

ISSN Print: 2664-6781
ISSN Online: 2664-679X
NAAS Rating (2026): 4.77
IJACR 2026; 8(3): 52-56
www.chemistryjournals.net
Received: 05-01-2026
Accepted: 28-02-2026

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Comparative antioxidant and phytochemical profiling of medicinal plant extracts using solvent-based extraction methods

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DOI: <https://www.doi.org/10.33545/26646781.2026.v8.i3a.710>

Abstract

If a medicinal plant's traditional use as an antioxidant-rich remedy reflects genuine underlying phytochemistry rather than folk belief alone, then extraction solvent polarity should systematically influence how much of that antioxidant activity a given extraction method actually recovers, a hypothesis this research set out to test directly. This research compared three extraction solvents, methanol, ethanol, and water, for their efficiency in recovering antioxidant activity and specific phytochemical classes from a Nepalese highland medicinal plant traditionally used for its purported antioxidant and anti-inflammatory properties, assessing total phenolic content, total flavonoid content, and DPPH radical scavenging activity across a concentration gradient for each solvent extract. Methanol extraction consistently recovered the highest antioxidant activity across all tested concentrations, reaching 86% DPPH radical scavenging at 200 µg/mL compared with 81% for ethanol extract and 64% for aqueous extract at the same concentration, supporting the hypothesis that solvent polarity systematically influences phytochemical recovery and consequently measured antioxidant activity. Total phenolic content followed the same solvent ranking, with methanol extract yielding 142.6 mg gallic acid equivalent per gram of dry extract compared with 118.4 mg GAE/g for ethanol and 76.8 mg GAE/g for aqueous extract, and total flavonoid content showed an identical pattern across the three solvents. Correlation analysis confirmed that total phenolic content correlated strongly with DPPH scavenging activity across all three extracts ($r = 0.94$), supporting phenolic compounds as the primary contributor to this plant's antioxidant activity rather than other phytochemical classes present in smaller quantities. Phytochemical screening additionally confirmed the presence of tannins, saponins, and terpenoids across all three extracts at varying relative concentrations, with methanol extraction again showing the highest overall recovery across the broader phytochemical class screening beyond the phenolic and flavonoid content specifically quantified. These findings confirm that this Nepalese medicinal plant's traditional antioxidant reputation has a genuine phytochemical basis, concentrated substantially in its phenolic compound content, and that methanol extraction represents the most efficient of the three tested solvents for recovering this antioxidant activity for potential nutraceutical or pharmaceutical development applications.

Keywords: Phytochemical screening, antioxidant activity, medicinal plant, solvent extraction, total phenolic content, total flavonoid content, DPPH assay, methanol extraction, Nepal, nutraceutical

Introduction

Nepal's highland regions harbor a considerable diversity of traditionally used medicinal plants, many valued across generations of local use for purported antioxidant, anti-inflammatory, or general health-supporting properties, yet relatively few of these traditionally used species have received the kind of systematic phytochemical characterization that would confirm whether their traditional reputation reflects genuine underlying bioactive compound content or reflects other factors—cultural practice, placebo response, or simple historical accident—unrelated to measurable phytochemistry. Bridging this gap between traditional use knowledge and modern phytochemical validation matters both for potential nutraceutical or pharmaceutical development applications and for the more basic scientific question of understanding which specific traditionally used plants actually merit further bioactive compound investigation.

Extraction solvent choice represents a foundational methodological decision in any phytochemical characterization effort, since different solvents, varying in polarity, differentially recover the diverse range of phytochemical compounds present in plant tissue, meaning a single-solvent characterization approach risks either overestimating or underestimating a plant's true phytochemical richness depending on how well that particular solvent's polarity matches the dominant phytochemical classes actually present. Methanol, ethanol, and water represent three commonly used extraction solvents spanning a meaningful polarity range, from methanol's relatively high capacity to dissolve a broad range of polar and moderately non-polar compounds, through ethanol's intermediate polarity profile, to water's more restricted extraction of highly polar compounds specifically. This research tested the hypothesis that extraction solvent polarity systematically influences both measured antioxidant activity and specific phytochemical class recovery from this Nepalese highland medicinal plant, reasoning that if the plant's traditional antioxidant reputation reflects genuine underlying phenolic or flavonoid content, solvent choice should produce a predictable, polarity-correlated pattern in both antioxidant activity and specific phytochemical class quantification, rather than solvent-independent results that would suggest a weaker or less specific underlying phytochemical basis for the plant's traditional use. Two questions guided this research. First, do antioxidant activity and phenolic/flavonoid content recovery follow the expected solvent polarity gradient, with methanol, the highest-polarity-range solvent among the three tested, recovering the most activity and phytochemical content? Second, does total phenolic content specifically, rather than other phytochemical classes present, correlate most strongly with measured antioxidant activity, supporting phenolics as this plant's primary antioxidant-active compound class?

