**BIBECHANA**
Vol. 23, No. 3, August 2026, 116–125

ISSN 2091-0762 (Print), 2382-5340 (Online)
Journal homepage: http://nepjol.info/index.php/BIBECHANA
Publisher: Dept. of Phys., Mahendra Morang A. M. Campus (Tribhuvan University), Biratnagar

# Study of antioxidant and $\alpha$-amylase inhibitory activity of *Aconogonon molle* (D. DON) and *Polygala arillata* buch, of Nepal

**Bimala Subba*, Priya Limbu, Ganga Bir Rai**
*Central Department of Chemistry, Tribhuvan University, Kirtipur, Nepal.*

*Corresponding author: Email: bimala.subba@cdc.tu.edu.np

**Abstract**

*Aconogonon molle* (leaves and stems) and *Polygala arillata* (roots) were selected for comparative investigation based on their ethnomedicinal relevance and their traditional use as plant ingredients in marcha, as reported by local Limbu community members during field collection. Methanolic extracts of both species were evaluated under identical experimental conditions for phytochemical composition, antioxidant activity, $\alpha$-amylase inhibition, and antibacterial activity. *A. molle* showed higher total phenolic content ($55.36 \pm 1.16$ mg GAE/g) and total flavonoid content ($11.02 \pm 0.32$ mg QE/g) than *P. arillata*. It also exhibited stronger antioxidant activity, with a DPPH $IC_{50}$ of $42.35 \pm 1.05$ $\mu$g/mL and FRAP value of $670.94 \pm 2.50$ mM $Fe^{2+}$/L. *A. molle* showed moderate $\alpha$-amylase inhibitory activity ($IC_{50}$ = 867.78 $\mu$g/mL) compared with acarbose ($IC_{50}$ = 26.28 $\mu$g/mL) and modest antibacterial activity against *Staphylococcus aureus* (7.75 mm inhibition zone), while no activity was detected against *Salmonella typhi*. *P. arillata* showed weak DPPH radical-scavenging and $\alpha$-amylase inhibitory activities, and its $IC_{50}$ values could not be determined within the tested concentration ranges. It also showed no detectable antibacterial activity against either test organism. Overall, *A. molle* exhibited stronger biological activities than *P. arillata* under the tested conditions. These findings provide comparative baseline information for further isolation and characterization of bioactive constituents from these ethnobotanically related medicinal plants.

**Keywords**
Marcha, Limbu community, ethnomedicine, phytochemical screening, DPPH radical scavenging, antidiabetic potential.

**Article information**
Manuscript received: November 01, 2026; Revised: August 28, 2026; Accepted: September 04, 2026
DOI: https://doi.org/10.3126/bibechana.v23i3.85926


## 1 Introduction

Nepal has diverse landforms and climates, ranging from tropical lowlands to alpine regions. These ecological conditions support a rich diversity of medicinal plants. More than 1,900 plant species are used in traditional healing, reflecting the country's strong ethnobotanical heritage [1]. Indigenous com-

munities have long used medicinal plants to manage infections, inflammation, digestive disorders, and chronic diseases. This traditional knowledge provides an important basis for scientific research and sustainable use of medicinal plant resources [2].

Studies on Nepalese medicinal plants have identified several classes of bioactive compounds, including flavonoids, phenolic acids, alkaloids, saponins, and terpenoids [3,4]. Many of these compounds exhibit antioxidant, antimicrobial, and enzyme-inhibitory activities, increasing interest in medicinal plants as potential sources of bioactive compounds for managing oxidative stress and metabolic disorders.

Diabetes mellitus is a major global health problem. In Nepal, unhealthy diets, physical inactivity, and changing lifestyles have contributed to the increasing prevalence of type 2 diabetes [5]. Persistent hyperglycemia promotes the generation of reactive oxygen species and oxidative stress, which can damage lipids, proteins, and DNA and contribute to complications such as cardiovascular disease, neuropathy, and kidney disorders [6].

Antidiabetic medicines, including metformin, sulfonylureas, and acarbose, are effective but may cause adverse effects [7]. Medicinal plants are therefore being investigated as complementary sources of bioactive compounds [8,9]. Polyphenol-rich plant extracts may reduce oxidative stress and inhibit carbohydrate-digesting enzymes such as $\alpha$-amylase and $\alpha$-glucosidase, thereby slowing carbohydrate digestion and postprandial glucose release [10].

*Aconogonon molle* and *Polygala arillata* are medicinal plants used in traditional practices for fever, infections, and digestive disorders [11, 12]. During field collection, local Limbu informants reported that both species are used as plant ingredients in marcha, a traditional starter culture used for alcoholic fermentation. The reported use of *A. molle* in marcha is also consistent with previous ethnobotanical literature [13], whereas the corresponding use of *P. arillata* was recorded from local informants during the present field collection. This shared traditional use provided the primary rationale for selecting the two species for comparative investigation.

