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Evaluation of antioxidant activity and identification of phyto-constituents in *Galium rotundifolium*

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ABSTRACT

The present study investigates the phytochemical composition, physicochemical properties, and antioxidant potential of Galium rotundifolium *extracts. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, and proteins, with notable variations between methanolic (GRM) and aqueous (GRA) extracts. Methanolic extracts exhibited higher levels of alkaloids (++), flavonoids (++), and moderate alcohol-soluble extractives (18.79 ± 2.03% w/w), while aqueous extracts demonstrated enhanced carbohydrate content (+++). Both extracts showed significant tannin and phenolic content (++), supporting their medicinal potential. Physicochemical analysis indicated a slightly acidic pH (6.16 ± 0.02), low moisture (4.94 ± 0.20% w/w), and high hydrophilic phytochemical content (37.90 ± 1.91% w/w), ensuring extract stability and suitability for pharmaceutical formulations. Antioxidant activity assessed using DPPH and ABTS assays confirmed the superior performance of GRM, with lower IC50 values (126.11 mg/ml and 168.47 mg/ml) compared to GRA. The findings align with previous studies on related* Galium *species, highlighting* G. rotundifolium *as a promising source of bioactive compounds with antioxidant, antimicrobial, and anti-inflammatory potential.*

Keywords: *Galium rotundifolium*, phytochemical analysis, antioxidant activity, flavonoids, tannins, phenolics, methanolic extract, aqueous extract, DPPH assay, ABTS assay, pharmaceutical potential, bioactive compounds

INTRODUCTION

Oxidative stress, caused by an imbalance between free radicals and antioxidants in the body, plays a pivotal role in the pathogenesis of various chronic diseases such as cancer, diabetes, and cardiovascular disorders. Natural antioxidants derived from plants have garnered significant attention due to their potential to neutralize free radicals and mitigate oxidative damage, making them promising candidates for therapeutic development (Pérez-Torres *et al.,* 2021, Gautam *et al.,* 2024, Binepal *et al.,* 2013 & Akbari *et al.,* 2022) Galium rotundifolium, a medicinal plant belonging to the Rubiaceae family, is known for its diverse bioactive compounds, including phenolics, flavonoids, and tannins, which contribute to its antioxidant properties (Fici, 1992 & Friščić *et al.,* 2018). Traditionally, members of the Galium genus have been employed in folk medicine for their anti-inflammatory, antimicrobial, and wound-healing properties. However, the antioxidant potential and detailed phytochemical composition of G. rotundifolium remain largely unexplored. Insights into traditional medicinal practices, such as those documented among the Gaddi shepherds and other communities, highlight the crucial role of high-altitude plants in ethnomedicine (Dutt *et al.,* 2015; Singh, 2012 & Amjad *et al.,* 2015). The diversity and conservation status of Himalayan flora further underscore the significance of plants like G. rotundifolium (Balkrishna *et al.,* 2020).

The current study focuses on evaluating the antioxidant activity and identifying the phytoconstituents of G. rotundifolium collected from the higher altitudes of the Almora district, Uttarakhand, India. The plant material was sourced from an elevation characterized by pristine environmental conditions conducive to the biosynthesis of secondary metabolites. Specifically, the collection site is located at latitude 29.5970°N and longitude 79.6568°E, providing a unique opportunity to study the phytochemical profile of plants thriving in high-altitude ecosystems (Agrawal, 2001). This investigation aims to establish a comprehensive understanding of the

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antioxidant potential of G. rotundifolium by employing in vitro assays, such as DPPH and ABTS radical scavenging methods, alongside enzymatic activity analyses. Furthermore, this study seeks to characterize its phytochemical constituents, including total phenolics, flavonoids, tannins, and carbohydrates, thereby contributing valuable insights into the medicinal significance of high-altitude flora.

Scientific Classification	
Plant Identification by	**Botanical Survey of India Dehradun**
Accession No.	**1377**
Botanical Name	***Galium rotundifolium* L.**
Kingdom	Plantae
Order	Gentianales
Family	Rubiaceae
Genus	Galium
Species	*Galium rotundifolium*

Fig 1: "Taxonomic Identification and Botanical Classification of *Galium rotundifolium* L.

