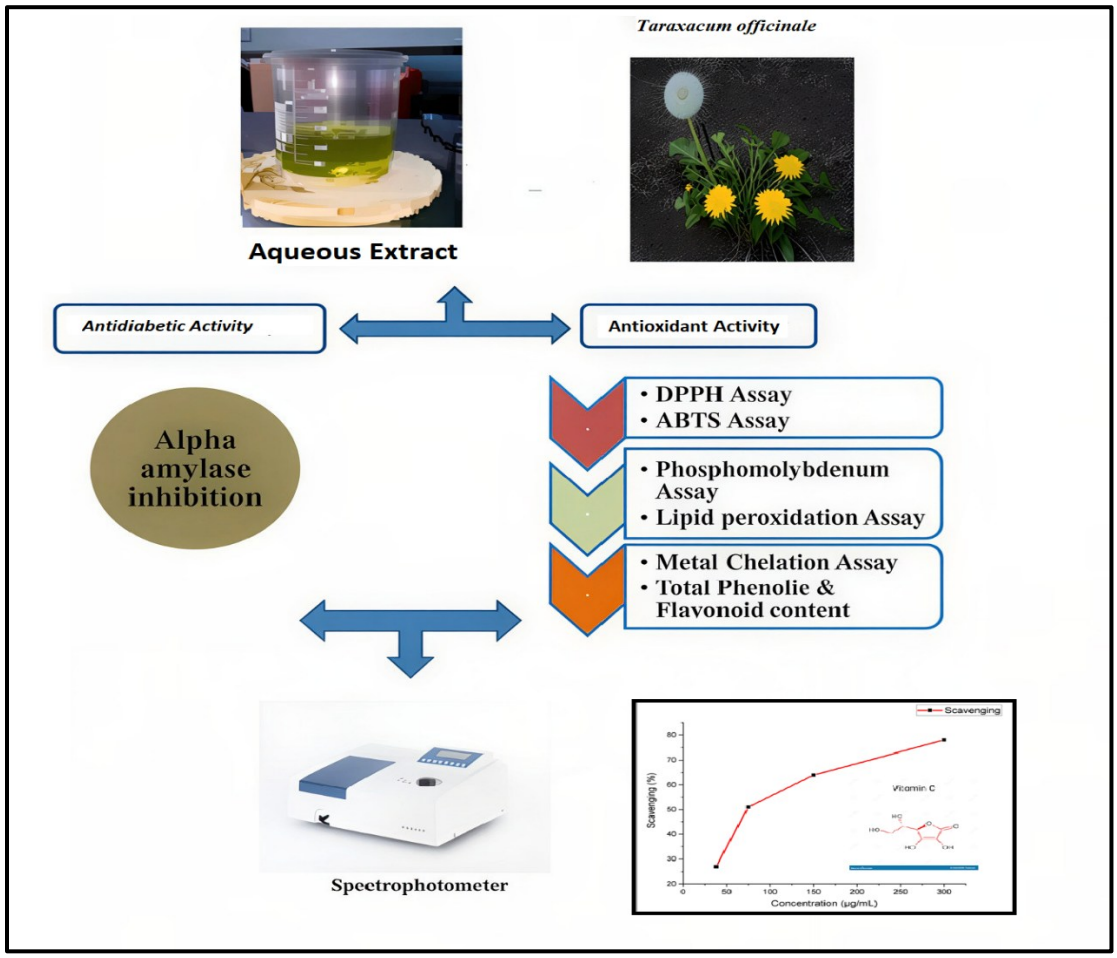
GRAPHICAL ABSTRACT



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Dandelion *(Taraxacum officinale)* is an edible plant belonging to the Asteraceae family, known for its therapeutic qualities. The antioxidant potential of dandelion leaves was assessed by 1.1-diphenyl-2-picrylhdrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), lipid peroxidation, phosphomolybdenum assay, and metal chelation assays. Results revealed a concentration-dependent antioxidant activity, with DPPH inhibition ranging from 27% to 78%, ABTS inhibition from 55% to 75%, and lipid peroxidation inhibition ranging from 31% to 70% against different prooxidants. Total antioxidant activity was evaluated by phosphomolybdenum reduction assay while metal chelation inhibition was observed between 23% and 58%. Bioactive chemicals in the aqueous extract included total flavonoid content (TFC), which was estimated as 20 mg/g, and total phenolic content (TPC), which is 41 mg/g. In addition to its antioxidant properties, the study also evaluated the antidiabetic potential of dandelion through an alpha-amylase inhibition assay, which exhibited inhibition values ranging from 32% to 77%. These findings suggest that *Taraxacum officinale* possesses considerable antioxidant and antidiabetic activities, presumably due to the high concentration of flavonoids and phenols in it.

