European Journal of Clinical Pharmacy

Print ISSN: 2385-409X **Online ISSN:** Applied

Website: https://farmclin.es/

Original Researcher Article

Phytochemical and Pharmacological Screening of *Goniocaulon indicum* (Klein ex Willd.): A Promising Wild Leafy Vegetable from Asteraceae Family

Article History:

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Received: 15-10-2025
Revised: 20-11-2025
Accepted: 23-12-2025
Published: 29-12-2025

Abstracts: *Goniocaulon indicum* (Klein ex Willd.), a lesser-known wild leafy vegetable of the Asteraceae family, represents an important yet underexplored taxon with considerable nutritional and pharmacological potential. Members of Asteraceae are widely recognized worldwide for their historical use as food and medicine, and they continue to serve as a valuable source of bioactive compounds. In the present investigation, phytochemical and pharmacological evaluations were carried out on ethanolic extracts of *G. indicum* collected from the Nanded District of Maharashtra, India, with the aim of assessing its therapeutic prospects. Extracts prepared from roots (GIRE), stems (GISE), and leaves (GILE) were subjected to qualitative, quantitative, antioxidant, and antibacterial assays. The phytochemical profile indicated the presence of alkaloids, flavonoids, phenols, triterpenoids, glycosides, and steroids, whereas saponins, tannins, and anthocyanins were absent. Quantitative estimation revealed that phenolic compounds were most abundant in GISE (665.89 µg/g) and GILE (410.33 µg/g), while flavonoid content was highest in GIRE (490.23 µg/g) and GILE (274.79 µg/g), measured as gallic acid equivalents (GAE). Antioxidant activity, assessed by DPPH and SOD radical scavenging assays, demonstrated strong concentration-dependent effects, with maximum DPPH inhibition in GISE (57.77%; IC_{50}=78.32µg/ml) and GIRE (48.70%; IC_{50}=92.43µg/ml), and peak SOD scavenging activity in both GIRE (48.54%; IC50=92.32µg/ml). and GISE (48.54%; IC50=92.22µg/ml). These results suggest that G. indicum extracts possess effective free radical–neutralizing capacity, supporting their role in preventing oxidative stress–associated conditions. Furthermore, antibacterial screening using the agar well diffusion method showed notable inhibitory action against Gram-negative strains (*Escherichia coli* NCIM 2832; *Pseudomonas aeruginosa* ATCC 15442) and Gram-positive strains (*Bacillus subtilis* ATCC 6633; *Staphylococcus aureus* NCIM 2654), with inhibition zones approaching those of the standard antibiotic streptomycin. Collectively, the study emphasizes the phytochemical richness, antioxidant potential, and antimicrobial efficacy of *G. indicum*, validating its

traditional use as a wild edible plant and highlighting its promise for nutraceutical and pharmaceutical applications.

Keywords: Wild leafy vegetable, G. indicum (Klein ex Willd.), pharmaceutical applications, Asteraceae family

INTRODUCTION

Wild edible plants have historically played a crucial role in sustaining rural and indigenous populations by providing essential dietary components, medicinal remedies, and preserving cultural traditions (Bharucha & Pretty, 2010; Teklehaymanot & Giday, 2020). These lesser-exploited resources are notable for their abundance of bioactive secondary metabolites, such as flavonoids, phenolics, alkaloids, terpenoids, and glycosides, which are known to exhibit multiple pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Kumar et al., 2021; Sharma et al., 2023). In the context of growing interest in functional foods and nutraceuticals, scientific attention has increasingly shifted toward evaluating the phytochemistry and therapeutic efficacy of these underutilized vegetables, both as a means of enhancing nutritional security and for drug discovery. Among such resources, the Asteraceae family stands out as one of the largest and most diverse groups of flowering plants, encompassing over 1,600 genera and approximately 23,000 species worldwide (Christenhusz & Byng, 2016). Several well-known members, including *Taraxacum officinale, Lactuca sativa,* and *Sonchus oleraceus*, are traditionally consumed as leafy vegetables or employed in herbal medicine owing to their richness in secondary metabolites (Hussain et al., 2019; Yeung et al., 2021). These phytoconstituents not only provide ecological advantages in plant defense but also confer significant therapeutic benefits for human health. The antioxidant activity of many Asteraceae species, in particular, has been strongly correlated with their phenolic and flavonoid contents, which aid in counteracting free radicals and reducing oxidative stress–related disorders (Huang et al., 2022).

Goniocaulon indicum (Klein ex Willd.), a lesser-known wild leafy vegetable of the Asteraceae family, is traditionally gathered and consumed seasonally in parts of India, particularly during the monsoon. Despite its ethnobotanical importance, scientific data on its phytochemical profile and pharmacological properties remain sparse. Previous investigations into related Asteraceae taxa have demonstrated considerable antioxidant and antibacterial activities, underlining their relevance in combating microbial pathogens and oxidative stress–associated ailments (Gupta et al., 2022; Nandhakumar et al., 2023). However, comprehensive studies on *G. indicum* are lacking. Considering the global health concerns linked to antimicrobial resistance and oxidative stress–driven diseases, exploring the bioactive potential of such wild leafy vegetables is of both nutritional and therapeutic value (WHO, 2021; Chen et al., 2023). The present study, therefore, undertakes qualitative and quantitative phytochemical assessments, along with antioxidant and antibacterial evaluations, of *Goniocaulon indicum* (Klein ex Willd.). The findings are expected to validate its ethnomedicinal applications and position it as a promising candidate for nutraceutical and pharmaceutical development.

METHODOLOGY

Study Area

The wild leafy vegetable was collected in Therban village, Bhokar taluka, Nanded District, Maharashtra, India. Nanded District extends roughly between 18°15′–19°55′ N latitude and 77°00′–78°25′ E longitude and covers about 10,332 km²; it occupies the eastern (south-eastern) part of Maharashtra within the Marathwada region (Government of Maharashtra; Nanded District, 2025). The district experiences a monsoon-dominated tropical climate conditions, with marked interannual rainfall variability documented by recent IMD assessments (IMD, 2025; Peel et al., 2007).

