RESEARCH ARTICLE

Phytochemical Characterization and Antioxidant Potential of *Cichorium intybus* Root Extracts

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Abstract

Cichorium intybus L. is a medicinal plant recognized for its rich phytochemical composition and diverse pharmacological properties. The present study comparatively evaluated the extraction yield, phytochemical composition, and antioxidant potential of aqueous, methanolic, and hydroethanolic (70%) root extracts of *C. intybus*. The extracts were analyzed for extraction yield, qualitative phytochemical constituents, total flavonoid content (TFC), total phenolic content (TPC), and nitric oxide (NO) radical scavenging activity. The aqueous extract exhibited the highest extraction yield (18.25 ± 0.56%), whereas the hydroethanolic extract showed the broadest phytochemical profile. Quantitative analysis demonstrated that the hydroethanolic extract contained the highest TFC (38.44 ± 0.33 mg QE/100 g DW) and TPC (250.75 ± 0.86 mg GAE/100 g DW). Consistently, this extract exhibited the strongest nitric oxide radical scavenging activity, with the lowest IC_{50} value (261.83 µg/mL), indicating superior antioxidant potential. These findings suggest that hydroethanolic extraction is an efficient approach for recovering bioactive phytochemicals from *C. intybus* roots and highlight its potential as a natural source of antioxidant compounds for nutraceutical and pharmaceutical applications.

Key words: *Cichorium intybus*, Hydroethanolic extract, Nitric oxide scavenging activity, Phenolic compounds, Phytochemical profiling.

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Introduction

Cichorium intybus L. (chicory), a perennial herb of the family Asteraceae, is widely used as a food, forage crop, coffee substitute, and medicinal plant. Traditionally, different parts of the plant have been employed for the management of gastrointestinal, hepatic, metabolic, and inflammatory disorders (Pathak *et al.*, 2023a,b). The root is particularly important because it contains inulin, fructooligosaccharides, sesquiterpene lactones, phenolic acids, flavonoids, coumarins, sterols, and other secondary metabolites with diverse biological activities (Street *et al.*, 2013; Nwafor *et al.*, 2017). Consequently, chicory has attracted increasing attention as a natural source of antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and nutraceutical agents (Adham and Naqishbandi, 2012; Pathak and Singh, 2022; Pathak *et al.*, 2022; Pathak *et al.*, 2024a,b). Major phenolic compounds, including caffeic acid derivatives, chlorogenic acid, cichoric acid, and caffeoylquinic acids, together with sesquiterpene lactones such as lactucin and lactucopicrin, are primarily responsible for its pharmacological properties (Street *et al.*, 2013; Janda *et al.*, 2021; Pathak *et al.*, 2022).

The antioxidant and anti-inflammatory activities previously reported for *C. intybus* roots support further investigation of their phytochemical composition and nitric oxide radical scavenging potential (Rizvi *et al.*, 2014; Matvieieva *et al.*, 2023; Pathak *et al.*, 2024a,b). The chemical composition and biological activity of medicinal plant extracts are strongly influenced by the extraction solvent. Water primarily extracts highly polar constituents, whereas organic solvents recover compounds with varying polarity. Hydroethanolic solvent systems are especially effective because water enhances plant-matrix swelling while ethanol improves extraction of moderately polar metabolites. Previous studies have shown that ethanol-

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water mixtures efficiently recover phenolic constituents, although extraction efficiency also depends on solvent concentration, extraction time, temperature, and plant matrix characteristics. Qualitative phytochemical screening and estimation of total phenolic and flavonoid contents provide valuable information on antioxidant constituents, whereas gas chromatography-mass spectrometry (GC-MS) enables tentative identification of volatile and semi-volatile compounds.

Although several studies have described the phytochemistry and biological properties of chicory (Adham and Naqishbandi, 2012; Street *et al.*, 2013; Pathak *et al.*, 2022; Pathak *et al.*, 2024), comparative evaluation of aqueous, methanolic, and hydroethanolic root extracts under a common experimental framework remains limited. Therefore, the present study comparatively evaluated these extracts for extraction yield, physical characteristics, phytochemical composition, total phenolic and flavonoid contents, and nitric oxide radical scavenging activity. The hydroethanolic extract was further characterized by GC-MS to identify its major constituents and provide a phytochemical basis for its antioxidant potential.

