

COMPARATIVE EVALUATION OF NUTRITIONAL AND PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL AND METAL SAFETY OF *CARALLUMA TUBERCULATA* AND *ASPARAGUS RACEMOSUS* FROM NAMAL VALLEY, PUNJAB, PAKISTAN: A MULTIVARIATE ANALYSIS

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Abstract

Medicinal plants are important natural sources of nutrients and bioactive compounds that have therapeutic potential. The aim of the study was to assess the *Caralluma tuberculata* and *Asparagus racemosus*, collected from two different locations in Namal Valley, Punjab, Pakistan for their nutritional composition, heavy metal accumulation, phytochemical content, antioxidant and antibacterial activities as well as metal associated health risks. A total of 12 samples of *C. tuberculata* and *A. racemosus* collected from two sites (Rikhi and Namal village) were analysed using a comparative experimental design. Proximate analysis revealed variation in nutritional parameters among the studied plants. *A. racemosus* had the highest crude protein (22.15%), crude fat (13.54%), and crude fibre (42.37%), whereas *C. tuberculata* had the highest carbohydrate (48.78%) and ash contents (14.19%). Heavy metal values were within permissible limits: Zn ranged from 34.49 to 55.64 mg/kg, Mn from 17.29 to 42.19 mg/kg, and Cu from 11.63 to 36.05 mg/kg. Risk assessment parameters including Estimated daily intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI) suggested their safe composition. Phytochemical analysis showed considerable levels of total phenolics (41.55–122.84 mg GAE/g) and flavonoids (15.60–69.04 mg QE/g), associated with strong antioxidant effect (31.55–73.01%). Antimicrobial testing demonstrated the inhibitory potential against *Escherichia coli* (clear inhibition zone beyond the 9mm disc: 1.94 mm to 21.91 mm) and *Staphylococcus aureus* (5.04 mm to 24.73 mm) although their antibacterial effects were generally lower than those of the positive control ciprofloxacin. Pearson correlation analysis, PCA and hierarchical clustering revealed the positive relationships between phytochemical constituents and biological activity. The overall findings suggest that the investigated medicinal plants meet the established guidelines for medicinal plants in terms of heavy metal concentrations and potential health risks, and demonstrated the promising phytochemical composition and biological activity. The results support their potential for development of natural therapeutic agents and highlighted the need for the conservation of these valuable traditional medicinal resources.

Key words: Antioxidant activity; Antimicrobial efficacy; Ethnobotany; Heavy metals; Medicinal plants; Phytochemical composition

Introduction

Ethno-medicinal research serves as an important tool for identifying novel pharmacological compounds with therapeutic potential (Edo *et al.*, 2023). In underdeveloped regions, 80% of the population relies upon herbal remedies for basic healthcare, and in developed countries, approximately 25% of the prescribed medicines come from wild sources (Miranda, 2021). Natural remedies based on plant products are widely accepted because they are easily available, safe to use and economical (Olafadehan *et al.*, 2020). Ethno-medicinal evaluation of natural herbs is necessary because people in developing countries depend upon herbal medicines due to limited access to conventional healthcare services (Fitzgerald *et al.*, 2020; Ahmad *et al.*, 2026).

Phytochemicals found in plants are crucial for sustaining the physiological functions of plants and

defending them against pathogen attack and environmental stressors, including UV exposure, pollution, and illness (Shaheen *et al.*, 2021). The nutritional value of medicinal plants plays an important role in determining the pharmacological efficacy of plants (Radha *et al.*, 2021; Chen *et al.*, 2025). A critical research gap exists in the evaluation of the pharmacological properties of herbal medicines (Shi *et al.*, 2022). Due to various toxicities found in herbal plants, it is necessary to determine the proper pharmacological profile of herbal medicines to make them reliable according to essential standards (Rezaei *et al.*, 2022). Systematic evaluation of metal accumulation in medicinal plants has become essential for developing evidence-based quality assurance protocols and establishing safe consumption guidelines (Chen *et al.*, 2022; Malematja *et al.*, 2023). Heavy metals present in elevated concentrations lead to serious health risks when used in raw form (Raza *et al.*, 2025; Akram *et al.*, 2026).

Medicinal plants are recognized for their strong antioxidant and antimicrobial activities (Chater *et al.*, 2026; Ed-Dahmani *et al.*, 2025). The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay has become one of the most widely employed *In vitro* methodologies for rapidly evaluating the antioxidant potential of plant extracts, enabling comparison across diverse plant species and extraction protocols (Madhuranga, 2023). Plant extracts have antimicrobial effects that arise from diverse mechanisms such as membrane damage that are caused by lipophilic phytochemicals and inhibition of bacterial cell wall formation (Rahman *et al.*, 2024). Natural phenolic-rich extracts have demonstrated particular promise as alternatives to synthetic antimicrobial agents (Oulahal & Degraeve, 2022). There is a need to focus on the regulation of traditional drugs (Barkat *et al.*, 2021). It is necessary to assess the quality of herbal medicines to make them safe to use. The hazardous effects of herbal products are caused by contamination toxins (Jitäreanu *et al.*, 2022; Li *et al.*, 2026).

Despite extensive documentation of medicinal plant use in Pakistan, several critical research gaps require urgent attention (Ralte *et al.*, 2024). The relationship between heavy metal accumulation and phytochemical production remains poorly understood (Ghafar *et al.*, 2021; Hlihor *et al.*, 2022). The current study evaluated the nutritional composition, phytochemical profile, heavy metal accumulation, antioxidant and antibacterial activities, and health risk indices of *Caralluma tuberculata* and *Asparagus racemosus* collected from Namal Valley, Punjab, Pakistan. It also examined relationships among nutritional, phytochemical, metal, and biological variables using Pearson correlation, principal component analysis, and hierarchical clustering to assess their medicinal potential.

The novelty of this study lies in the integrated assessment of nutritional and phytochemical profile, heavy metal accumulation, antioxidant and antibacterial potential, and associated health risks of *Caralluma tuberculata* and *Asparagus racemosus* from the unexplored ecological regions of Namal Valley, Punjab, Pakistan. *Caralluma tuberculata* and *Asparagus racemosus* were selected because of their significance as underutilized edible plants that serve both as dietary vegetables and traditional medicinal resources, with long-standing ethno-medicinal applications and potential bioactive properties. Unlike previous studies focusing on individual biological activities, this research combines nutritional, phytochemical, safety, and pharmacological evaluations along with multivariate statistical approaches to provide a comprehensive understanding of their medicinal potential.

Materials and Methods

Sampling of plant material: Three independent biological samples of each of the two selected wild medicinal plants, namely, whole plant of *Caralluma tuberculata* and *Asparagus racemosus*, were collected randomly in the month of November 2024 from two sites (Rikhi and Namal Village) in Namal Valley, district Mianwali, Punjab, Pakistan. Collected plants were stored in paper bags. The collected plant specimens were identified and authenticated. To ensure proper identification and documentation voucher specimens of the plant species that were collected are prepared and voucher numbers are assigned to them. These

specimens were deposited in the herbarium of the Department of Botany, University for future reference. The plant samples were washed with distilled water just to remove the dust and other impurities and then air dried for 5 to 7 days under ambient conditions and then finely powdered for further laboratory investigations (Ullah *et al.*, 2023). Coordinates of studied ecological sites are given in Table 1.

Table 1. Latitude and longitude of studied ecological sites.