MATERIALS AND METHODS

Plant material collection and extraction

Galium rotundifolium was collected from Almora (2212 m altitude) and identified by the Botanical Survey of India, with a voucher specimen (No. 1377) deposited at BSI, Dehradun. The plant material was washed, shade-dried, and ground into powder. A 50 g sample was extracted with 80% methanol using a Soxhlet apparatus or maceration for 48 hours, then filtered, concentrated under reduced pressure, and stored at 4°C for further analysis.

Physicochemical parameters and preliminary phytochemical screening

Physicochemical parameters, including moisture content, ash values, and extractive values, were assessed following WHO (2011) guidelines. Phytochemical screening was performed qualitatively to detect alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, and glycosides using protocols by Harborne (1998) and Kokate (1994). All tests were conducted in triplicate for accuracy and reproducibility.

Phytochemical analysis

Total phenols and flavonoids contents in the plant extracts were analyzed by UV spectroscopy following the standard procedures outlined by WHO (1998), McDonald (2001), and Chang *et al.* (2003). Total carbohydrates were determined according to the method by Yemm and Willis (1954), while total tannins were quantified based on the procedure described by Scalbert (1992).

Antioxidant activity assays

Antioxidant activity was assessed using DPPH and ABTS assays. For DPPH, 0.1 mL of extract (20–120 mg/mL) was mixed with 3 mL of 0.1 mM DPPH solution, incubated for 30 minutes in the dark, and absorbance measured at 517 nm. For ABTS, an ABTS cation solution was prepared, incubated in the dark for 16 hours, and diluted to an absorbance of 0.7 at 734 nm. Extracts were mixed with the diluted ABTS solution, and absorbance was measured after 6 minutes at 734 nm. Ascorbic acid was used as the standard in both assays.

$$\%\ Radical\ Scavenging = \frac{(\text{Acontrol} - \text{Asample})}{(\text{Acontrol})} \mathbf{X\ 100}$$

Where, A control and A sample are the absorbances of the control and sample, respectively.

Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed and expressed

as mean ± standard deviation (SD). Statistical analysis and plotting were performed using GraphPad Prism software.

RESULTS AND DISCUSSION

The phytochemical screening of *Galium rotundifolium* extracts, namely methanolic extract (GRM) and aqueous extract (GRA), revealed significant differences in the presence of bioactive constituents (Table 1).

Preliminary Phytochemical Screening

Phytochemical analysis revealed that both extracts exhibited the presence of alkaloids, with a higher concentration in GRM (++ compared to + in GRA). Saponins were detected in both extracts with comparable intensity (+). Carbohydrates showed a higher concentration in GRA (+++) compared to GRM (++), while glycosides were present in both extracts with similar intensity (+). Flavonoids were more abundant in GRM (++ compared to + in GRA). Both extracts lacked steroids and triterpenoids (-), as well as fats and oils (-). Proteins and amino acids were detected in both extracts (+), and tannins and phenolics were abundant in both (++) (Aslantürk *et al.,* 2017; Abrar *et al.,* 2023). These results illustrate the solvent-dependent extraction efficiency of different phytoconstituents from *Galium rotundifolium*. The higher extraction of carbohydrates in the aqueous extract (GRA) is attributed to the water-solubility of polysaccharides and simple sugars (Hanganu *et al.,* 2018). Conversely, GRM exhibited higher levels of flavonoids and alkaloids, likely due to the ability of methanol to solubilize more nonpolar or slightly polar compounds (Ilina *et al.,* 2019; Shynkovenko *et al.,* 2017). The significant presence of alkaloids, flavonoids, glycosides, and tannins/phenolics highlights the medicinal potential of *G. rotundifolium* for pharmacological applications, including antioxidant, antimicrobial, and anti-inflammatory activities (Laanet *et al.,* 2023; Kanso *et al.,* 2024; Dutt *et al.,* 2015). Additionally, the absence of steroids and fats suggests a lesser role for lipophilic constituents in these extracts (Ghita *et al.,* 2012).