Plant Collection and Authentication

Mature specimens of *Goniocaulon indicum* (Klein ex Willd.) were collected from their natural stands in Nanded District, Maharashtra, India, located between 18°15′–19°55′ N latitude and 77°00′–78°25′ E longitude. Voucher specimens were prepared following standard herbarium protocols (Mann & David, 1997; Bridson & Forman, 1999) on herbarium sheets measuring 41 × 29 cm (16.5 × 11.5 inches). Taxonomic authentication was carried out in the Department of Botany by comparing the prepared specimens with authenticated reference

collections. The scientific nomenclature of G. indicum was cross-verified using multiple authoritative botanical databases and floras, including Plants of the World Online (POWO, 2025), World Flora Online (WFO, 2025), International Plant Names Index (IPNI, 2025), Indian Flora Online (Indian Flora, 2025), and The Flora of Marathwada (Naik, 1998). All consulted sources confirmed the currently accepted name as Goniocaulon indicum (Klein ex Willd.) (WFO-ID: wfo-0000005657). To evaluate the ethnobotanical relevance of the species, the Frequency Index (FI) of use was determined following the modified approach of Madikizela et al. (2012), with further refinements drawn from subsequent ethnobotanical research (Tugume et al., 2019; Bhogaonkar et al., 2023). The FI was calculated using the formula: ; where FC represents the number of respondents citing the plant, N is the total number of respondents surveyed, and FI denotes the frequency index of species utilization.

Taxonomy of *Goniocaulon indicum* (Klein ex Willd.) (Karat-Kusumba)

Goniocaulon indicum (Klein ex Willd.), belonging to the family Asteraceae, is an erect, glabrous annual herb ranging from 40–120 cm in height. The stem is distinctly angled and longitudinally grooved. Leaves are alternate, oblanceolate, measuring 6–15 × 1–2 cm, sessile or subsessile, deeply serrate, acute, and glabrous. The capitula are narrow (1–2 cm long), few-flowered, and arranged in compound corymbs; bracts are linear, with very short pedicels. The receptacle is convex and paleaceous, with palea linear-oblong and glistening. The involucre is many-seriate, comprising lanceolate, acute bracts, white with pink or brownish apices. The corollas are tubular, 5-fid, pink, and longer than the involucre, with segments linear. Anthers are sagittate at the base with short tails. Style arms are filiform. Achenes are oblanceolate, 5–6 mm long, closely ribbed, and glabrous. The pappus scales are very unequal, linear-oblanceolate. Flowering and fruiting typically occur between November and December in peninsular India (Naik, 1998; Karthikeyan et al., 2009; POWO, 2025; WFO, 2025).

Preparation of Plant Extracts

Freshly collected leaves of the wild vegetable were thoroughly washed to remove surface impurities and subsequently shade-dried at room temperature for about seven days to ensure the preservation of heat-sensitive phytoconstituents. Once dried, the material was ground into a fine powder using a mechanical grinder and stored in airtight containers to avoid moisture uptake and microbial growth (Azwanida, 2015; Saha et al., 2022). For extraction, the powdered material was subjected to Soxhlet extraction using ethanol as the solvent, in accordance with established phytochemical methodologies (Harborne, 1998; Sasidharan et al., 2011). Ethanol was selected due to its proven ability to solubilize a wide range of polar and non-polar phytochemicals, as supported by contemporary pharmacognostic investigations (Do et al., 2014; Ali et al., 2023). The resulting extracts were concentrated, transferred into sterile screw-capped vials, protected from light with aluminum foil to minimize photodegradation, and preserved under refrigeration at 3–4 °C until further experimental use (Azwanida, 2015; Saha et al., 2022).

PHYTOCHEMICAL ANALYSIS:

1. Qualitative analysis:

Qualitative phytochemical tests were conducted following standard protocols described in earlier works (Tyler et al., 1981; Pharmacopoeia, 1996; Mukherjee, 2002; Khandelwal, 2002, 2003; Kokate et al., 2004; Ammor et al., 2018), with minor modifications to suit the experimental conditions. These procedures are widely employed for the detection of alkaloids, flavonoids, tannins, phenols, saponins, steroids, and other secondary metabolites in plant extracts. To ensure reliability, the methods were cross-referenced with recent phytochemical analysis guidelines and validation studies (Tiwari et al., 2011; Ekor, 2014; Akinmoladun et al., 2020; Sahu et al., 2022).

Alkaloids

Mayer's test: Addition of Mayer's reagent (potassium mercuric iodide) to the extract resulted in a cream-colored precipitate, suggestive of alkaloids.

Wagner's test: Treatment with Wagner's reagent (iodine in potassium iodide) produced a brown precipitate, confirming alkaloid presence.

Hager's test: A yellow precipitate developed upon reaction with saturated picric acid solution, indicating alkaloids.

Flavonoids

Ferric chloride test: The extract gave a bright green coloration with ferric chloride, showing flavonoids.

Shinoda test: Magnesium ribbon and concentrated HCl caused the appearance of a pink to magenta-red color, confirming flavonoids.

Zinc–HCl reduction test: Addition of zinc powder and hydrochloric acid yielded a magenta-red color, indicating flavonoids.

Alkaline reagent test: Sodium hydroxide turned the extract yellow, which became colorless upon acidification, confirming flavonoids.

Lead acetate test: Treatment with 10% lead acetate formed a yellow precipitate, further suggesting flavonoids.

Phenolic Compounds

Ferric chloride test: Mixing the ethanolic extract with $FeCl_3$ solution produced characteristic blue, green, red, or purple shades, indicating phenols.

Triterpenoids and Sterols

Salkowski test: Concentrated sulfuric acid produced a red coloration in the lower layer, indicative of sterols/triterpenoids.

Liebermann–Burchard test: Acetic anhydride followed by H_2SO_4 produced a brown ring at the junction and a green coloration in the upper layer, confirming triterpenoids.

Saponins

Foam test: Vigorous shaking of the extract with water produced stable froth persisting for ≥15 min, indicating saponins.

Raymond's test: Hot methanolic alkali and dinitrobenzene generated a violet coloration, confirming saponins.

Tannins

Gelatin test: A white precipitate appeared when the extract was treated with gelatin and NaCl solution, indicating tannins.