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Keywords: Taraxacum officinale; DPPH activity; ABTS assay; Metal chelation; Lipid peroxidation

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INTRODUCTION

Oxidative stress is a condition characterized by an imbalance between oxidants and antioxidants. This imbalance results in the excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as hydrogen peroxide, hydroxyl radicals, superoxide, nitric oxide, and various hydroperoxides (Jafri *et al.* 2022). Free radicals can be neutralized by antioxidants, which scavenge them and act as metal chelators. Through eliminating free radicals from the body—such as superoxide anions, hydrogen peroxide, nitric oxide, and hydroxyl radicals—antioxidants prevent oxidation through multiple biological mechanisms. Antioxidants function as reducing agents, terminating oxidative

processes by undergoing oxidation themselves (Biel *et al.* 2017). The most potent antioxidant compounds are typically phenolics and flavonoids (Hatano *et al.* 1988; Duh *et al.* 1999). *Taraxacum officinale* exhibits notable antioxidant properties and serves as an effective source of dietary fiber and natural antioxidants (Ishfaq *et al.* 2018)

Insufficient insulin production by the pancreas or the body's inability to effectively utilize insulin results in a chronic condition known as diabetes. It is primarily characterized by polyuria, excessive thirst, persistent hunger, sudden weight loss, fatigue, and blurred vision. Diabetes encompasses a group of disorders defined by hyperglycemia and altered metabolism of lipids, proteins, and carbohydrates. As the disease progresses, organs, such as the eyes, kidneys, nerves, blood vessels, and heart, are exposed to increased risk (Wirngo *et al.* 2016). In addition to their antioxidant properties, dietary polyphenols have been shown to interact with glucose transporters, producing anti-hyperglycemic effects (Lacroix and Li-Chan 2014). Plant-based compounds exhibit antidiabetic activity by inhibiting enzymes responsible for carbohydrate breakdown—including α-amylase, β-galactosidase, and α-glucosidase—by blocking renal glucose reabsorption and modulating potassium channel activity.

Over recent decades, research on medicinal plants and their applications worldwide has gained considerable attention, as plants produce numerous secondary metabolites that serve as valuable sources of active pharmaceutical ingredients (APIs) (Mahboubi and Mahboubi 2020). The botanical species *Taraxacum officinale* belongs to the family Asteraceae, subfamily Cichorioideae. Originally native to Eurasia, dandelions have become globally distributed, thriving in temperate regions of North and South America, Africa, and Australia. With a global representation of over 1,100 genera and 19,000 species, *Taraxacum officinale*, commonly known as dandelion, is particularly abundant in temperate and subtropical regions (Faria *et al.* 2019). The species possesses extensive medicinal properties due to a diverse array of phytochemicals found in its leaves, stems, roots, and flowers. The main bioactive compounds include phenolic acids, carotenoids, and flavonoids (Khan *et al.* 2024); triterpenes, polysaccharides (inulin); sterols (taraxasterol and stigmasterol); and sesquiterpene lactones (Di Napoli and Zucchetti 2021). The annual species *Taraxacum officinale* is widely recognized for its numerous therapeutic properties as well as its nutritional significance (Elcik and Kirkin 2024). All parts of the dandelion exhibit biological activity against various diseases, due to the broad spectrum of phytochemicals present in its leaves, stems, flowers, and roots (Perumal *et al.* 2022).

Given the well-documented benefits of *Taraxacum officinale*, the present study aimed to evaluate its antioxidant and antidiabetic activities to validate its traditional (folkloric) use. Although herbal and plant-based medicines remain strong sources of antioxidant compounds, their non-scientific and unregulated use poses major hurdle to reliable therapeutic outcomes. One of the most persistent issues is lack of standardization the concentration of phytochemical compounds in plant extracts can vary significantly depending on species, soil composition, climate, harvesting season, and extraction method (Girish and Koner 2018). This variability complicates reproducibility across research and clinical settings. As the antioxidant activities vary with plant species, geographical conditions and climatic conditions it is important to explore *Taraxacum officinale* from Rawalakot, Pakistan to provide additional data from all over the world. Hot water extraction was preferred, as it is traditionally used in different formulations such as teas and beverages and data is not available on it.