Table 1: Preliminary Phytochemical screening in Aqueous and Methanolic extract of *Galium rotundifolium*

Phytochemical constituents	GRM	GRA
Alkaloids (Mayer's test/ Dragendorff's test)	++	+
Saponins (Frothing test)	+	++
Carbohydrates (Molisch's test/ Fehling's test)	++	+++
Glycosides (Borntrager's test/Legal's test)	+	++
Flavonoids (Shinoda test)	++	++
Steroids and triterpenoids	-	+
Test for Proteins/Amino acids	+	++
Tannins/Phenolics	++	++
Fats/oils	-	-

(+) present; (-) absent: *Galium rotundifolium* Methanolic (GRM); *Galium rotundifolium Aqueous* (GRA)

Physicochemical Parameters

The physicochemical analysis of the plant extract revealed a pH of 6.163 ± 0.021 in a 10% aqueous solution, indicating a slightly acidic nature typical of herbal extracts, suitable for medicinal applications. The moisture content, measured at 105°C, was 4.940 ± 0.197% w/w, suggesting good storage stability and resistance to microbial growth. The total ash content, representing the mineral composition, was 3.813 ± 0.211% w/w, aligning with quality standards for *Galium* species, while the acid-insoluble ash content was minimal at 0.377 ± 0.453% w/w, indicating high purity and low contamination. A high water-soluble extractive content of 37.897 ± 1.909% w/w reflects abundant hydrophilic phytochemicals, making the extract well-suited for aqueous-based formulations. Additionally, the alcohol-soluble extractive content of 18.790 ± 2.032% w/w suggests a significant presence of moderately polar bioactive compounds such as flavonoids and phenolics, further supporting its potential in pharmaceutical applications.

The findings for *Galium rotundifolium* align closely with those of related species such as *Galium aparine* and *Galium odoratum*. Previous studies have documented pH values in the range of 5.5 to 6.5 for 10% aqueous solutions of *Galium* species, consistent with the pH of *G. rotundifolium* (Gayibova and Aripov 2019 & Kahkeshani, *et al.,* 2013). Similarly, the total ash content of *G. aparine* at 4.0 ± 0.3% (Al-Snafi 2020) is comparable to the 3.813% found in *G. rotundifolium*, while its low acid-insoluble ash content, under 1%, reflects good processing quality (Al-Snafi 2020). The strong extraction yields are evident from water-soluble extractive values for *G. odoratum*, which exceed 35% in some analyses, paralleling those of *G. rotundifolium* (León *et al.,* 2018), while alcohol-soluble extractive contents ranging between 15-20% across related species are similarly consistent with the values reported for *G. rotundifolium* (Mocan *et al.,* 2016; Bradic *et al.,* 2021).

Table 2: Physiochemical parameters of *Galium rotundifolium*

Parameters	Results
pH (10% aqueous solution (v/w)	6.163±0.021
Loss on drying at 105°C (% w/w)	4.940±0.197
Total Ash (% w/w)	3.813±0.211
Acid-insoluble ash (% w/w)	0.377±0.453
Water-soluble extractive (% w/w)	37.897±1.909
Alcohol-soluble extractive (% w/w)	18.790±2.032

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD)

Phytochemical Analyses

The Phytochemical profile of *Galium rotundifolium* reveals distinct characteristics in its carbohydrate, protein, and bioactive compound content. The carbohydrate content of 7.26 ± 0.20 g/100g aligns with values reported for related species like *G. aparine* (6.80–8.50 g/100g), suggesting functional parallels within the genus (Yildirim *et al.,* 2001). The protein content, measured at 8.88 ± 1.38 g/100g, surpasses that of *G. odoratum* (5.45 ± 0.85 g/100g), potentially reflecting ecological or genetic factors influencing nitrogen metabolism (Fici, 1992). Notable bioactive compound levels include tannins (33.20 ± 0.0020 mg/g) and phenolics (35.31 ± 0.0025 mg/g), slightly exceeding the ranges reported for *G. verum* (25.0–30.0 mg/g for tannins and 32.0–35.0 mg/g for phenolics) (Semenescu *et al.,* 2023), highlighting its enhanced antioxidant potential and traditional medicinal value (Singh, 2012). Moreover, the flavonoid content (116.24 ± 0.030 mg/g) significantly surpasses that of species like *G. aparine* (80.0–95.0 mg/g), likely due to environmental or genetic influences favoring secondary metabolite accumulation (Balkrishna *et al.,* 2020).

