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RESEARCH ARTICLE

Phytochemical Properties, Antioxidant and Antimicrobial Activities of *Nardostachys jatamansi* Root Extract from Rasuwa, Nepal

Sanjaya Paudel[1], Pawan Kumar Kurmi[2], Dinesh Yadav[2], Shankar Kharel[2], Khagendra Prasad Bohara[2*], Mahesh Kumar Joshi[1,2*]

[1]*Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal*

[2]*Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal*

[*]*Corresponding E-mail: mahesh.joshi@trc.tu.edu.np*, khagendra.bohara@cdc.tu.edu.np

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Abstract

Nardostachys jatamansi (D. Don) DC is a high-altitude Himalayan herb traditionally valued in Ayurveda and Tibetan medicine. This study assessed the phytochemical profile, antioxidant potential, and antimicrobial activity of its ethanolic root extract collected from Rasuwa, Nepal. Soxhlet extraction yielded 2.857% (w/w), and phytochemical screening confirmed the presence of alkaloids, phenolics, flavonoids, and tannins, while steroids, terpenoids, and saponins were absent. The extract contained 34.064 ± 1.774 mg gallic acid equivalent per gram dry extract of phenolics and 23.148 ± 0.005 mg quercetin equivalent per gram dry extract of flavonoids. Antioxidant activity, measured by the DPPH assay, showed moderate free radical scavenging with an IC_{50} of 134.4 ± 2.97 µg/mL, which is weaker than that of quercetin. Antimicrobial testing revealed inhibition of two bacterial strains (Escherichia coli and Staphylococcus aureus) and one fungal strain (Candida albicans), though less effective than standard antibiotics. These findings demonstrate that N. jatamansi is a phenolic-rich plant with measurable bioactivity, supporting its traditional use and indicating potential for further pharmacological development.

Keywords: *Nardostachys jatamansi*; Phytochemicals; Antioxidant; Antimicrobial;

Introduction

Nepal, although it covers approximately 0.1% of the global land area, is rich in plant diversity. The country has approximately 13,067 known plant species, including around 7,000 flowering plants, and nearly 2,500 of these are used for medicinal purposes [1]. This tradition of using plants for healing is an important part of Nepal's culture, with more than 125 ethnic groups preserving ancient knowledge of herbal medicine and natural remedies [2]. The Himalayan region of Nepal alone is home to nearly 2,000 medicinal and aromatic plant species [3], and over 1,400 of these are still in use today. Among them, *Nardostachys jatamansi* (jatamansi) is a valuable herb native to high altitudes [4]. It is traditionally collected from Nepal's alpine regions and is well known for its essential oil and various therapeutic benefits [5].

Nardostachys jatamansi (D. Don) DC, often referred to as Jatamansi, muskroot [6] or Indian spikenard [7], is in grave danger of extinction, a high-altitude medicinal herb deeply entrenched in the traditional pharmacopoeias of South Asia [8]. Renowned for its neuroprotective, sedative, and antimicrobial properties, its aromatic rhizomes have been used for millennia in Ayurveda, Tibetan medicine, and local healing practices to treat ailments ranging from insomnia to microbial infection [9]. *N. Jatamansi* is commonly found in the subalpine and alpine regions of India, Pakistan, Nepal, China, and Yunnan [10]. The therapeutic efficacy of the

plant is attributed to its rich phytochemical profile, which exhibits potent bioactivity. However, the phytochemical composition and pharmacological potential of *N.jatamansi* are profoundly influenced by its geographical provenance, growth conditions, and post-harvest processing, factors that remain underexplored in populations from remote Himalayan regions [11].

