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Defining Solvent and Concentration Type to Maximise Polyphenol Extraction and Antioxidant Activity in *Rubia cordifolia* L. and *Taraxacum officinale* Weber

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ABSTRACT

This investigation aims to determine the most suitable solvent system and concentration for extracting different polyphenols and the antioxidant activity from different plant parts of *Rubia cordifolia* and *Taraxacum officinale*. The results revealed that the highest polyphenolic content and antioxidant activity were observed in 70% ethanol and methanol extracts, irrespective of the plant part. Notably, *R. cordifolia* exhibited high phenolic content in its leaves and roots, whereas *T. officinale* showed higher content in its roots. With respect to antioxidant and anti-mutagenic activity, the extract of different plant parts showed varied responses. The highest antioxidant activity was observed in the root extracts of both plants, whereas the leaves of *R. cordifolia* and the roots of *T. officinale* exhibited higher anti-mutagenic activity. Given the high polyphenol content and strong antioxidant and anti-mutagenic activities, these plants have the potential to be used in phyto-nutraceutical and pharmaceutical applications.

1 | Introduction

Polyphenols, comprising approximately 8000 molecules, are primarily synthesised as secondary metabolites by plants and are an important source of natural antioxidants due to their ability to scavenge reactive oxygen species and free radicals (Pandey and Rizvi 2009; B. Singh et al. 2026). These are predominantly found in fruits, vegetables, medicinal plants and drinks such as beer, wine, tea, coffee and hot chocolate (Stromsnes et al. 2021). The plant-derived polyphenols are categorised into diverse groups, such as phenols, flavonoids, tannins, alkaloids, terpenoids, lignins and stilbens (Pandey and Rizvi 2009; Tiwari, Kewlani, et al. 2023). These polyphenols are known to possess various therapeutic activities, including anticancer, anti-mutagenic, anti-diabetic, anti-ageing, anti-hypertension and antioxidant and are extensively utilised in pharmaceutical, nutraceutical, cosmeceutical and

functional foods (Ajila et al. 2011; L. Singh et al. 2024; Stromsnes et al. 2021; Kewlani et al. 2022).

Extraction is the first and most important step in discovering potential phenolic compounds from plant materials. Among others, solvent extraction is widely used to extract phenolic compounds. This extraction medium is mainly influenced by various factors such as the type of solvents and their concentration, extraction time, temperature, pH, solid-liquid ratio and pressure (Humbal et al. 2024). Besides these, different solvent systems, including polar, nonpolar and biphasic mixtures, are frequently utilised for the extraction of metabolites from plant matrices. Temperature plays a crucial role in solvent extraction to enhance solubility, diffusion and cell wall permeability. However, excessive heating may lead to thermal degradation of

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many phytochemicals, such as phenolics, flavonoids and volatile compounds. In conventional solid–liquid extraction, temperatures are generally kept below 40°C–60°C to improve solvent penetration and solubility with minimal degradation of heat-sensitive compounds (Akowuah and Zhari 2010). Moreover, the yield and purity of phytonutrients depend on a thorough understanding of the physicochemical interactions between solvents and target compounds. Careful selection of a suitable extraction medium, based on the specific characteristics of the metabolites of interest, is therefore essential for efficient and effective extraction processes.

Rubia cordifolia Linn. (family: Rubiaceae) and *Taraxacum officinale* Weber (family: Asteraceae) are considered potent anticancer plants and used in traditional medicinal systems to cure several ailments (Table S1). Both the selected species exhibit a broad spectrum of pharmacological activities, including antioxidant, anticancer, anti-inflammatory, antimicrobial, neuro-protective and immunostimulatory (Table S1). Moreover, the selected plant species contain various phenolic compounds such as phenols, resins, tannins, sterols, terpenes, anthraquinones, alkaloids, glycosides, saponins and flavonoids (Tiwari, Bhatt, et al. 2023; Tiwari et al. 2024). However, the evaluation of suitable extraction media and the anti-mutagenic activity of *R. cordifolia* and *T. officinale* has not been investigated to date. Although several studies have reported the phytochemical composition and pharmacological activities of *R. cordifolia* and *T. officinale*, comparative evaluation of solvent systems influencing the recovery of polyphenolic compounds and their associated antioxidant and anti-mutagenic activities across different plant parts remains limited. Understanding solvent effects is important for efficient extraction of bioactive compounds for nutraceutical and pharmaceutical applications. Therefore, the present study systematically evaluates different solvent types and concentrations to determine their effectiveness in extracting polyphenols and associated bioactivities from these two medicinally important species.

2 | Materials and Methods

2.1 | Plant Material

Whole plants of *R. cordifolia* and *T. officinale* were collected from Dunagiri (N 29°48'53"; E 79°26'17"; 2162 m asl), Uttarakhand, India. The plant parts (leaves, stems and roots) were separated and thoroughly washed with tap water. All the plant parts were initially dried at room temperature, followed by oven drying until a constant weight was achieved. Dried plant parts were ground into a fine powder and stored at 4°C separately till analysis.