Table 3: Amount of Phytoconstituent in *Galium rotundifolium*

Phytoconstituents	Amount of Phytoconstituents
Carbohydrate (g/100g)	7.26±0.20
Tannin (mg/g)	33.20±0.0020
Phenolic (mg/g)	35.31±0.0025
Protein (g/100g)	8.88±1.38
Flavonoid (mg/g)	116.24±0.030

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD).

Antioxidant Activity of Galium rotundifolium Extracts

The antioxidant activity of *Galium rotundifolium* was assessed using DPPH and ABTS radical scavenging assays, with methanolic and aqueous extracts tested at concentrations ranging from 20 to 120 mg/ml. In both assays, the methanolic extract consistently demonstrated higher antioxidant activity than the aqueous extract, with percentage inhibition of free radicals increasing with concentration. For the DPPH method, the methanolic extract showed inhibition ranging from 26.19% to 48.67%, while the aqueous extract ranged from 22.18% to 44.66%, with IC50 values of 126.11 mg/ml and 146.01 mg/ml, respectively (Aslantürk *et al.,* 2017). Similarly, in the ABTS assay, the methanolic extract exhibited percentage inhibition ranging from 27.74% to 44.52%, compared to 27.91% to 37.75% for the aqueous extract, with IC50 values of 168.47 mg/ml and 198.67 mg/ml, respectively (Friščić *et al.,* 2016).

Table 4: Free Radical scavenging activity of *Galium rotundifolium* by DPPH and ABTS method

Sample Name	*Galium rotundifolium* Methanolic	*Galium rotundifolium* Aqueous
Amount mg/ml	% Inhibition in DPPH method	
20 mg/ml	26.19	22.18
40 mg/ml	30.20	26.32
60 mg/ml	34.25	31.76
80 mg/ml	39.30	35.16
100 mg/ml	44.66	39.48
120 mg/ml	48.67	44.66
IC 50 mg/ml	126.11	146.01
Amount mg/ml	% Inhibition in ABTS method	
20 mg/ml	27.74	27.91
40 mg/ml	30.76	29.31
60 mg/ml	32.77	30.65
80 mg/ml	35.35	32.94
100 mg/ml	39.49	34.51
120 mg/ml	44.52	37.75
IC 50 mg/ml	168.47	198.67

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD)

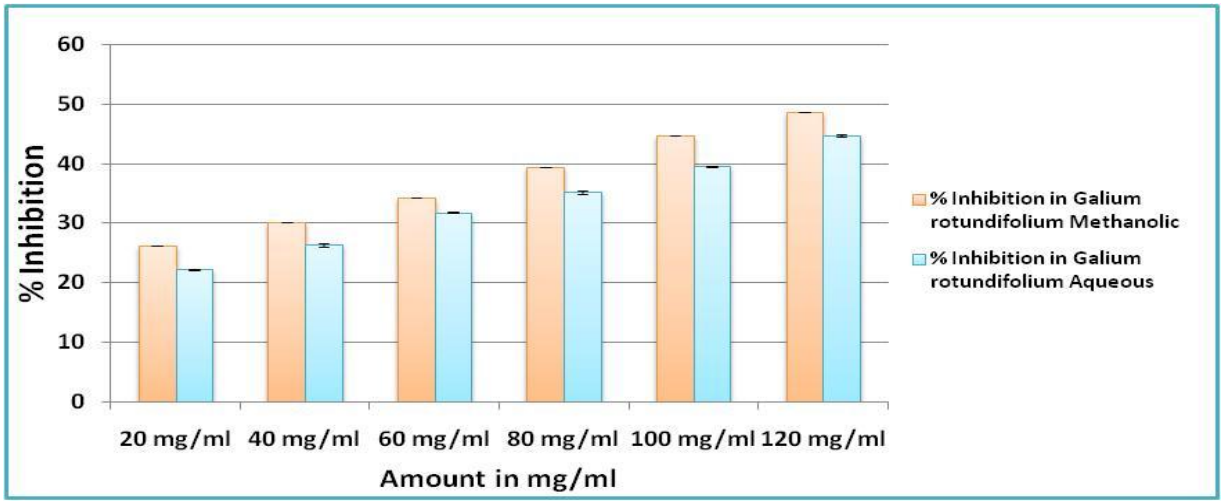

Fig 1: Antioxidant activity in *Galium rotundifolium* using DPPH assay in different solvent