EXPERIMENTAL

Materials

Ethanol, 1.1-diphenyl-2-picrylhdrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ammonium molybdate, iron sulphate ($FeSO_4$), sodium nitroprusside (SNP), 3,5-dinitrosalicylic acid (DNSA), o-phenonthroline, ferric chloride ($FeCl_3$), acarbose, EDTA, gallic acid, quercetin, and sodium hydroxide (NaOH) were purchased from Sigma Aldrich. Analytical grade chemicals were used.

Preparation of Plant Extracts

Taraxacum officinale (dandelion) were collected from the nearby areas of Rawalakot Azad Kashmir Pakistan and were identified by a botanist of department of Botany University of Poonch, Rawalakot where the voucher number 215 was deposited. The leaves were extracted with hot water for 30 min and were stored at low temperature.

DPPH Radical Scavenging Activity

The plant extract's antioxidant capacity was assessed using a free radical scavenging test with DPPH (Fatema *et al.* 2025; Sharna *et al.* 2025). Briefly, 0.25 mM solution of DPPH radical (0.5 mL) was added to the sample solution in ethanol (1 mL) at different concentrations (25 to 200 µg/mL) of aqueous extracts. The mixture was vigorously stirred and left to stand for 30 min in the dark, and the absorbance was measured at 517 nm. Ascorbic acid was used as a standard compound in the assay.

ABTS Assay

The ABTS assay was performed using the discoloration method, following a previously reported protocol (Re *et al.* 1999). An aqueous solution of ABTS was prepared by dissolving 8 mg of ABTS in 1 mL of distilled water (Solution A). Then, 1.32 mg of potassium per sulphate was dissolved in 1mL of distilled water (Solution B). Subsequently, 2 mL of solutions A and B were mixed well. The solution was kept in dark for 16 h. Afterwards, 1 mL of this solution was taken and serial dilutions were prepared with ethanol until the absorbance reached 0.7. Five different dilutions were made by adding ABTS, ethanol and plant extract in five test tubes. For about 10 min tubes were allowed to stand. Absorbance was measured spectrophotometrically at 734 nm. Ascorbic acid was used as a standard compound in the assay.

Phosphomolybdenum Assay

The reduction potential of the *Taraxacum officinale* leaves was determined by the phosphomolybdenum method (Fatema *et al.* 2026). At acidic pH, a green-colored complex phosphate/Mo (V) was formed. The reagent solution was prepared by mixing 0.6 M H_2SO_4 (0.83 mL of H_2SO_4 in 24.1 mL water), 28 mM sodium phosphate (0.193 g in 50 mL water), and 4 mM ammonium molybdate (0.247 g in 50 mL water). Then, 0.1 mg/mL of the extract was added to 3 mL of previously prepared solution. For about 90 min, all the tubes were subjected to incubation at 95 ºC. The reaction mixture was allowed to cool down at room temperature. Absorbance was measured spectrophotometrically (DB-20; Dynamica) at 695 nm. The results were expressed as mg/g of ascorbic acid equivalent.

Lipid Peroxidation Assay

The ability of *Taraxacum officinale* leaves to inhibit lipid peroxidation was studied following a previously reported method (Khaliq *et al.* 2015). An egg yolk weighing approximately 1 g was used and its pH was adjusted to 7.4 by adding 0.1M phosphate buffer saline. After that, 10 µM of $FeSO_4$ solution and 5 µM of sodium nitroprusside (SNP) were added to the previous solution. The tubes were heated for an hour at 37 °C in a water bath. Afterwards, 600 microliters of TBA (Thiobarbituric acid) and acetic acid was added to the reaction mixture to allow formation of MDA-TBA complex and then heated at 100 °C for 12 min. After adding 2 mL of n-butanol, the tubes were centrifuged. Absorbance was determined at 532 nm spectrophotometrically. Ascorbic acid was used as a standard compound in the assay.

Metal Chelation Assay

Metal chelation assay was performed following a previously reported method (Fatema *et al.* 2025). Different concentrations of extract were added to 150 µL of freshly prepared 2 mM $FeSO_4$ solution, 168 µL of 0.1 M Tris-HCl solution, and 218 µL of 0.9% NaCl. After 5 min of incubation, 13µL of o-phenanthroline was added to each test tube. The absorbance was measured in spectrophotometer at 510 nm. EDTA was used as a standard compound in the assay.

