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Quantitative Analysis of Antioxidant Constituents in Some Wild Plants of Kangchup Chingkhong, Senapati District Manipur, Northeast India

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Abstract

The present study, conducted from March, 2024 to May, 2025, at the laboratory of the Institutional Biotech Hub, Lilong Haoreibi College, Lilong, Manipur, India, aimed to evaluate the antioxidant constituents of selected wild medicinal and edible plants collected from various localities of Senapati District, Manipur, Northeast India. Quantitative estimations of total phenolics, flavonoids, tannins, and ascorbic acid were performed using standard spectrophotometric methods. The findings revealed substantial variation in antioxidant compound levels among the 32 plant species analysed.Total phenolic content (TPC) ranged from 6.33–95.24 mg g^{-1}, with the highest values observed in *Wendlandia grandis* flower (95.24 mg g^{-1}) and *Clerodendrum serratum* flower (90.24 mg g^{-1}). Total flavonoid content (TFC) varied from 0.88–12.76 mg g^{-1}, peaking in *Leucaena leucocephala* seed (12.76 mg g^{-1}) and pulp (11.36 mg g^{-1}). Total tannin content (TTC) ranged between 0.18–13.98 mg g^{-1}, with *C. serratum* flower (13.98 mg g^{-1}) and *Brachycorythis obcordata* leaves (10.34 mg g^{-1}) being the richest sources. Ascorbic acid levels were relatively low, ranging from 1.21 to 2.80 mg g^{-1}, with the highest concentration recorded in *Maranta arundinaceae* (2.80 mg g^{-1}). Overall, flowers, leaves, and seeds exhibited superior antioxidant profiles, particularly in *W. grandis*, *C. serratum*, *L. leucocephala*, and *B. obcordata*. These findings highlighted the phytochemical diversity of the wild flora of Senapati District and supported their traditional medicinal use. The results also emphasized the potential of several species as promising candidates for natural antioxidant formulations, nutraceuticals, and phytopharmaceutical development.

Keywords: Antioxidant, flavonoids, phenolics, ascorbic acid, tannins, wild plants

1. Introduction

Natural antioxidants such as flavonoids, tannins, ascorbic acid, and phenolic compounds play a pivotal role in protecting biological systems against oxidative stress by scavenging reactive oxygen species (ROS) and preventing cellular damage (Dai and Mumper, 2010; Shahidi and Ambigaipalan, 2015). These bioactive compounds are widely distributed in plants and contribute significantly to their pharmacological and therapeutic potential, including anti-inflammatory, anticancer, and antimicrobial activities (Panche et al., 2016; Altemimi et al., 2017). Phenolics and flavonoids, in particular, are considered the primary determinants of total antioxidant capacity in plants, functioning as reducing agents, hydrogen donors, and singlet oxygen quenchers (Shahidi and Zhong, 2015; Bisso et al., 2022). In recent years, there has been a growing scientific interest in exploring the antioxidant constituents of wild medicinal plants, especially those used in traditional medicine, to validate their ethnopharmacological importance (Singh et al., 2021). The Northeastern region of India, located within the Indo-Burmese biodiversity hotspot, is recognized as one of the richest reservoirs of medicinal plant diversity and associated traditional knowledge (Biona et al., 2025). The Senapati District of Manipur, in particular, harbors a wide range of wild plant species that have been traditionally utilized by indigenous communities for the treatment of various ailments. Several ethnobotanical surveys have documented extensive indigenous knowledge related to plant selection, preparation methods, dosage, and therapeutic applications, highlighting the cultural and medicinal significance of these resources (Kom et al., 2021; Biona et al., 2025; Gangurde et al., 2025). Despite this wealth of ethnomedicinal heritage, limited quantitative studies have been conducted to estimate key antioxidant constituents in wild plants of this district.Flavonoids and tannins are polyphenolic compounds known for their strong radical scavenging and chelating abilities (Ayele et al., 2002; Serrano

