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# Quantitative phytochemical analysis and antioxidant potential of medicinal plants used in traditional healing practices in Lower Dir, Pakistan

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**Abstract**

It is advantageous to use plant chemicals and ethnobotanical techniques while looking into new medications. One essential measure to distinguish the bioactive components of medicinal plants used in formal therapy is to identify the phytochemicals. In this study, 20 traditionally used medicinal plants were taken from Lower Dir, Pakistan. The medicinal plant species were identified and grouped into appropriate families. Standard procedures were used to screen these medicinal plants for the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phlobatannis, saponins, steroids, tannins, terpenoids, and triterpenes. The phytochemical screening of 20 medicinal plant species revealed notable interspecific variation in secondary metabolites. It has been shown that nine different plant species' leaves, the whole plant body of 10 plant species and the flowers of one plant species contained major groups detected, that included alkaloids (57.1%) from 12 plants species, saponins (61.1%) from 13 plant species, tannins (95.2%) from all 20 species, steroids (52.4%) from 11 species, phenols (52.4%) from 11 species, flavonoids (76.2%) from 16 species, terpenoids (81%) from 17 species, triterpenoids (81%) from 17 species, and phlobatannins (42.9%) from 19 species. Tannins, flavonoids, and terpenoids were the most widely distributed, while alkaloids and saponins were also prominent in several species. Rich profiles were observed in *Chenopodium album, Ficus carica,* and *Vitex negundo,* whereas *Justicia adhatoda, Withania somnifera,* and *Silybum marianum* showed bioactive constituents consistent with their reported therapeutic uses. These research findings confirm traditional medicinal healing practices and the pharmacological potency of the studied florist diversity.

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## Introduction

Medicinal plant species can produce a collection of bioactive substances that modify healing potency, determine physiological states, and address specific health issues via established pharmacological pathways. These botanicals form the foundation of ethnological medicines, utilized widely as preventive and therapeutic measures, especially in developing countries (Akhtar et al., 2019). The importance of plant-derived chemicals in modern pharmaceutical treatment is considerable; empirical evidence shows that several licensed medications are either directly sourced from plants or are synthetic equivalents based on botanical-led compounds (Seidel, 2020). A revived interest in medicinal plants has been driven by the growing costs of synthetic medications, both for direct use in alternative medicine and as a source of new therapeutic compounds. Growing

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globalization and consumer demand for natural health products are further factors driving this paradigm shift (Chaachouay et al., 2024). In order to isolate and create novel chemical entities for pharmaceuticals and chemotherapeutics from both established ethnobotanical sources and creative plant candidates, the pharmaceutical industry is gradually devoting resources to the bioprospecting of these species (Porras et al., 2020).

Phytochemistry examines the diverse chemical substances synthesized by plants. Specific chemical compounds present in medicinal species, including tannins, alkaloids, carbohydrates, flavonoids, terpenoids, and steroids, exert distinct physiological effects on various parts of the human body (Saxena et al., 2013). The comprehensive screening reveals that the presence of these chemical compounds is markedly unique in biological, taxonomic, and chemical contexts, aiding in the documentation of their aromatic characteristics (Mathe et al., 2024). It encompasses the chemical composition, production, turnover, metabolism, natural distribution, and biological function of these chemicals (Manzetti et al., 2014). Environmental elements and their consequences connected to desert climates, overhead salinity, nutrient adequacy, and water scarcity have highlighted the significance of medicinal plants engaged in the process of the pharmaceutical business and alternative therapies (Jangpangi et al., 2025). These plants have been utilized traditionally as a source of welfare throughout human history. Many of the medicinal attributes of plant species have been passed down through generations. Plant-derived secondary substances are utilized as drugs to address basic human and animal needs. Because of their prominent diversity, proper sustainability, and simplicity of obtaining natural production, these plant species are widely acknowledged (Elshafie et al., 2023; Ahmad et al., 2025). Plants with proven therapeutic efficacy that are frequently used in traditional medicine may include compounds that could be used as pharmaceuticals. Moreover, the distribution of these chemicals varies throughout the plant body. Traditional medicine practice uses a variety of plant species parts, including bark, which is a basic part, the photosynthetic part leaves, the reproductive parts flowers, the mechanical supportive parts stems, the fruits, rhizomes, resins, seeds, and the basic supportive parts, i.e., roots. Nonetheless, these plants are also used for specialized purposes to treat a few serious disorders (Mohiuddin, 2019).