Materials and Methods

Plant Material Collection and Authentication

Fresh roots of *Cichorium intybus* L. were collected from the Polyhouse, Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar, Uttarakhand, India. The plant material was authenticated at the CSIR–National Botanical Research Institute (NBRI), Lucknow, India, and a voucher specimen (Accession No. 109668) was deposited in the institute herbarium. The roots were washed, shade-dried, powdered, and stored in airtight containers until extraction.

Preparation of Root Extracts and Qualitative Phytochemical Screening

Powdered root material (20 g) was extracted separately with distilled water, methanol, and 70% ethanol (hydroethanol) in a 1:10 (w/v) ratio by cold maceration for 24 h at room temperature. The extracts were filtered through Whatman No. 1 filter paper and concentrated. The filtrates were dried at 37 °C in a fan-assisted incubator until a constant weight was obtained, and the dried extracts were stored in airtight containers at −4 °C until further analysis (Pathak *et al.,* 2021).

The aqueous, methanolic, and hydroethanolic extracts were qualitatively screened for major phytochemical groups, including alkaloids, glycosides, flavonoids, carbohydrates, tannins, saponins, proteins, amino acids, anthraquinones, phenolics, and fixed oils/fats using standard procedures described by Harborne (1998).

Determination of Total Flavonoid (TF) and Total Phenolic (TC) Content

Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric method following the method of Ordonez *et al.* (2006). Results were calculated from a quercetin calibration curve and expressed as mg quercetin equivalents (QE)/100 g dry weight (DW).

Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay according to Arabshahi-Delouee and Urooj (2007). Gallic acid was used as the standard, and the results were expressed as mg gallic acid equivalents (GAE)/100 g dry weight (DW).

Nitric Oxide Radical Scavenging Assay

Nitric oxide radical scavenging activity was evaluated according to Ebrahimzadeh *et al.* (2008) using sodium nitroprusside as the nitric oxide donor and the Griess reagent for detection. Gallic acid served as the reference antioxidant. The percentage inhibition was calculated, and the IC_{50} values were determined from dose-response curves. All analyses were performed in triplicate and expressed as mean ± standard error (SE).

GC-MS Analysis of Hydroethanolic Root Extract

Gas chromatography-mass spectrometry (GC-MS) analysis of hydroethanolic extract of *Cichorium intybus* roots was performed using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass selective detector equipped with a Triple-Axis HED-EM detector. Mass spectra were acquired over a scan range of 50-600 m/z under electron ionization (EI) mode at 70 eV, with a solvent delay of 3.0 min. The chromatographic peaks were tentatively identified by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST 8). Mass Spectral Library and compounds were characterized based on their retention time, molecular mass and spectral matching, as described in previous GC–MS-based phytochemical studies (Sparkman *et al.,* 2011).

Results and Discussion

Extraction Yield and Physical Characteristics

The aqueous extract showed the highest extraction yield (18.25 ± 0.56%), followed by the hydroethanolic (16.45 ± 0.34%) and methanolic (7.60 ± 0.11%) extracts. All extracts were semi-solid with a characteristic odour. The aqueous and hydroethanolic extracts were brown, whereas the methanolic extract was dark brown. The higher yield obtained with distilled water can be attributed to its greater

ability to extract highly polar constituents, including inulin, fructooligosaccharides, soluble polysaccharides, sugars, proteins, amino acids, and organic acids, which constitute a substantial proportion of *Cichorium intybus* roots. These hydrophilic compounds contribute significantly to the total extractable mass, resulting in a higher overall yield. Although 70% hydroethanol efficiently extracts both polar and moderately polar phytochemicals, its lower polarity than water reduces the extraction of highly water-soluble polysaccharides and carbohydrates, resulting in a slightly lower yield. In contrast, methanol preferentially extracts phenolic compounds and flavonoids, which are present in relatively lower quantities, leading to the lowest overall extraction yield. The darker colour of the methanolic extract further indicates enrichment of phenolic constituents rather than a greater recovery of total solids. Similar solvent-dependent variations in extraction yield and extract characteristics have been reported for *C. intybus* roots (Rizvi *et al.,* 2014; Nwafor *et al.,* 2017; Ferioli *et al.,* 2019; Matvieieva *et al.,* 2023).