The ecological importance of *N. jatamansi* in alpine and subalpine zones extends well beyond its medicinal utility. As a perennial rhizomatous herb, it plays a role in stabilising fragile mountain soils and contributes to the overall biodiversity of Himalayan ecosystems[12,13]. Overharvesting, however, has led to severe population decline, since the species has recently been classified as "critically endangered" by the IUCN Red List, restricting its international trade[14]. In Nepal, the species is largely wild-harvested; policy measures (e.g., trade controls, community forestry) aim to curb unsustainable extraction, and *N. jatamansi* is among the species restricted for collection, transport, and export[15]. The chemistry of N. jatamansi varies by region and processing method. Plants from different parts of Nepal exhibit variations in essential oil yield, as well as in phenolic and flavonoid content, which impact their antioxidant activity. This shows the importance of studying plants from specific locations[16]. Aamachhodingmo Rural Municipality in Rasuwa District is a region characterised by its pristine alpine ecosystems and unique climatic conditions. Despite the recognition of this area as a biodiversity hotspot, *N. jatamansi* grown in this region remained unexplored.

Recent studies have highlighted that *Nardostachys jatamansi* contains a diverse array of phytochemicals, including phenolics, flavonoids, and terpenoids, which contribute to its antioxidant and antimicrobial properties. Extracts from different solvents have shown notable variations in total phenolic and flavonoid content, correlating with free-radical scavenging capacity[17]. Essential oils rich in jatamansone and related sesquiterpenes also exhibit antimicrobial potential and synergistic effects with antibiotics[18]. Furthermore, molecular analyses confirm key compounds such as gallic acid, rutin, and kaempferol, reinforcing the plant's pharmacological importance[19,20]. These findings emphasise the need to examine populations from unexplored alpine habitats like Aamachhodingmo for unique bioactive profiles.

This study presents a comprehensive analysis of the phytochemical constituents, antioxidant capacity, and antimicrobial efficacy of *N. jatamansi* root extract from Aamachhodingmo Rural Municipality in the Rasuwa district. Using ethanol as a solvent, we employed Soxhlet extraction to obtain the bioactive fraction. The antioxidant potential of the extract was determined using the DPPH radical scavenging method, while the antimicrobial activity was examined against representative bacterial strains, including a gram-positive species (*Staphylococcus aureus*) and a gram-negative species (*Escherichia coli*).

Classification of *Nardostachys jatamansi* (D. Don) DC

The systematic classification of *Nardostachys jatamansi* (D. Don) DC is given as;

Taxonomic Classification

Plantae
Tracheophytes
Angiosperms
Eudicots
Asterids
Dipsacales
Caprifoliaceae
Nardostachys
N. jatamansi (D. Don) DC.
Synonyms: N. grandiflora DC.
N. chinensis Batalin
Patrina jatamansi D. Don

Nepali name: Jatamasi, Bhultye
English - Himalayan spikenard

Materials and Methods

Sample Collection

The plant's underground rhizome of *Nardostachys jatamansi* (D. Don) is locally referred to as Jatamansi, which was collected from the Aamachhodingmo Rural Municipality in Rasuwa District (28°10′16″N, 85°18′53″E) during October. This region, situated in the Himalayan belt, is recognised for its unique biodiversity and serves as a traditional habitat for *N. jatamansi*, a critically endangered species valued in Ayurvedic and Tibetan medicine. The collection timing (October) aligns with the post-monsoon growth phase of the plant, a period associated with peak accumulation of secondary metabolites in rhizomes due to seasonal physiological changes. The plant material used in this study was selected based on morphological similarity to a taxonomically authenticated herbarium specimen recorded at the Department of Plant Resources (DPR), Nepal.

Plant Extract Preparation

Ethanolic extraction was carried out in a Soxhlet apparatus, which enables efficient recovery of bioactive constituents. Ethanol was selected for its ability to solubilise a broad spectrum of medium-polarity phytoconstituents, including sesquiterpenoids, lignans, and phenolic acids, which are characteristic of *N. jatamansi*. The crushed rhizomes underwent continuous extraction until solvent exhaustion, followed by evaporation on a water bath to obtain the dried extract[21].

Percentage yield = $\frac{\text{Weights of the plant extracts}}{\text{weights of raw material used to plant extract.}} \times 100\%$

Soxhlet extraction was employed in this study due to its ability to provide exhaustive and reproducible extraction of phytochemicals using continuous solvent reflux. This method is particularly suitable for crude extract preparation when the objective is to obtain a broad spectrum of bioactive compounds. Although time-consuming, Soxhlet extraction ensures consistent extraction efficiency, making it appropriate for comparative biological evaluation.