2.2 | Extract Preparation and Experimental Design

Before the analysis, the finely ground powder samples (0.25 g) were extracted separately with different solvent systems, including distilled water, ethanol, methanol, and acetone (20 mL). Each sample extract was heated at 60°C for 60 min in a water bath and allowed to stand on a rotary shaker (24 h; 160 rpm) at room temperature. The homogenized mixture was then centrifuged at room temperature for 20 min at 10,000 rpm. The supernatant was collected and filtered by Whatman filter paper

number 2 and subsequently stored at 4°C until phytochemical analysis. The experiment was designed to obtain an extraction medium for higher total phenolic (TP), total flavonoid (TF), total tannin (TT) content, and antioxidant activities through 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and ferric reducing antioxidant power (FRAP) assays. The extraction methodology was designed to maximize extraction efficiency while minimizing the thermal degradation of analytes. A detailed overview of the methodology adopted is provided in Figure 1.

2.3 | Quantification of Total Polyphenolics and Antioxidant Activities

Quantification of the total polyphenolics and antioxidant activities was conducted following previously described methods (Kewlani et al. 2022; Brand-Williams et al. 1995; Cai et al. 2004).

2.4 | Anti-Mutagenic Activity

Plasmid (pBR322) DNA was tested for DNA damage activity based on the method previously described (L. Singh et al. 2022). The plasmid DNA (2 µL, 180 ng) was damaged under UV radiation for 15 min in the presence of H₂O₂ (3.5%). Indicators like conversion of the plasmid supercoiled (S) DNA to the open-circular (OC) or linear (L) are marked as a sign of DNA damage. The extent of DNA damage and the preventive effect of the test samples were analysed on agarose gel (1.0%) in 1X TAE buffer run over 2 hours at 50 V at room temperature. The band density was calculated using the gel documentation system (UVI pro Platinum ver. 12.9, UK). The following equation was used to calculate the supercoiled DNA intensity:

$$S (\%) = [S \text{ band density} / \text{density of } (S + L + OC)] \times 100.$$

The recovery of supercoiled DNA was also calculated with reference to the positive control as relative supercoiled DNA (%) = S (%) of the test sample/S (%) of the positive control.

2.5 | Statistical Analysis

Statistical analysis was carried out using IBM Statistical Package for the Social Sciences (SPSS) version 26. All measurements were carried out in triplicates, and the results were expressed as mean ± standard deviation. Multivariate analysis of variance (MANOVA) was applied to assess the effects of solvent type, solvent concentration and plant part on polyphenolic content and antioxidant activities. Before conducting MANOVA, assumptions of normality and homogeneity of variance–covariance matrices were evaluated. Significant differences among treatments were determined using Duncan's multiple range test (DMRT) at $p < 0.05$. Pearson correlation analysis was also performed to evaluate the relationships between total phenolic content and antioxidant activities.

3 | Results and Discussion

In this study, phenolic compounds were extracted from *R. cordifolia* (leaf, stem and root) and *T. officinale* (leaf and root) using four solvents with different polarities (water > methanol > ethanol > acetone) at three concentrations (100%, 70% and 40%) prepared with distilled water.