Qualitative Phytochemical Screening

The qualitative phytochemical profile of aqueous, methanolic, and hydroethanolic (70%) extracts of *C. intybus* roots is presented in Table 1. All extracts tested positive for alkaloids, flavonoids, tannins, saponins, proteins, and phenolic compounds, indicating their widespread occurrence irrespective of the extraction solvent. Glycosides and carbohydrates/ reducing sugars were detected in the aqueous and hydroethanolic extracts but were absent in the methanolic extract. Amino acids were present in the methanolic and hydroethanolic extracts, whereas fixed oils and fats were detected exclusively in the hydroethanolic extract. Anthraquinones were absent in all extracts. These variations are primarily attributed to differences in solvent polarity. Water efficiently extracts highly polar constituents, methanol preferentially recovers moderately polar metabolites, while the hydroethanolic solvent system extracts both polar and moderately polar phytochemicals, resulting in the broadest phytochemical profile.

Similar observations have been reported for *Cichorium intybus* roots, where hydroethanolic extracts exhibited greater phytochemical diversity than single-solvent extracts (Street *et al.,* 2013; Ferioli *et al.,* 2019). The presence of phenolics, flavonoids, alkaloids, tannins, and saponins supports the reported antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and cytoprotective activities of chicory roots. The absence of anthraquinones is consistent with previous reports (Street *et al.,* 2013). The detection of fixed oils and fats exclusively in the hydroethanolic extract suggests that the hydroethanolic solvent system was more suitable for extracting these moderately lipophilic constituents than either distilled water or methanol under the conditions employed (Ferioli *et al.,* 2019). This observation highlights the advantage of hydroethanolic extraction in recovering a broader spectrum of phytochemicals with varying polarities, thereby enhancing the overall phytochemical profile of the extract.

Table 1: Qualitative phytochemical profile of different *Cichorium intybus* root extracts

Phytochemical group	Aqueous extract	Methanolic extract	Hydroethanolic extract
Alkaloids	+	+	+
Glycosides	+	–	+
Flavonoids	+	+	+
Carbohydrates / Reducing sugars	+	–	+
Tannins	+	+	+
Saponins	+	+	+
Proteins	+	+	+
Amino acids	–	+	+
Anthraquinones	–	–	–
Phenolic compounds	+	+	+
Fixed oils and fats	–	–	+

(+) indicates the presence and (–) indicates the absence of the respective phytochemical group.

Quantitative Analysis of TFC and TPC of Different *Cichorium intybus* Root Extracts

The total flavonoid content (TFC) and total phenolic content (TPC) of aqueous, methanolic, and hydroethanolic (70%) *Cichorium intybus* root extracts are presented in Table 2. The hydroethanolic extract exhibited the highest TFC (38.44 ± 0.33 mg QE/100 g DW) and TPC (250.75 ± 0.86 mg GAE/100 g DW), followed by the methanolic extract (28.75 ± 0.75 mg QE/100 g DW and 144.97 ± 0.56 mg GAE/100 g DW). The aqueous extract contained the lowest TFC (17.12 ± 0.31 mg QE/100 g DW) and TPC (80.93 ± 0.92 mg GAE/100 g DW). The superior recovery of flavonoids and phenolics in the hydroethanolic extract is attributed to the balanced polarity of the ethanol-water solvent system, which efficiently extracts both polar and moderately polar phytochemicals. In contrast, water mainly extracts highly polar constituents, while methanol preferentially recovers moderately polar metabolites. Similar findings have been reported for *C. intybus* and other medicinal plants, where hydroethanolic solvents consistently yielded higher concentrations of phenolic compounds than aqueous or absolute organic solvents (Alara *et al.,* 2021; Ferioli *et al.,* 2019; Matvieieva *et al.,* 2023; Pathak *et al.,* 2023).