Screening of Extracts for Phytochemicals

The extracts underwent qualitative phytochemical analysis according to the methods outlined by Shaikh (2020)[22], to identify the presence of alkaloids, terpenoids, flavonoids, phenolic compounds, tannins, saponins and glycosides.

Determination of TPC

To determine total phenolic content (TPC), the ethanolic extract of *N.jatamansi* was analysed using the Folin-Ciocalteu procedure[23]. A 100-ppm stock was prepared by dissolving 1 mg of the sample in 1 mL of methanol. In each well of a 96-well plate, 20 µL of extract or gallic acid reference solution (10-80 µg/mL) was combined with 100 µL of Folin-Ciocalteu reagent (10%). Absorbance was taken at 765 nm before adding 80 µL of 1 M sodium carbonate. After a 30-minute incubation at room temperature, the absorbance was recorded again at 765 nm. Phenolic concentration was calculated as gallic acid equivalents (mg Gal per g dry weight).

Determination of TFC

Total flavonoids were quantified using the aluminium chloride colourimetric assay [24,25]. A stock solution of the ethanolic extract (1.00 mg/mL, equivalent to 1000 ppm) was prepared in methanol. In a 96-well microplate, 20 µLof the stock solution was mixed with 100 µL of distilled water and 60 µL of ethanol. An initial absorbance reading at 415 nm was recorded on a multimode microplate reader (Bio-Tec Synergy LX) to correct for any intrinsic simple colour. Then 10 V of 10% (w/v) $AlCl_3$ and 10 µL of 1 M potassium acetate were added to each well. After incubation at room temperature for 30 minutes, absorbance was measured again at 415 nm. Quercetin calibration standards were run in parallel, and the total flavonoid content was expressed as mg quercetin equivalents (QE) per g of dry sample.

Antioxidant activity

The ability of the extracts to quench free radicals was measured using the DPPH

assay[26,27]. DPPH solution (0.1 mM) was prepared in methanol, with quercetin used as the positive control. Plant extracts were diluted to different concentrations and mixed with DPPH in a 96-well plate. Following a 30-minute incubation in the dark, absorbance was read at 517 nm. The percentage of radical scavenging was determined, and IC_{50} values were calculated using GraphPad software.

Antioxidant Activity

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Antimicrobial Study of the Extract

The ethanolic extract of *N. jatamansi* was evaluated for antimicrobial efficacy against two bacterial strains and one fungal strain by the agar well diffusion method [28,29]. The liquid broth (LB) was prepared, sterilised, and used to culture bacterial inoculum for 24 hours. Mueller–Hinton agar (MHA) plates were subsequently prepared, sterilised, and inoculated with 150 µL of microbial suspension. Wells were created in the agar medium, into which 100 µL of the plant extract (100 mg/mL) was dispensed. Kanamycin (5 mg/mL, 10 µL) was used as the positive control, while the solvent used for extract preparation (100 µL) served as the negative control. The plates were incubated at 37 °C for 24 hours, after which antimicrobial activity was assessed by measuring the diameter of the zones of inhibition.

Results and Discussion

An ethanol-based extract of *Nardostachys jatamansi* (JM) was prepared using a Soxhlet apparatus and concentrated by evaporation on a water bath. Starting with a sample weight of 140.52 g of plant material, the process yielded 4.014 g of dried ethanolic extract. The extract yield was calculated as 2.857% (w/w). This result indicates the efficiency of the extraction process in isolating bioactive compounds from the plant material under the specified experimental conditions. The ethanolic extract obtained from Rasuwa, Nepal, is substantially lower than several published yields for Nardostachys jatamansi: Sharma & Singh reported 14.32 g from 200 g (≈7.16% w/w) using 70% ethanol Soxhlet extraction [30]. Mude et al. recorded about 6% yield from 90% ethanol Soxhlet maceration [31] Other studies have shown yields as high as 20%, which highlights the role of several influencing factors, including solvent composition, extraction time, temperature, plant source, and geographical conditions. In addition to these variables, altitude differences can also play a significant role, as plants growing at higher elevations often accumulate different levels of secondary metabolites due to environmental stress, temperature variation, and soil composition. Such ecological variations may partly explain the lower extractive value observed in the Rasuwa sample.