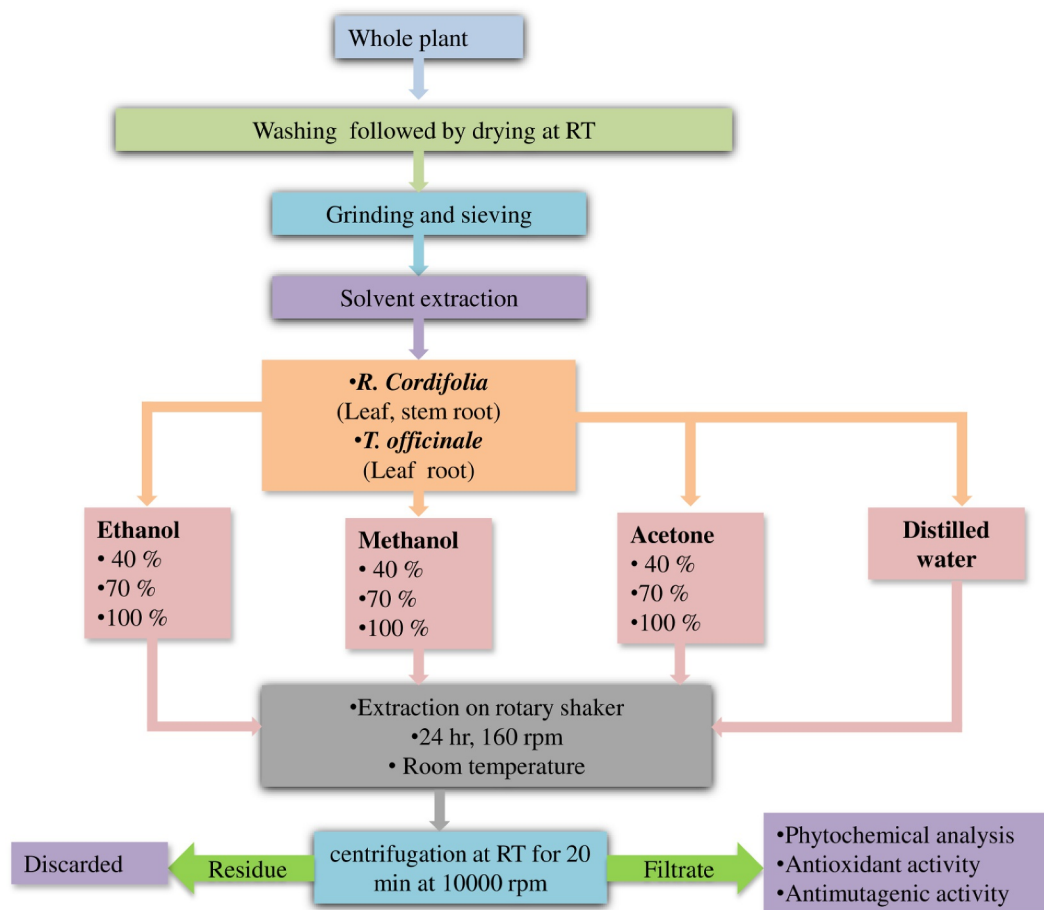


FIGURE 1 | Detailed flow diagram of the extraction methodology adopted in this study.

3.1 | Effect of Different Extraction Media and Concentrations on Polyphenols of Different Plant Parts

The analysis of total phenolics (TP), total flavonoids (TF) and total tannins (TT) revealed significant variations in response to different solvent systems and concentrations ($p < 0.05$). In *R. cordifolia*, significantly ($p < 0.05$) higher recovery of TP in leaf (35.11 ± 0.11 mg GAE/g DW) and stem (21.98 ± 0.90 mg GAE/g DW) was recorded in 70% methanol. In contrast, maximum TP content in the root (30.40 ± 0.24 mg GAE/g DW) was recorded in 70% acetone (Figure 2A). In *T. officinale*, maximum TP (leaf: 7.06 ± 0.40 mg GAE/g and root: 16.11 ± 0.23 mg GAE/g DW) was recorded in 70% ethanol and 70% methanol, respectively (Figure 3A). Further analysis demonstrated significantly higher TF content in the leaves (65.10 ± 0.23 mg QE/g DW), stem (33.83 ± 0.61 mg QE/g DW) and root (48.70 ± 0.11 mg QE/g DW) of *R. cordifolia* showing optimal yields in 70% ethanol, 40% acetone and 70% acetone, respectively (Figure 2B). In *T. officinale*, significantly ($p < 0.05$) higher TF in leaf (61.24 ± 0.4 mg QE/g DW) and root (31.28 ± 1.38 mg QE/g DW) was found in 70% and 40% ethanol, respectively (Figure 3B). Significantly ($p < 0.05$) higher TT in *R. cordifolia* (leaf: 14.97 ± 0.05 mg TAE/g, stem: 8.16 ± 0.06 mg TAE/g and root: 10.10 ± 0.02 mg TAE/g DW) and *T. officinale* (leaf: 11.78 ± 0.03 mg TAE/g and root: 6.13 ± 0.03 mg TAE/g DW) was found in 70% ethanol (Figures 2C and 3C).

The high recovery of these phenolic compounds may also be attributed to the 60°C extraction temperature used in this study. Although increasing temperature can enhance solvent penetration and solubility of phenolic compounds, many polyphenols, particularly flavonoids and tannins, are thermolabile and may undergo oxidative degradation or structural transformation at elevated temperatures (Khoddami et al. 2013). Therefore, 60°C was selected to balance efficient extraction kinetics with the preservation of heat-sensitive compounds, preventing the decline in total phenolic content often observed at higher temperatures (Ferreira et al. 2023). Furthermore, most phenolic compounds present in selected species are polar due to the presence of a hydroxyl group; therefore, polar solvents facilitate better extraction according to the principle of ‘like dissolves like’ (Ozturk et al. 2018). In the present study, the plant parts showed varying responses towards the solvents to recover maximum phenolic content as reported elsewhere (Allothman et al. 2009; Khoddami et al. 2013; Akinmoladun et al. 2010; Shahrajabian and Sun 2023). The noticeable diversity in chemical composition and antioxidant activity in different plants is generally attributed to different compositions of phenolic compounds in plant parts, growth period, sampling time, extraction solvent, extraction method employed and environmental factors for the study (Sylvie et al. 2014; Monteiro et al. 2020; Liu et al. 2022; Humbal et al. 2024).