The high phenolic and flavonoid contents are consistent with the presence of chlorogenic acid, caffeic acid derivatives, cichoric acid, and other polyphenols, which contribute to the antioxidant, anti-inflammatory,

Table 2: Total flavonoid and total phenolic contents of different *Cichorium intybus* root extracts (Mean ± SE, n=3).

Phytochemical	Aqueous extract	Methanolic extract	Hydroethanolic extract
Total flavonoid content (mg QE/100 g DW)	17.12 ± 0.31[a]	28.75 ± 0.75[b]	38.44 ± 0.33[c]
Total phenolic content (mg GAE/100 g DW)	80.93 ± 0.92[c]	144.97 ± 0.56[b]	250.75 ± 0.86[a]

QE = Quercetin equivalent; GAE = Gallic acid equivalent; DW = Dry weight

antimicrobial, hepatoprotective and free radical scavenging activities of chicory (Street *et al.,* 2013). As phenolics and flavonoids protect against oxidative damage through free radical scavenging, hydrogen donation, and metal chelation, the higher levels observed in the hydroethanolic extract indicate its superior antioxidant potential and support its use in pharmacological and antioxidant studies.

Nitric Oxide Radical Scavenging Activity

The nitric oxide (NO) radical scavenging activity of aqueous, methanolic, and hydroethanolic (70%) *Cichorium intybus* root extracts is presented in Figure 1. All extracts exhibited concentration-dependent NO radical scavenging activity. The hydroethanolic extract showed the strongest antioxidant activity with the lowest IC_{50} value, followed by the methanolic and aqueous extracts, whereas gallic acid, used as the reference antioxidant, displayed the highest scavenging activity. The superior activity of the hydroethanolic extract is attributed to its higher total phenolic and flavonoid contents. Phenolic compounds neutralize reactive nitrogen species through hydrogen atom or electron donation, while flavonoids stabilize reactive intermediates and inhibit oxidative chain reactions. The lower activity of the aqueous extract and the intermediate activity of the methanolic extract corresponded to their comparatively lower polyphenolic contents. A positive relationship between total phenolic content and nitric oxide scavenging activity has also been reported by Street *et al.* (2013) and Ferioli *et al.* (2019) for *Cichorium intybus* and other medicinal plants, confirming the major role of polyphenols in antioxidant activity.

GC–MS Analysis of Hydroethanolic *Cichorium intybus* Root Extract

The GC+MS chromatogram of the hydroethanolic extract of *C. intybus* roots revealed the presence of multiple phytoconstituents, indicating the chemical complexity of the extract (Fig. 2). A total of 15 major compounds were identified by comparing the obtained mass spectra with those available in the NIST mass spectral library, and their retention times and relative peak area percentages are

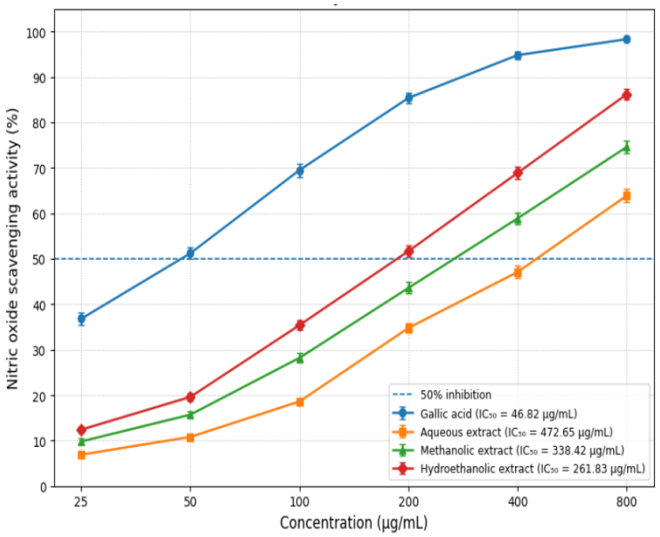


Fig. 1: Nitric oxide radical scavenging activity of aqueous, methanolic and hydroethanolic (70%) *Cichorium intybus* root extracts