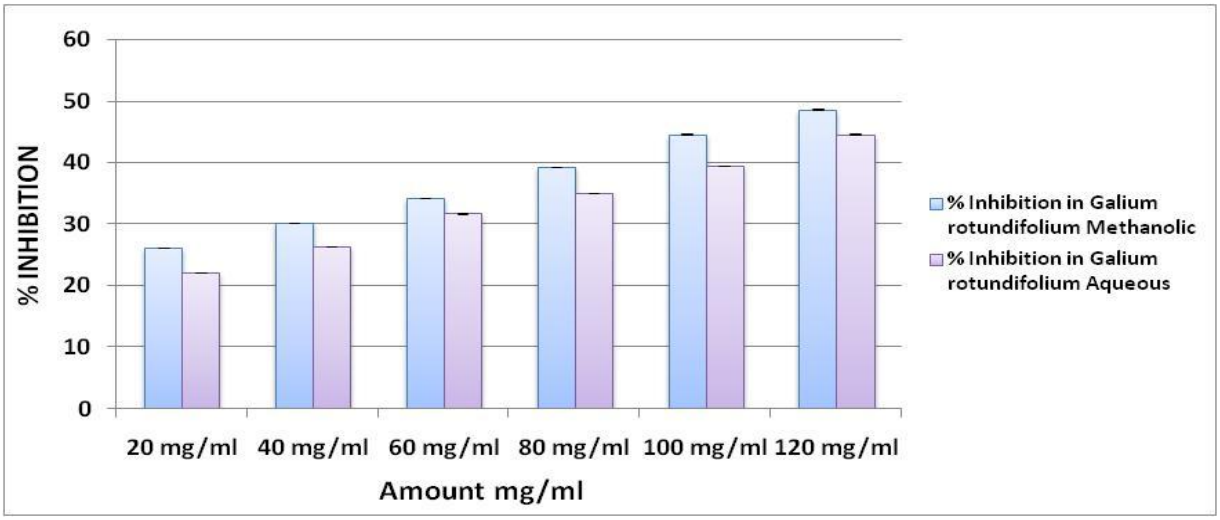

Fig 2: Antioxidant activity in *Galium rotundifolium* using ABTS assay in different solvents

Conclusion

The phytochemical and physicochemical analysis of *Galium rotundifolium* extracts underscores the medicinal potential of the plant, marked by a rich profile of bioactive compounds. Methanolic and aqueous extracts reveal complementary profiles, with the methanolic extract showing a higher concentration of flavonoids and alkaloids, while the aqueous extract exhibits higher carbohydrate levels. The significant presence of glycosides, tannins, phenolics, and proteins in both extracts highlights their suitability for pharmacological applications such as antioxidant, antimicrobial, and anti-inflammatory therapies. Additionally, the absence of steroids and fats emphasizes the hydrophilic and non-lipophilic nature of the extracts, supporting their relevance for aqueous- and alcohol-based formulations.

The physicochemical properties, including a slightly acidic pH, low moisture and ash contents, and high extractive yields, indicate good storage stability, processing quality, and suitability for medicinal use. Comparative analysis with related species like *G. aparine* and *G. odoratum* confirms the quality and reliability of the findings. Furthermore, higher levels of flavonoids and phenolics in *G. rotundifolium* reflect superior antioxidant activity, with methanolic extracts exhibiting greater free radical scavenging efficiency in DPPH and ABTS assays.

Future Prospects

Further studies should focus on isolating and characterizing the individual bioactive constituents responsible for the observed antioxidant activity. Investigating the synergistic effects of these phytochemicals could unlock enhanced pharmacological applications. Additionally, evaluating the antimicrobial and anti-inflammatory properties of *G. rotundifolium* extracts in vitro and in vivo will expand its medicinal potential. Application-driven research should explore its integration into drug delivery systems and nutraceutical formulations, particularly emphasizing flavonoid-rich compounds. Comparative analysis across various ecological conditions and genetic variants of *G. rotundifolium* can provide insights into the influence of environmental factors on its phytochemical profile.

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