Previous studies have reported antioxidant phenolic constituents in *A. molle* and antioxidant and $\alpha$-amylase inhibitory activities in *P. arillata* [12–14]. However, the two species have not been directly compared under identical extraction and assay conditions, and collection-site information was not clearly documented in some earlier reports. This is relevant because phytochemical composition may vary with geographical location, altitude, climate, soil conditions, season, plant part, and extraction method [15–17].

Therefore, the present study compared the phytochemical composition and biological activities of *A. molle* and *P. arillata* collected from documented locations in Nepal. Methanolic extracts were evaluated under identical experimental conditions for phytochemical composition, total phenolic and flavonoid contents, DPPH and FRAP antioxidant activities, $\alpha$-amylase inhibition, and antibacterial activity. The study provides a standardized comparison of these traditionally related plants and new $\alpha$-amylase inhibitory data for *A. molle* leaves and stems.

## 2 Materials and methods

### 2.1 Chemicals and reagents

Analytical-grade ethanol, methanol, and dimethyl sulfoxide (DMSO) were obtained from Thermo Fisher Scientific, India. Porcine pancreatic $\alpha$-amylase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), gallic acid, quercetin, and ascorbic acid were purchased from Sigma-Aldrich, USA. Acarbose and neomycin were obtained from Canvax Biotech, Germany. Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) were purchased from HiMedia, Mumbai, India. *Staphylococcus aureus* (ATCC 25923) and *Salmonella typhi* (ATCC 14028) were obtained from the Central Department of Chemistry, Tribhuvan University, Kirtipur, Nepal, and used for antibacterial assays.

### 2.2 Selection and collection of plant materials

*Aconogonon molle* (D. Don) H. Hara and *Polygala arillata* Buch.-Ham. ex D. Don were selected based on their ethnomedicinal relevance in Nepal [2,12]. During plant collection, informal consultation with local Limbu community members indicated that both species are traditionally used as plant ingredients in Marcha, a traditional starter culture for alcoholic fermentation. This ethnobotanical information provided an additional basis for selecting the two species for comparative investigation.

*A. molle* was collected from Tehrathum District (27.12° N, 87.60° E), while *P. arillata* was collected from Makawanpur District (27.40° N, 85.03° E) during the flowering season, Figure 1. The collected materials consisted of leaves and stems of *A. molle* and roots of *P. arillata*. Species identification was based on morphological characteristics and comparison with descriptions provided in Flora of Nepal [13] and Flowers of the Himalaya [17,18].

Collected samples were washed with distilled water to remove surface debris and then shade-dried at

room temperature to minimize heat-related degradation. The dried materials were ground into a fine powder using a mechanical grinder and stored in airtight containers until extraction.

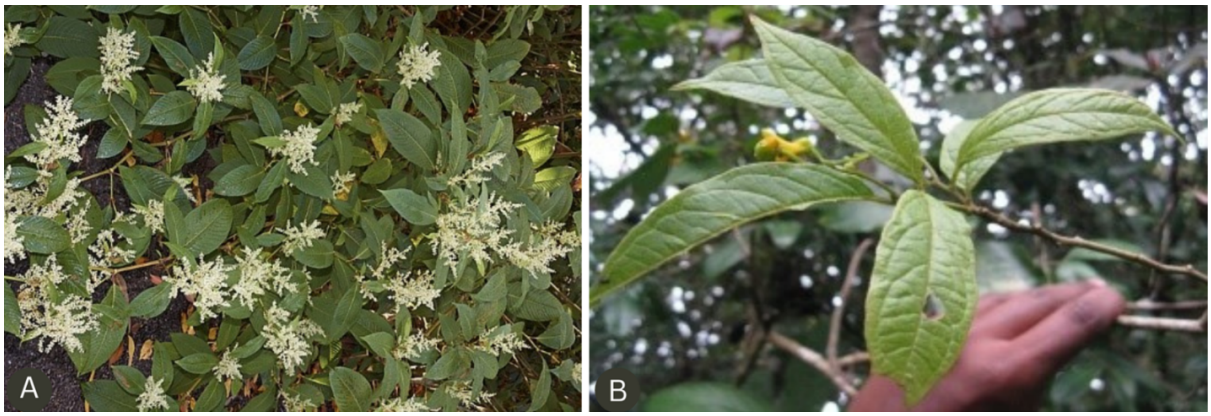


Figure 1: Photographs of the study plants: (A) *Aconogonon molle* and (B) *Polygala arillata*.

## 2.3 Extraction procedure

Methanolic extracts of *A. molle* (leaves and stems) and *P. arillata* (roots) were prepared using cold percolation. Briefly, 150 g of air-dried powdered plant material was soaked in 500 mL methanol at room temperature. The samples were extracted for five days with occasional shaking and then filtered through Whatman No. 1 filter paper. Extraction was repeated three times using fresh methanol until the solvent became visibly lighter. The combined filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator (Heidolph, Germany) at 150 rpm. The concentrated extracts were air-dried and stored at 4°C until further analysis.