Phytochemical Screening

Preliminary phytochemical screening of the ethanol-derived extract of *Nardostachys jatamansi* revealed multiple bioactive constituents. Compounds such as alkaloids, flavonoids, phenols, and tannins were identified, whereas terpenoids, saponins, and steroids were absent. These results indicate that the extract contains important phytochemicals that may contribute to its biological activities.

Total phenolic content

The ethanolic extract of *Nardostachys jatamansi* showed a total phenolic content (TPC) of 34.064 ± 1.774 mg GAE/g dry extract, determined using a gallic acid calibration curve shown in **Figure 1** ($y = 0.0083x + 0.0584$, $R^2 = 0.994$. This value is slightly lower than the phenolic content reported for Soxhlet-extracted

rhizome, which was 41.34 ± 2.30 mg GAE per gram of dry extract [30]. In another study, ultrasonic-assisted extraction with chloroform yielded 49.53 mg GAE per gram, while ethyl acetate extraction yielded 44.41 mg GAE per gram of dry extract. These variations demonstrate that the extraction technique, solvent polarity, and environmental factors significantly influence the phenolic content of *Nardostachys jatamansi*. The value obtained from the Rasuwa sample lies within the reported range, suggesting that although lower than some methods, it still represents a considerable amount of phenolics with potential biological activity.

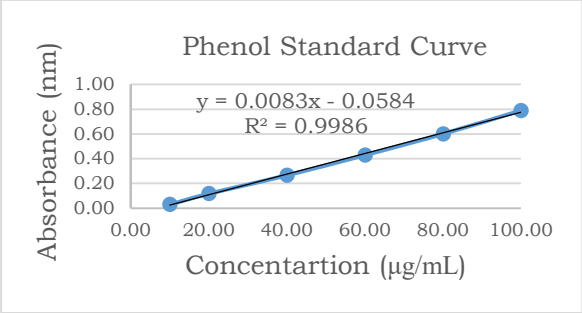

Figure 1: Standard calibration curve constructed using gallic acid.

Total Flavonoid Content

TFC was quantified using a calibration curve constructed from quercetin standards (linear regression: y = 0.0027x + 0.0035, R^2 = 0.9968), shown in **Figure 2**, which yielded 23.148 ± 0.005 mg quercetin equivalents (QE)/g dry extract.

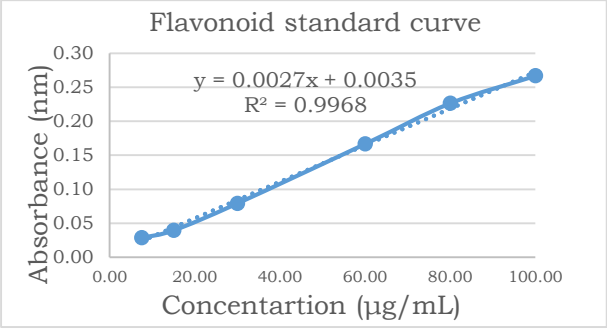

Figure 2. Calibration curve for standard gallic acid and standard quercetin

This value is comparable with previous reports, where seventy per cent ethanol extracts showed around 25 mg flavonoid equivalents per gram, and other solvent fractions ranged from about 10 to 31 mg per gram, depending on the extraction method and polarity documented in the literature[17]. The variation among reported values could arise from differences in solvent selection, extraction technique, calibration standards, and environmental factors such as altitude, which influence flavonoid biosynthesis. Overall, the measured content suggests that the Rasuwa extract has a moderate to high flavonoid level, supporting its potential antioxidant activity.The disparity in phenolic and flavonoid yields between studies highlights critical methodological and ecological considerations, including plant provenance, developmental stage, and environmental stressors, which collectively modulate secondary metabolite synthesis. Flavonoids, recognised for their antioxidant and anti-inflammatory bioactivity[32], suggest the extract's potential utility in advanced therapeutic formulations, such as nanofiber-based delivery systems. These findings reinforce the necessity of standardised extraction protocols and species-specific validation in phytochemical research.