Total Phenolic Content

Total phenolic content was estimated by following a previously reported method (Prior *et al.* 2005). Gallic acid was used as a standard compound in the assay. Based on the calibration curve, the linear equation as follows was used to determine the total phenolic content:

$$y = 0.0063\,x + 0.0396$$

The absorbance at 765 nm is represented by y, whereas the total phenolic content of various *Taraxacum officinale* extracts is represented by x.

Total Flavonoid Content

Total flavonoid content was estimated from previous work (Lee *et al.* 2011). Quercetin was used as a standard compound in the assay. Based on the calibration curve, the following linear equation was used to determine the total flavonoid content:

$$y = 0.0026\,x + 0.0114$$

Here, x is the total flavonoid content of various extracts of *Taraxacum officinale* and y is the absorbance at 765 nm.

α-Amylase Inhibitory Assay

The alpha amylase inhibition assay was followed (Miller 1959). Through dissolving 1 g of starch in 100 mL of water, boiling, and shaking, 1% starch was obtained. The DNSA reagent was prepared by integrating 0.2 g of phenol, 1 g of 3,5-dinitrosalicylic acid, 1 g of sodium hydroxide, and 1 g of sodium sulphite with 100 mL of water. A total of 10 mL of buffer was utilized to dilute 5 mg of alpha amylase (0.272 g of buffer and 0.035 g of sodium chloride should be added to 100 mL of distilled water to yield KH_2PO_4 buffer). A pair of test tubes were employed; the control tube included 20 µL of enzyme, 850 µL of phosphate buffer, and 130 µL of distilled water. A total of 20 µL of enzyme,

100 µL of plant extract, 850 µL of phosphate buffer and 30 µL of distilled water were mixed in the actual test tube. Incubation of the tubes lasted for 15 min at 370 °C, followed by the addition of 200 µL of 1% starch and the mixture was incubated at 37 °C for 30 min. Following the incubation period, 100 µL of DNSA reagent was added to the tubes followed by heating for 10 min at 100 °C. Tubes were diluted to 900 µL and the absorbance was measured at 540 nm. Acarbose was used as a standard compound in the assay.

The test sample's α-amylase inhibition percentage was determined using the following formula:

$$Inhibition\ (\%) = 100 \times (AC - AS)/(AC)$$

where the sample and control absorbance are denoted by AS and AC, respectively.

Statistical Analysis

The results were expressed as means. The data was subjected to one way analysis of variance (ANOVA)in SPSS followed by Duncan`s multiple range test where necessary.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Activity

For free radicals, the most commonly used method to assess a plant's antioxidant capacity is the DPPH assay. The DPPH radical, originally purple, is reduced to bright yellow diphenylpicrylhydrazine upon interaction with antioxidants, a change that can be monitored spectrophotometrically by a decrease in absorbance at 517 nm (Dedić *et al.* 2022). The extract of *Taraxacum officinale* leaves demonstrated high antioxidant activity. With increasing concentrations of the plant extract, the purple color of the DPPH radical gradually turned yellow. The extent of this color change reflects the scavenging capacity of the antioxidants.

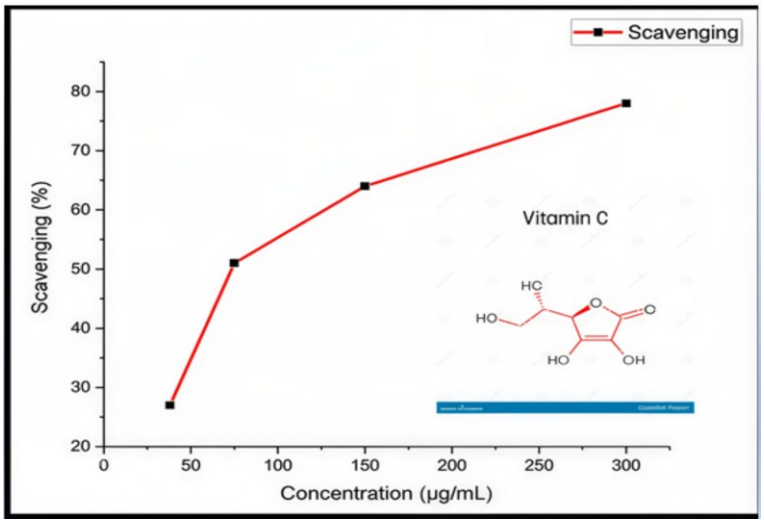

Fig. 1. DPPH activity of *Taraxacum officinale* leaves extract; Tested concentrations showed significant differences (P < 0.05) according to DMR tests