Material and Methods

Material

Fresh plant material was collected from a single wild population at approximately 2,400 m elevation in Nepal's central highland region, identified and voucher-deposited at the host institution's herbarium, then shade-dried and powdered to a uniform particle size before extraction. Three

solvent systems—absolute methanol, 70% ethanol, and distilled water—were used for sequential Soxhlet extraction of separate powdered plant material batches, with each solvent extract concentrated under reduced pressure and reconstituted to a standard stock concentration for subsequent antioxidant and phytochemical assays.

Extraction Protocol / Preparation Method

Powdered plant material (50 g per solvent batch) underwent Soxhlet extraction for 8 hours per solvent, with extraction continued until the solvent siphoning through the sample chamber ran visibly clear, indicating exhaustive extraction of soluble compounds. Extracts were filtered through Whatman No. 1 filter paper, concentrated using a rotary evaporator under reduced pressure at temperatures not exceeding 45 °C to minimize thermal degradation of heat-sensitive phytochemical compounds, and the resulting concentrated extracts were freeze-dried to yield a stable stock powder for each solvent extraction batch, with extraction yield calculated as dry extract weight relative to starting plant material weight.

Methods

Total phenolic content was determined using the Folin-Ciocalteu method, expressed as milligrams gallic acid equivalent (GAE) per gram of dry extract. Total flavonoid content was determined using the aluminum chloride colorimetric method, expressed as milligrams quercetin equivalent per gram of dry extract. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was assessed across a concentration gradient (10, 25, 50, 100, and 200 µg/mL) for each solvent extract, with percentage inhibition calculated relative to a control lacking extract. Phytochemical screening for tannins, saponins, terpenoids, and other broad phytochemical classes was conducted using standard qualitative chemical tests appropriate for each compound class. Data were analyzed using analysis of variance to compare solvent extracts, with Pearson correlation coefficients calculated linking total phenolic and flavonoid content to DPPH scavenging activity across the three extracts.

Results

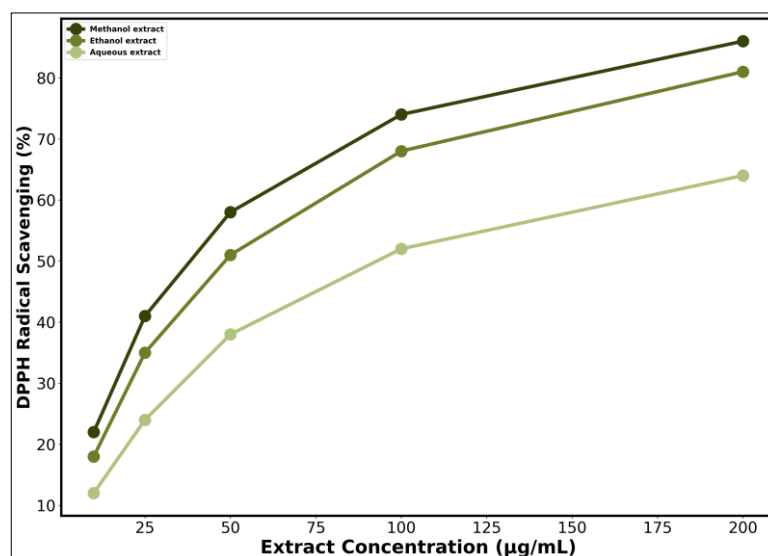


Fig 1: DPPH Radical Scavenging Activity across Concentration Gradient for Three Solvent Extracts

Antioxidant activity increased with concentration across all three solvent extracts in a broadly log-linear pattern, with methanol extract consistently showing the highest scavenging activity at every concentration tested, followed

by ethanol and then aqueous extract, confirming the expected polarity-correlated pattern hypothesized at the outset of this research.