NaOH test: Treatment with 10% NaOH produced an emulsion, confirming tannins.

Glycosides

Keller–Killiani test: Addition of glacial acetic acid with $FeCl_3$ followed by H_2SO_4 yielded a reddish-brown interface and bluish-green upper layer, indicating cardiac glycosides.

Raymond's test: A violet color appeared after treatment with hot methanolic alkali and dinitrobenzene, confirming glycosides.

Legal's test: Pyridine and sodium nitroprusside developed a pink-to-red coloration, showing glycosides.

Steroids

Chloroform test: Dissolution of extract in chloroform followed by H_2SO_4 addition resulted in a red upper layer and greenish-yellow fluorescence below, confirming steroids.

Salkowski test: Addition of concentrated H_2SO_4 produced a reddish-brown interface, indicative of steroids.

Carbohydrates

Molisch's test: Formation of a purple ring at the junction after treatment with Molisch's reagent and H_2SO_4 confirmed carbohydrates.

Barfoed's test: Boiling with Barfoed's reagent produced a brick-red precipitate, showing monosaccharides.

Benedict's test: Reaction with Benedict's reagent upon heating gave a reddish-brown precipitate, suggesting reducing sugars.

Proteins and Amino Acids

Millon's test: Treatment with Millon's reagent followed by heating developed a yellow color, confirming proteins.

Xanthoproteic test: Boiling with concentrated nitric acid produced a yellow precipitate, indicative of aromatic amino acids.

Ninhydrin test: Reaction with ninhydrin reagent resulted in a blue coloration, confirming proteins and free amino acids.

Starch

Iodine test: Treatment with iodine solution after heating with NaCl yielded a blue-purple coloration, confirming starch.

Anthocyanins

Acid–alkali test: Treatment with HCl produced a pink coloration, which shifted to bluish-violet upon addition of ammonia, confirming anthocyanins.

2. Quantitative Tests:

2.1 Total Phenolic Content (TPC)

TPC was estimated using the Folin–Ciocalteu assay (Singleton & Rossi, 1965; Ainsworth & Gillespie, 2007). Extracts of GIRE, GISE, and GILE (300–900 µg/mL) were mixed with FCR, followed by sodium carbonate, and adjusted to 25 mL. After 90 min incubation at room temperature, absorbance was read at 550 nm against blanks. Gallic acid served as the standard, and results were expressed as µg gallic acid equivalents (GAE)/g extract (Chang et al., 2002; Everette et al., 2010).

2.2 Total Flavonoid Content (TFC)

TFC was determined using the aluminium chloride colorimetric method (Chang et al., 2002; Ashok et al., 2017). Extracts were sequentially treated with sodium nitrite, $AlCl_3$, and NaOH, and final volume adjusted to 10 mL. Absorbance was measured at 510 nm. Quercetin was used as the standard, and results were expressed as µg quercetin equivalents (QE)/g extract (Djeridane et al., 2006; Kumar & Pandey, 2013).

TPC and TFC values were calculated using: ; where C = content (µg GAE or QE/g extract), C_1 = concentration from calibration curve (µg/mL), V = volume of extract (mL), and M = weight of extract (g). All assays were performed in triplicate and reported as mean ± SD.

3. Antioxidant activity

3.1 DPPH: The antioxidant potential of GIRE, GISE, and GILE was determined using two in vitro assays. Free radical scavenging activity was assessed through the DPPH method, where extracts (300–900 µg/mL) were mixed with 0.1% DPPH solution and incubated for 30 min in the dark. The reduction in absorbance at 517 nm indicated radical quenching activity (Brand-Williams et al., 1995; Baliyan et al., 2022).

3.2 SOD: Superoxide scavenging ability was evaluated using the NBT reduction assay, which mimics superoxide dismutase (SOD) activity. The reaction mixture contained phosphate buffer, NADH, NBT, and extracts (300–900 µg/mL), initiated with phenazine methosulfate. After 5 min of incubation at 25 °C, absorbance was measured at 560 nm, and scavenging percentage was calculated relative to the control (Naskar et al., 2010; Mishra et al., 2017).

4. Antibacterial Activity

Antibacterial activity was tested using the agar well diffusion method (Balouiri et al., 2016). Nutrient agar plates inoculated with bacterial suspensions received 6 mm wells filled with 100 µL of extract (100 µg/mL in DMSO). Streptomycin (1 mg/mL) and DMSO served as positive and negative controls, respectively. After 24 h incubation at 37 °C, inhibition zones were measured. Tests were performed in triplicate, and results reported as mean ± SD.

Results and Discussions

The wild leafy vegetable Goniocaulon indicum (Klein ex Willd.) of the family Asteraceae was collected from its natural habitats within the study region during the rainy season, which aligns with its traditional period of consumption. Seasonal patterns strongly influence the use of wild edible plants, as many leafy vegetables are harvested and consumed fresh during monsoon months due to their abundance and enhanced nutritional quality (Uprety et al., 2019; Grivetti & Ogle, 2020). The frequency index calculated for G. indicum highlighted its cultural and dietary importance, since the leaves are widely gathered for household use and are also marketed locally, thereby serving both subsistence and income-generating purposes. Comparable ethnobotanical findings indicate that wild leafy vegetables make a vital contribution to rural food security and livelihood systems (Kumar et al., 2021; Singh et al., 2023). For phytochemical analysis, collected leaves were shade-dried, finely powdered, and subjected to ethanol extraction. Approximately 20–25 g of dried plant material yielded 2–3 gm of crude extract through Soxhlet extraction. Ethanol was employed as the extraction solvent owing to its ability to dissolve a broad spectrum of bioactive compounds, including phenolics, flavonoids, alkaloids, and terpenoids, which are frequently associated with the antioxidant and antimicrobial potential of medicinal plants (Alara et al., 2018; Do et al., 2022).

Table 1: Season of availability and extraction yield of G. indicum leafy vegetable.