et.al., 2009). Ascorbic acid (vitamin C), a non-enzymatic antioxidant, works synergistically with phenolic compounds to regenerate other antioxidants and prevent lipid peroxidation (Zhu et al., 2020). Phenolic compounds, on the other hand, form the largest group of natural antioxidants, contributing significantly to total reducing capacity and playing critical roles in plant defense, pigmentation, and growth regulation (Dai and Mumper, 2010; Perez et al., 2023). Together, these molecules constitute the biochemical foundation of the antioxidant defense system in plants. Studies conducted in related regions have shown that wild and underutilized plant species possess considerable antioxidant potential. For example, Singh et al. (2021) reported that wild edible plants from the Loktak Lake wetland ecosystem in Manipur contained high levels of total phenolics and flavonoids, which correlated strongly with DPPH and FRAP antioxidant activities. Similarly, phytochemical screening of *Euryale ferox*, *Eupatorium birmanicum*, and other wild medicinal plants from Manipur revealed total phenolic contents ranging from 21.95 to 119.80 mg GAE g^{-1} extract and total flavonoid contents between 2.57 and 7.27 mg QE g^{-1} extract (Devi et al., 2024). These findings reinforce the idea that indigenous wild plants of Manipur represent a valuable yet under explored source of natural antioxidants. Despite such evidence, there remains a distinct gap in comprehensive, multi-parameter antioxidant analyses of medicinal plants specifically from Senapati District. Most prior research in the region has been limited to qualitative phytochemical screening or antioxidant assays without detailed quantification of individual biochemical constituents (Singh et al., 2021). Therefore, the present study was undertaken to conduct a quantitative estimation of flavonoids, tannins, ascorbic acid, and phenolic compounds in selected wild medicinal plants from Senapati District, Manipur, employing standardized analytical methods. This study aimedto provide baseline biochemical data that wouldnot only supported the validation of traditional ethnomedicinal claims but also enhanced the understanding of antioxidant diversity among wild plants in this biogeographically significant region. The outcomes wereexpected to contribute to the scientific basis for future pharmacological screening, nutraceutical development, and conservation planning.

2. Materials and Methods

The experiment was conducted from March, 2024 to May, 2025 at the laboratory of the Institutional Biotech Hub, LilongHaoreibi College, Lilong, Manipur, India

2.1. Plant material collection and preparation of extracts

Various parts of 22 plant species were collected from the KangchupChingkhong area, Senapati district of Manipur. The plant samples were cleaned by using tap water followed by double-distilled water to remove all the dust and oven-dried at 60°C. The dried samples were ground into powder using a grinder. 100 mg of each sample was taken in a mortar and pestle, and to it, approximately 10 ml of 80% ethanol was added and kept pasting until a clear plant solution was observed. Then the solutions were centrifuged at 4000 rpm for 12 min, and the final supernatants were collected in respective tubes.

2.2. Quantitative estimation of antioxidant constituents

2.2.1. Total phenolic content (TPC)

Total phenolic content was quantified using the Folin–Ciocalteu reagent (FCR). 1 ml of the extract was mixed with 5 ml of 10-fold diluted FCR. After incubation for 5 min, 4 ml of 7.5% sodium carbonate (Na_2CO_3) solution was added. The mixture was vortexed and incubated in the dark for 30 minutes at room temperature. Absorbance was measured at 765 nm against the reagent blank. A standard curve was generated using gallic acid solutions (20–100 µg ml^{-1}). TPC was calculated from the calibration curve and expressed as mg gallic acid equivalents (GAE) g^{-1} of extract (mg GAE g^{-1}).

2.2.2. Total flavonoid content (TFC)

Total flavonoid content of the extracts was estimated using the aluminium chloride colorimetric method with quercetin as the reference standard. An aliquot of the plant extract (1 ml) was mixed with 4 ml of distilled water, followed by the addition of 0.3 ml of 5% sodium nitrite ($NaNO_2$). After 5 min, 0.3 ml of 10% aluminium chloride ($AlCl_3$) solution was added. The mixture was allowed to stand for another 6 min, after which 2 ml of 1 M sodium hydroxide (NaOH) was added, and the final volume was made up to 10 ml with distilled water. The reaction mixture was incubated at room temperature for 15 min, and the absorbance was recorded at 510 nm against a reagent blank. A calibration curve was constructed using quercetin (10–100 µg ml^{-1}), and the results were expressed as mg quercetin equivalents (QE) g^{-1} of extract (mg QE g^{-1}).