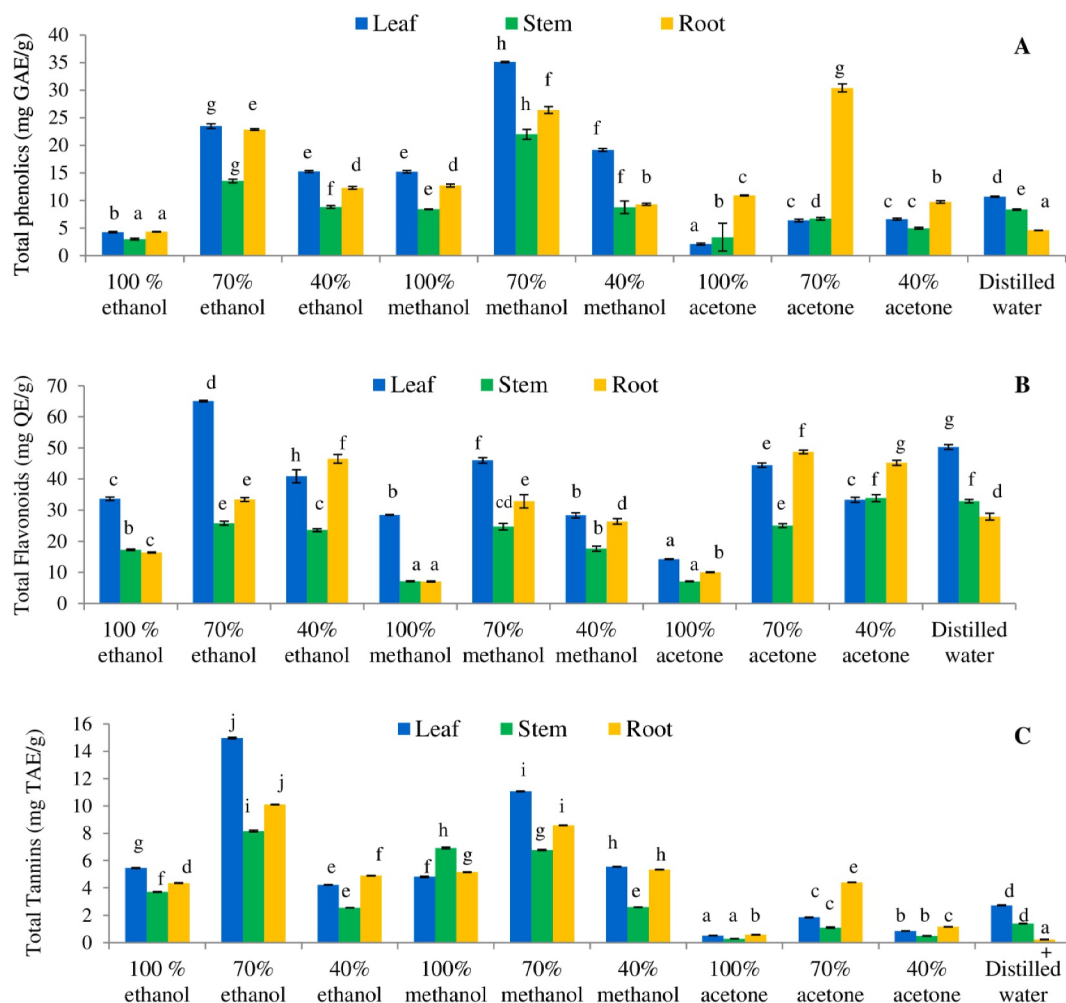


FIGURE 2 | Effect of different solvents and solvent concentrations on (A) total phenolics, (B) total flavonoids and (C) total tannins content in different plant parts of *Rubia cordifolia*. Means with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test (DMRT).

3.2 | Effect of Different Extraction Media and Concentrations on the Antioxidant Activity of Different Plant Parts

Significantly ($p < 0.05$) higher antioxidant activity in *R. cordifolia* using ABTS (leaf: 22.83 ± 0.13 mM AAE/g, stem: 22.27 ± 0.18 mM AAE/g and root: 28.12 ± 0.02 mM AAE/g DW) and DPPH (leaf: 3.4 ± 0.02 mM AAE/g, stem: 3.08 ± 0.02 mM AAE/g and root: 3.63 ± 0.02 mM AAE/g DW) was found in ethanol at the concentration 70% and 40%, respectively (Figure 4A,B), whereas significantly ($p < 0.05$) higher FRAP activity (leaf: 5.12 ± 0.03 mM AAE/g, stem: 3.29 ± 0.01 mM AAE/g DW and root: 5.53 ± 0.01 mM AAE/g DW) was found in 70% methanol (Figure 4C). Similarly, in *T. officinale*, significantly ($p < 0.05$) higher antioxidant activity using ABTS (leaf: 21.53 ± 0.21 mM AAE/g and root: 22.73 ± 0.10 mM AAE/g DW) and DPPH (leaf: 2.71 ± 0.02 mM AAE/g and root: 3.10 ± 0.01 mM AAE/g) was recorded in 70% and 40% ethanol, respectively (Figure 5A and B). In contrast, FRAP activity was found to be significantly ($p < 0.05$) higher (leaf: 2.99 ± 0.02 mM AAE/g and root: 1.87 ± 0.01 mM AAE/g DW) in 100% methanol (Figure 5C).

Maximum antioxidant activity using ABTS and DPPH was found in ethanol at concentrations of 70% and 40% for

R. cordifolia and *T. officinale*, respectively. In contrast, higher FRAP activity was found in methanol (70% methanol—*R. cordifolia* and 100% methanol—*T. officinale*). Notably, 70% ethanol provides maximum recovery of phenolic compounds and the strongest antioxidant activity. According to previous studies, aqueous ethanol solvents are most suitable for obtaining higher phenolic compounds and antioxidant activity in plant extracts (Horozic et al. 2023; Ozdemir et al. 2024). Moreover, ethanol is a less toxic solvent, exhibits relatively low environmental impacts and is acceptable for human consumption compared to other solvents (Durling et al. 2007).