Sr. No.	Botanical Name/Common Name	Plant Part	Season of Availability	Frequency Index (%)	Commercially Sold	Solvent System	Extract Yield	Plant Extract Code
1	*Goniocaulon indicum* L. (Karat-Kusumba)	Root	Rainy and Winter	81	Yes	Ethanol	2-3 gm/ extraction	**GIRE**
2		Stem						**GISE**
3		Leaves						**GILE**

1. Qualitative Analysis:

The outcomes of the preliminary phytochemical screening are presented in Table 2. Qualitative

analysis of the ethanolic extracts prepared from the root (GIRE), stem (GISE), and leaf (GILE) of *Goniocaulon indicum* revealed the presence of multiple classes of secondary metabolites, including alkaloids, flavonoids, phenols, triterpenoids, glycosides, steroids, carbohydrates, proteins, and starch. However, compounds such as saponins, tannins, and anthocyanins were not detected in any of the tested extracts. The identification of alkaloids and flavonoids is of particular interest, as these phytochemicals are widely recognized for their antioxidant, antimicrobial, and anti-inflammatory roles (Odhav et al., 2020; Ghimire et al., 2022). Similarly, phenolic compounds—found consistently across all extracts—are well documented for their free radical neutralizing ability, thereby contributing to the reduction of oxidative stress and prevention of chronic degenerative disorders (Saxena et al., 2021). The detection of triterpenoids and steroids further strengthens the therapeutic potential of *G. indicum*, since these constituents have been linked to anti-cancer, hepatoprotective, and immunomodulatory effects (El-Sayed et al., 2020; Rahman et al., 2023). On the other hand, the absence of saponins and tannins indicates a distinctive phytochemical profile for this species. While saponins are often associated with hemolytic and detergent-like properties, their absence could lower the risk of cytotoxicity, thereby favoring dietary safety (Kumar et al., 2021). Likewise, the lack of tannins—which are known to interfere with nutrient absorption by binding proteins and minerals—enhances the nutritional suitability of the plant for consumption (Awasthi et al., 2021; Singh et al., 2023).

In addition to secondary metabolites, the presence of proteins and starch highlights the nutritional value of *G. indicum*. Such findings reflect the concept of the "food–medicine continuum," wherein wild leafy vegetables serve both as sources of essential nutrients and as reservoirs of pharmacologically active compounds (Shrestha et al., 2022; Sharma et al., 2024). This observation aligns with previous reports on leafy vegetables of the Asteraceae family, where carbohydrate and protein fractions have been shown to contribute not only to dietary energy but also to functional health benefits (Uprety et al., 2019). Taken together, the broad phytochemical diversity of *G. indicum* supports its traditional use as a seasonal leafy vegetable and reinforces its nutritional and ethnomedicinal relevance. Moreover, the plant's profile suggests promising prospects for pharmacological exploration, warranting comparative investigations with other underutilized Asteraceae taxa to establish its relative richness in bioactive compounds and potential therapeutic value.

Table 2: Qualitative Phytochemical Analysis of *G. indicum* L.

Sr. No.	Chemical Constituents	Tests	GIRE	GISE	GILE	Observation
1	Alkaloids	Mayer's test	--	++	++	Present
		Wagner's test	++	++	++	
		Hager's test	++	++	--	
2	Flavonoids	Lead acetate solution test	++	--	++	Present
		Shinoda test	++	++	++	
		Ferric chloride test	++	++	++	
		Zinc-HCL-reduction test	--	--	--	
		Alkaline reagent test	--	--	--	
3	Phenol	Fecl3 test	++	++	++	Present
4	Triterpenoids	Salkowaski test	++	++	--	Present
		Liebermann Burchard test	++	++	--	
5	Saponins	Foam test	--	--	--	Absent
		Raymond's test	--	--	--	
6	Tannins	Gelatin test	--	--	--	Absent
		NaoH Test	--	--	--	
7	Glycosides	Raymond's test	--	++	++	Present
		Keller killiani test	--	++	++	
		Legal's test	--	--	--	
8	Steroids	Chloroform test	--	++	--	Present
		Salkowski test	++	++	--	
9	Carbohydrates	Molisch's test	++	--	++	Present
		Benedict's test	++	++	++	
		Leagal's test test	--	--	--	
10	Proteins	Xanthoproteic Test	++	++	++	Present
		Million's Test	++	--	--	
		Ninhydrin Test	--	--	--	
11	Starch	Starch Reagent	++	--	--	Present
12	Anthocyanin	Hcl Test	--	--	--	Absent

2. Quantitative Analysis

Secondary metabolites such as phenolics and flavonoids are widely recognized as characteristic phytoconstituents of the Asteraceae family.

Although not directly nutritive, these compounds are crucial contributors to the antioxidant, antimicrobial, and anti-inflammatory efficacy of edible and medicinal plants (Shrestha et al., 2022; Sharma et al., 2024). In the present investigation, quantitative assays were conducted on root (GIRE), stem (GISE), and leaf (GILE) extracts of *Goniocaulon indicum* to assess their total phenolic and flavonoid contents, both of which are regarded as key markers of pharmacological potential.

2.1 Total Phenolic Content (TPC)

The total phenolic content was determined spectrophotometrically at 550 nm (Figure 1; Table 3). Among the examined plant parts, the stem extract (GISE) recorded the maximum phenolic concentration (655.89 µg/g), followed by the leaf extract (GILE, 410.33 µg/g). In contrast, the root extract (GIRE) contained comparatively lower levels (325.99 µg/g). Such differential accumulation across plant parts aligns with prior observations in wild leafy vegetables, where aerial tissues like stems and leaves typically exhibit elevated phenolic levels due to continuous exposure to environmental stressors such as ultraviolet radiation, oxidative pressure, and herbivore interactions (Ouyang et al., 2020; Singh et al., 2023). Phenolic compounds are well documented for their potent antioxidant activity, metal ion chelation, and their ability to regulate enzymes linked to oxidative stress and chronic disease progression (Saxena et al., 2021; Rahman et al., 2023). The relatively high phenolic content in GISE and GILE underscores the potential of *G. indicum* as a natural source of dietary antioxidants, particularly during its seasonal abundance when it is consumed as a leafy vegetable.