presented in Table 3. Among the identified constituents, 8-methyl-6-nonenamide exhibited the highest relative peak area (0.987%), followed by dipalmitin (0.927%), methyl linolenate (0.907%), 4H-pyran-4-one (0.883%), 9,12-octadecadienoic acid (0.872%), propanoic acid (0.869%), lupeol (0.851%) and others. The occurrence of fatty acids, phytosterols, terpenoids and oxygenated heterocyclic compounds indicates that the hydroethanolic solvent effectively extracted chemically diverse metabolites from *C. intybus* roots, which is consistent with previous phytochemical investigations of chicory (Street *et al.,* 2013; Ferioli *et al.,* 2019). Several of the detected compounds have well-documented biological activities. Lupeol is a pentacyclic triterpenoid reported to possess antioxidant, anti-inflammatory, antimicrobial, hepatoprotective and anticancer properties (Saleem, 2009). Similarly, β-sitosterol and stigmasterol have been widely investigated for their antioxidant, anti-inflammatory, immunomodulatory and hypocholesterolemic activities (Babu and Jayaraman, 2020). In addition, 4H-pyran-4-one derivatives have been reported to exhibit free radical scavenging and antimicrobial activities, thereby enhancing the biological significance of the extract (Ferioli *et al.,* 2019).

The predominance of fatty acid derivatives, phytosterols and terpenoids in the hydroethanolic extract is in agreement with the higher total phenolic content, total flavonoid content and antioxidant activity observed in the present investigation. Hydroethanolic solvents possess intermediate polarity, enabling simultaneous extraction of both hydrophilic and moderately lipophilic phytochemicals, which generally results in greater phytochemical diversity than aqueous or absolute organic solvents (Street *et*