## 2.4 Phytochemical screening

Preliminary phytochemical screening of *A. molle* leaf and stem extract and *P. arillata* root extract followed standard procedures [19, 20]. Alkaloids were tested using Dragendorff's and Mayer's reagents. Orange-brown or cream precipitates indicated positive reactions. Flavonoids were tested using the alkaline reagent test. Yellow coloration that disappeared after acidification indicated a positive result. Phenolic compounds and tannins were tested using ferric chloride solution. Blue, green, or dark coloration indicated their presence.

Saponins were detected by persistent foam formation in the froth test. Terpenoids were tested using the Salkowski test, with a reddish-brown interface indicating a positive reaction. Sterols were tested using the Liebermann-Burchard reaction, with blue-green coloration indicating their presence. Glycosides were tested using the Keller-Killiani test, where a brown ring at the interface indicated a positive reaction. Quinones were identified by red or pink coloration after adding alkali. Reducing sugars were tested using Benedict's or Fehling's reagent, with a brick-red precipitate indicating a positive reaction.

## 2.5 Determination of total phenolic content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu method [21]. Briefly, 20 $\mu$L of extract or gallic acid standard was mixed with 100 $\mu$L of 2N Folin-Ciocalteu reagent and 80 $\mu$L of 1 N $Na_2CO_3$ in a 96-well microplate. After incubation in the dark for 15 min at room temperature, absorbance was measured at 765 nm using a microplate reader (Synergy LX, BioTek, USA). Gallic acid was used to prepare the calibration curve. Results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

## 2.6 Determination of total flavonoid content (TFC)

Total flavonoid content was determined using the aluminum chloride colorimetric method [22]. Each well contained 20 $\mu$L of extract or quercetin standard, 120 $\mu$L distilled water, 60 $\mu$L ethanol, 5 $\mu$L of 10% $AlCl_3$, and 5 $\mu$L of 1 M potassium acetate. After incubation for 30 min at room temperature, absorbance was measured at 415 nm. Quercetin was used to prepare the calibration curve. Results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

## 2.7 Antioxidant assays

The antioxidant capacity of the methanolic extracts was assessed using two complementary in vitro assays:

### DPPH radical scavenging assay

The DPPH radical-scavenging assay was performed according to Brand-Williams et al. (1995) [23]. Both methanolic extracts were initially screened for DPPH radical-scavenging activity. *P. arillata* showed weak activity during preliminary screening and did not reach 50% inhibition within the tested concentration range. Therefore, its $IC_{50}$ could not be determined, and a full concentration-response analysis was performed only for *A. molle*.

Equal volumes (100 $\mu$L) of extract at different concentrations range (10-500 $\mu$g/mL) and 0.1 mM DPPH solution were mixed and incubated in the dark for 30 min. Absorbance was measured at 517 nm, with ascorbic acid used as the positive control. DPPH radical-scavenging activity was calculated as

$$\text{Scavenging } (\%) = \frac{A_0 - A_t}{A_0} \times 100$$

where $A_0$ is the absorbance of the control, and $A_t$ is the absorbance of the test sample.

### Ferric reducing antioxidant power (FRAP) assay

The reducing power of the extracts was analyzed following Benzie and Strain (1996) [24]. The FRAP reagent, freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in 40 mM HCl), and $FeCl_3 \cdot 6H_2O$ (20 mM), was added to 100 $\mu$L of extract or $FeSO_4$ standard. After 4 min incubation at 37°C, absorbance was measured at 593 nm. Results were expressed as mM Fe (II) equivalents.

## 2.8 $\alpha$-amylase inhibition assay

The $\alpha$-amylase inhibitory activity was determined using a colorimetric assay modified from Sudha et al. in 2011 [25]. Both methanolic extracts were initially screened for $\alpha$-amylase inhibitory activity. *P. arillata* showed weak inhibition and did not reach 50% inhibition within the tested concentration range (up to 2000 $\mu$g/mL). Therefore, its $IC_{50}$ could not be determined within this range. A full concentration-response analysis was performed for *A. molle*.

For the assay, 50 $\mu$L of phosphate buffer (50 mM, pH 6.9), 10 $\mu$L of $\alpha$-amylase solution (9 U/mL), and 20 $\mu$L of extract were mixed and pre-incubated at 37°C for 15 min. Then, 20 $\mu$L of soluble starch (0.05%) was added, and the reaction mixture was incubated for another 15 min. The reaction was terminated by adding 20 $\mu$L of 1 N HCl, followed by 100 $\mu$L of iodine reagent (5 mM $I_2$ and 5 mM KI). Absorbance was measured at 620 nm using a multimode reader (Synergy HT, BioTek). Acarbose was used as the positive control. The percentage inhibition was calculated as

$$\text{Inhibition } (\%) = \frac{A_c - A_s}{A_c} \times 100$$

where $A_c$ = Control absorbance and $A_s$ = Sample absorbance. $IC_{50}$ values were determined using GraphPad Prism 8.