S. No.	Site name	Location	Site code
1	Rikhi	32°38'43.9"Nand 71°47'56.2"E	S1
2	Namal village	32°39'56.8"Nand 71°48'44.0"E	S2

Extraction of plant material: Plant Extracts were prepared by simple maceration (Tzanova *et al.*, 2024). Methanol was selected as the extraction solvent because it has high efficiency in extracting a broad range of polar and moderately polar bioactive compounds, including phenolics, flavonoids, tannins, and other secondary metabolites (Ghaffar & Perveen, 2025). Dried plant powder (2 g) was soaked in 20 mL of methanol and kept at room temperature for 72 hours to facilitate the extraction of bioactive compounds. After the extraction of bioactive compounds, the mixture was then filtered through Whatman filter paper to remove the plant debris. The filtrate was then concentrated under vacuum using a rotatory evaporator until complete removal of solvent. The resulting crude extract was then dried and resulted in airtight amber coloured glass vials at 4°C for further analysis. The extraction yield was calculated through following formula (Mehmood *et al.*, 2022).

$$\text{Extraction yield (\%)} = \frac{\text{Weight of extraction}}{\text{Weight of dry sample}} \times 100$$

Proximate analysis: Proximate analysis was carried out by Anon., (2010) protocols. The moisture content was determined by drying 2 g of the sample in a petri dish at 100°C for 2 hours until a constant weight was achieved. The ash content was determined by heating 2 g of sample in a platinum crucible at 500°C for 3 hours. The fibre content was determined by boiling 2 g of sample with H₂SO₄ and NaOH solutions, filtering, drying, and incinerating the residue. Protein content was determined by the Kjeldahl method, followed by titration with hydrochloric acid. The fat content was determined by extracting 5 g of dried sample using hexane in a Soxhlet apparatus for 4-6 hours. Carbohydrate content was calculated as 100 minus the sum of moisture, protein, ash, fat, and fibre percentages.

Metal analysis of plant samples: Atomic absorption spectrophotometry was used to estimate the following metal contents in plant samples. Zinc (Zn), Manganese (Mn), and Copper (Cu).

Digestion of plant and soil samples: Three replicates of powdered samples, each weighing 1 g, were properly measured with an electrical balance and deposited in crucibles for further investigation. Plant samples were digested using an acid combination to guarantee thorough solubilization. Initially, 1 g of powdered plant material was treated with 10 mL of concentrated (65%) HNO₃ and allowed to stand overnight without heating. The mixture was cooked on a hotplate at 70 degrees Celsius until it evaporated. After cooling the digestion mixture to 25°C, 5

mL of concentrated perchloric acid (70%) was added. The mixture was then reheated at 70°C until complete broad fumes formed, indicating that the sample had been fully oxidized and digested. The digest sample was then placed into volumetric plastics to ensure full sample recovery. The digested samples were filtered through Whatman Filter Paper #42. The filtered samples were placed in 50 mL volumetric flasks and filled to the mark with deionized water. Deionized water was added drop wise until the lower meniscus reached the flask's mark, after which the flask was shaken thoroughly. The solution's concentration was determined by monitoring the meniscus to ensure that it was similar at the bottom (Khan *et al.*, 2020). For digestion of soil samples, 1g of sample was placed in 250mL flask and 10 ml of Nitric acid was added to it. The mixture was incubated overnight. The samples were digested on a hot plate by adding H₂O₂ drop by drop until colourless processed mixture was obtained. The mixture was diluted by 50ml of double distilled water and filtered through Whatman No. 42 filter paper. The concentration of metals in purified sample was measured (Iqbal *et al.*, 2024).

Standard preparation and metal analysis: Standard solutions were prepared for the analysis of zinc, copper, and manganese in plant samples using an atomic absorption spectrophotometer (Shimadzu Co., Ltd., Japan). Standard solutions were prepared to set particular curves.

Estimation of dietary intake of metals: The estimated average daily intake (EDI) was determined by the following formula (Ebrahimzadeh *et al.*, 2024).

$$EDI = \frac{(EF \times ED \times IR \times MC)}{(BW \times AT)}$$

where EF is the exposure frequency, which was set to 90 days per year (Luo *et al.*, 2021), ED is the exposure days over a lifetime (20 years), IR is the daily ingestion rate per capita, which is 0.5 g·d⁻¹ (Luo *et al.*, 2021), MC is the calculated metal concentration (mg/kg), BW is the body weight, the average adult body weights were considered to be 60 (Sohrabi & Majidifar, 2015) and AT is the average lifetime (day) (AT = 60 years × 365 days per year).

The target hazard quotient (THQ) and hazard index (HI): The target hazard quotient (THQ) represents the quantitative evaluation of the potential noncarcinogenic effects of individual toxic metals and was calculated by the following formula:

$$THQ = \frac{EDI}{RfD}$$

where EDI is the daily average exposure dose (mg/kg/day) and RfD represents the oral reference dose, which is 0.3 mg/kg/day for Zn, 0.14 mg/kg/day for Mn, and 0.04 mg/kg/day for Cu (Council *et al.*, 2014).

The hazard index was calculated by the following formula:

$$HI = THQ_1 + THQ_2 + THQ_3$$

Total phenolic and flavonoid content assays: The Folin-Ciocalteu reagent, which is a commonly used reagent in the determination of phenolic compounds, was used to determine the total phenolic content of the samples. Simultaneously, the aluminium chloride colorimetric assay was used to determine the total flavonoid content of the sample, which is a colorimetric method specific to flavonoid-related absorbance. The results were expressed as gallic acid equivalents (GAE) for total phenolics and quercetin equivalents (QE) for total flavonoids (Pandey & Pandey, 2023).

Antioxidant assay: The plant extracts were tested on the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay with slight modifications to determine their antioxidant activity (Rehman *et al.*, 2022). The methanol extracts were kept at a concentration level of 500 µg/mL. The absorbance was obtained at 518 nm by UV-visible spectrophotometer. Methanol mixed with extract was used as the blank, and DPPH solution mixed with methanol was used as the control. The reference standard was Gallic acid. The percentage scavenging activity of the DPPH radical was determined by the following equation:

$$\text{Scavenging activity (\%)} = [(A_c) - (A_s)] / (A_c) \times 100$$

Antimicrobial activity: The plant extracts' antibacterial activity was assessed using disc diffusion and MIC (minimum inhibitory concentration) techniques in Mueller-Hinton II agar (Beckton Dickinson) under standard conditions, following CLSI recommendations (Kozłowska *et al.*, 2022). Bacterial strains (*E. coli* and *S. aureus*) were obtained from laboratory culture collection and cultivated overnight in nutrient broth and then adjusted to 0.5 McFarland turbidity to standardize the bacterial inoculum. After being impregnated with 2 mg of methanol plant extract, sterile Whatman No. 3 filter paper discs (9 mm diameter) were placed on agar plates previously infected with the test bacteria. The inhibition zone extension beyond the edge of the 9 mm disc was measured after 18 h of incubation at 35 ± 2.5°C. The negative and positive controls were methanol and ciprofloxacin (0.03 mg/disc), respectively. Twofold serial dilutions of the extracts were made in DMSO and added to molten agar to produce final concentrations ranging from 31.3 to 2000 mg/L in order to determine the minimum inhibitory concentration (MIC). Each plate was inoculated with 2 µL of bacterial solution following solidification. Negative controls were plates with only DMSO. The lowest extract concentration that totally stopped observable bacterial growth during a 24-hour incubation period at 35°C was known as the minimum inhibitory concentration, or MIC. The zone of inhibition was measured as the clear zone extending beyond the edge of the 9 mm antibiotic disc. MIC values for ciprofloxacin were calculated in accordance with CLSI recommendations.

Statistical analysis

Experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analysis was performed by using MS Excel and Python. The effects of plant species, collection site, and their interaction were evaluated using two-way analysis of variance (ANOVA). Normality and homogeneity assumptions were tested before applying ANOVA. Pearson correlation

analysis was performed to examine the relationships among proximate composition, heavy metal concentrations, phytochemical contents, antioxidant activity, and antibacterial activity. Principal component analysis (PCA) was conducted using standardized (z-score transformed) variables to reduce dimensionality and identify the major sources of variation among nutritional composition, heavy metal concentrations, phytochemical contents, antioxidant activity, and antibacterial activity. Variables were standardized before analysis to eliminate differences in measurement scales. Principal components with eigenvalues greater than 1.0 were retained, and the percentage of variance explained by each component was calculated. The loading coefficients of the retained principal components were examined to identify the variables contributing most strongly to sample differentiation. Hierarchical cluster analysis (HCA) was performed using standardized data to evaluate similarities among the measured variables. Euclidean distance was used as the dissimilarity measure, and Ward's minimum variance method was applied as the linkage criterion to generate the dendrogram. The resulting clusters were interpreted based on similarities in nutritional, phytochemical, heavy metal, antioxidant, and antibacterial characteristics. All statistical analyses were performed using XLSTAT 2024 (Addinsoft, Paris, France), and differences were considered statistically significant at $p < 0.05$.