In recent decades, there has been an increased demand for herbal treatments due to growing public awareness of the benefits of reverting to natural methods for a healthy lifestyle. The goal of this survey was to identify alternative metabolites, in particular traditional, long-used medicinal plants, and look into any possible relationships between these metabolites' concentrations and the plants' ethnomedical effectiveness in a specific area, Lower Dir, a district in the Khyber Pakhtunkhwa (KP) province of Pakistan, characterized by rich flora (Shuaib et al., 2014). Consequently, an investigation into these plants was felt essential for a comprehensive understanding of their attributes, safety measures, and applications.

## Materials and Methods

### Research area

This research study was carried out in the wide area of Lower Dir, Khyber Pakhtunkhwa province, Pakistan. The location of the district Lower Dir is between 34° 37′ to 35° 07′ North Latitudes and 71° 31′ to 72° 14′ East Longitude with an altitude of 820 m above sea level. The topography of Lower Dir is diverse, featuring plains, undulating, and mountainous areas covered by grasses, shrubs, and trees. The topography is further characterized by a mountainous landscape with peaks reaching up to 4,876 m on the north side and 3,048 m along the Swat River on the east. The average minimum temperature in December is 5.2 ˚C, while the mean maximum in July reaches 35.8 ˚C (Waris, 2020; Ullah, 2025) (**Figure 1**).

### Collection of medicinal plants

Twenty indigenous medicinal plant species were collected from the Lower Dir district based on their morphological and ecological characteristics. The plant species were identified accurately and documented at the Center of Plant Biodiversity and Conservation, University of Peshawar, Pakistan, following the procedures described elsewhere (Hedberg, 1993; Pandey and Das, 2014). Plant species and their components (leaves, flowers, and whole plant bodies) were collected throughout the study area. Leaves were collected and utilized from the plant species, *Chenopodium murale* L., *Dodonaea viscosa* Jacq., *Eriobotrya japonica* (Thunb.) Lindl., *Ficus carica* L., *Fumaria indica (*Hausskn.) Pugsley. *Justicia adhatoda* L., *Vitex negundo* L., *Withania somnifera* (L.) Dunal, *Zanthoxylum armatum* DC., while whole plants were used from the species, i.e., *Chenopodium album* L., *Conyza canadensis* (L.) Cronq., *Euphorbia helioscopia* L., *Mentha longifolia* (L.) L., *Parthenium hysterophorus* L., *Ranunculus arvensis* L., *Sonchus asper* (L.) Hill, *Solanum nigrum* L., *Viola canescens* Wall., and *Verbascum thapsus* Wall.; and the final species, *Silybum marianum* (L.) Gaertn was used just for its flowers (**Figure 1**). The plant species were verified at the PCSIR Lab in Peshawar, Khyber Pakhtunkhwa, and the plant samples were cleaned with water to eliminate contamination. The collected plant components were thereafter dried in the shade and pulverized using a mortar and pestle (**Table 1**).