The study revealed a dose-dependent increase in DPPH activity, with scavenging percentages of 27%, 51%, 64%, and 78%, respectively (Fig. 1). Table 1 shows the IC50 values for sample and standard ascorbic acid. These results indicate that higher concentrations of dandelion extract exhibit greater radical scavenging activity. Analysis of the aqueous extracts of *Taraxacum officinale* confirms that this plant is a rich source of antioxidant compounds. The current findings are consistent with previous studies (Xue *et al.* 2017; Gholami *et al.* 2025).

ABTS Scavenging Potential of the *Taraxacum officinale*

The ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation assay is a widely used method for evaluating antioxidant activity. The assay involves the generation of the ABTS radical cation (ABTS•+), which is typically produced by reacting ABTS with an appropriate oxidant, such as potassium persulfate (Kim *et al.* 2008). The ABTS•+ radical cation exhibits a green-blue color and strongly absorbs at 734 nm. Generally, the ABTS radical cation is generated a day prior to the assay (García-Carrasco *et al.* 2015). Upon addition of antioxidants, a gradual discoloration occurs, shifting from blue to colorless. The ABTS radical cation scavenging activity of the dandelion plant extract was evaluated at various concentrations. The scavenging activity increased with the concentration of the extract: 25 µg/mL showed 55% activity, 50 µg/mL exhibited 60%, 75 µg/mL reached 67%, and the highest tested concentration, 100 µg/mL, demonstrated 75% scavenging activity (Fig. 2). Table 1 shows the IC50 values for sample and standard ascorbic acid. These results indicate that higher concentrations of the extract are more effective at neutralizing ABTS radicals, highlighting its potential as a source of natural antioxidants capable of preventing oxidative stress-related diseases. The current findings are consistent with previous studies (Sun *et al.* 2014).

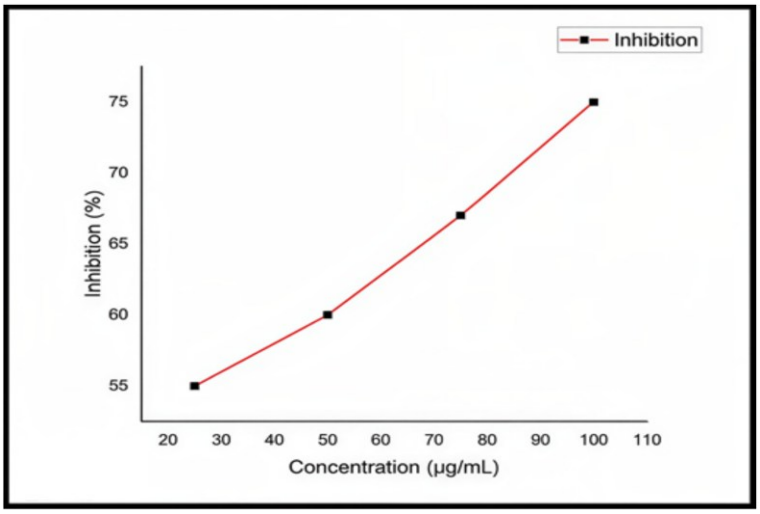

Fig. 2. ABTS activity of *Taraxacum officinale* leaves extract; Tested concentrations showed significant differences (P < 0.05) according to DMR tests

Lipid Peroxidation Assay

Lipid peroxidation refers to the oxidative degradation of lipids, a process that damages cell membranes and serves as a marker of oxidative stress. Assessing the lipid peroxidation inhibitory activity of plant extracts provides insights into their potential as

protective agents against oxidative damage (Majewski *et al.* 2020). Oxidative stress contributes to more than 200 diseases and accelerates the aging process (Ghazanfari *et al.* 2006). The production of thiobarbituric acid reactive substances (TBARS) often leads to damage of biomolecules such as DNA (Chen *et al.* 2005). Reactive oxygen species generated by free radicals and metal ions not only damage DNA and proteins but also attack other cellular components, including polyunsaturated fatty acid residues in phospholipids, which are particularly susceptible to oxidation. Iron overload promotes the formation of lipid peroxidation products, which have been detected in liver and kidney tissues (Houglum *et al.* 1990). Pro-oxidant agents, such as Fe_2SO_4 and sodium nitroprusside (SNP), enhance lipid peroxidation in egg yolk homogenates. In this study, the dandelion leaf extract demonstrated a dose-dependent protective effect, with lipid peroxidation decreasing as extract concentration increased. Specifically, 25 µg/mL of extract inhibited lipid peroxidation by 31%, and this inhibition increased progressively to 70% at 100 µg/mL (Fig. 3). Table 1 shows the IC50 values for sample and standard ascorbic acid. These findings indicate that the extract effectively mitigates iron-induced lipid peroxidation in egg yolk homogenates.