Results

Proximate and nutritional analysis: The proximal analysis was demonstrated showing notable variation in the nutritional composition of *C. tuberculata* and *A. racemosus* that are collected from two different sites in Namal Valley (Table 2). Moisture content, ranged from $3.11 \pm 0.105\%$ to $8.47 \pm 0.471\%$ indicates the fluctuation among samples. The highest moisture levels were detected among two sites. *C. tuberculata* collected from site 2 exhibited higher moisture content whereas *A. racemosus* have relatively lower levels.

The highest crude protein content ($22.15 \pm 0.090\%$) was present in *A. racemosus* at site 2. In contrast, the lowest crude protein content of *C. tuberculata* ($5.61 \pm 0.120\%$) was observed at site 1. The maximum total carbohydrate content ($48.78 \pm 0.120\%$) was determined in *C. tuberculata* at site 2, indicating its comparatively higher energy reserves and mineral accumulation potential compared to *A. racemosus*, which showed the minimum total carbohydrate content ($18.03 \pm 0.150\%$) at site 2.

The mean values of the crude fat content ranged between 2.94 ± 0.290 in *C. tuberculata* and $13.54 \pm 0.040\%$ in *Asparagus racemosus*, suggesting their role as an energy source. The maximum value of crude fibre ($42.37 \pm 0.130\%$) was found in *A. racemosus* at site 1, while the minimum

value of crude fibre ($20.91 \pm 0.190\%$) was reported in *C. tuberculata* at site 1, indicating the nutritional importance of digestive and metabolic regulation. The ash content varied from $3.33 \pm 0.100\%$ in *A. racemosus* to $14.19 \pm 0.160\%$ in *C. tuberculata*, representing inorganic mineral elements, including both essential micronutrients and potentially toxic heavy metals. The variation observed among species and sites may be explained by genetic differences, physiological differences, environmental differences, soil differences and differences in the microclimate affecting nutrient build-up and metabolism.

Two-way ANOVA and indicated that plant species significantly affected all nutritional parameters, including moisture ($F=835.03$), protein ($F=38901.51$), carbohydrate ($F=7003.23$), fat ($F=4849.46$), fibre ($F=50102.53$), and ash content ($F=19786.97$), with $p < 0.001$. Site also had a significant effect on moisture, protein, fat, fibre, and ash ($p < 0.001$), whereas carbohydrate content was not significantly affected by site ($F=4.01$, $p=0.0801$). Significant species $\times$ site interactions were observed for moisture ($p=0.0287$), protein ($p < 0.001$), carbohydrate ($p=0.0025$), fat ($p < 0.001$), fibre ($p < 0.001$), and ash ($p=0.0191$). These results revealed that both species and collection site have a considerable influence on the nutritional composition of the studied plants. The interaction effect further suggests differential responses of the two species to environmental conditions (Table 3).

Metal accumulation and health risk assessment: Table 4 presents the concentrations of zinc, manganese, and copper in all plant samples. The zinc concentration in all plant samples varied from 34.49 ± 0.111 mg/kg in *Asparagus racemosus* to 55.64 ± 0.157 mg/kg in *Caralluma tuberculata*. The results revealed that the metal is present below permissible limits, indicating minimal risk of toxic exposure. High zinc accumulation in *C. tuberculata* observed at site 2 reflected the corresponding metal enrichment in soil. The highest concentration of manganese was found in *A. racemosus* at site 1, while the lowest concentration was found in *C. tuberculata* (17.29 ± 0.111 mg/kg) at site 2. The observed concentrations of manganese indicate that the metal is present under the permissible limit given by the World Health Organization. The concentration of copper was also under the permissible limit given by WHO/FAO, with the highest concentration recorded in *A. racemosus* (36.05 ± 0.095 mg/kg) at site 2, and the lowest concentration was also observed at site 2 in *C. tuberculata* (11.63 ± 0.066 mg/kg). The variation among the values may be due to the physical and chemical properties of the soil and climatic conditions of an area that affect the availability of heavy metals.

Table 2. Nutritional composition of selected plants collected from Namal Valley

Site	Plants	Total moisture content %	Crude protein %	Total carbohydrates %	Crude fat %	Crude fibre %	Total ash content %
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
1.	<i>Caralluma tuberculata</i>	8.05 ± 0.221	5.61 ± 0.120	47.97 ± 0.840	3.27 ± 0.350	20.91 ± 0.190	14.19 ± 0.160
	<i>Asparagus racemosus</i>	3.11 ± 0.105	18.92 ± 0.130	20.24 ± 0.850	11.23 ± 0.070	42.37 ± 0.130	4.13 ± 0.080
2.	<i>Caralluma tuberculata</i>	8.47 ± 0.471	5.7 ± 0.170	48.78 ± 0.120	2.94 ± 0.290	21.13 ± 0.160	12.98 ± 0.130
	<i>Asparagus racemosus</i>	4.36 ± 0.110	22.15 ± 0.090	18.03 ± 0.150	13.54 ± 0.040	38.59 ± 0.110	3.33 ± 0.100

Table 3. Two-way ANOVA for nutritional components in plant samples.

Parameter	Source of variation	df	SS	MS	F-value	p-value	Significance
Moisture	Species	1	61.3816	61.3816	835.03	<0.001	***
Moisture	Site	1	2.1000	2.1000	28.57	<0.001	***
Moisture	Species x Site	1	0.5208	0.5208	7.09	0.0287	*
Moisture	Residual	8	0.5881	0.0735	-	-	
Protein	Species	1	664.2432	664.2432	38901.51	<0.001	***
Protein	Site	1	8.2668	8.2668	484.15	<0.001	***
Protein	Species x Site	1	7.3947	7.3947	433.07	<0.001	***
Protein	Residual	8	0.1366	0.0171	-	-	
Carbohydrate	Species	1	2564.9328	2564.9328	7003.23	<0.001	***
Carbohydrate	Site	1	1.4700	1.4700	4.01	0.0801	Ns
Carbohydrate	Species x Site	1	6.8403	6.8403	18.68	0.0025	**
Carbohydrate	Residual	8	2.9300	0.3662	-	-	
Fat	Species	1	258.3552	258.3552	4849.46	<0.001	***
Fat	Site	1	2.9403	2.9403	55.19	<0.001	***
Fat	Species x Site	1	5.2272	5.2272	98.12	<0.001	***
Fat	Residual	8	0.4262	0.0533	-	-	
Fibre	Species	1	1136.0748	1136.0748	50102.53	<0.001	***
Fibre	Site	1	9.5052	9.5052	419.19	<0.001	***
Fibre	Species x Site	1	12.0000	12.0000	529.22	<0.001	***
Fibre	Residual	8	0.1814	0.0227	-	-	
Ash	Species	1	291.3631	291.3631	19786.97	<0.001	***
Ash	Site	1	3.0301	3.0301	205.78	<0.001	***
Ash	Species x Site	1	0.1261	0.1261	8.56	0.0191	*
Ash	Residual	8	0.1178	0.0147	-	-	

Note: SS = Sum of squares; MS = Mean square; df = Degrees of freedom; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = Nonsignificant. Residual rows represent within-group error and therefore do not have F-values or p values

Table 4. Concentration of metals among selected plants collected from Namal Valley.