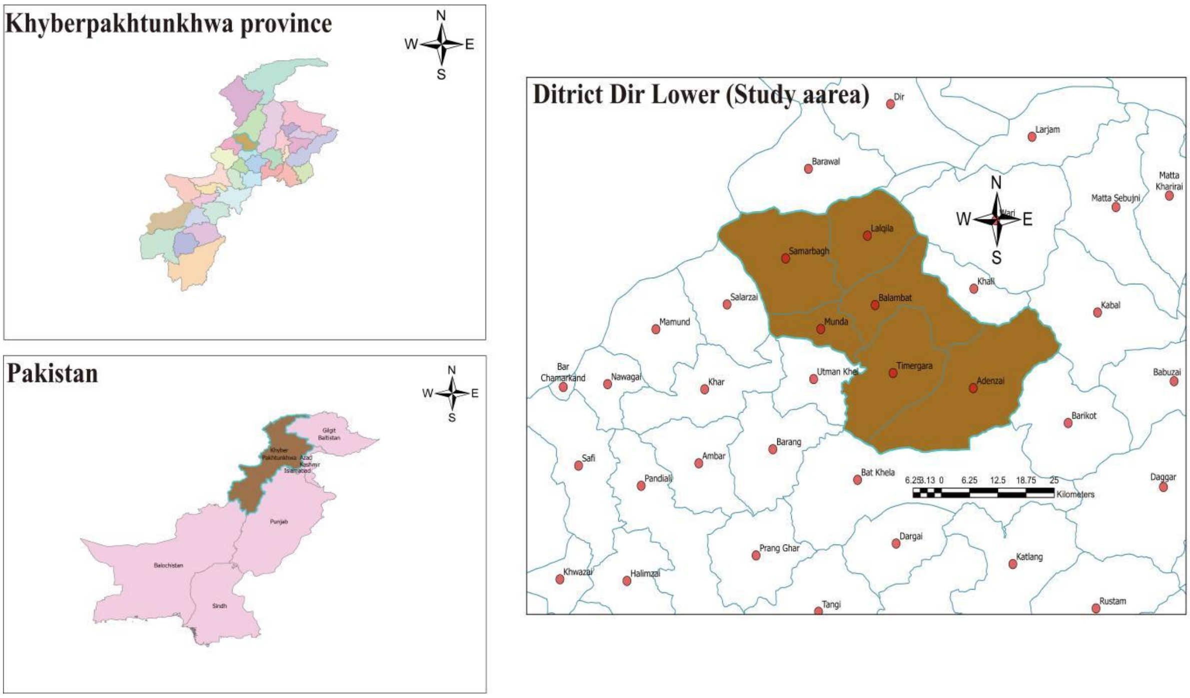


**Figure 1: This spatial figure shows the study area map, representing different provinces in Pakistan, various districts in the Khyber Pakhtunkhwa province, and the specific study area district of Lower Dir, with its sub-regions within the study area.**

**Table 1: List of medicinal plants in the research area, District Lower Dir, Khyber Pakhtunkhwa, Pakistan**

| Plant species | Part used | Family | Habit |
|---|---|---|---|
| *Chenopodium album* L. | Whole plant | Amaranthaceae | Annual herb |
| *Conyza canadensis* (L.) Cron. | Whole plant | Asteraceae | Annual herb |
| *Chenopodium murale* (L.) S.Fuentes, Uotila and Borsch | Leaves | Amaranthaceae | Annual herb |
| *Dodonaea viscosa* Jacq. | Leaves | Sapindaceae | Shrub |
| *Euphorbia helioscopia* L. | Whole plant | Euphorbiaceae | Annual herb |
| *Eriobotrya japonica* L. | Leaves | Rosaceae | Small tree |
| *Ficus carica* L. | Leaves | Moraceae | Small tree |
| *Fumaria indica* (Hausskn.) Pugsley | Leaves | Papaveraceae | Annual herb |
| *Justicia adhatoda* L. | Leaves | Acanthaceae | Shrub |
| *Mentha longifolia* (L.) L. | Whole plant | Lamiaceae | Perennial herb |
| *Parthenium hysterophorus* L. | Whole plant | Asteraceae | Annual herb |
| *Ranunculus arvensis* L. | Whole plant | Ranunculaceae | Annual herb |
| *Sonchus asper* (L.) Hill | Whole plant | Asteraceae | Annual herb |
| *Silybum marianum* (L.) Gaertn. | Flower | Asteraceae | Annual herb |
| *Solanum nigrum* L. | Whole plant | Solanaceae | Annual herb |
| *Viola canescens* L. | Whole plant | Violaceae | Perennial herb |
| *Vitex negundo* L. | Leaves | Lamiaceae | Shrub |
| *Verbascum thapsus* L. | Whole plant | Scrophulariaceae | Biennial herb |
| *Withania somnifera* (L.) Dunal | Leaves | Solanaceae | Perennial herb |
| *Zanthoxylum armatum* DC. | Leaves | Rutaceae | Shrub |

* All species belong to Class Magnoliopsida (Dicots)