2.2.3. Tannin content

Tannin content was estimated following the Folin–Denis colorimetric method using tannic acid as the standard. One millilitre of the sample extract was mixed with 7.5 ml of distilled water, followed by 0.5 ml of Folin–Denis reagent. Then 1 ml of saturated sodium carbonate solution (35%) was added, and the volume was made up to 10 ml. The mixture was vortexed and incubated for 30 min at room temperature to allow colour development. The absorbance was measured at 700 nm using a UV–Vis spectrophotometer. A standard curve was prepared using tannic acid (20–100 µg ml^{-1}). Tannin content was calculated from the standard curve and expressed as mg tannic acid equivalents (TAE) g^{-1} of extract (mg TAE g^{-1}).

2.2.4. Ascorbic acid content

Ascorbic acid content was determined using the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method. Plant sample was extracted with 4% oxalic acid and filtered to remove interfering substances. The filtrate was titrated against standard DCPIP dye solution until a persistent light pink colour was observed, lasting for at least 15 sec. The amount of dye consumed was used to calculate the ascorbic

acid content using a standard ascorbic acid calibration. Results were expressed as mg ascorbic acid 1 g^{-1} of fresh weight (mg g^{-1} FW).

2.3. Statistical analysis

All experiments were conducted in triplicate, and results were expressed as mean±standard deviation. Statistical analysis and graphical representation were performed using Microsoft Excel 2019.

3. Results and Discussion

The present study revealed significant variability in total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and ascorbic acid across different wild edible plants and their plant parts. These variations werelargely due to species-specific metabolic activities, environmental factors, and physiological roles of plant tissues. The findings aligned with previous research suggesting that plant tissues such as flowers, seeds, and leaves tend to accumulate higher levels of secondary metabolites as protective compounds against environmental stressors (Singh et al., 2016).

3.1. Total phenolic content (TPC)

The total phenolic content showed extensive variability among the 32 plant samples, ranging from 6.33 mg g^{-1} to 95.24 mg g^{-1}. The highest TPC was recorded in *Wendlandia grandis* flower (95.24 mg g^{-1}), followed by *Clerodendrum serratum* flower (90.24 mg g^{-1}). Other phenolic-rich samples included *Clerodendrum serratum* root (62.65 mg g^{-1}), stem (68.10 mg g^{-1}), *Leucaena leucocephala* seed (61.43 mg g^{-1}), pulp (60.80 mg g^{-1}), and *Brachycorythis obcordata* leaves (61.74 mg g^{-1}). In contrast, *Clerodendrum serratum* stem (6.33 mg g^{-1}) and *Maranta arundinaceae* (8.17 mg g^{-1}) exhibited the lowest phenolic content.

Phenolic compounds werewidely recognized as major contributors to antioxidant capacity due to their redox properties, which enabled them to scavenge free radicals and chelate metals (Singh et al., 2016). The exceptionally high phenolic levels in flowers and leaves mightbe linked to their protective roles against UV stress and pathogen attack. The results aligned with earlier studies showing that phenolic accumulation wasparticularly high in reproductive structures and photosynthetic tissues (Muflihah et al., 2021). These findings suggested that species such as *W. grandis* and *C. serratum* were promising sources of natural antioxidants for nutraceutical applications.

3.2. Total flavonoid content (TFC)

Flavonoid content displayed considerable differences among the samples, with values ranging from 0.88 mg g^{-1} to 12.76 mg g^{-1}. The highest flavonoid concentration occurred in *Leucaena leucocephala* seed (12.76 mg g^{-1}), followed by its pulp (11.36 mg g^{-1}). Significant flavonoid levels were also observed in *Clerodendrum serratum* leaves (8.80 mg g^{-1}), *Wendlandia grandis* flower (7.92 mg g^{-1}), *Zanthoxylum oxyphyllum* leaves (7.04 mg g^{-1}), and *Dysoxylum excelsum* leaves (6.16 mg g^{-1}). Many rhizomes, however, showed uniformly low values (0.88 mg g^{-1}), indicating that flavonoids accumulate more in aerial plant parts exposed to environmental stress.