Site	Plants	Metals in soil			Metals in plants		
		Zn	Mn	Cu	Zn	Mn	Cu
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
1.	<i>Caralluma tuberculata</i>	100.3 $\pm$ 0.974	79.53 $\pm$ 0.729	44.99 $\pm$ 0.948	49.44 $\pm$ 0.121	22.91 $\pm$ 0.085	13.15 $\pm$ 0.092
	<i>Asparagus racemosus</i>	99.45 $\pm$ 1.388	77.89 $\pm$ 1.386	45.80 $\pm$ 0.313	34.49 $\pm$ 0.111	42.19 $\pm$ 0.542	32.19 $\pm$ 0.121
2.	<i>Caralluma tuberculata</i>	113.3 $\pm$ 0.846	63.47 $\pm$ 0.387	46.76 $\pm$ 0.587	55.64 $\pm$ 0.157	17.29 $\pm$ 0.111	11.63 $\pm$ 0.066
	<i>Asparagus racemosus</i>	118.2 $\pm$ 0.603	52.27 $\pm$ 1.328	53.57 $\pm$ 0.601	38.95 $\pm$ 0.075	39.86 $\pm$ 0.640	36.05 $\pm$ 0.095

The estimated average daily intake (EDI) of zinc (Zn), manganese (Mn) and copper (Cu) from *C. tuberculata* and *A. racemosus* across both sites is presented in Table 6. Low EDI values indicated that these plants contain trace amounts of metals and are not expected to cause any risk of exceeding safe daily intake levels. Target hazard quotient (THQ) calculations showed that the chronic daily dose of these medicinal plants would generally not result in metal exposure levels above regulatory guidance for general consumption, but it is important to note that vulnerable groups (children and pregnant women or individuals with impaired renal function) would require more conservative recommendations on consumption (Table 7). Graphical representation of estimated average daily intake (EDI), Target hazard quotient (THQ) and hazard index (HI) of studied metals from selected plants across both sites is given in Fig. 1.

Analysis of soil samples demonstrated that metal concentrations varied between both collection sites. Among the sites, Site 1 consistently showed the highest accumulation of manganese, while site 2 exhibited

elevated concentrations of zinc and copper. Larger differences among different sampling sites emphasize the role of environmental conditions and geospatial characterization and social geochemical evaluation into ethnobotanic investigations.

The relationship between species, sampling site and their interaction significantly affects the concentrations of metals detected in plant samples (Table 5). Zinc, manganese and copper concentrations were significantly affected by species ($F = 52230.76$, $p < 0.001$ for Zn, $F = 7264.30$, $p < 0.001$ for Mn and $F = 155241.50$, $p < 0.001$ for Cu), site ($F = 5928.81$, $p < 0.001$ for Zn, $F = 262.14$, $p < 0.001$ for Mn and $F = 450.05$, $p < 0.001$ for Cu), and species x site interaction ($F = 157.96$, $p < 0.001$ for Zn, $F = 44.89$, $p < 0.001$ for Mn and $F = 2378.99$, $p < 0.001$ for Cu). Statistical analysis indicated that both species that play an important role in determining metal accumulation. The significant interaction between species, site further demonstrated that the plant species differ in their capacity to accumulate metals under the same environmental conditions.

Table 5. Two-way ANOVA for Zn, Mn and Cu concentrations in plant samples.

Metal	Source of variation	df	SS	MS	F-value	p-value	Significance
Zn (mg/kg)	Species	1	750.8172	750.8172	52230.76	<0.001	***
Zn (mg/kg)	Site	1	85.2267	85.2267	5928.81	<0.001	***
Zn (mg/kg)	Species x Site	1	2.2707	2.2707	157.96	<0.001	***
Zn (mg/kg)	Residual/Error	8	0.1150	0.0144	-	-	
Mn (mg/kg)	Species	1	1313.5669	1313.5669	7264.30	<0.001	***
Mn (mg/kg)	Site	1	47.4019	47.4019	262.14	<0.001	***
Mn (mg/kg)	Species x Site	1	8.1181	8.1181	44.89	<0.001	***
Mn (mg/kg)	Residual/Error	8	1.4466	0.1808	-	-	
Cu (mg/kg)	Species	1	1416.5787	1416.5787	155241.50	<0.001	***
Cu (mg/kg)	Site	1	4.1067	4.1067	450.05	<0.001	***
Cu (mg/kg)	Species x Site	1	21.7083	21.7083	2378.99	<0.001	***
Cu (mg/kg)	Residual/Error	8	0.0730	0.0091	-	-	

Note: df = Degrees of freedom; SS = Sum of squares; MS = Mean square. Significance codes: *** p<0.001; ** p<0.01; * p<0.05; ns = Nonsignificant. Residual/error rows are included to show the error term used in the F-ratio calculation

Table 6. Estimated average daily intake (EDI) of metals from selected plants collected from Namal Valley.

Sites	Plants	Zn	Mn	Cu	HI
1.	<i>Caralluma tuberculata</i>	1.13×10^{-4}	1.12×10^{-4}	2.25×10^{-4}	4.50×10^{-4}
	<i>Asparagus racemosus</i>	7.87×10^{-5}	2.06×10^{-4}	5.50×10^{-4}	8.35×10^{-4}
2.	<i>Caralluma tuberculata</i>	1.27×10^{-4}	8.43×10^{-5}	1.99×10^{-4}	4.10×10^{-4}
	<i>Asparagus racemosus</i>	8.90×10^{-5}	1.95×10^{-4}	6.18×10^{-4}	9.02×10^{-4}

Table 8. Extraction yield, total phenolic and flavonoid contents of methanol extracts of selected plants collected from selected sites.

Sites	Plants	Extraction yield	TPC	TFC
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
1.	<i>Caralluma tuberculata</i>	11.00 $\pm$ 1.50	41.55 $\pm$ 0.659	15.6 $\pm$ 0.825
	<i>Asparagus racemosus</i>	20.67 $\pm$ 2.08	107.65 $\pm$ 1.768	55.55 $\pm$ 1.441
2.	<i>Caralluma tuberculata</i>	13.33 $\pm$ 1.61	59.71 $\pm$ 1.524	21.67 $\pm$ 1.261
	<i>Asparagus racemosus</i>	23.33 $\pm$ 1.04	122.84 $\pm$ 0.554	69.04 $\pm$ 1.218

Table 9. Two-way ANOVA for TPC and TFC in plant samples.

Parameter	Source of variation	df	SS	MS	F-value	p-value	Significance
TPC	Species	1	12525.2947	12525.2947	8092.58	<0.001	***
TPC	Site	1	834.1669	834.1669	538.95	<0.001	***
TPC	Species x Site	1	6.6157	6.6157	4.27	0.0725	Ns
TPC	Residual	8	12.3820	1.5477	-	-	
TFC	Species	1	5718.5868	5718.5868	3922.95	<0.001	***
TFC	Site	1	286.9452	286.9452	196.84	<0.001	***
TFC	Species x Site	1	41.2923	41.2923	28.33	<0.001	***
TFC	Residual	8	11.6618	1.4577	-	-	

Note: SS = Sum of squares; MS = Mean square; df = Degrees of freedom; * p<0.05; ** p<0.01; *** p<0.001; ns = Nonsignificant. TPC = Total phenolic content; TFC = Total flavonoid content. Residual rows represent within-group error and therefore do not have F-values or p values

Table 10. Antioxidant activity of selected plants collected from two selected sites.

Sites	Plants	Antioxidant activity %
		Mean $\pm$ SD
1.	<i>Caralluma tuberculata</i>	31.55 $\pm$ 0.455
	<i>Asparagus racemosus</i>	60.91 $\pm$ 0.809
	Gallic acid	94.02 $\pm$ 0.207
2.	<i>Caralluma tuberculata</i>	54.08 $\pm$ 0.115
	<i>Asparagus racemosus</i>	73.01 $\pm$ 0.197
	Gallic acid	94.02 $\pm$ 0.207

Enrichment of phenolic and flavonoid content: The methanol extraction yield varied among the plant samples and collection sites. *Asparagus racemosus* showed a higher extraction yield than *Caralluma tuberculata*, indicating greater recovery of methanol soluble constituents. The extracts revealed significant variation in TPC and TFC

values among the studied plant samples (Table 8). *Asparagus racemosus* showed the highest TPC value (122.84 $\pm$ 0.554 mg GAE/g) at site 2. These findings suggest increased accumulation of secondary metabolites in this species under the environmental conditions that are present at site 2. The observed values indicated a considerable accumulation of phenolic compounds, which are recognized for their antioxidant and protective biological activities. A variation in phytochemical composition was observed in the studied medicinal plants. The lowest accumulation of total phenolic content was observed in (41.55 $\pm$ 0.659 mg GAE/g) in *C. tuberculata* and highest flavonoid content (69.04 $\pm$ 1.218 mg QE/g) was observed in *Asparagus racemosus* from site 2. The lowest TFC value was recorded (15.60 $\pm$ 0.825 mg QE/g) showed in *C. tuberculata*, suggesting that production and accumulation of both compounds are

influenced by both species identity and environmental conditions that include geographic location, climates, soil characteristics, availability of light and different stress factors. The result showed that species affect both phenolic and flavonoid contents.