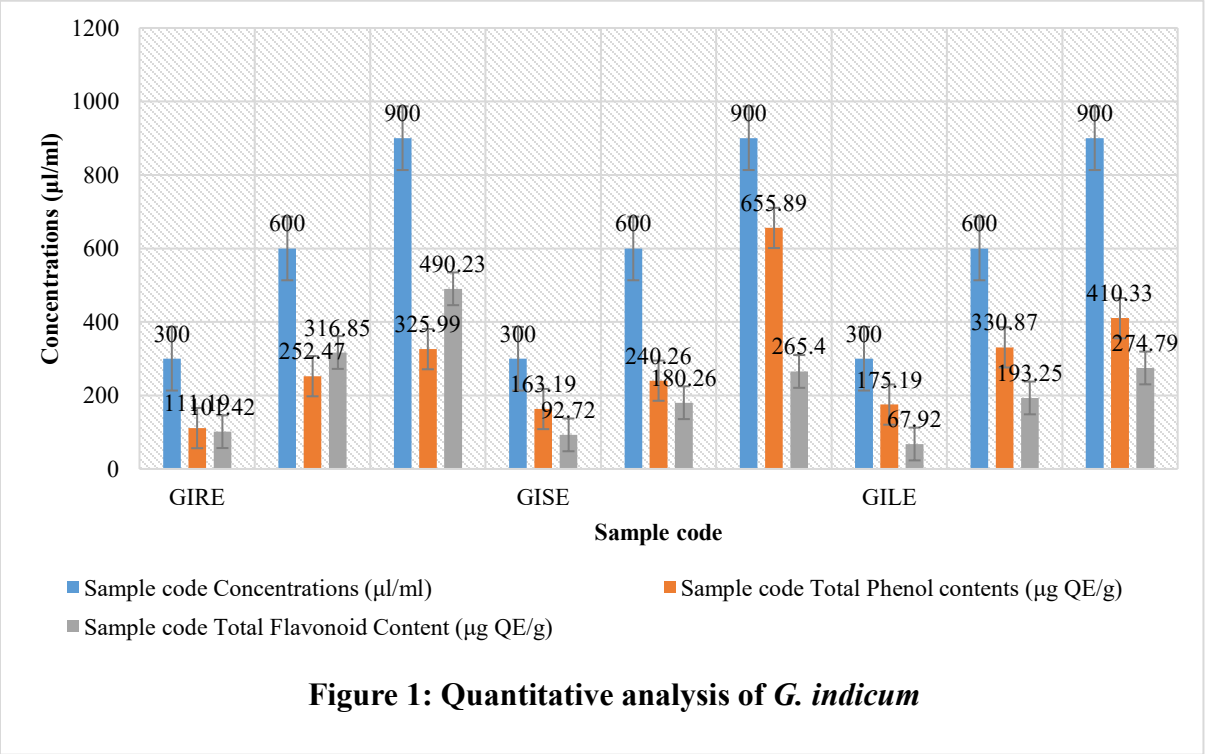


Figure 1: Quantitative analysis of *G. indicum*

2.2 Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using a spectrophotometric assay (Figure1; Table 3). The highest flavonoid concentrations were recorded in the root (GIRE, 490.23 µg/g) and leaf extracts (GILE, 274.79 µg/g), whereas the stem extract (GISE) showed the lowest value (265.40 µg/g). These findings highlight a differential accumulation pattern, where roots serve as important reservoirs of flavonoids. Such distributional patterns have also been reported in other Asteraceae species, where flavonoids accumulate in underground tissues as protective molecules against microbial invasion and oxidative damage (Ghimire et al., 2022; Uprety et al., 2019). Flavonoids are structurally diverse polyphenolic compounds with extensive pharmacological significance, including anti-carcinogenic, cardioprotective, hepatoprotective, and neuroprotective activities (El-Sayed et al., 2020; Liu et al., 2022). The high flavonoid concentration in GIRE and GILE reinforces the therapeutic potential

of *G. indicum*, especially considering its traditional use as a seasonal wild leafy vegetable.

The quantitative analysis of *G. indicum* revealed appreciable levels of phenolics and flavonoids across different plant parts, with the stem being particularly rich in phenolics and the root in flavonoids. Such part-specific distribution is ecologically and physiologically significant and has implications for both nutritional utilization and medicinal exploitation. Comparisons with other Asteraceae wild vegetables, such as *Sonchus oleraceus, Vernonia amygdalina*, and *Lactuca sativa*, indicate that *G. indicum* holds comparable or even superior phytoconstituent levels (Awasthi et al., 2021; Singh et al., 2023). Thus, it represents an underutilized but potentially valuable plant resource for dietary diversification and functional food development.

3. Antioxidant Activity

Leafy vegetables are well recognized as important sources of bioactive compounds that strengthen antioxidant defense systems by scavenging free radicals, reducing lipid peroxidation, and stimulating endogenous antioxidant enzymes (Sharma et al., 2023; Patel et al., 2024). In particular, phenolics and flavonoids play a central role due to their hydroxyl groups, which serve as efficient hydrogen donors and metal chelators (Liu et al., 2022; Rahman et al., 2023). Antioxidant activity in plants is typically assessed using in vitro assays such as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide dismutase (SOD) methods, both of which provide complementary insights into radical-scavenging potential (Omar et al., 2020). In the present study, ethanolic extracts of Goniocaulon indicum roots (GIRE), stems (GISE), and leaves (GILE) were examined for antioxidant efficiency using these two assays.

3.1 DPPH Radical Scavenging Assay

The free radical scavenging capacity of the extracts was first evaluated with the DPPH assay, which measures the ability of phytochemicals to donate electrons or hydrogen atoms (Ouyang et al., 2020). All extracts showed substantial activity when compared with the standard ascorbic acid (73.57% inhibition). Among them, the stem extract (GISE, 57.77%) exhibited the strongest scavenging effect, followed by the root extract (GIRE, 48.70%), while the leaf extract (GILE, 44.55%) displayed the lowest inhibition (Figure 2; Table 3). Among the tested extracts, GISE exhibited the lowest DPPH IC50=78.32µg/ml, indicating stronger radical scavenging potential compared to GIRE and GILE. Antioxidant potential increased progressively with higher extract concentrations, reflecting a dose-dependent response. These findings agree with previous reports on wild Asteraceae vegetables, such as Sonchus oleraceus and Vernonia amygdalina, where stem tissues often contained higher levels of phenolic acids and tannins, thereby displaying greater antioxidant activity than leaves (Singh et al., 2023; Ghimire et al., 2022). The strong radical scavenging property of GISE can thus be linked to its elevated phenolic content, supporting the established correlation between phenolics and antioxidant efficacy (Saxena et al., 2021).