## Phytochemical analysis

The primary categories of bioactive chemicals in the medicinal plants under study were identified using phytochemical screening. Following established protocols, the tests were carried out on raw powdered plant material as well as aqueous and organic solvent extracts, delineated as described elsewhere (Alabri et al., 2014; Egbuna et al., 2018). Qualitative phytochemical screening was performed to identify major classes of bioactive constituents. The analysis was conducted on three distinct sample forms: (i) raw dried plant powder, (ii) an aqueous extract, and (iii) a single, defined organic extract prepared using a ternary solvent blend of chloroform, methanol, and ethyl acetate in a standardized ratio of 4:4:2. This combined solvent system was specifically chosen to facilitate the concurrent extraction of a broad polarity range of secondary metabolites. All assays followed the referenced standard protocols (Harborne, 1973; Sofowora, 1993; O'Neill, 1996; Sasidharan et al., 2011).

## Crude extraction

Plants were chopped, crushed, and dried in the shade before being sent to the Pakistan Council of Scientific and Industrial Research Laboratories Complex (PCSIR) in Peshawar for phytochemical examination following procedures as described elsewhere (Alabri et al., 2014; Egbuna et al., 2018). To the powdered material (150 g) of each plant, 500 mL of methanol, ethyl acetate, and chloroform in a standardized ratio of 4:4:2. was addedly. The flasks containing plant material were heated between 40 and 50 °C in a vacuum cyclical evaporator.

## Determination of extracts

The resulting extract was heated at 30-45 °C in a rotary evaporator and concentrated at reduced pressure. After concentrating to a semi-solid form, the extract was removed from the round-bottom flask. A reweighed China dish was used to dry the product in a water bath set at 45 °C. The following equation was used to find the extraction percent yield, and then it was dried and weighed again.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of ground plant material}} \times 100$$

## Phytochemical screening

Powdered material of each plant species was soaked in 300 mL solvent of chloroform, ethyl acetate, and methanol in a standardized ratio of 4:4:2 for 2 weeks. Then each material was shaken well twice a day and then filtered. The filtrates were then evaporated under reduced pressure to obtain gummy residues with the help of a rotary evaporator. All extracts were stored in sterile glass bottles at room temperature until screened. The extracts were tested for flavonoids, phenols, glycosides, alkaloids, cardiac glycosides, terpenoids, saponins, and tannins. The subjective or qualitative screening was executed using the standard methodology (Peiris et al., 2023).

## Tannins

The method described by Loman and Ju (2017) was followed for the qualitative determination of tannins. Two mL of 5% ferric chloride solution were added to 1 mL of each concentrated extract in a test tube. In the experiment, the appearance of a greenish color confirmed the presence of tannins.

## Flavonoids

The presence of flavonoids in the chosen plants was confirmed by adding 0.2 mL of each extract to the test tube. This was followed by the addition of 10 mL of deionized water and 5 mL of diluted ammonia to the mixture. After that, 2 mL of concentrated $H_2SO_4$ was added. The appearance of a yellow color indicated the presence of flavonoids.

## Alkaloids

In a test tube, 2 mL of concentrated hydrochloric acid (HCl) and 2 mL of each of the plant extracts were combined with a couple of drops of Mayer's reagent. The presence of alkaloids was indicated by the appearance of a yellow color.

## Terpenoids

The Salkowski's method was followed to determine terpenoids in all plant extracts. The quantity of terpenoids in the plant sample was determined by shaking 0.2 mL of the extract in 5 mL of methanol and filtering the mixture. The filtrate was thoroughly combined with 4 mL of chloroform. A crimson coating formed after adding 3 mL of concentrated $H_2SO_4$, which was a sign of the existence of terpenoids.

### Phenols

When 1 mL of the concentrate was mixed with 2 mL of distilled water and a few drops of 10% ferric chloride, a greenish hue was produced, indicating the presence of phenols.

### Glycosides

Two to three drops of α-naphthol reagent were added to 2 mL of the test solution in a test tube. The test tubes were inclined, and one mL of conc. $H_2SO_4$ was added. The appearance of a purple ring in the junction of two layers indicated the presence of glycosides.