The species effect was highly significant for TPC ($F = 8092.58$, $p < 0.001$), indicating that *A. racemosus* contained a higher phenolic content than *C. tuberculata*. The site effect was also highly significant for TPC ($F = 538.95$, $p < 0.001$), showing that phenolic accumulation differed significantly between Site 1 and Site 2. However, the species $\times$ site interaction for TPC was nonsignificant ($F = 4.27$, $p = 0.0725$), which suggested that the difference between the two plant species remained broadly consistent across both collection sites. For TFC, species, site, and their interaction were all statistically significant. The species effect was highly significant ($F = 3922.95$, $p < 0.001$), confirming strong species-level variation in flavonoid content. The site effect was also highly significant ($F = 196.84$, $p < 0.001$), indicating that the collection site significantly influenced the flavonoid concentration. In addition, the species $\times$ site interaction was significant ($F = 28.33$, $p < 0.001$), meaning that the magnitude of site-related change in TFC differed between *C. tuberculata* and *A. racemosus*. Overall, *A. racemosus* showed greater phytochemical enrichment than *C. tuberculata*, and site 2 generally supported higher TPC and TFC values (Table 9).

Antioxidant potential of medicinal plants: Table 10 demonstrates the antioxidant activity assessment using the DPPH radical scavenging assay. The results revealed that *Asparagus racemosus* possessed the highest inhibition ($73.01 \pm 0.197\%$) at site 2 against DPPH, and *Caralluma tuberculata* showed the lowest percentage inhibition ($31.55 \pm 0.455\%$). The high antioxidant values indicate that both species have antioxidant potential. Variations recorded among the antioxidant potential of studied plants from both sites may be due to the environmental and physiological variations that affect the formation of bioactive compounds responsible for the antioxidant activity of plants. Figure 2 illustrates the graphical representation of variations in antioxidant activity among selected plants across both sites.

Antibacterial efficacy against human pathogens: Antimicrobial testing against pathogenic bacteria revealed differential susceptibility patterns (Table 11). Maximum antibacterial activity against *E. coli* was shown by *Asparagus racemosus* (21.91 ± 0.459 mm) at site 2 with an MIC of 0.25 mg/mL in comparison to ciprofloxacin (24.92 ± 0.288 mm). The minimum activity (1.94 ± 0.115 mm) was shown by *C. tuberculata* at site 1, with an MIC of >2 mg/mL. The antibacterial activity of selected plants against *S. aureus* varied from 5.04 ± 0.261 mm to 24.73 ± 0.354 mm in comparison to the positive control ciprofloxacin (30.76 ± 0.167 mm). The highest antibacterial activity was shown by *A. racemosus* (24.73 ± 0.354 mm), with an MIC of 0.125 mg/mL extract at site 2. The lowest activity was shown by *C. tuberculata* (5.04 ± 0.261 mm) at site 1, with an MIC of >2 mg/mL. Antibacterial activity varied significantly among species and collection sites, likely reflecting differences in the concentration and specificity of antimicrobial phytochemicals (Fig. 3).

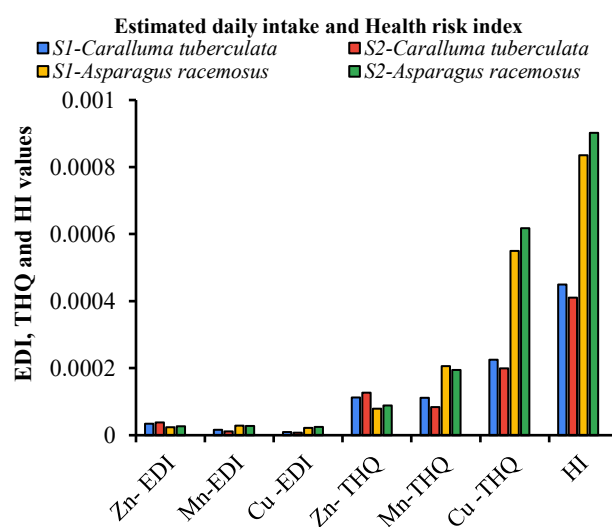


Fig. 1. Estimated average daily intake (EDI), Target hazard quotient (THQ) and hazard index (HI) metals from selected plants collected from Namal Valley

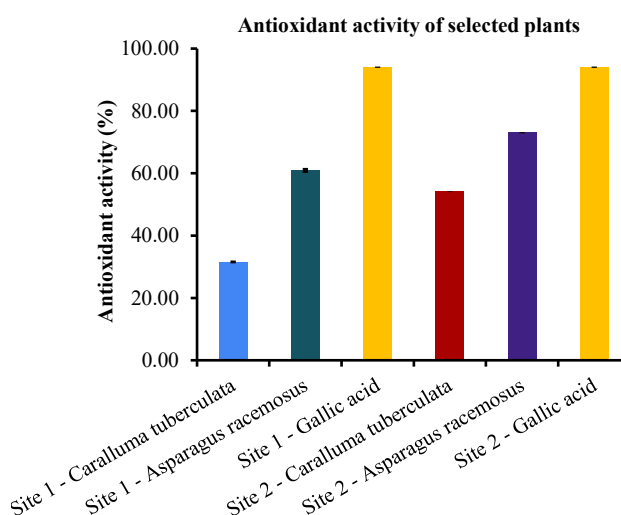


Fig. 2. Antioxidant activity of selected plants collected from two selected sites.

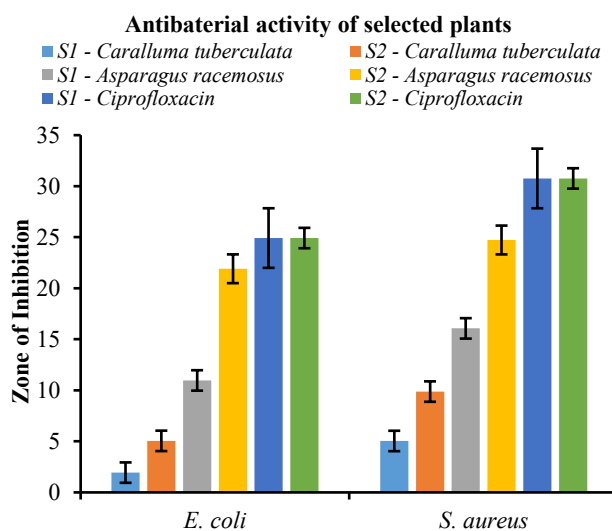


Fig. 3. Antibacterial activity of selected plants collected from two selected sites.

Table 11. Antibacterial activity of selected plants collected from two selected sites.

Sites	Plants	<i>E. coli</i>		<i>S. aureus</i>	
		ZOI (mm)	MIC (mg/mL)	ZOI (mm)	MIC (mg/mL)
1.	<i>Caralluma tuberculata</i>	1.94 ± 0.115	>2	5.04 ± 0.261	>2
	<i>Asparagus racemosus</i>	10.97 ± 0.368	>1	16.07 ± 0.477	0.125
	Ciprofloxacin	24.92 ± 0.288	0.0125	30.76 ± 0.167	0.0125
2.	<i>Caralluma tuberculata</i>	5.05 ± 0.231	>2	9.88 ± 0.286	1
	<i>Asparagus racemosus</i>	21.91 ± 0.459	0.25	24.73 ± 0.354	0.125
	Ciprofloxacin	24.92 ± 0.288	0.0125	30.76 ± 0.167	0.0125