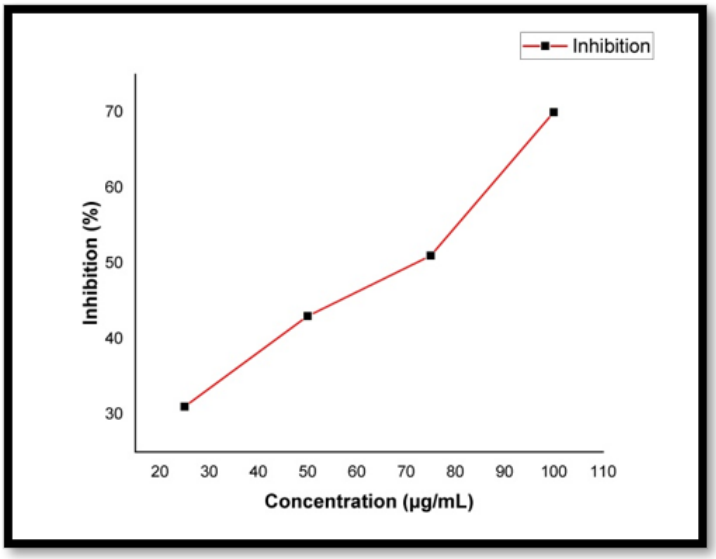

Fig. 3. Lipid peroxidation induced by iron in egg yolk homogenate (Fe_2SO_4) of *Taraxacum officinale* leaves; Tested concentrations showed significant differences ($P < 0.05$) according to DMR tests

This trend suggests that *Taraxacum officinale* leaves extract contains bioactive compounds that are effective in neutralizing reactive oxygen species and/or chelating iron ions, thus mitigating lipid peroxidation. These results are in line with previous studies (Hu and Kitts 2005).

The SNP in egg yolk formulation was employed to initiate lipid peroxidation and the extract from dandelion (*Taraxacum officinale*) leaves was additionally assessed for its antioxidant effects. Varying concentration of plant extract was used in lipid peroxidation induced by SNP in egg yolk mixtures. The control test tube containing only egg yolk mix without dandelion extract remains light yellow, indicating no lipid peroxidation (Fig. 4). Lipid peroxidation can be assessed qualitatively using the color changes observed in the

test tubes. Oxidative stress causes lipids to break down and MDA is produced, which is inhibited by the phenolics of the extract.

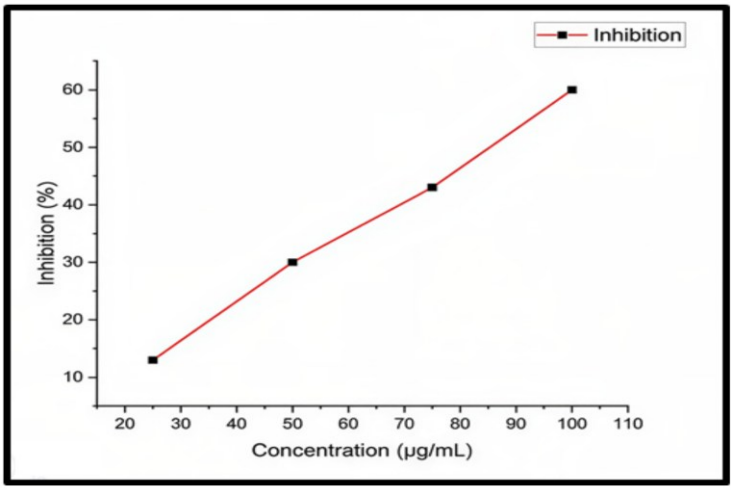

Fig. 4. Lipid peroxidation induced by SNP in egg yolk homogenate of *Taraxacum officinale* leaves; Tested concentrations showed significant differences (P < 0.05) according to DMR tests

Total Antioxidant Activity of *Taraxacum officinale* by Phosphomolydenum Assay

The phosphomolybdenum assay is a method for assessing a sample's total antioxidant capacity. The methodology is based on the antioxidants present in the sample reducing Mo(VI) to Mo(V) and then forming a green phosphate/Mo(V) complex at an acidic pH (Prieto *et al.* 2010). Figure 5 shows that as the concentration increased, the absorbance value of the sample was also increased. This indicates that the extract can reduce the Mo(VI) to Mo(V) at this concentration due to the presence of high concentration of antioxidants. These results are in line with the previous study (Tetty *et al.* 2014).