### Cardiac glycosides

Qualitative analysis of cardiac glycosides in all plant extracts was carried out according to the Keller Killiani test as described elsewhere (Leicach and Chludil, 2014). Acetic acid (1 mL) and ferric chloride (2 drops) were added to 2 mL of each extract. Then 2 mL of concentrated sulfuric acid was added to each test tube. Appearance of reddish-brown color confirmed the presence of cardiac glycosides.

## Results and Discussion

The collected medicinal plants of District Lower Dir, Pakistan, were likely to provide many secondary metabolites, including flavonoids, glycosides, alkaloids, phenols, and phytosterols. The proximity of these alternative metabolites enhances the therapeutic capacity of the chosen medicinal plants. These phytochemicals provide medicinal evaluations of the examined plants. Different types of phytochemicals, such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phlobatannins, saponins, steroids, tannins, terpenoids, and triterpenes, were identified and presented in **Table 2** and **Figure 2**.

**Table 2: Phytochemical screening of selected 20 plants ((+) present, (−) absent)) of the district Lower Dir, Khyber Pakhtunkhwa, Pakistan**

| Plant species | Part used | A | CG | S | T | S | P | F | T | Tr | Ph |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chenopodium album* L. | Whole plant | + | + | +++ | + | + | + | ++ | + | +++ | + |
| *Conyza canadensis* (L.) Cron. | Whole plant | - | + | + | ++ | + | | ++ | ++ | + | + |
| *Chenopodium murale* (L.) S.Fuentes, Uotila and Borsch | Leaves | +++ | ++ | + | - | - | + | + | + | ++ | - |
| *Dodonaea viscosa* Jacq. | Leaves | - | - | + | ++ | ++ | - | - | ++ | +++ | ++ |
| *Euphorbia helioscopia* L. | Whole plant | + | - | - | ++ | ++ | + | +++ | + | + | + |
| *Eriobotrya japonica* L. | Leaves | - | + | - | ++ | + | + | + | ++ | ++ | + |
| *Ficus carica* L. | Leaves | - | ++ | +++ | +++ | +++ | - | ++ | +++ | +++ | ++ |
| *Fumaria indica* (Hausskn.) Pugsley | Leaves | ++ | - | + | +++ | - | - | +++ | ++ | ++ | - |
| *Justicia adhatoda* L. | Leaves | ++ | - | ++ | ++ | - | + | ++ | + | + | - |
| *Mentha longifolia* (L.) L. | Whole plant | ++ | - | - | ++ | - | + | +++ | + | + | + |
| *Parthenium hysterophorus* L. | Whole plant | ++ | + | ++ | ++ | + | + | ++ | + | - | - |
| *Ranunculus arvensis* L. | Whole plant | ++ | + | - | +++ | + | - | - | - | + | + |
| *Sonchus asper* (L.) Hill | Whole plant | +++ | ++ | + | - | - | + | + | + | ++ | - |
| *Silybum marianum* (L.) Gaertn. | Flowers | - | - | + | ++ | ++ | - | - | ++ | +++ | ++ |
| *Solanum nigrum* L. | Whole lant | + | - | - | ++ | ++ | + | +++ | + | + | + |
| *Viola canescens* L. | Whole plant | - | + | - | ++ | + | + | + | ++ | ++ | + |
| *Vitex negundo* L. | Leaves | - | ++ | +++ | +++ | +++ | - | ++ | +++ | +++ | ++ |
| *Verbascum thapsus* L. | Whole plant | ++ | - | + | +++ | - | - | +++ | ++ | ++ | - |
| *Withania somnifera* (L.) Dunal | Leaves | ++ | - | ++ | ++ | - | + | ++ | + | + | - |
| *Zanthoxylum armatum* DC. | Leaves | ++ | + | ++ | ++ | + | + | ++ | + | - | - |

A = Alkaloids; CG = Cardiac glycosides; S = Saponins; T = Tannins; S = Steroids; P = Phenols; F = Flavonoids; T = Terpenoids; Tr = Triterpenoids; Ph = Phlobatannins

The diversity of phytochemical contents has been reported to be influenced by environmental conditions such as soil and climate (Ogwu et al., 2025), therefore accounting for the disparity in compound presence among species. The evidence is in favor of these plants being of benefit to traditional medicine, but certain species appear to provide limited phytochemical diversity, suggesting differing therapeutic potential (Alamgeer et al., 2018; Aziz et al., 2018). This study shows that the phytochemical screening of the twenty medicinal plants reveals distinct profiles that provide a scientific basis for their traditional uses. To identify possible sources of particular bioactive substances, the study can be efficiently classified by the plant portion used, such as the entire plant, leaves, or flowers (**Figure 3**).