3.2 Superoxide Dismutase (SOD) Activity Assay

To further validate antioxidant performance, extracts were analysed for superoxide radical scavenging activity using the SOD assay, with curcumin (66.56%) as the reference control. Both root (GIRE, 48.54%) and stem (GISE, 48.54%) extracts demonstrated the highest activity, while the leaf extract (GILE, 47.45%) recorded slightly lower inhibition (Figure 2; Table 3). SOD assays results were relatively similar across extracts, with IC50 values ranging between 92.22-94.83µg/ml, suggesting moderate superoxide radical scavenging activity. Similar to the DPPH test, activity increased with extract concentration, suggesting that G. indicum phytochemicals effectively neutralize reactive oxygen species (ROS). Superoxide radicals are considered highly damaging due to their role in DNA mutations, lipid peroxidation, and protein oxidation, processes strongly linked to chronic diseases including cardiovascular disorders, cancer, and neurodegeneration (El-Sayed et al., 2020; Liu et al., 2022). Therefore, the observed SOD-like activity highlights the therapeutic potential of *G. indicum* in counteracting oxidative stress. Overall, the combined DPPH and SOD assays provided a detailed profile of the antioxidant strength of *G. indicum*. The close agreement between phenolic/flavonoid concentrations and antioxidant responses indicates that these metabolites are the primary contributors to its radical scavenging activity. Comparisons with other wild Asteraceae vegetables further emphasize the species' potential as a nutraceutical resource and as a candidate for functional food development (Shrestha et al., 2022; Sharma et al., 2024).

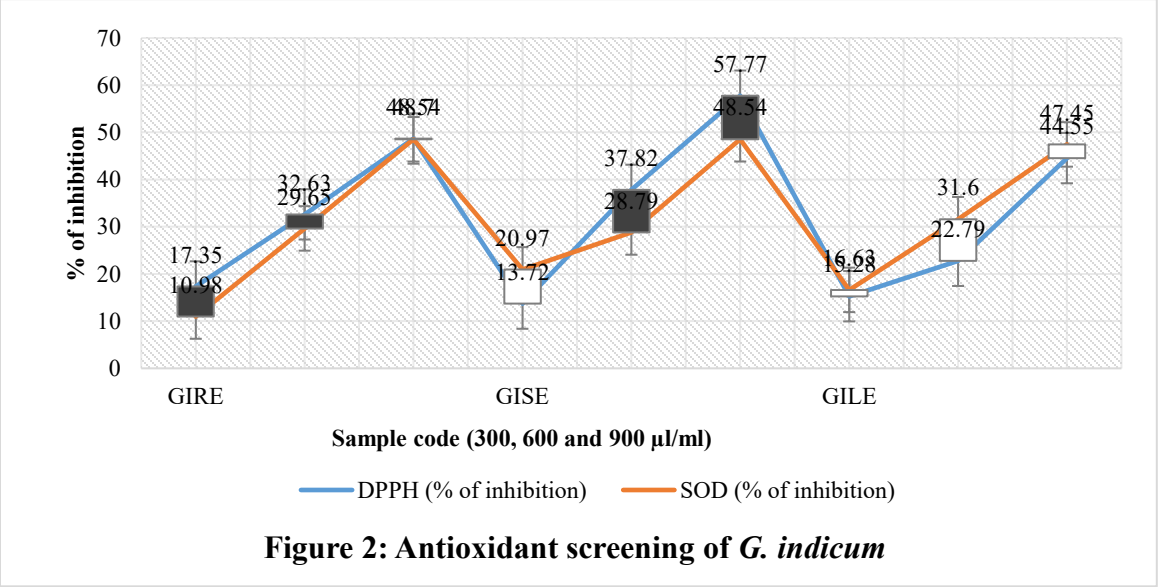


Figure 2: Antioxidant screening of *G. indicum*

Table 3. Quantitative and antioxidant analysis of *G. indicum* a leafy vegetable of family Asteraceae.

Sr. No.	Sample code	Extract Concentrations for Phenol and Flavonoid (µl/ml)	Quantitative Analysis		Extract Concentrations for DPPH and SOD (µl/ml)	Antioxidant analysis			
			Total Phenol contents (µg QE/g)	Total Flavonoid Content (µg QE/g)		DPPH (% of inhibition)	IC50 (µg/ml)	SOD (% of inhibition)	IC50 (µg/ml)
1	GIRE	300	111.19	101.42	30	17.35		10.98	
		600	252.47	316.85	60	32.63	**92.43**	29.65	**92.32**
		900	325.99	490.23	90	48.70		48.54	
2	GISE	300	163.19	92.72	30	13.72		20.97	
		600	240.26	180.26	60	37.82	**78.32**	28.79	**92.22**
		900	655.89	265.40	90	57.77		48.54	
3	GILE	300	175.19	67.92	30	15.28		16.63	
		600	330.87	193.25	60	22.79	**97.51**	31.60	**94.83**
		900	410.33	274.79	90	44.55		47.45	