## 2.9 Antimicrobial activity

The antimicrobial activity of the extracts was evaluated using the agar well diffusion method according to CLSI (2018) [26,27]. Mueller-Hinton agar plates were inoculated with *S. aureus* (Gram-positive) and *S. typhi* (Gram-negative) bacterial suspensions ($10^8$ CFU/mL). Wells (6 mm diameter) were filled with 50 $\mu$L of extract solution. Neomycin and DMSO served as positive and negative controls, respectively. Plates were pre-diffused for 30 min and incubated at 37°C for 24 h. Antimicrobial activity was expressed as the diameter of the zone of inhibition (mm).

## 2.10 Statistical analysis

All experiments were performed in triplicate, and results are presented as mean ± standard error of the mean (SEM). $IC_{50}$ values were determined by nonlinear regression analysis using Graph Pad Prism 8.0, while descriptive statistics were performed using Python (version 3.12.3).

# 3 Results and discussion

## 3.1 Phytochemical composition

Phytochemical screening revealed the presence of alkaloids, glycosides, terpenoids, saponins, sterols, phenolic compounds, and reducing sugars in both species, whereas flavonoids, tannins, and quinones varied between them (Table 1). *A. molle* exhibited a broader phytochemical profile than *P. arillata*. Although *P. arillata* showed only a weak positive reaction for flavonoids during qualitative screening, the quantitative assay confirmed their presence (8.95 ± 0.20 mg QE/g). This difference may reflect the greater sensitivity of the aluminum chloride spectrophotometric method compared with qualitative color-based tests.

The phytochemical profile of *A. molle* is consistent with previous reports describing phenolic compounds and flavonoids in this species and related Polygonaceae plants [28]. Likewise, the detection of alkaloids and saponins in *P. arillata* agrees with previous reports on Polygalaceae species [29, 30] and with Subba and Chemjong [13], who also reported these constituents in *P. arillata*. Differences

in phytochemical profiles among studies may arise from geographical origin, altitude, climate, soil conditions, plant part, developmental stage, extraction solvent, and extraction method [19, 31]. These factors may partly explain variations between the present findings and earlier reports.

Overall, both extracts contained several classes of secondary metabolites with potential biological relevance. *A. molle* showed a broader qualitative phytochemical profile, together with higher phenolic and flavonoid contents and stronger antioxidant activity in the present study. However, the contribution of individual phytochemicals to these biological activities requires further investigation.

Table 1: Results of phytochemical analysis

| S.N. | Phyto-chemicals | *P. arillata* | *A. molle* |
|---|---|---|---|
| 1 | Flavonoids | Trace (+) | + |
| 2 | Alkaloids | + | - |
| 3 | Glycosides | + | + |
| 4 | Phenolic compounds | - | + |
| 5 | Terpenoids | + | + |
| 6 | Sterols | - | + |
| 7 | Saponins | + | + |
| 8 | Tannins | - | + |
| 9 | Quinones | + | - |
| 10 | Reducing sugar | + | + |
| 11 | Volatile Oils | + | - |

"+" = present; "-" = absent.

## 3.2 Total phenolic and flavonoid contents (TPC and TFC)

*A. molle* showed significantly higher total phenolic content (55.36 ± 1.16 mg GAE/g) and total flavonoid content (11.02 ± 0.32 mg QE/g) than *P. arillata* (26.38 ± 1.53 mg GAE/g and 8.95 ± 0.20 mg QE/g, respectively) (Figure 2; $p < 0.001$ and $p < 0.01$, respectively).

Phenolic compounds and flavonoids can contribute to antioxidant activity through hydrogen donation, electron transfer, and metal chelation [32, 33]. The higher TPC and TFC of *A. molle* therefore suggest a greater abundance of polyphenolic constituents in its methanolic extract.

The present results differ from those reported by Subba and Chemjong [13] for *P. arillata* collected from Panchthar at an elevation of 3000-6000 ft. They reported a higher TPC (44.27 ± 2.97 mg GAE/g) but a lower TFC (5.99 ± 1.00 mg QE/g) than the values obtained in the present study (26.38 ± 1.53 mg GAE/g and 8.95 ± 0.20 mg QE/g, respectively). Such differences may be associated with geographical origin, altitude, plant part, climate, soil conditions, harvest season, solvent system, and extraction method, all of which can influence the phytochemical composition of medicinal plants [19, 31].

The higher phenolic and flavonoid contents of *A. molle* were accompanied by stronger DPPH and FRAP activities in the present study. This pattern suggests a possible contribution of polyphenolic constituents to its antioxidant activity, although a statistical correlation was not established. Polyphenols have been reported to interact with $\alpha$-amylase through non-covalent interactions, which can alter enzyme activity and reduce starch hydrolysis [34]. Therefore, the moderate $\alpha$-amylase inhibition observed for *A. molle* may partly be associated with its polyphenolic constituents; however, this mechanism was not directly investigated in the present study.

## 3.3 Antioxidant activity (DPPH and FRAP assays)

The antioxidant activity of the methanolic extracts was evaluated using DPPH radical-scavenging and ferric reducing antioxidant power (FRAP) assays. In the DPPH assay, *A. molle* showed an $IC_{50}$ value of 42.35 ± 1.05 $\mu$g/mL, which was significantly higher ($p < 0.05$) than that of ascorbic acid (31.14 ± 2.45 $\mu$g/mL) (Figure 3). Thus, *A. molle* was less potent than the reference antioxidant but showed considerable radical-scavenging activity. In contrast, *P. arillata* showed less than 20% inhibition at the highest tested concentration (500 $\mu$g/mL); therefore, its $IC_{50}$ could not be determined within the tested concentration range.