Different plant parts of both species showed varied polyphenolic content and antioxidant activity. In *R. cordifolia*, maximum TP content was found in the leaf, followed by root and stem (Figure 2A), while *T. officinale* root had maximum TP content compared to the leaf (Figure 3A). The higher TF and TT content were exhibited in the leaves of both species, *R. cordifolia* (Figure 2B and C) and *T. officinale* (Figure 3B,C). The higher phenolic content (TP, TF and TT) in the leaf was also previously reported in medicinal plants (Senguttuvan et al. 2014; Xue et al. 2017). The higher antioxidant activity using ABTS, DPPH and FRAP was found in the root of *R. cordifolia*, followed by the leaf

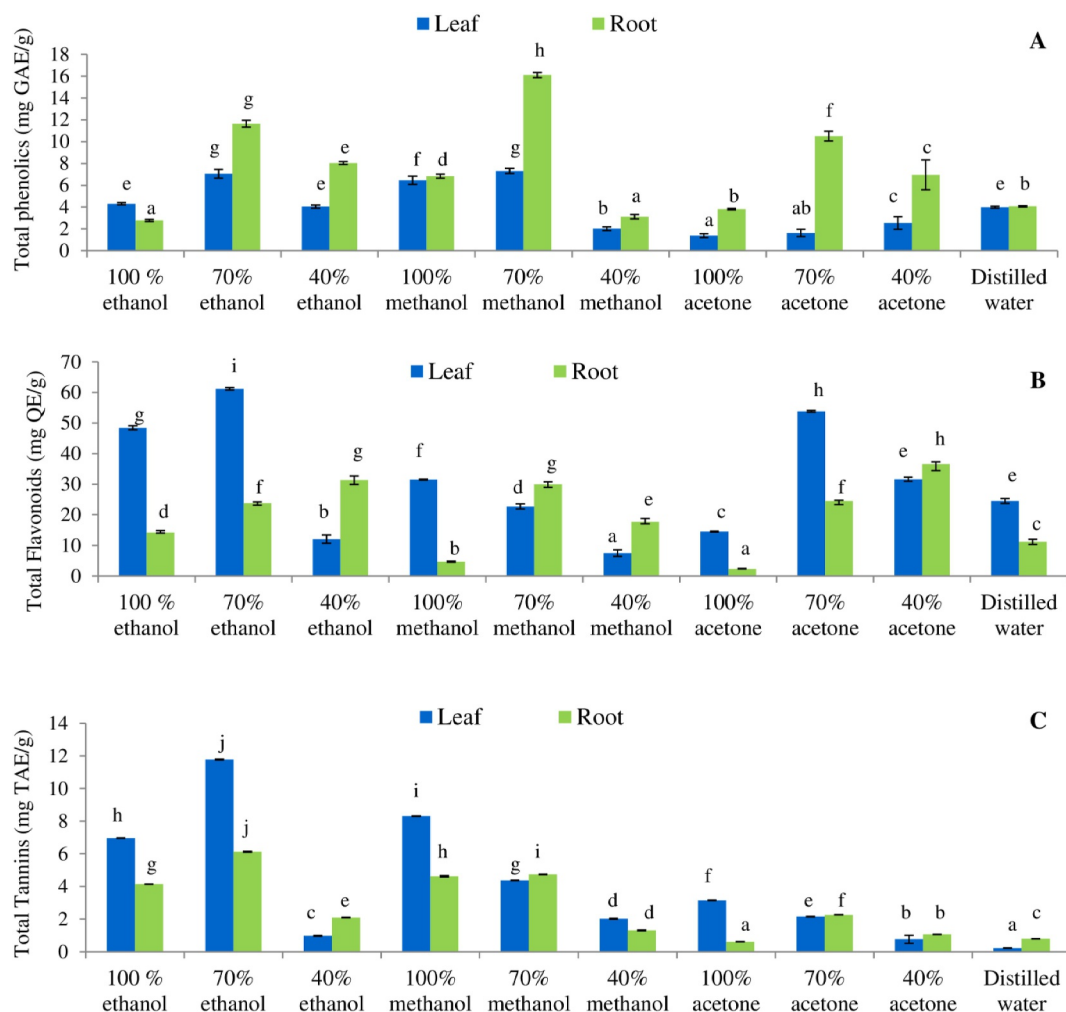


FIGURE 3 | Effect of different solvents and solvent concentrations on (A) total phenolics, (B) total flavonoids and (C) total tannins content in different plant parts of *Taraxacum officinale*. Means with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test (DMRT).

and stem (Figure 4A–C). In agreement with our results, Humbare et al. (2022) reported a similar variation in antioxidant activities in various parts of *R. cordifolia*. In *T. officinale*, maximum ABTS and DPPH activity was shown in the leaf, whereas higher FRAP activity was found in the leaf (Figure 5A–C). The earlier study also reported higher FRAP activity in the leaf (Aremu et al. 2019).

Several studies suggest that phenolic compounds such as anthocyanins, flavonoids, phenolic acids, and tannins are the major contributors to antioxidant activity in medicinal plants (Djeridane et al. 2006; Muanda et al. 2011; Hossain et al. 2020).