Flavonoids play crucial roles in UV protection, oxidative stress mitigation, and defense against pathogens (Muflihah et al., 2021). Their strong antioxidant potential stems from the presence of hydroxyl groups that act as effective hydrogen donors. The high levels observed in *Leucaena leucocephala* and *C. serratum* suggested superior radical-quenching capacity. Similar patterns of elevated leaf-based flavonoids have been reported in earlier studies (Singh et al., 2016), reinforcing that leaf and seed tissues werehotspots for flavonoid biosynthesis.

3.3. Total tannin content (TTC)

Tannin content varied widely, ranging from 0.18 mg g^{-1} to 13.98 mg g^{-1}. The highest TTC was found in *Clerodendrum serratum* flower (13.98 mg g^{-1}), followed by *Brachycorythis obcordata* leaves (10.34 mg g^{-1}) and *Parkia timoriana* flesh (10.20 mg g^{-1}). Elevated tannin concentrations were also present in *Dysoxylum excelsum* stem (8.92 mg g^{-1}) and *Paedaria foetida* leaves (7.72 mg g^{-1}). On the other hand, *Clerodendrum serratum* stem (0.24 mg g^{-1}) and *Smallanthus sonchifolius* tuber (0.24 mg g^{-1}) exhibited the lowest tannin levels (Table 1).

Tannins werewell-known for their antimicrobial, anti-inflammatory, and astringent properties due to their ability to precipitate proteins and interfere with microbial enzymes (Ayele et al., 2022). Their abundance in flowers and leaves highlighted their ecological roles in herbivore deterrence and defense. High tannin content, particularly in *C. serratum* flower, suggests strong potential for therapeutic uses such as wound-healing and antimicrobial formulations. The results were consistent with previous phytochemical studies indicating that tannin-rich plant species exhibited strong bioactivity (Singh et al., 2016).

3.4. Ascorbic acid (Vitamin C)

Ascorbic acid content among the samples ranged from 1.21 to 2.80 mg g^{-1}. The highest levels were recorded in *Maranta arundinaceae* (2.80 mg g^{-1}), *Clerodendrum colebrookianum* leaves (2.49 mg g^{-1}), *Curcuma amada* (2.28 mg g^{-1}), and *Dysoxylum excelsum* flower (2.28 mg g^{-1}). The lowest ascorbic acid concentration appeared in *Siphonochilus aethiopicus* (1.21 mg g^{-1}).

Although lower in concentration than phenolics and tannins, vitamin C (ascorbic acid) remaineda crucial antioxidant capable of scavenging reactive oxygen species (ROS) and regenerating oxidized polyphenols, thereby enhancing the overall antioxidant capacity of plant tissues (Kazmierczak Baranska et al., 2020). This function madevitamin C an essential component in maintaining redox homeostasis and complementing the activity of other plant-derived antioxidants.

Table 1: Antioxidant constituents of some wild edible plants of Kangchup Chingkhong