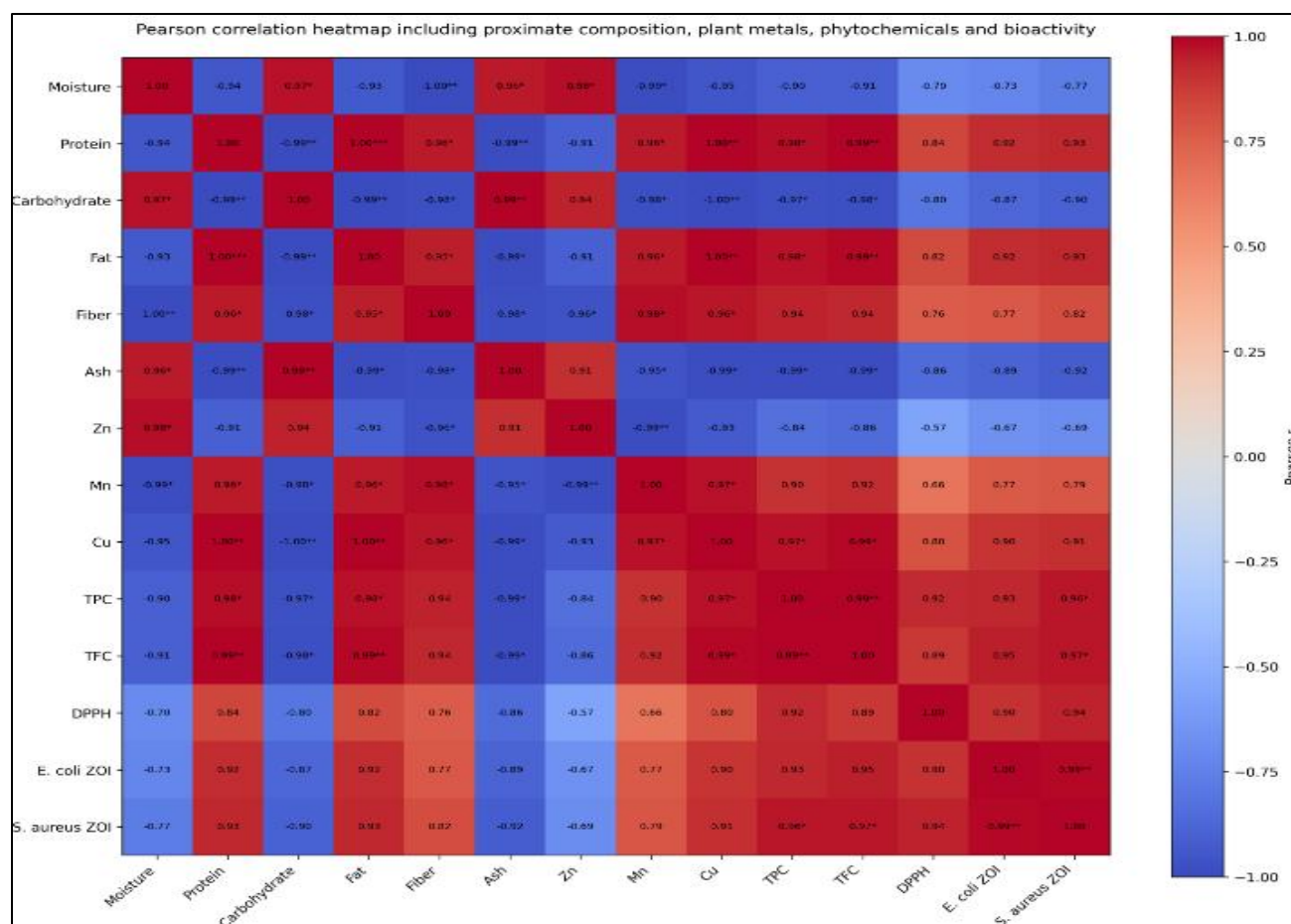


Fig. 4. Pearson correlation heatmap of biochemical and bioactivity variables.

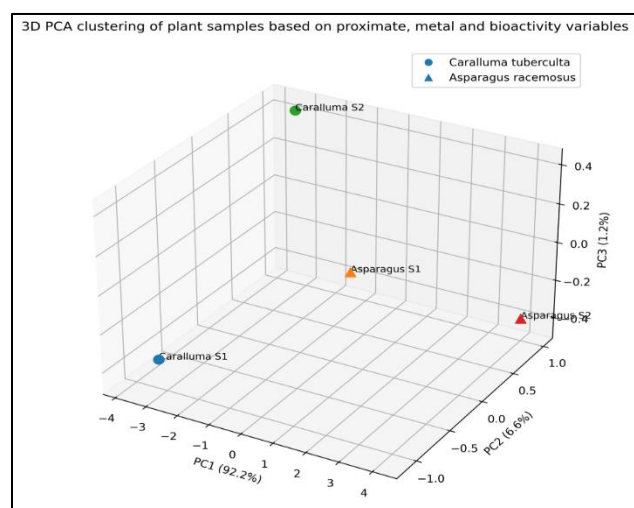


Fig. 5. Three-dimensional principal component analysis of studied variables.

Relationship among phytochemicals and biological activities: Pearson correlation analysis showed associations among proximate composition, plant metals, phytochemicals and bioactivity variables. TPC and TFC were positively correlated with DPPH activity and antibacterial zones of inhibition. Protein, fat, Mn and Cu also showed positive associations with TPC, TFC and bioactivity variables, while carbohydrate, ash, moisture and Zn generally showed negative associations with antioxidant and antimicrobial responses. The observed association suggests that phenolic and flavonoid compounds may contribute to antioxidant and antimicrobial activity (Fig. 4).

Multivariate differentiation of samples: Three-dimensional PCA showed clear clustering of plant samples based on proximate composition, plant metal concentration, phytochemical content, antioxidant activity and antimicrobial activity. PC1 explained 92.2% of the total variation, PC2 explained 6.6%, and PC3 explained

1.2%. The largest positive loadings on PC1 were observed for total phenolic content (TPC), total flavonoid content (TFC), DPPH antioxidant activity, protein, fibre, Cu, Mn, and antibacterial activity, whereas carbohydrate content, ash, moisture, and Zn showed negative loadings, indicating that PC1 primarily represented variation associated with phytochemical richness and biological activity. PC2 contributed comparatively less variation and mainly reflected differences associated with sampling sites. Component analysis differentiated the two plant species *C. tuberculata* and *A. racemosus* that have distinct regions of the PCA. Plant sites related clustering was also apparent especially between site 1 and site 2 samples that play the role of local environmental factors that significantly influenced plant composition and biological activity.

Overall, PCA revealed patterns consistent with Pearson correlation analysis (Fig. 5).

Cluster analysis: HCA grouped variables into two major clusters. The first cluster comprised carbohydrate, ash, moisture and Zn, indicating a distinct compositional pattern among these variables. The second major cluster included DPPH antioxidant activity, *E. coli* and *S. aureus* zones of inhibition, fibre, Mn, Cu, protein, fat, TPC and TFC. Within this cluster, TPC and TFC were closely associated, while the antibacterial variables were grouped with DPPH activity, demonstrating relationships between phytochemical composition and biological activity. These findings support the Pearson correlation and PCA results (Fig. 6).

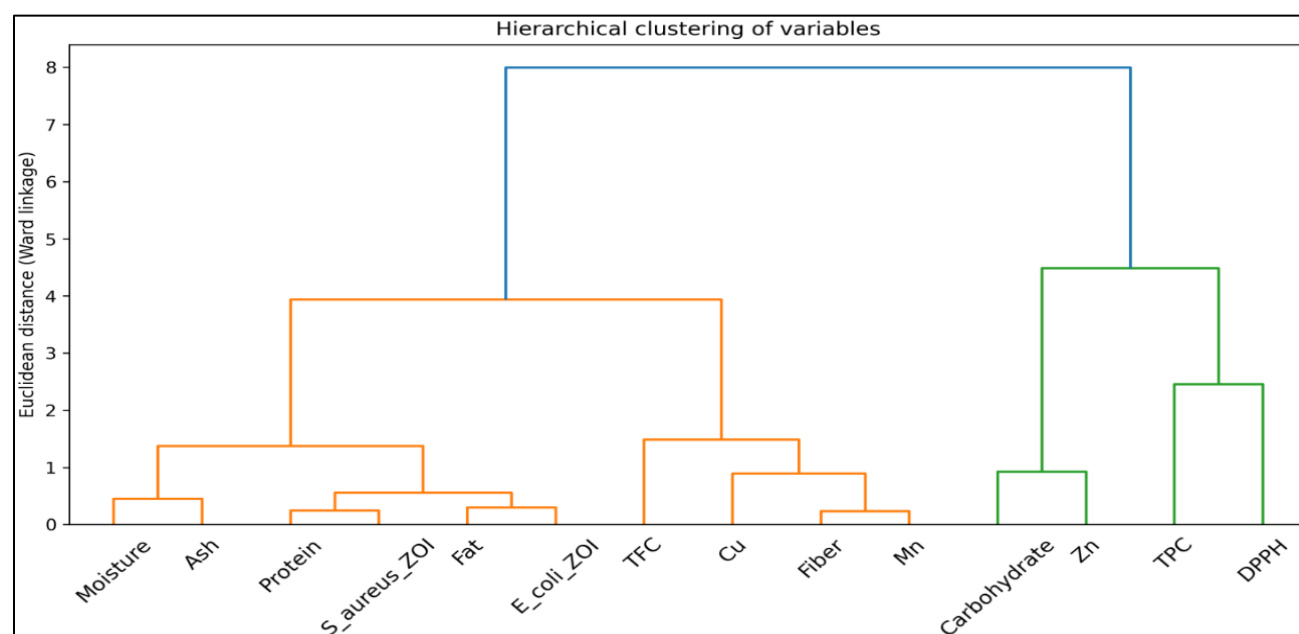


Fig. 6. Hierarchical clustering dendrogram of biochemical and bioactivity variables.