The antioxidant activity of *A. molle* is consistent with previous phytochemical studies reporting quercetin, quercetin glycosides, gallic acid, chlorogenic acid, and other phenolic constituents in this species [29]. Several of these compounds have shown strong DPPH radical-scavenging activity, suggesting that phenolic constituents may contribute to the antioxidant activity observed in the present study. Antioxidant constituents have previously been reported from both the leaves and flowers of *A. molle* [12, 28].

Differences between the present results and earlier reports may arise from geographical origin, altitude, climate, plant part, harvest season, extraction solvent, and extraction conditions, which can influence the accumulation and recovery of secondary metabolites [19, 31]. The present study examined methanolic extracts of *A. molle* leaves and stems collected from a documented location in Nepal. Dif-

ferences in plant material and collection conditions may therefore partly explain variation from previously reported antioxidant values.

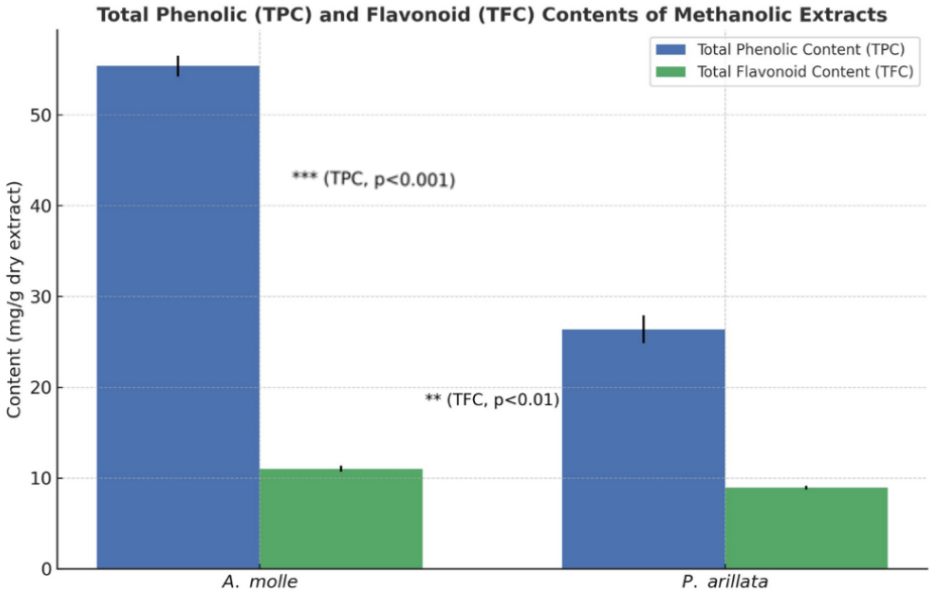


Figure 2: Total phenolic content (TPC) and total flavonoid content (TFC) of methanolic extracts of *A. molle* and *P. arillata*, expressed as mg GAE/g and mg QE/g dry extract, respectively. Error bars represent the standard error of the mean (SEM; $n = 3$). Statistical significance was determined using an independent-samples t-test ($p < 0.05$; $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***).

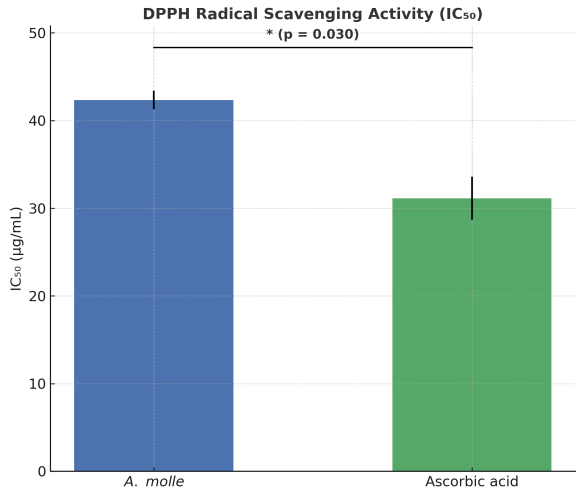


Figure 3: DPPH radical scavenging activity ($IC_{50}$) of the methanol extract of *A. molle* compared with the reference antioxidant, ascorbic acid. Error bars indicate the standard error of the mean (SEM, $n = 3$). Statistical significance was determined using an independent-samples t-test ($p < 0.05$).

The FRAP assay showed a similar pattern. *A. molle* exhibited significantly higher ferric reducing antioxidant power ($670.94 \pm 2.50$ mM $Fe^{2+}$/L) than *P. arillata* ($160.07 \pm 1.04$ mM $Fe^{2+}$/L) ($p < 0.001$) (Figure 4). This pattern was consistent with the higher total phenolic and flavonoid contents observed in *A. molle*. Polyphenolic compounds can act as electron donors and contribute to the reduction of $Fe^{3+}$ to $Fe^{2+}$ [21, 29].