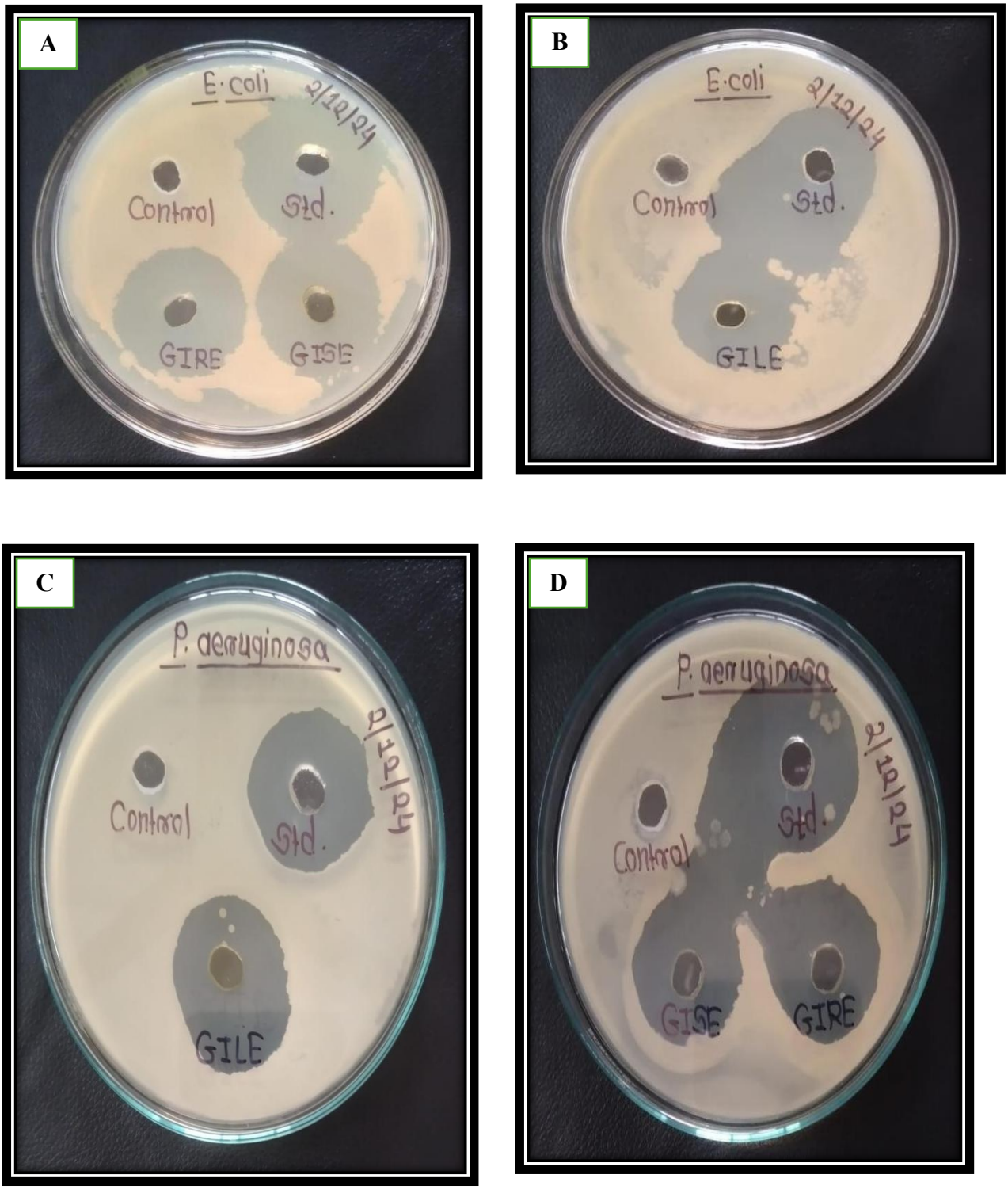
4. Antibacterial Activity

The antibacterial potential of ethanolic extracts of *Goniocaulon indicum* (Klein ex Willd.)—roots (GIRE), stems (GISE), and leaves (GILE)—was investigated using the agar well diffusion technique against selected Gram-negative (*Escherichia coli* NCIM 2832, *Pseudomonas aeruginosa* ATCC15442) and Gram-positive (*Bacillus subtilis* ATCC6633, *Staphylococcus aureus* NCIM 2654) bacteria (Table 4). The inhibitory effect was evaluated by measuring the zone of inhibition (ZI), a standard method for preliminary antimicrobial screening of plant-derived bioactives (Balouiri et al., 2016; Khatoon et al., 2022). All three extracts displayed appreciable antibacterial effects, with inhibition zones ranging from 18–21 mm against *E. coli*, 14–19 mm against *P. aeruginosa*, 13–21 mm against *B. subtilis*, and 18–20 mm against *S. aureus*. Among the tested extracts, GILE exhibited the strongest effect, producing maximum inhibition against *B. subtilis* (21 mm) and *E. coli* (21 mm). These results indicate that ethanol-soluble phytoconstituents are likely responsible for the antibacterial activity observed. Although slightly less effective than the standard antibiotic streptomycin, the extracts still showed considerable

inhibitory potential (Figure 3). The findings are consistent with earlier studies reporting that Asteraceae members contain abundant antimicrobial metabolites such as flavonoids, phenolic acids, and terpenoids (Salehi et al., 2018; Yadav et al., 2021). Flavonoids and phenolics are particularly known to impair bacterial survival by disrupting membrane integrity, interfering with nucleic acid synthesis, and hindering energy metabolism (Cushnie & Lamb, 2011; Tungmunnithum et al., 2018). The comparatively higher inhibition against Gram-positive strains, especially *B. subtilis* and *S. aureus*, may be linked to differences in cell wall architecture; Gram-positive bacteria possess a less complex cell envelope, making them more vulnerable to phenolic compounds, whereas Gram-negative species are protected by an additional outer membrane (Rather et al., 2021). Overall, the results suggest that *G. indicum* extracts possess noteworthy antibacterial activity, supporting its traditional use as a medicinally valuable leafy vegetable. Future investigations involving bioassay-guided fractionation, phytochemical isolation, and molecular docking are recommended to identify and characterize the active principles responsible for these antimicrobial effects.

Table 4: Antibacterial activity by Agar well diffusion method.