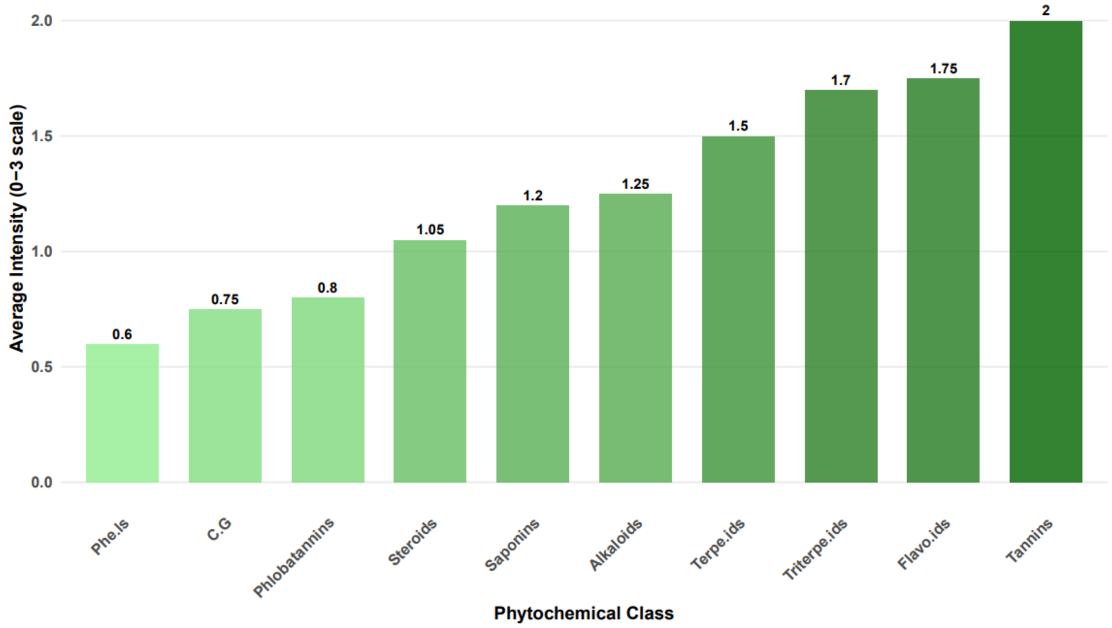


**Figure 2: Average phytochemical intensity across species, and mean intensity score (0–3 scale) for each phytochemical class.**