DPPH Free Radical Scavenging Activity

The ethanolic fraction of *N. jatamansi* demonstrated a moderate free radical quenching effect in the DPPH assay, with an IC_{50} value determined as 134.4 ± 2.97 µg/mL, which is markedly higher than the potent standard quercetin (IC_{50} = 3.431 ± 0.89 µg/mL). This reduced antioxidant efficacy aligns with its total flavonoid content (TFC = 23.148 ± 0.005 mg QE/g), suggesting that the phenolic and flavonoid composition of the extract may lack synergistic radical-quenching mechanisms or specific bioactive constituents critical for enhanced activity. The accompanying graph depicts the DPPH free radical scavenging activity of the *N. jatamansi* extract and the standard Quercetin. Different concentration ranges were employed for the plant extract and the standard due to their differing activity profiles. The concentration of the sample is

plotted on the x-axis, and the percentage inhibition of DPPH is shown on the y-axis in **Figure 3.**

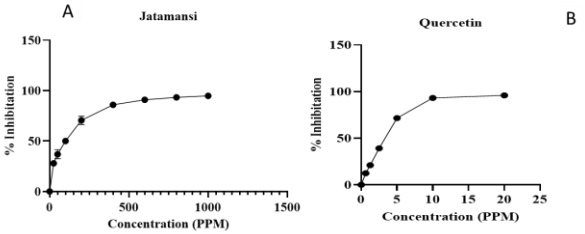

Figure 3. Graph showing IC_{50} value of DPPH of ethanolic extract of (a) N. jatamansi and (b) Quercetin

The moderate DPPH radical scavenging activity of the ethanolic extract of *N. jatamansi* suggests that while the extract contains significant flavonoid and phenolic compounds, their composition or concentration may not be sufficient to achieve highly potent free radical neutralisation compared to standard antioxidants like quercetin. Similar trends have been reported in other studies, where ethanolic or hydroalcoholic extracts of *N. jatamansi* showed appreciable but moderate IC_{50} values, indicating that factors such as solvent type, extraction method, and plant origin strongly influence antioxidant potential. Furthermore, altitude and environmental conditions at the collection site, such as Rasuwa in Nepal, may affect the biosynthesis of secondary metabolites, including phenolics and flavonoids, thereby modulating antioxidant activity. These results suggest that while the extract demonstrates biologically relevant antioxidant effects, optimisation of extraction parameters or fractionation could potentially enhance its radical scavenging efficiency. Due to the preliminary nature of this study, no statistical correlation analysis was conducted to directly relate phytochemical content with antioxidant or antimicrobial activity; therefore, the observed associations should be interpreted qualitatively.

Antimicrobial Activity

Antimicrobial screening against *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Candida albicans* (fungi) revealed broad-spectrum inhibitory effects, albeit with modest zone of inhibition (ZOI) diameters of 1.8 cm, 1.5 cm, and 1.8 cm, respectively, shown in **Figure 4**. These values were consistently lower than positive controls (ZOI = 2.4cm, 2.8, 2.5 cm), indicating suboptimal efficacy compared to conventional antimicrobial agents. The observed activity likely stems from secondary metabolites such as terpenoids and flavonoids, though their low concentration or incomplete extraction may limit potency.

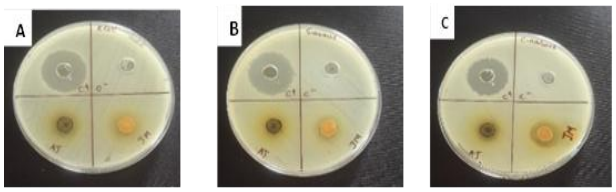

Figure 4. Antimicrobial activities are shown by the ethanolic extract of N.jatamansi with (A) Escherichia coli, (B) Staphylococcus aureus, and (C) Candida albicans