3.3 | Anti-Mutagenic Activity of Different Plant Parts Under Suitable Extraction Conditions

The extracts of different plant parts of both species showed considerable anti-mutagenic activities under suitable extraction conditions (70% ethanol). The damaged DNA, when treated with different concentrations of extracts (2, 4 and 8 $\mu\text{g}/\mu\text{L}$), showed significant recovery. The leaf extract of *R. cordifolia* had a maximum anti-mutagenic activity with recovery percentage

44.89% (2 $\mu\text{g}/\mu\text{L}$), 47.72% (4 $\mu\text{g}/\mu\text{L}$) and 49.21% (8 $\mu\text{g}/\mu\text{L}$) followed by stem extract with 38.50% (2 $\mu\text{g}/\mu\text{L}$), 40.61% (4 $\mu\text{g}/\mu\text{L}$) and 46.50% (8 $\mu\text{g}/\mu\text{L}$) and root extract with 26.59% at 2 $\mu\text{g}/\mu\text{L}$, 28.86% at 4 $\mu\text{g}/\mu\text{L}$ and 31.79% at 8 $\mu\text{g}/\mu\text{L}$, respectively (Table 1). The damaged DNA recovery percentage was compared with that of standard marker antioxidant compounds such as epigallocatechin and gallic acid. Of these, gallic acid showed higher anti-mutagenic activity (leaf: 72.59%, stem: 71.18% and root: 71.53%) than epigallocatechin (leaf: 69.04%, stem: 69.63% and root: 68.07%) at the concentration 4 $\mu\text{g}/\mu\text{L}$ (Table 1). On the contrary, the maximum anti-mutagenic activity in *T. officinale* was exhibited by the root with recovery percentages 52.30% (2 $\mu\text{g}/\mu\text{L}$), 56.65% (4 $\mu\text{g}/\mu\text{L}$) and 66.05% (8 $\mu\text{g}/\mu\text{L}$) compared to the leaf with 32.02%, 34.53% and 39.66% at 2, 4 and 8 $\mu\text{g}/\mu\text{L}$, respectively (Table 2). Between the standard compounds, gallic acid was found to exhibit higher anti-mutagenic activity (leaf –70.21% and root—74.52%) than epigallocatechin (leaf: 68.02% and root: 73.01%). Mutagens are chemical substances that increase the frequency of DNA modifications of an organism, resulting in a mutation, which is considered the initial stage of carcinogenesis (Hope Smith et al. 2004; Gerba 2019). Plants produce naturally occurring polyphenols with anti-mutagenic

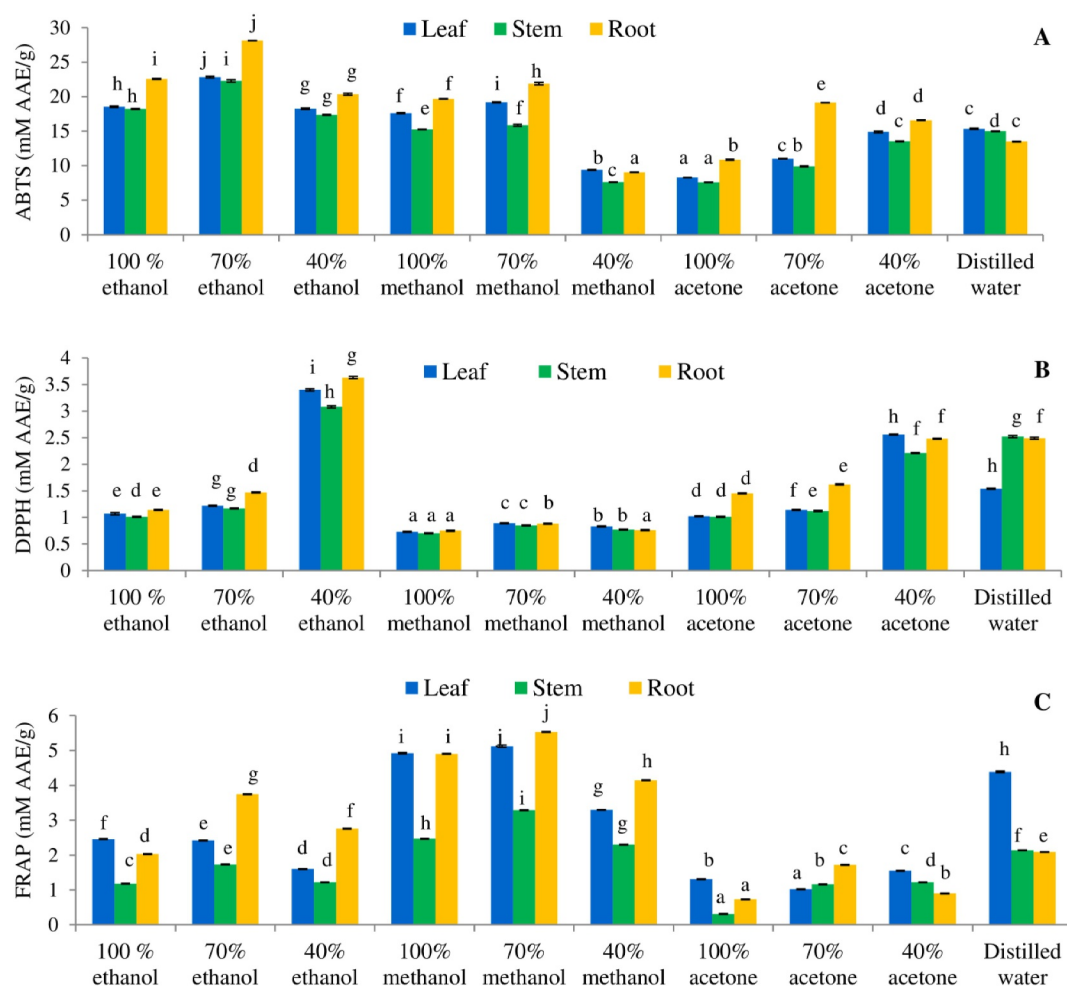


FIGURE 4 | Effect of different solvents and solvent concentrations on antioxidant activity using (A) ABTS, (B) DPPH and (C) FRAP in different plant parts of *Rubia cordifolia*. Means with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test (DMRT).