Overall, *A. molle* showed higher phenolic and flavonoid contents together with stronger DPPH and FRAP activities than *P. arillata*. This pattern suggests a possible association between polyphenolic content and antioxidant activity. However, no statistical correlation analysis was performed because only two plant species were evaluated and a DPPH $IC_{50}$ value could not be determined for *P. arillata*. The relationship should therefore be regarded as a descriptive association rather than a quantitative correlation.

## 3.4 $\alpha$-amylase inhibition

The methanolic extract of *A. molle* showed concentration-dependent $\alpha$-amylase inhibition, with an $IC_{50}$ value of 867.78 $\mu$g/mL (Figure 5). Acarbose showed substantially stronger inhibitory activity, with an $IC_{50}$ value of 26.28 $\mu$g/mL. These results indicate that *A. molle* had moderate $\alpha$-amylase inhibitory activity but was considerably less potent than the reference inhibitor. In con-

trast, *P. arillata* showed less than 20% inhibition at the highest tested concentration (2000 $\mu$g/mL); therefore, its $IC_{50}$ could not be determined within the tested concentration range.

The moderate $\alpha$-amylase inhibition of *A. molle* may partly be associated with its phenolic and flavonoid constituents. Polyphenols have been reported to interact with $\alpha$-amylase through non-covalent interactions, which can alter enzyme activity and reduce starch hydrolysis [35]. However, the active compounds and their interactions with $\alpha$-amylase were not investigated in the present study. Therefore, this mechanism should be considered a plausible explanation rather than a confirmed mode of action.

Overall, the results provide preliminary evidence of $\alpha$-amylase inhibitory activity in the crude methanolic extract of *A. molle*. Its relatively high $IC_{50}$ compared with acarbose indicates modest potency. Further studies involving isolation of active constituents, enzyme kinetic analysis, and in vivo evaluation are required before conclusions can be drawn regarding its potential role in glycemic control.

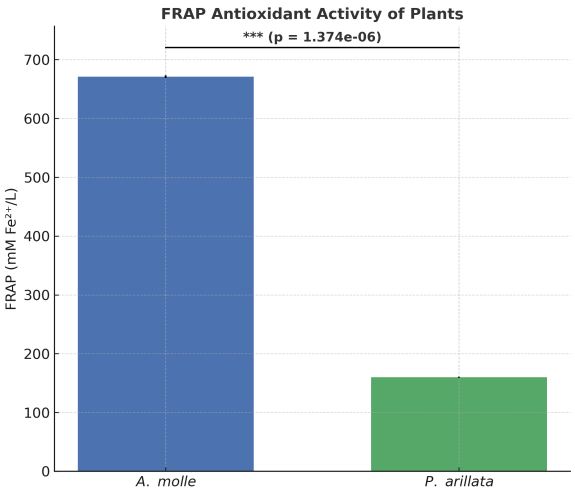


Figure 4: FRAP antioxidant activity of methanol extracts of *A. molle* and *P. arillata* expressed in mM Fe(II)/L. Error bars represent the standard error of the mean (SEM, $n = 3$). Statistical analysis was performed using an independent-samples t-test ($p < 0.001$).

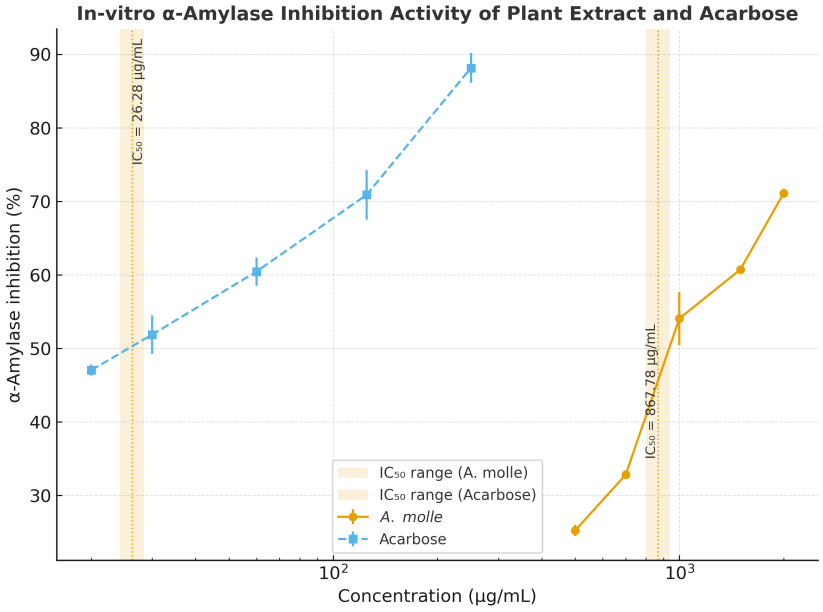


Figure 5: In vitro $\alpha$-amylase inhibition by the methanol extract of *A. molle* compared with the standard inhibitor acarbose. Data are presented as mean $\pm$ SEM ($n = 3$). Vertical dashed lines with shaded bands represent $IC_{50}$ values and ranges (867.78 $\mu$g/mL for *A. molle* and 26.28 $\mu$g/mL for acarbose).