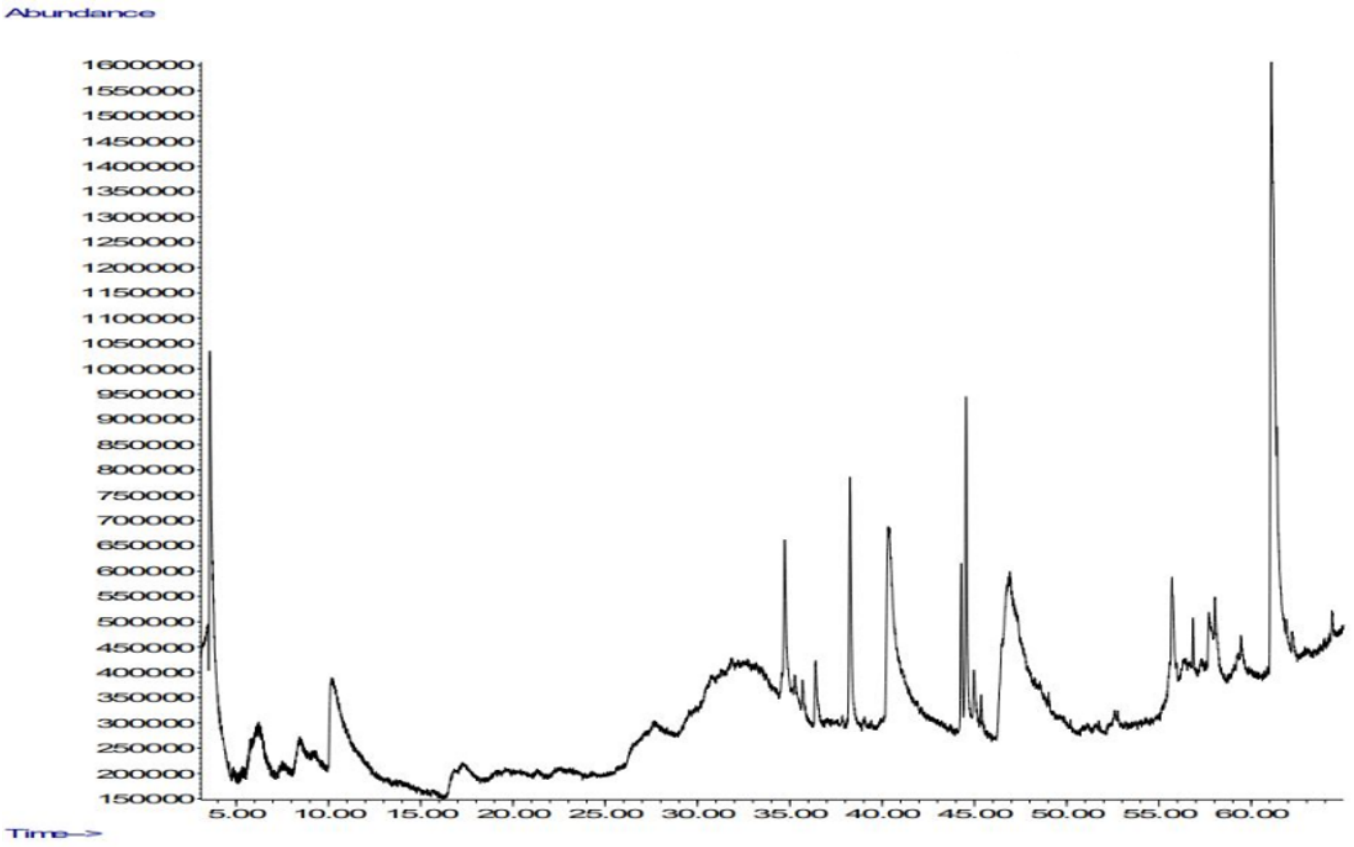


Fig. 2: Chromatogram of hydroethanolic extract of root of *Cichorium intybus* obtained from GC-MS analysis

Table 3: Phytochemicals identified in hydroethanolic extract of root of *Cichorium intybus* through GC-MS analysis

Name of the compounds	Retention time (min)	Peak area (%)
Tetradecanoic acid	5.725	0.362
4H-Pyran-4-one	10.189	0.883
2-Butanone	34.733	0.712
Lupeol	35.126	0.851
3,7,11,15-tetramethyl-2-hexadecen-1-ol	36.419	0.842
n-Hexadecanoic acid	37.125	0.121
Methyl linolenate	40.348	0.907
Stigmasterol	44.297	0.183
Linolenic acid	44.558	0.004
Dipalmitin	46.856	0.927
Propanoic acid	55.702	0.869
9,12-octadecadienoic acid	57.703	0.872
Beta-sitosterol	58.026	0.624
8-Methyl-6-nonenamide	61.091	0.987
Verrucarol	62.289	0.296

al., 2013; Alara *et al.,* 2021). This finding supports the superior antioxidant activity of the hydroethanolic extract demonstrated in the nitric oxide radical scavenging assay, and GC-MS findings of Street *et al.* (2013). Such differences are expected because the phytochemical profile of medicinal plants is influenced by both genetic and environmental factors.

Conclusion

The present study demonstrated that the extraction solvent significantly influenced the phytochemical composition and antioxidant activity of *Cichorium intybus* root extracts. The hydroethanolic (70%) extract exhibited the richest phytochemical profile, the highest total flavonoid and phenolic contents, and the strongest nitric oxide radical scavenging activity, indicating superior antioxidant potential. GC-MS analysis further revealed the presence of several biologically important compounds, which are known to possess antioxidant, anti-inflammatory and pharmacological activities. Collectively, these findings suggest that hydroethanolic extraction is the most effective method for recovering bioactive constituents from *C. intybus* roots and support its potential application as a natural source of antioxidant phytochemicals for nutraceutical and pharmaceutical use. Further studies on compound isolation, quantitative profiling, and *in vivo* evaluation are required to confirm its therapeutic potential.

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