Discussion

Proximate analysis revealed the species-specific differences in tissue water content that directly influence the extraction efficiency and shelf-life stability of prepared herbal medicines (Table 2). The highest moisture content of *Caralluma tuberculata* ($8.47 \pm 0.471\%$) at site 2 was supported by the value of 10.51% reported by Usman *et al.*, (2020). The reasonably high moisture contents show that there might also be a higher activity of water-soluble enzymes (Stadlmayr *et al.*, 2011). Conversely, the lowest moisture content recorded in *Asparagus racemosus* ($3.11 \pm 0.105\%$) at site 1 was comparable to the slightly higher value reported by Olayinka *et al.*, (2024). The high moisture content reflects the succulent nature of plants, with rapid drying rates critical for preventing fungal contamination and maintaining phytochemical integrity during storage (Ali *et al.*, 2024). The highest crude protein content ($22.15 \pm 0.090\%$) were found in *A. racemosus* at site 2 was comparable to the values (29.4%) recorded by Stoyanova and Angelova-Romova (2024). High crude protein values suggest the nutritional superiority of plants (Shahid *et al.*, 2023). In contrast, the lowest crude protein

content of *C. tuberculata* ($5.61 \pm 0.120\%$) at site 1 was comparable to that (3.79%) reported by Bouzid *et al.*, (2024). The difference between the observed values of protein contents may be due to various intrinsic factors that affect the concentration of protein in plants, as reported by Bakhtiar *et al.*, (2024). Agroclimatic variations also lead to variation among the protein contents of different plant species (Rehman & Adnan, 2018; Mobeen *et al.*, 2026).

The maximum total carbohydrate content ($48.78 \pm 0.120\%$) was determined in *C. tuberculata* at site 2, and the minimum total carbohydrate content ($18.03 \pm 0.150\%$) was found in *A. racemosus* at site 2. The obtained carbohydrate values were comparable to those described by Okonwu *et al.*, (2020) and Añibarro-Ortega *et al.*, (2019). A considerable amount of carbohydrates suggests that the plant can be a good source of energy and can also help in digestion and the assimilation of other nutrients (Kashyap *et al.*, 2022). The reason for the difference in the carbohydrate content could be the difference in physiological functions of the studied parts of the plants reported by Begum *et al.*, (2018). The mean values of the crude fat content ranging between 2.94 ± 0.290 in *C. tuberculata* and $13.54 \pm 0.040\%$ in *A. racemosus* were

close to the values of 2.01% and 18.59% observed by Ibrahim *et al.*, (2024) and Rao & Poonia (2023), respectively. The considerable amount of crude fat in selected plant species suggests their role as an energy source as well as in boosting the absorption of fat soluble vitamins (Radha *et al.*, 2021). The difference in crude fat values may be due to different types of cultivars (Tura *et al.*, 2023). The maximum value of crude fibre ($42.37 \pm 0.130\%$) found in *A. racemosus* at site 1 was close to the value (52.08%) reported by Aroyehun *et al.*, (2020). The minimum value of crude fibre ($20.91 \pm 0.190\%$) reported in *C. tuberculata* at site 1 was comparable to the value (25.57%) reported by Audu *et al.*, (2024). The presence of a reasonable amount of crude fibre suggests its nutritional importance, as it plays a significant role in digestive and metabolic regulation (Bera *et al.*, 2026). The ash content ranged from $3.33 \pm 0.100\%$ in *A. racemosus* to $14.19 \pm 0.160\%$ in *C. tuberculata*. The obtained values were supported by the value of 3.008% calculated by Ilechukwu *et al.*, (2025). In addition, 13.38% was reported by Khan *et al.*, (2025) in wild medicinal plants. The ash content represents inorganic mineral elements, including both essential micronutrients and potentially toxic heavy metals (Yiblet & Adamu, 2023). Variation in nutritional components among different plants and sites may be due to differences in phytochemical content, harvesting time and environmental influences, including temperature and humidity (Al-Qahtani *et al.*, 2020; Hou *et al.*, 2024). Moreover, the variations might be due to spatiotemporal variations, geographical variations and variations in environmental parameters that affect the nutritional composition of plants (Shahzad *et al.*, 2022; Pirasteh-Anosheh *et al.*, 2023). In addition, different plant parts have different nutritional profile which may be the reason of variation in proximate composition of *A. racemosus* and *C. tuberculata* (Ghafar *et al.*, 2022; Biel *et al.*, 2023).

The species-specific accumulation patterns of zinc, manganese, and copper demonstrate that differential metal uptake capabilities correlate with plant taxon-specific differences in root morphology, metal transporter expression, and soil-to-plant translocation efficiency (Table 4). The zinc concentration in all plant samples varied from 34.49 ± 0.111 mg/kg in *A. racemosus* to 55.64 ± 0.157 mg/kg in *C. tuberculata*. The recorded values were supported by the values reported by Khan *et al.*, (2020). While zinc is an essential cofactor for hundreds of metalloenzymes involved in protein synthesis, energy metabolism, and immune function, concentrations exceeding 200 mg/kg in plant tissues may impose toxicological risks for chronic dietary consumers (Zhong *et al.*, 2024). The highest concentration of manganese (42.19 ± 0.542 mg/kg) found in *A. racemosus* at site 1 was close to the value (44 mg/kg) reported by Lanjwani *et al.*, (2018). The lowest concentration (17.29 ± 0.111 mg/kg) found in *C. tuberculata* was supported by the value (13.2 mg/kg) found by Jyothsna *et al.*, (2020). The concentration of Zinc in both of the medicinal plants is under the permissible limit of 100 mg/Kg (Sulaiman *et al.*, 2024). Manganese, while essential for photosynthetic oxygen evolution and antioxidant defence enzyme (manganese superoxide dismutase) function, exhibits a narrow margin between nutritional sufficiency and toxicity, with excessive

accumulation potentially inducing manganese toxicosis characterized by neurological dysfunction and impaired cognitive performance. According to the results, the concentration of manganese is under the permissible limit (200 mg/kg) given by the World Health Organization (Baba & Mohammed, 2021). The concentration of copper varied from 11.63 ± 0.066 mg/kg in *C. tuberculata* to 36.05 ± 0.095 mg/kg in *A. racemosus*. The observed range was supported by the range (13.278–42.586 mg/kg) calculated by Karahan (2023). The difference in previously reported values and studied values may be due to spatiotemporal variations (Jing *et al.*, 2025).