## 3.5 Antibacterial activity

The methanolic extract of *A. molle* produced a 7.75 mm inhibition zone against *S. aureus* (Table 2). Neither plant extract showed detectable activity against *S. typhi*. Neomycin produced a 19.5 mm inhibition zone under the same conditions (Figure 6). The smaller inhibition zone of *A. molle* indicates modest antibacterial activity compared with neomycin. This activity may be associated with phenolic and terpenoid compounds, which have been reported to affect bacterial membranes and other cellular processes [29]. However, the mechanism was not investigated in the present study.

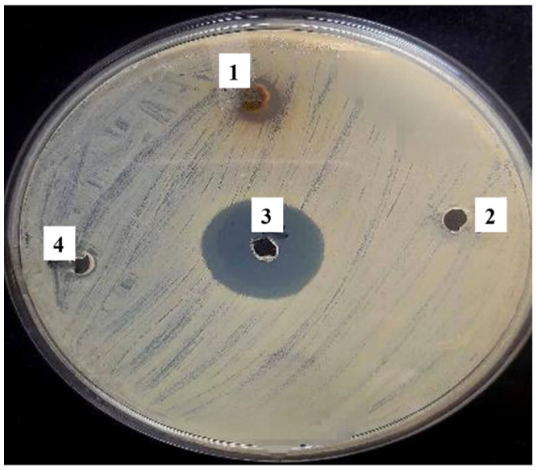


Figure 6: Antimicrobial activity of methanol extracts of *P. arillata* and *A. molle* against *Staphylococcus aureus*. (1) *P. arillata*, (2) DMSO (negative control), (3) neomycin (positive control), and (4) *A. molle*. Clear inhibition zones around the wells indicate bacterial growth suppression, with *A. molle* exhibiting moderate activity relative to the standard antibiotic neomycin.

Table 2: Values of zone of inhibition for antimicrobial activity of methanol extracts of *P. arillata* and *A. molle* against *Staphylococcus aureus* and *Salmonella typhi*

| | **Zone of inhibition** (mm) *S. aureus* | **Zone of inhibition** (mm) *S. typhi* |
|---|---|---|
| *P. arillata* (50 $\mu$g/mL) | - | - |
| *A. molle* (50 $\mu$g/mL) | 7.75 | - |
| Neomycin (10 $\mu$g/mL) | 19.5 | 19.5 |

The absence of activity against *S. typhi* may be related to the outer membrane of Gram-negative bacteria, which can reduce the entry of many plant-derived compounds [36]. This explanation remains plausible rather than confirmed. A previous Nepalese study also reported no antibacterial activity of methanolic *P. arillata* extract against *S. typhi* under similar conditions [12].

These findings should be considered preliminary. Only two bacterial strains were tested, and minimum inhibitory concentration (MIC) values were not determined. The use of crude extracts may also limit interpretation of the active constituents. Future studies should include additional bacterial strains, MIC and MBC determination, lower DMSO concentrations, fractionation of the extracts, and isolation of active compounds.

## 4 Conclusion

Between the two medicinal plants, *A. molle* (leaves and stems) showed higher phenolic and flavonoid contents, stronger antioxidant activity, moderate $\alpha$-amylase inhibition, and modest antibacterial activity against *S. aureus*. In contrast, *P. arillata* (roots) showed weaker biological activities under the tested conditions. These findings suggest that *A. molle* may be a useful source of antioxidant and $\alpha$-amylase inhibitory constituents.

The present study provides a direct comparison of two ethnobotanically related medicinal plants evaluated under identical extraction and assay conditions. Their shared traditional use as ingredients in marcha, as reported by local Limbu community members, provided the main basis for selecting the species together. The antibacterial activity of *A. molle* should be considered preliminary because only two bacterial strains were tested and MIC values were not determined. Future studies should isolate and characterize the active compounds, determine MIC values, examine mechanisms of action, and validate the observed activities through broader biological and in vivo studies.

## Conflicts of interest

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

## Funding statement

This research received no external funding.

## Author's contribution

BS: Conceptualization, methodology, supervision, and writing, review, and editing. PL: Investigation, methodology, data collection and formal analysis. GBR: Writing original draft, writing, reviewing and editing.

## References

[1] Kunwar RM, Bussmann RW. Ethnobotany in the nepal himalaya. Journal of Ethnobiology and Ethnomedicine. 2008;4(1):24.

[2] Uprety Y, Bhattarai HD. From the field into the lab: contribution of traditional knowledge to modern medicine. Annapurna Journal of

Health Sciences. 2022;2(2):63-5.

[3] Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: advances and opportunities. Nature Reviews Drug Discovery. 2021;20(3):200-16.