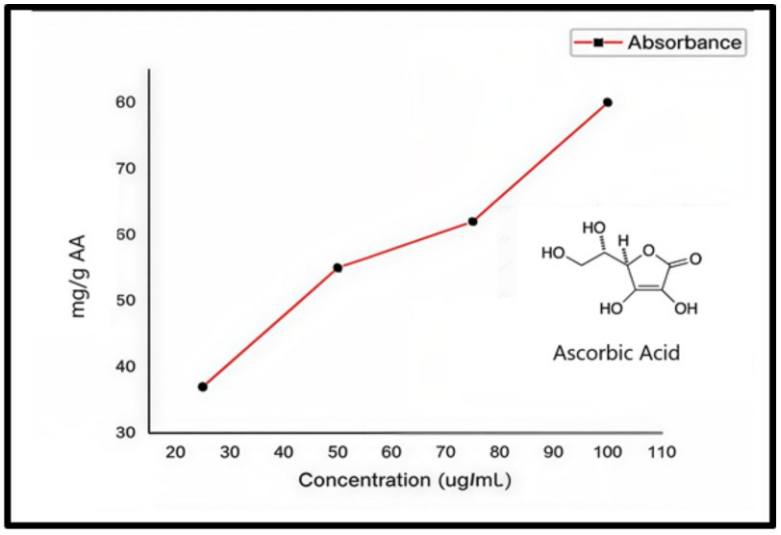

Fig. 5. Total antioxidant activity assessed by phosphomolydenum assay; Tested concentrations showed significant differences (P < 0.05) according to DMR tests

Iron Chelating Activity of *Taraxacum officinale*

The metal chelating potential of a molecule can be assessed using a metal chelation assay. Several plant extracts have been reported to exhibit iron-chelating activity (Bucher *et al.* 1983; Minotti and Aust 1987). This method is based on the extract's ability to reduce free Fe^{3+} to Fe^{2+}, which subsequently forms a colored complex with ortho-phenanthroline. The results indicate a dose-dependent increase in metal chelation activity with increased concentrations of dandelion extract. Specifically, the extract exhibited 23% inhibition at the lowest concentration (25 µg/mL), which progressively increased to 58% at the highest concentration tested (100 µg/mL) (Fig. 6). Table 1 shows the IC50 values for sample and standard EDTA. These findings suggest that dandelion extract possesses metal-chelating properties, potentially enhancing its overall antioxidant capacity by reducing the availability of free metal ions that promote free radical generation. These results are in line with the previous studies (Puntel *et al.* 2005; Aremu *et al.* 2019).

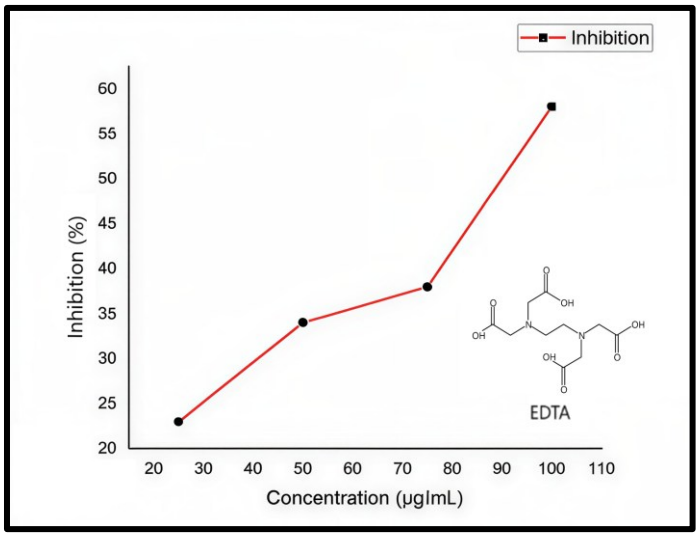

Fig. 6. Metal chelation activity of *Taraxacum officinale* leaves extract; Tested concentrations showed significant differences ($P < 0.05$) according to DMR tests

Total Phenolic and Flavonoid Contents

The extract contained high content of total phenolics 40 mg/g of gallic acid equivalent and flavonoid contents 25 mg/g as quercetin equivalent, which may be responsible for high antioxidant activity of the plant extract, in a similar way to previously reported results (Chang *et al.* 2002). Khan *et al.* (2019) reported the phenolic content of aqueous extract 41.47 mg/g and 691.6 mg/g for hydro-alcoholic extract.