Copper is essential for cytochrome c oxidase and laccase enzyme function yet is highly toxic when it accumulates excessively due to its potent generation of hydroxyl radicals (Feng *et al.*, 2022). The results showed that the concentration of copper is under the permissible limit (40 mg/Kg) given by the WHO/FAO (Dado, 2023). The variation among the values may be due to plant physiology, and agro climatic conditions of an area can also affect the concentration of heavy metals in plants (Radha *et al.*, 2021). Pollution indices for heavy metal toxicity also showed variations among different plants and metals (Tables 6 and 7; Fig. 1). Low EDI values reflect that these plants contain trace amounts of these essential metals, and their consumption is unlikely to pose any risk of exceeding safe daily intake levels (Rajih & Oudah, 2026). The target health quotient (under the permissible limit of less than 1) indicated a low risk of non-carcinogenic health effects that are associated with the chronic consumption of the studied medicinal plants as metal exposure levels remained below regulatory benchmarks. More conservative intake recommendations may be necessary that includes children, pregnant women and individuals that have reduced renal susceptibility to the toxicity of metals (Buqaj *et al.*, 2026). Overall, the result suggested low non-carcinogenic risk under the assumed intake scenario but it is necessary to screen other toxic metals to ensure the safe consumption of plants as edible plants with high concentration of heavy metals can pose serious threat to human health even at low doses as reported by Ahmad *et al.* (2026).

The concentrations of total phenolic content (TPC) and total flavonoid content (TFC) in methanol extracts of the selected medicinal plants have been determined (Table 8). Among the studied samples, *A. racemosus* collected from Site 2 exhibited the highest TPC (122.84 ± 0.554 mg GAE/g). The observed values indicate a considerable accumulation of phenolic compounds, which are recognized for their antioxidant and protective biological activities. The highest TPC value was lower than that reported by Abolhasani *et al.*, (2015). The lowest concentration of total phenolic content was observed in *C. tuberculata* (41.55 ± 0.659 mg GAE/g). The obtained value was close to the value (33.14) reported by Dragusha *et al.*, (2023). Medicinal plants showed considerable concentration of essential bioactive compound which may associated with their biological activity as reported by Abdullah *et al.* (2026). Mean values of total flavonoid content showed that *A. racemosus* contained the highest concentration of total flavonoid content (69.04 ± 1.218 mg QE/g) at site 2. The value observed was close to the value

(63.90) reported by Gorski *et al.* (2019). The lowest concentration of total flavonoid content in methanol extracts was observed in $(15.6 \pm 0.825 \text{ mg QE/g})$. The obtained value was comparable to the previously reported value (11.92) in edible medicinal plants (Ralte *et al.*, 2021). The previous study performed on *C. tuberculata* revealed that considerable amount of TPC ($83.12 \pm 1.26 \text{ mg GAE/g}$) and TFC ($47.45 \pm 1.37 \text{ mg QE/g}$) was present in extracts with considerable antioxidant and antibacterial potential (Alodaini *et al.*, 2025). In case of *A. racemosus*, it has been reported that the plant extract contains $80.92 \pm 0.57 \text{ mg GAE/g}$ of TPC and $58.22 \pm 1.62 \text{ mg QE/g}$ of TFC along with significant antioxidant and antimicrobial properties (Oli *et al.*, 2024). Both the reported results support our findings with minor variations which may be attributed to differences in environmental conditions, including geographical location, climatic factors, light intensity, soil characteristics, and other abiotic and biotic stresses that influence secondary metabolite production in plants (Pratyusha, 2022). Most of the plant samples contained considerable concentrations of total flavonoids, which impart multiple pharmaceutical properties to plants (Shahrajabian *et al.*, 2022).

The antioxidant activity assessment using the DPPH radical scavenging assay revealed that the studied plants possess considerable antioxidant potential (Table 10, Figure 2). Among both plants, *A. racemosus* possessed the highest inhibition ($73.01 \pm 0.197\%$) at site 2 against DPPH. The obtained value was close to the value (79.21) calculated by Pinipay *et al.*, (2022). The lowest percentage inhibition, recorded in *C. tuberculata* ($31.55 \pm 0.455\%$), was comparable to the value (34.2%) reported by Ahmed *et al.*, (2019). The high antioxidant values indicate that both species represent valuable therapeutic resources for managing oxidative stress-related pathologies, including cardiovascular disease, diabetes mellitus complications, neurodegenerative disorders, and malignant neoplasm prevention (Nazir *et al.*, 2021).

Antimicrobial testing against pathogenic bacteria revealed differential susceptibility patterns (Table 11, Fig. 3). Maximum antibacterial activity against *E. coli* was shown by *A. racemosus* ($21.91 \pm 0.459 \text{ mm}$) at site 2 with an MIC of 0.25 mg/mL in comparison to ciprofloxacin ($24.92 \pm 0.288 \text{ mm}$). The value was close to the value (27 mm) observed by Al-Ashoor *et al.*, (2023). The minimum activity ($1.94 \pm 0.115 \text{ mm}$) was shown by *C. tuberculata* at site 1, with an MIC of $>2 \text{ mg/mL}$. The observed value was close to the value (1.23) reported by Seif (2022). The antibacterial activity of selected plants against *S. aureus* varied from $5.04 \pm 0.261 \text{ mm}$ to $24.73 \pm 0.354 \text{ mm}$ in comparison to the positive control ciprofloxacin ($30.76 \pm 0.167 \text{ mm}$). The highest antibacterial activity was shown by *A. racemosus* ($24.73 \pm 0.354 \text{ mm}$), with an MIC of 0.125 mg/mL extract at site 2. The value was close to the value (27.8 mm) observed by Battu and Kumar (2010). Minimum activity was shown by *C. tuberculata* ($5.04 \pm 0.261 \text{ mm}$) at site 1, with an MIC of $>2 \text{ mg/mL}$. The observed value was close to the value (5 mm) reported by Ullah *et al.*, (2022). Antibacterial activity varied significantly among species and collection sites, likely reflecting differences in the concentration and specificity

of antimicrobial phytochemicals (Li *et al.*, 2020; Shang *et al.*, 2024). The higher antibacterial activity was recorded in the study plants that may be associated with their elevated levels of phenolic and flavonoid compounds that impair bacterial cell structures and inhibit critical metabolic pathways (Ngolo *et al.*, 2025). Previous studies have also demonstrated that plant extracts can act synergistically with conventional antibiotics that enhance the efficacy of microbial activity against both gram positive and negative bacteria including *E. coli* and *S. aureus* (Rahman *et al.*, 2024). Further investigation of antioxidant and antimicrobial potential of studied plants may lead to the discovery of new drugs for the treatment of cancer, tumors, diabetes and inflammation (Cuevas-Cianca *et al.*, 2023; Wu *et al.*, 2024). Higher concentration of phytochemicals and higher biological activity of *A. racemosus* as compared to *C. tuberculata* may be due to the environmental and physiological variations that affect the formation of bioactive compounds responsible for the biological activity of plants, as reported by Zargoosh *et al.*, (2019). Investigations are needed to identify the phytochemical constituents that are responsible for antibacterial activity and their contributions to antibacterial effectiveness. The above study revealed that studied plants have rich nutritional and phytochemical composition along with low non-carcinogenic risk profile and safe daily intake levels so the plants can be the potential candidates for further pharmacological investigation.

Conclusion

The study presents a thorough nutritional and phytochemical analysis of selected medicinal plants in terms of their biological activities, as natural sources of bioactive compounds. The nutritional composition was estimated by proximate analysis emphasizing the nutritional importance of studied plants. The results of heavy metal analysis indicated that these plants have low non-carcinogenic risk under the tested conditions. Strong correlation between total phenolic and flavonoids content of selected plants and antioxidant activity was found, indicating that these compounds might have role as antioxidant agents. Furthermore, the plant extracts demonstrated antibacterial activity against clinically relevant pathogens showing their potential antimicrobial properties under in-vitro conditions. Overall, the results suggested that the studied plants have potential value as a source of antioxidants and antimicrobials. Further investigations, including isolation of bioactive compounds, toxicity testing, mechanism of action and in-vivo validation, are encouraged to further evaluate their development into therapeutic products.

Ethics approval and consent to participate: This study did not involve human participants, animals, or protected species. Plant sampling was conducted with verbal permission from local landowners; therefore, formal ethical approval was not needed.

Conflict of Interest: All authors declare that there are no competing interests.

Author's Contributions: H.S., collected and analysed the samples; K.A., and Z.I.K., supervised the study; M.N., and M.N.Q., revised the original draft; A.A., M.A.G reviewed the article, prepared the graph and tables, revised the manuscript and worked on methodology. All authors contributed to the manuscript.

Availability of Data and Materials: Data will be provided by the author upon reasonable request.

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