S/N	Sample Code	Zone of Inhibition (ZI mm)			
		Gram-Negative Bacteria		Gram-Positive Bacteria	
		E. coli (Strain - NCIM 2832)	***P. aeruginosa*** (Strain - ATCC15442)	***B. subtilis*** (Strain - ATCC6633)	***S. aureus*** (Strain - NCIM 2654)
	Control	-	-	-	-
	Streptomycin (Standard)	28	28	28	28
1	**GIRE**	18	14	13	18
2	**GISE**	21	16	20	19
3	**GILE**	20	19	21	20



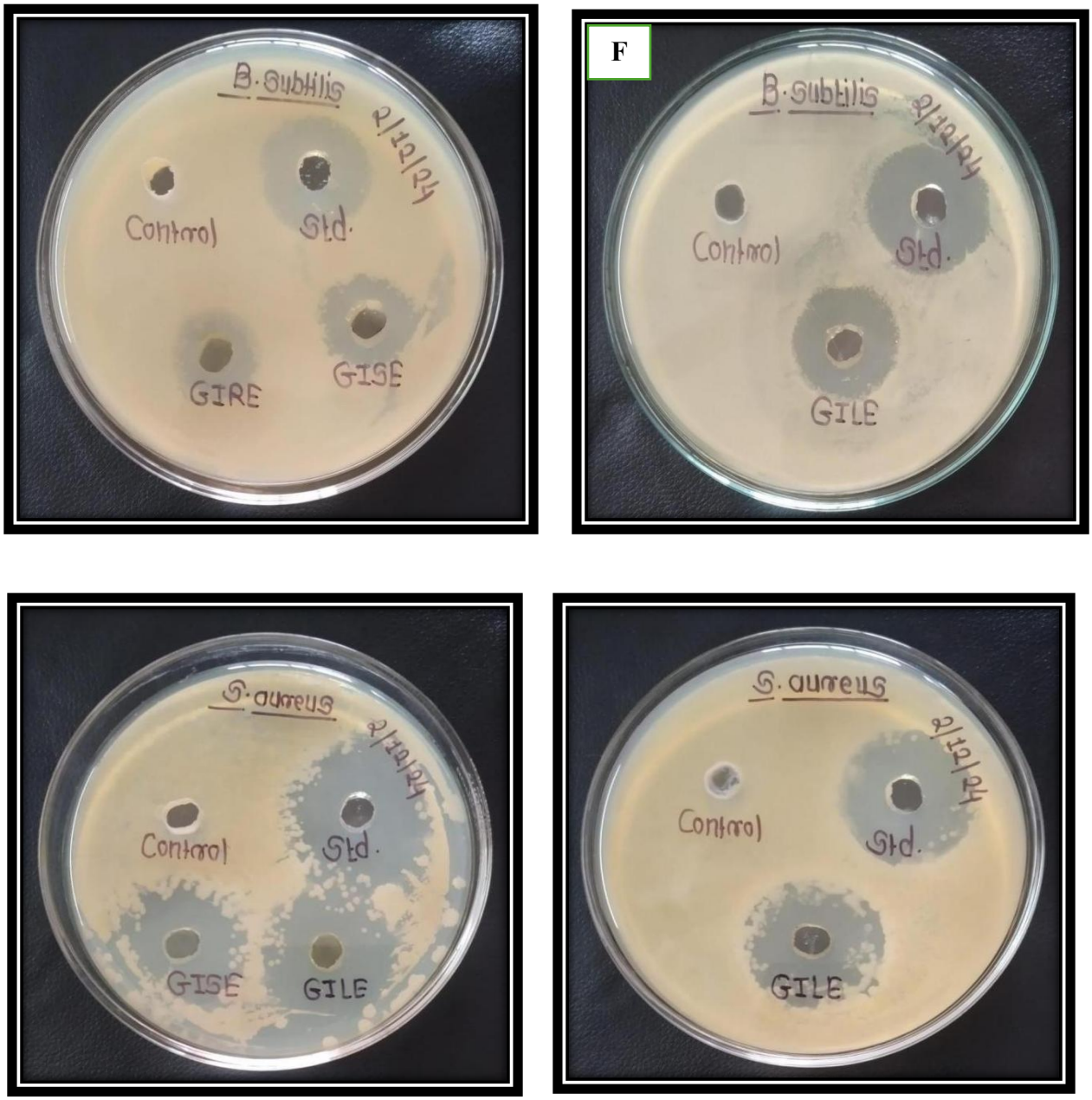


Figure 3. Antibacterial activity of ethanolic extracts against *Escherichia coli* (A–B), *Pseudomonas aeruginosa* (C–D), *Bacillus subtilis* (E–F), and *Staphylococcus aureus* (G–H) using the agar well diffusion method.

CONCLUSION

This study demonstrates the phytochemical diversity and biological potential of *Goniocaulon indicum* (Klein ex Willd.), a lesser-known leafy vegetable from the family Asteraceae. Phytochemical screening confirmed the presence of phenolics, flavonoids, alkaloids, glycosides, terpenoids, and steroids—classes of compounds widely associated with nutritional and pharmacological benefits. Quantitative analysis revealed higher concentrations of phenolics and flavonoids in stem and leaf extracts,

which corresponded to strong antioxidant activities in DPPH and SOD assays. These outcomes reinforce earlier reports that phenolic-rich vegetables can mitigate oxidative stress and reduce the risk of chronic and degenerative disorders (Tanase et al., 2019; Singh et al., 2021). In addition, ethanolic extracts of *G. indicum* exhibited significant antibacterial activity against both Gram-positive (S. aureus, B. subtilis) and Gram-negative (E. coli, P. aeruginosa) bacteria. The broad inhibitory spectrum observed parallels the activity of other Asteraceae taxa traditionally recognized for antimicrobial efficacy (Balouiri et al., 2016; Ríos & Recio, 2019; Jangra et al., 2022). Together, these findings validate the ethnomedicinal relevance of *G. indicum* and highlight its dual value as a nutrient-rich food source and a potential reservoir of bioactive metabolites. Given the growing global interest in functional foods and plant-derived therapeutics, *G. indicum* shows promise for nutraceutical development and as a natural antimicrobial alternative. Nonetheless, additional investigations, including bioassay-guided isolation of active constituents, in vivo pharmacological testing, and toxicity assessments, are essential to establish its therapeutic applications. Conservation strategies and sustainable harvesting practices should also be prioritized to safeguard this underutilized but valuable wild leafy vegetable (Khatoon et al., 2022; Pandey et al., 2022; Popović-Djordjević et al., 2023).

ACKNOWLEDGMENT

The authors gratefully acknowledge the SLS, SRTM University, Nanded; Department of Botany, NES Science College, Nanded; and Infinite Research Lab, Sangli, for their support and for providing the facilities required to complete this study.

Conflict of interest: None

Financial support: Nil.

Authors Contribution: All authors contributed equally.

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