[4] Ata A. Bioactive natural products from medicinal plants. In: Drug Discovery and Design Using Natural Products. Springer; 2023. p. 417-34.

[5] Shrestha N, Karki K, Poudyal A, Aryal KK, Mahato NK, Gautam N, et al. Prevalence of diabetes mellitus and associated risk factors in Nepal: findings from a nationwide population-based survey. BMJ Open. 2022;12(2):e060750.

[6] Vikram A, Tripathi DN, Kumar A, Singh S. Oxidative stress and inflammation in diabetic complications. International Journal of Endocrinology. 2014;2014:679754.

[7] Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, et al. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. Frontiers in Endocrinology. 2017;8:6.

[8] Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. Biology. 2020;9(9):252.

[9] Rybak M, Wojdylo A. Inhibition of $\alpha$-Amylase, $\alpha$-Glucosidase, pancreatic lipase, 15-Lipooxygenase and acetylcholinesterase modulated by polyphenolic compounds, organic acids, and carbohydrates of Prunus domestica fruit. Antioxidants. 2023;12(7):1380.

[10] Neagu E, Paun G, Albu C, Eremia SAMV, Radu GL. Artemisia abrotanum and Symphytum officinale polyphenolic compounds-rich extracts with potential application in diabetes management. Metabolites. 2023;13(3):354.

[11] Mehta PS, Negi KS, Ojha SN, Rayal A, Verma SK. Herbal based traditional practices used by the Bhotias and Gangwals of the central Himalayan region, Uttarakhand, India. J Nanotechnol. 2013;2(1):83-96.

[12] Joshi KR, Devkota HP, Watanabe T, Yahara S. Phenolic compounds from the flowers of Nepalese medicinal plant Aconogonon molle and their DPPH free radical-scavenging activities. Natural Product Research. 2014;28(23):2208-10.

[13] Chemjong K, Subba B. Scientific evaluation of Buddleja asiatica, Camellia sinensis, and polygala arillata of Nepal. Journal of Institute of Science and Technology. 2022;27(2):67-76.

[14] Watson MF, Akiyama S, Ikeda H, Pendry CA, Rajbhandari KR, Shrestha KK. Flora of Nepal. vol. 3. Royal Botanic Garden Edinburgh Edinburgh, UK; 2011.

[15] Verma N, Shukla S. Impact of various factors responsible for fluctuation in plant secondary metabolites. Journal of Applied Research on Medicinal and Aromatic Plants. 2015;2(4):105-13.

[16] Li Y, Kong D, Fu Y, Sussman MR, Wu H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiology and Biochemistry. 2020;148:80-9.

[17] Polunin O, Stainton A. Flowers of the Himalayas: Oxford University Press. New Delhi, India. 1984:79.

[18] Shrestha KK, Watson MF, Akiyama S, Pendry C, editors. Flora of Nepal. Royal Botanic Garden Edinburgh; 2018. Available from: https://floraofnepal.org.np.

[19] Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998.

[20] Evans WC. Trease and Evans' pharmacognosy. Elsevier Health Sciences; 2009.

[21] Vl S. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. 1999;299:152-78.

[22] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002;10(3).

[23] Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995;28(1):25-30.

[24] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry. 1996;239(1):70-6.

[25] Zinjarde SS, Bhargava SY, Kumar AR, et al. Potent $\alpha$-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complementary and Alternative Medicine. 2011;11(1):5.

[26] Schuetz AN, Ferrell A, Hindler JA, Humphries R, Bobenchik AM. Overview of changes in the clinical and laboratory standards institute performance standards for antimicrobial

susceptibility testing: M100 32nd and 33rd editions. Journal of Clinical Microbiology. 2025;63(9):e01623-3.

[27] Perez C. Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp. 1990;15:113-5.

[28] Rokade PD, Sonawane PL, Waje PB, Rajesh L, Bhoye GSK, Bachhav RS, et al. Analgesic and Anti-Inflammatory Potential of Nardostachys Jatamansi and Pongamia Pinnata: Phytochemical and Pharmacological Perspectives. 2026.

[29] Cushnie TPT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. International Journal of Antimicrobial Agents. 2011;38(2):99-107.

[30] Singh B, Sharma RA. Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. 3 Biotech. 2015;5(2):129-51.

[31] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale Pharmaceutica Sciencia. 2011;1(1):98-106.

[32] Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997;2(4):152-9.

[33] Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-52.

[34] Kaeswurm JAH, Claasen B, Fischer MP, Buchweitz M. Interaction of structurally diverse phenolic compounds with porcine pancreatic $\alpha$-amylase. Journal of Agricultural and Food Chemistry. 2019;67(40):11108-18.

[35] Ćorković I, Gašo-Sokač D, Pichler A, Šimunović J, Kopjar M. Dietary polyphenols as natural inhibitors of $\alpha$-amylase and $\alpha$-glucosidase. Life. 2022;12(11):1692.

[36] Vaara M. Antibiotic-supersusceptible mutants of Escherichia coli and Salmonella typhimurium. Antimicrobial Agents and Chemotherapy. 1993;37(11):2255-60.