and anti-cancer activities, inhibiting multistage carcinogenesis pathways (B. Singh et al. 2026). In the present study, the extracts of plant parts of *R. cordifolia* and *T. officinale* possessed considerable protection activity against DNA damage. Similar results were also reported using the plant extracts of various Himalayan plants such as *Prinsepia utilis*, *Polygonatum verticillatum*, *Valeriana jatamansi* and *Valeriana himalayana* and in the different plants of Ashtvarga (Giri et al. 2017; Jugran et al. 2017; Suyal et al. 2021; Kewlani et al. 2022). The DNA damage protection activity of the plant extracts may be attributed to the presence of health-promoting polyphenolic compounds consistent with the previously reported study (Tang et al. 2013).

Free radicals, recognised as potent carcinogenic agents that induces oxidative damage to DNA strands, resulting in mutagenesis, genomic instability, and the initiation, promotion and progression tumorigenesis (Waris and Ahsan 2006). As numerous mutagens act via the generation of reactive oxygen species (ROS), the removal of these reactive molecules plays a crucial role in the process of anti-mutagenesis (Shay et al. 2009; Tian et al. 2012). An imbalance between the generation of ROS (singlet oxygen, anion radical, hydroxyl radical and hydrogen peroxide) and cellular antioxidant capacity can

lead to oxidative stress (Waris and Ahsan 2006; Tang et al. 2013). These ROS can oxidise cellular biomolecules such as carbohydrates, proteins, lipids and nucleic acids (Ozcan and Ogun 2015) and are associated with various degenerative diseases, including inflammatory diseases, ischaemic diseases and several neurological disorders such as Alzheimer's and Parkinson's diseases, immunodeficiency syndrome and many others (Lobo et al. 2010). There is increasing evidence that compounds with antioxidant properties can remove ROS before these molecules react with DNA, resulting in a mutation (Lee et al. 2011; Tian et al. 2012).

4 | Conclusion

In this study, the extraction medium significantly influenced the phenolic content and antioxidant activity in both selected species (*R. cordifolia* and *T. officinale*). Among the solvents tested, ethanol, methanol and acetone at different concentrations yielded the highest levels of total phenolics (TP), total flavonoids (TF) and total tannins (TT). The strongest antioxidant activity was observed in aqueous ethanol and methanol extracts of both species. Variation in phenolic composition was also noted among different plant parts, with leaves exhibiting the highest TP, TF and TT contents, whereas roots showed higher