Plant Sample	Total Phenol content (mg g^{-1} dry weight)	Total Flavonoid content (mg g^{-1} dry weight)	Total Tannin Content (mg g^{-1} dry weight)	Total Ascorbic acid content (mg g^{-1} fresh weight)
Curcuma caesia rhizome	29.96±0.91	0.88±0.05	0.84±0.07	2.04±0.10
Kaempfera parviflora rhizome	13.62±0.91	0.88±0.05	1.32±0.10	1.68±0.08
Curcuma amada rhizome	9.08±0.45	4.40±0.22	2.76±0.10	2.28±0.08
Alpina officinarum leaves	16.34±0.91	0.88±0.05	2.40±0.10	2.04±0.06
Siphonochilus aethiopicus rhizome	18.16±0.45	0.88±0.05	1.94±0.06	1.21±0.05
Dysoxylum excelsum leaves	55.35±0.91	6.16±0.31	2.42±0.09	1.90±0.10
Dysoxylum excelsum flower	40.86±0.91	3.52±0.18	1.02±0.06	2.28±0.08
Dysoxylum excelsum stem	22.09±0.69	1.76±0.09	8.92±0.68	1.73±0.08
Parkia timoriana seed	14.23±0.26	2.64±0.13	1.08±0.04	1.50±0.07
Parkia timoriana flesh	38.10±0.45	0.88±0.05	10.20±0.89	1.68±0.08
Leucaena leucocephala seed	61.43±0.50	12.76±0.84	0.84±0.06	1.63±0.08
Leucaena leucocephala pulp	60.80±0.91	11.36±0.67	1.08±0.07	2.71±0.03
Hodgsonia heteroclita fruit	19.98±0.45	4.40±0.22	3.60±0.06	1.97±0.06
Wendlandia grandis flower	95.24±1.00	7.92±0.40	3.67±0.05	1.68±0.08
Paedaria foeteda gall	12.71±0.45	0.88±0.05	1.92±0.05	1.68±0.08
Paedaria foeteda leaves	22.40±0.69	4.96±0.75	7.72±0.10	2.19±0.07
Paedaria foeteda stem	10.90±0.91	0.88±0.05	0.24±0.01	1.24±0.05
Accacia pennata leaves	30.87±0.91	2.64±0.13	1.50±0.03	1.77±0.08
Accacia pennata stem	23.00±0.95	0.88±0.05	1.26±0.04	2.07±0.06
Brachycorythis obcordata leaves	61.74±0.45	6.72±0.84	10.34±0.73	1.68±0.08
Clerodendrum serratum leavs	41.47±0.69	8.80±0.44	4.74±0.05	2.28±0.08
Alpinia galanga rhizome	30.87±0.45	0.88±0.05	0.48±0.03	1.30±0.05
Maranta arundinaceae rhizome	8.17±0.45	1.76±0.09	3.78±0.04	2.80±0.07
Smallanthus sonchifolius tuber	28.15±0.45	1.64±0.05	0.24±0.03	1.50±0.07
Clerodendrum colebrookianum leaves	9.99±0.79	1.44±0.09	0.30±0.05	2.49±0.07
Clerodendrum colebrookianum stem	6.33±0.91	1.16±0.05	1.23±0.07	1.75±0.05
Zinziber striolatum	16.34±0.91	2.64±0.13	1.20±0.07	1.84±0.10
Zanthoxylum oxyphyllum leaves	46.31±0.91	7.04±0.35	0.18±0.02	1.78±0.10
Clerodendrum serratum root	62.65±0.45	7.04±0.35	3.54±0.07	1.63±0.08
Clerodendrum serratum stem	68.10±0.91	1.76±0.09	0.24±0.02	1.63±0.08
Clerodendrum serratum flower	90.24±1.36	6.16±0.31	13.98±1.00	1.68±0.08
Albizia myriophylla bark	22.40±0.45	1.28±0.05	2.44±0.06	1.74±0.03

An integrated analysis of the four antioxidant parameters clearly indicatedthat flowers, leaves, and seeds werethe richest sources of antioxidant compounds among the 32 samples. *Wendlandia grandis* flower and *Clerodendrum serratum* (flower, root, and leaves) consistently appeared among the top-performing species. Similarly, *Leucaena leucocephala* (seed and pulp) showed strong flavonoid and phenolic profiles, making it a promising nutraceutical candidate. *Brachycorythis obcordata* and *Zanthoxylum oxyphyllum* also displayed robust antioxidant profiles.

These findings agreed with earlier reports demonstrating that

antioxidant compounds tend to localize in reproductive and photosynthetic tissues due to their involvement in protective biochemical pathways (Muflihah et al., 2021; Singh et al., 2016). Overall, the results confirmed that several of the evaluated wild edible plants possess significant potential for use in natural antioxidant formulations, functional foods, and phytopharmaceutical development.

4. Conclusion

Several species exhibited high levels of phenolics, flavonoids, and ascorbic acid, indicating their strong antioxidant potential and pharmacological relevance. These findings supported the traditional use of these plants as nutritionally and medicinally valuable resources. The study concluded that these wild edible plants could serve as promising sources of natural antioxidants, and further investigations on compound isolation and bioactivity evaluation were warranted to identify potential therapeutic candidates.

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