Antidiabetic Activity of *Taraxacum officinale*

Alpha-amylase is an enzyme responsible for breaking down starch into sugars. The alpha-amylase inhibition assay is a widely used method to evaluate the ability of plant extracts to inhibit this enzyme. Bioactive compounds, such as phenolic acids, terpenoids, and flavonoids, are thought to contribute to the antidiabetic effects of dandelion leaves (Mir *et al.* 2015). In the present study, samples at concentrations of 25, 50, 75, and 100 µg/mL were tested (Fig. 7). Table 1 shows the IC50 values for sample and standard acarbose. A gradual color change from yellow to orange was observed, and absorbance was subsequently measured spectrophotometrically at 540 nm. The results indicate that

dandelion leaf extract inhibits alpha-amylase in a concentration-dependent manner, suggesting the presence of biologically active compounds capable of modulating enzyme activity (Nnamdi *et al.* 2012).

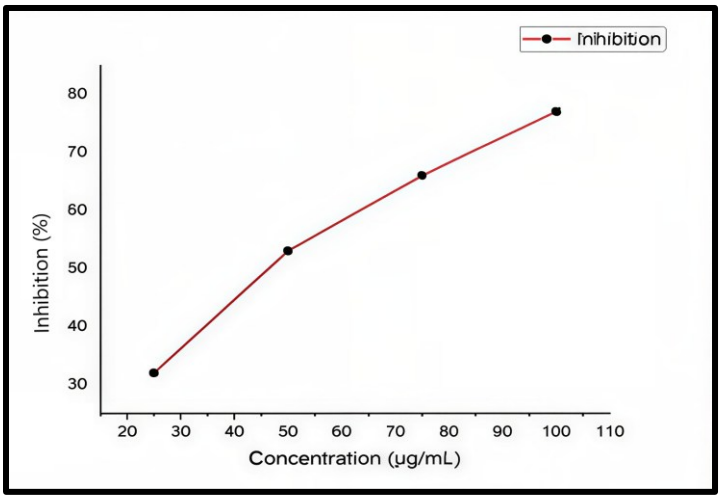

Fig. 7. Alpha amylase inhibition of *Taraxacum officinale* leaves extract

Table 1. Calculated IC50 Values for Various Antioxidant Activities of *Taraxacum officinale*

Activity	IC50 Value (µg/mL) for Hot Water Extract	IC50 Value (µg/mL) Standard Compounds
DPPH activity	91.2 ±0.4	4.5 ±0.02
ABTS activity	21.1±0.1	11.1±0.1
Lipid peroxidation against iron	71.1±0.5	45.1±0.6
Lipid peroxidation against SNP	92.1±1.2	55.1±0.7
Total antioxidant activity	45.1±0.3	7.1±0.1
Metal chelating activity	90.5±1.1	17.2±0.2
Alpha amylase inhibition	48.1±1.2	67.1±0.7

CONCLUSIONS

1. The crude aqueous extract of *Taraxacum officinale* (dandelion) possesses significant antioxidant activity, including effective free radical scavenging, inhibition of reactive oxygen species, and protection against lipid peroxidation. These results highlight the potential of dandelion as a functional food and as a natural agent for managing oxidative stress-related disorders.
2. The extract exhibited notable α-amylase inhibitory activity, indicating its promise as a candidate for diabetes management. These observations are consistent with previous reports showing that dandelion flavonoids can inhibit pancreatic α-amylase, thereby contributing to its antidiabetic effects.
3. Future *in vivo* studies are warranted to elucidate the mechanisms underlying these antioxidant and antidiabetic activities. Additionally, identifying the specific bioactive compounds responsible will further clarify the therapeutic potential of dandelion extracts.

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Conflict of Interest

The authors declare no conflict of interest.

Use of Generative AI

No AI was employed for the preparation of this manuscript

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