The ethanolic root extract of *Nardostachys jatamansi* from Rasuwa, Nepal, exhibited moderate antimicrobial activity against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans*, with smaller inhibition zones compared to standard antibiotics. Similar studies from India and Nepal reported varying degrees of antimicrobial activity, highlighting the influence of geographical origin, extraction methods, and solvent choice on bioactivity. The extracts from West Bengal showed significant inhibition against *E. coli, S. aureus*, and *Klebsiella pneumonia* [33], while Uttarakhand extracts displayed moderate zones against *S. aureus, E. coli*, and *C. albicans* [34]. Other studies from Nepal also reported broad-spectrum antimicrobial effects against multiple bacteria and fungi, though efficacy varied depending on plant origin and assay conditions reported in the literature[7]. These comparisons suggest that while the Rasuwa extract has moderate potency, optimising extraction and fractionation methods could enhance the yield of bioactive compounds and improve

antimicrobial efficacy. The ethanolic extract of *N. jatamansi* demonstrated activity, showing that it possesses broad-spectrum inhibitory effects despite lower potency compared to standard antimicrobial agents. This moderate activity can be correlated with the phenolic and flavonoid content of the extract. While total phenolic and flavonoid contents were substantial, they appear insufficient to produce strong antimicrobial effects on their own. Comparable findings have been reported in other studies, where extracts containing moderate amounts of phenolics and flavonoids exhibited antimicrobial activity against various bacterial and fungal strains, though their potency remained lower than that of standard antibiotics. This underscores the importance of both concentration and composition in influencing bioactivity.

The relationship between antioxidant activity and antimicrobial potential is also noteworthy. Compounds responsible for radical scavenging, such as phenolics and flavonoids, may contribute to antimicrobial mechanisms through oxidative stress modulation or disruption of microbial membranes. However, the moderate DPPH activity observed indicates that these compounds may not act synergistically at concentrations sufficient for strong antimicrobial effects. These findings suggest that further fractionation or optimisation of extraction methods could enhance the yield of specific bioactive constituents, potentially improving both antioxidant and antimicrobial efficacy. In practical terms, *N. jatamansi* extracts could serve as a natural adjunct in therapeutic formulations, food preservatives, or cosmetic applications, and future studies should explore the isolation of active compounds and combination strategies to maximise biological effects.

The use of crude plant extract is a limitation of the present study, as the observed biological activities may arise from the combined or synergistic effects of multiple phytochemical constituents rather than a single active compound. Therefore, the specific compounds responsible for the activity could not be individually identified. Future studies involving fractionation and compound-level characterisation would help to better elucidate the active components. This study represents a preliminary investigation aimed at establishing the feasibility of incorporating *Nardostachys jatamansi* crude extract into electrospun nanofibrous scaffolds, and the results should be interpreted accordingly.

Conclusions

The results obtained indicated the chemical composition and biological potential of the ethanolic root extract of *Nardostachys jatamansi* collected from Rasuwa, Nepal. The research confirmed that the extract contains substantial phenolics and moderate flavonoids, which contribute to its measurable antioxidant activity and broad-spectrum antimicrobial effects. While the crude extract showed lower potency compared to standard antioxidants and antibiotics, the observed activities are likely due to the combined effects of the plant's bioactive constituents, including phenolics, flavonoids, and terpenoids. These results highlight the therapeutic potential of N. jatamansi and support its traditional medicinal use. Additional research is required to separate and characterise individual bioactive compounds, evaluate their mechanisms of action, and optimise extraction and formulation strategies, including advanced delivery systems, to enhance bioavailability and clinical applicability. Although the findings are preliminary, the study demonstrates the potential of extract-loaded electrospun nanofibers as a promising platform for future biomedical applications

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Author's contribution statement

S. Paudel: Conceptualisation, Plant collection, Methodology, Plant extraction, Data analysis, Writing: original manuscript, Review and editing; **P. K. Kurmi:** Plant collection, Plant extraction, Methodology, Writing; **D. Yadav:** Plant collection, Plant extraction, Methodology, Writing; **S. Kharel:** Plant collection, Plant extraction, Methodology; **K. P. Bohara:** Supervision, Conceptualization, Data analysis, Writing: review and editing. **M. K. Joshi:** Supervision, Conceptualization, Data analysis, writing: review and editing.

Conflict of interest

The authors declare that there are no conflicts of interest associated with this research.

Data availability statement

All data supporting the conclusions of this research are available from the corresponding authors on reasonable request.

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