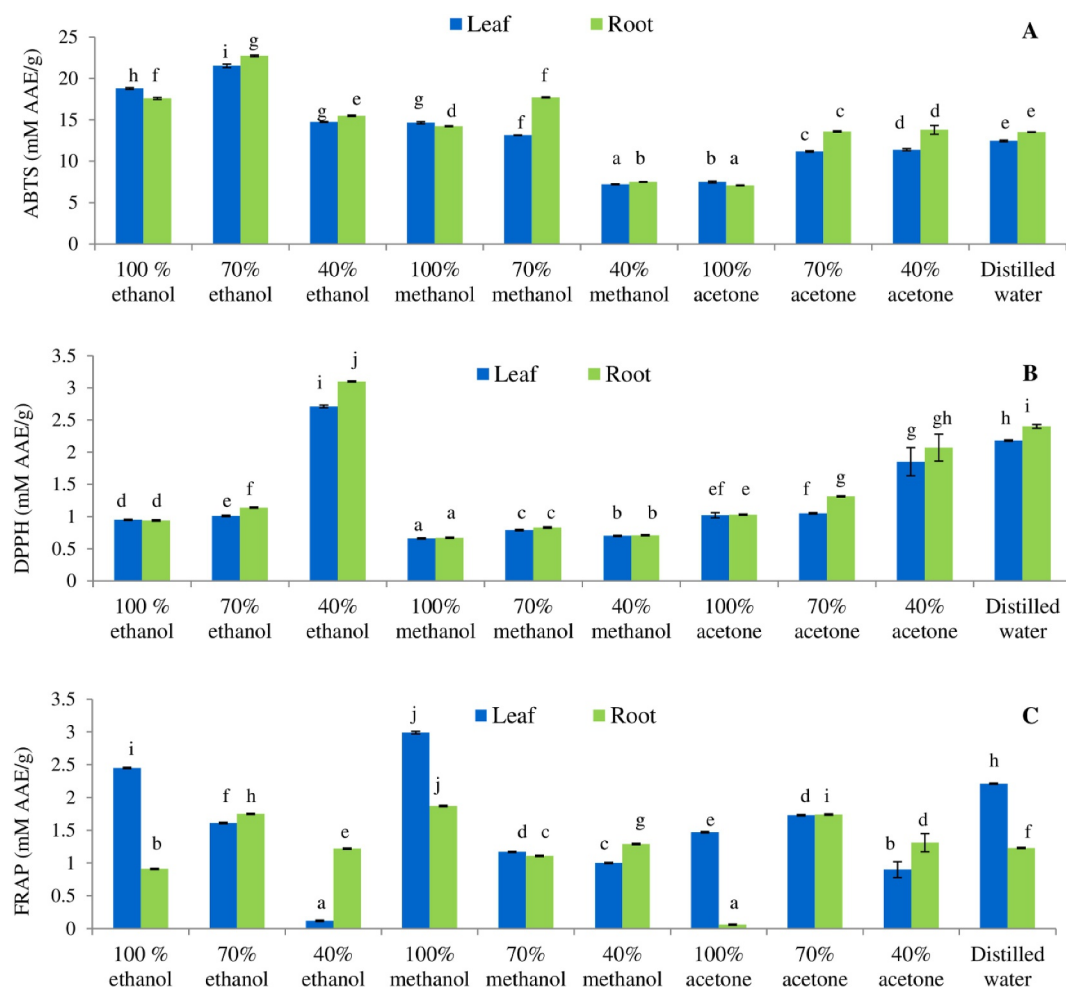


FIGURE 5 | Effect of different solvents and solvent concentrations on antioxidant activity using (A) ABTS, (B) DPPH and (C) FRAP in different plant parts of *Taraxacum officinale*. Means with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test (DMRT).

TABLE 1 | Anti-mutagenic activity of *Rubia cordifolia* (leaf, stem, and root) extract in optimal conditions.

Plant part	DNA form and recovery	Positive control (+C)	Negative control (+C)	Extract concentration			Polyphenolic standards	
	Percent			2 $\mu\text{g}/\mu\text{L}$	4 $\mu\text{g}/\mu\text{L}$	8 $\mu\text{g}/\mu\text{L}$	EP (4 $\mu\text{g}/\mu\text{L}$)	GA (4 $\mu\text{g}/\mu\text{L}$)
Leaf	Supercoiled (%)	79.33	0	35.60	37.85	39.04	54.77	57.58
	Recovery (%)			44.89	47.72	49.21	69.04	72.59
Stem	Supercoiled (%)	76.13	0	29.31	30.92	35.41	53.01	54.20
	Recovery (%)			38.50	40.61	46.50	69.63	71.18
Root	Supercoiled (%)	77.84	0	20.70	22.46	24.75	52.99	55.69
	Recovery (%)			26.59	28.86	31.79	68.07	71.53

Note: + C (positive control) = PBR322 alone and -C (negative control) = H_2O_2 treatment.
Abbreviations: EP, epigallocatechin and GA, gallic acid.

antioxidant activity. Both species demonstrated notable anti-mutagenic activity in 70% ethanol extracts, indicating this solvent as the most suitable extraction medium. These findings suggest that the studied species are promising sources of

polyphenols and may serve as natural antioxidants with potential therapeutic applications. Further studies are required to isolate and characterise the bioactive compounds responsible for the observed anti-mutagenic activity.

TABLE 2 | Anti-mutagenic activity of *Taraxacum officinale* (leaf and root) extract in optimal conditions.

Plant part	DNA form and recovery Percent	Positive control (+C)	Negative control (+C)	Extract concentration			Polyphenolic standards	
				2 µg/µL	4 µg/µL	8 µg/µL	EP (4 µg/µL)	GA (4 µg/µL)
Leaf	Supercoiled (%)	77.94	0	24.95	26.91	30.92	53.02	54.72
	Recovery (%)			32.02	34.53	39.66	68.02	70.21
Root	Supercoiled (%)	82.17	0	42.98	46.55	54.27	60.00	61.23
	Recovery (%)			52.30	56.65	66.05	73.01	74.52

Note: + C (positive control) = PBR322 alone and –C (negative control) = H₂O₂ treatment.
Abbreviations: EP, epigallocatechin; GA, gallic acid.

Author Contributions

Deepti Tiwari: conceptualization, investigation, writing – original draft, methodology, validation, formal analysis, data curation, software.
Pushpa Kewlani: methodology, validation, formal analysis. **Laxman Singh:** investigation, writing – original draft, validation, writing – review and editing, formal analysis. **Indra Dutt Bhatt:** conceptualization, funding acquisition, visualization, writing – review and editing, project administration, supervision, resources. **R. C. Sundriyal:** conceptualization, investigation, project administration, supervision.

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Ethics Statement

Plant samples were collected with prior authorization from the relevant authorities and identified. All analyses were conducted in accordance with established laboratory protocols. The authors declare no conflict of interest, whether financial or personal. This study, being a preliminary phytochemical investigation, did not involve human participants, animals or clinical trials; ethical approval was not applied in this case study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used and/or analysed during the current study are available upon reasonable request to the corresponding author.

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Supporting Information

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

Supporting Information S1: pfm270010-sup-0001-suppl-data.docx.

Table S1: Scientific name, traditional uses and pharmacological activities of selected medicinal plants.