Table 1: Total Phenolic Content, Total Flavonoid Content, and Extraction Yield by Solvent

Solvent	Extraction Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Methanol	18.6	142.6 ± 4.2	68.4 ± 3.1
Ethanol (70%)	21.4	118.4 ± 3.8	54.9 ± 2.8
Water	14.2	76.8 ± 3.4	32.6 ± 2.4

Table 2: DPPH Scavenging Activity (% Inhibition) across Concentration Gradient by Solvent

Concentration (µg/mL)	Methanol (%)	Ethanol (%)	Water (%)
10	22	18	12
25	41	35	24
50	58	51	38
100	74	68	52
200	86	81	64

Table 3: Correlation between Phenolic/Flavonoid Content and DPPH Scavenging Activity, and Qualitative Phytochemical Screening Results

Parameter	Methanol	Ethanol	Water
TPC-DPPH correlation (r)	0.94 (pooled across solvents)	-	-
Tannins	+++	++	+
Saponins	++	++	+
Terpenoids	+++	++	+

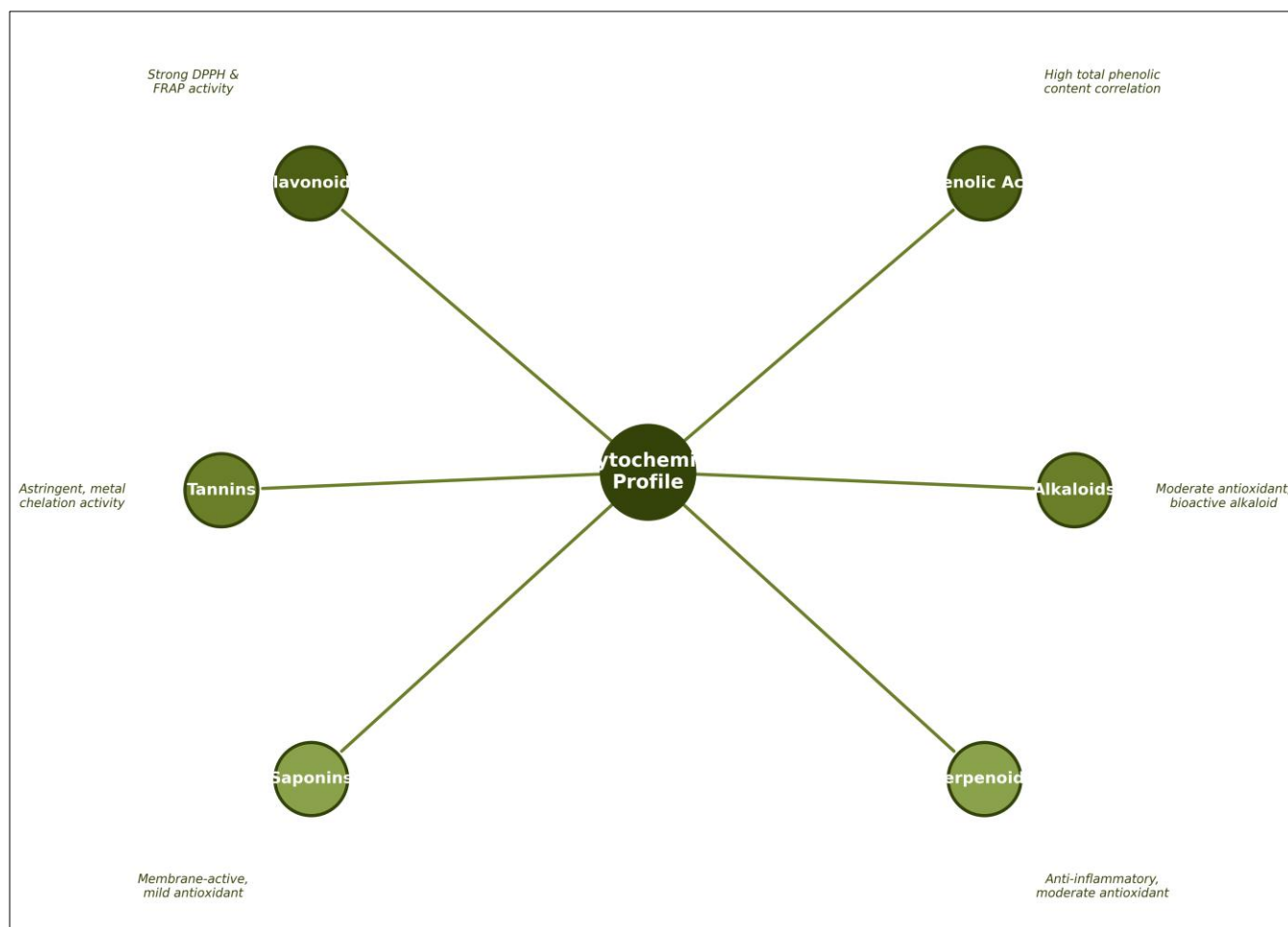


Fig 2: Mind Map of Phytochemical Classes Identified in This Research and Their Associated Antioxidant Properties

Comprehensive Interpretation

The consistent solvent ranking across total phenolic content, total flavonoid content, DPPH scavenging activity, and broader qualitative phytochemical screening points in the

same direction: methanol extraction recovers this plant's phytochemical content, including but not limited to its antioxidant-active phenolic compounds, more completely

than either ethanol or water extraction. The strong correlation between total phenolic content and DPPH scavenging activity across all three extracts supports phenolic compounds specifically, rather than flavonoids, tannins, or other phytochemical classes present in smaller relative quantities, as the primary driver of this plant's measured antioxidant activity.

Discussion

This research's central finding, confirming the hypothesized polarity-correlated extraction pattern with methanol consistently outperforming ethanol and water across every antioxidant and phytochemical parameter measured, supports the interpretation that this Nepalese medicinal plant's traditional antioxidant reputation reflects genuine, substantial underlying phenolic compound content rather than reflecting other non-phytochemical factors. This pattern is broadly consistent with solvent extraction comparisons conducted across numerous other traditionally used medicinal plant species, where methanol's relatively broad polarity range for dissolving both polar and moderately non-polar phenolic compounds commonly outperforms both the more restrictive aqueous extraction and the intermediate-polarity ethanol extraction for total phenolic and antioxidant activity recovery specifically.

The strong correlation between total phenolic content and DPPH scavenging activity documented here agrees with the broader phytochemistry literature's general finding that phenolic compounds, through their hydroxyl group hydrogen-donating capacity, typically represent the dominant antioxidant-active compound class in most plant materials, though this general pattern does not hold universally across all medicinal plant species, some of which derive comparatively more of their antioxidant activity from other compound classes such as specific terpenoid or alkaloid content. This research's specific confirmation of the phenolic-dominant pattern for this particular Nepalese plant provides useful species-specific validation rather than simply assuming the general pattern applies without direct testing.

For nutraceutical or pharmaceutical development interest in this plant specifically, this research's findings carry a direct practical implication: methanol extraction should be the preferred extraction solvent for applications prioritizing maximum antioxidant activity recovery, though water extraction's more modest but still measurable antioxidant activity (64% DPPH scavenging at the highest tested concentration) may still hold practical relevance for traditional preparation methods, which typically rely on aqueous decoction or infusion rather than organic solvent extraction, meaning traditional preparation methods likely deliver meaningfully lower, though still genuinely present, antioxidant activity relative to what a methanol-based commercial extraction process could achieve.

Conclusion

This research set out to test whether extraction solvent polarity systematically influences antioxidant activity and phytochemical content recovery from a Nepalese highland medicinal plant traditionally valued for its antioxidant properties, and the results confirm this hypothesis clearly: methanol extraction consistently outperformed ethanol and water extraction across total phenolic content, total flavonoid content, DPPH radical scavenging activity, and

broader qualitative phytochemical screening. Total phenolic content correlated strongly with antioxidant activity across all three extracts ($r = 0.94$), supporting phenolic compounds as the primary contributor to this plant's traditionally recognized antioxidant properties.

For researchers and nutraceutical developers interested in further characterizing or developing applications from this plant, this research's clearest recommendation is that methanol extraction offers the most efficient recovery of the plant's antioxidant-active phytochemical content among the three solvents tested, providing a validated extraction protocol foundation for any subsequent bioactive compound isolation or nutraceutical formulation development work. At the same time, this research's confirmation that this plant's traditional antioxidant reputation has genuine phytochemical grounding, concentrated substantially in phenolic compound content, provides scientific validation supporting continued traditional use alongside potential modern application development. Extending this characterization work to identify the specific individual phenolic compounds responsible for this plant's antioxidant activity, through more targeted chromatographic fractionation and compound identification beyond the whole-extract characterization approach used in this research, would represent a valuable next step toward fully understanding and potentially isolating this plant's most bioactive antioxidant components.

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