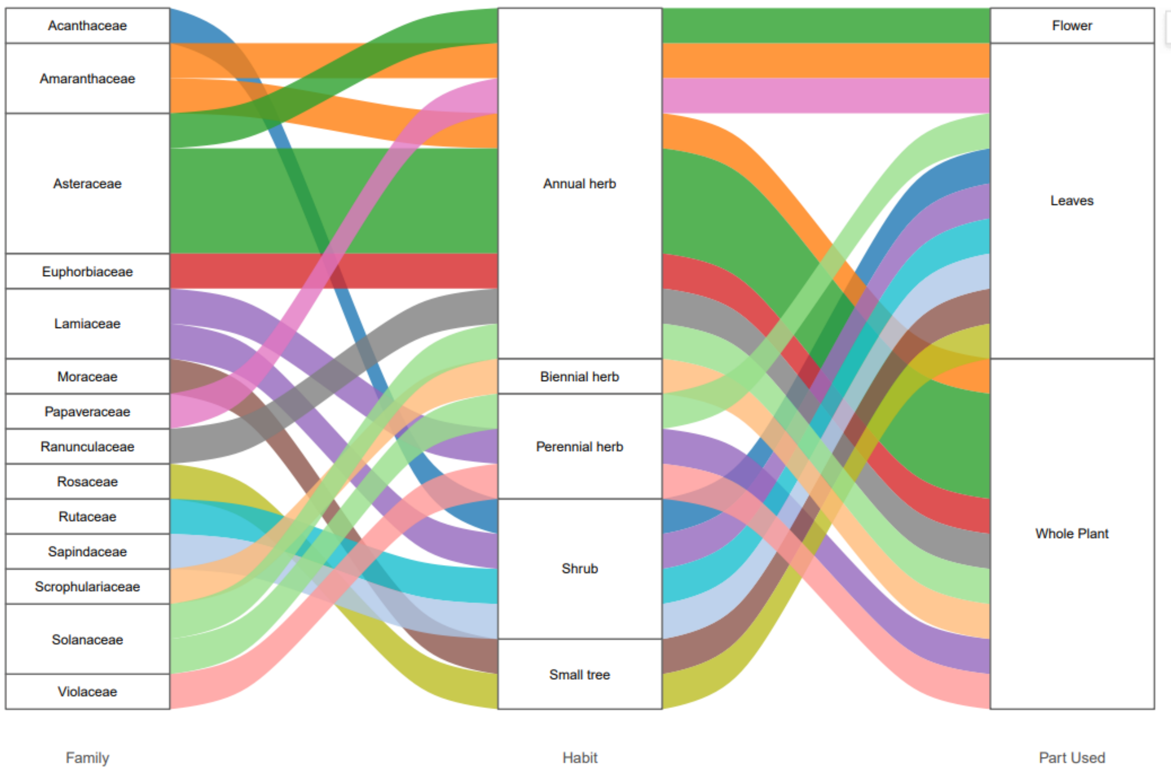


**Figure 3: Alluvial flow diagram shows taxonomic and functional relationships in medicinal plants (relationships between plant families, growth habits, and parts used).**

A significant number of species in this study were utilized in their entirety, suggesting a synergistic combination of metabolites across different tissues (Petrovska, 2012; Richards et al., 2016). *Chenopodium album* and *Parthenium hysterophorus*. exhibited a broad spectrum of compounds, including alkaloids, which are often associated with antimicrobial and insecticidal properties. Species like *Euphorbia helioscopia*, *Mentha longifolia*, and *Solanum nigrum*. were characterized by a very high concentration of flavonoids, supporting their traditional use as anti-inflammatory and antioxidant agents (Ullah et al., 2020). *Conyza canadensis* and *Viola canescens* presented more moderate profiles, while *Ranunculus arvensis* and *Sonchus asper* were notable for their very high concentrations of tannins and alkaloids, respectively, indicating potential astringent and bioactive properties. *Verbascum thapsus* also showed a potent combination of high tannins and flavonoids.

The analysis of leaves revealed several species with exceptionally potent and complex phytochemical profiles. *Ficus carica* and *Vitex negundo* were particularly outstanding, both showing very high concentrations of saponins, tannins, steroids, flavonoids, and triterpenoids, making them strong candidates for multi-target therapeutic applications such as wound healing, inflammation, and microbial infections. *Chenopodium murale* and *Justicia adhatoda* were rich in alkaloids, corroborating their use for respiratory and spasmodic conditions. *Dodonaea viscosa* was marked by a very high concentration of triterpenoids, compounds known for their anti-arthritic and hepatoprotective potential (Alanazi et al., 2023). Other species like *Eriobotrya japonica* (Zhu et al., 2022), *Fumaria indica* (Gupta et al., 2012), *Withania somnifera* (Saleem et al., 2020), and *Zanthoxylum armatum* (Alam et al., 2018) displayed robust profiles rich in tannins, flavonoids, and saponins, aligning with their reputations as tonics and remedies for various metabolic and inflammatory disorders, and a single species, *Silybum marianum,* was analyzed for its flowers. The profile was distinct, showing a very high concentration of triterpenoids and a complete absence of alkaloids and phenols. This specific chemoprofile is highly significant as it directly points to the source of its well-documented hepatoprotective compound, silymarin, a complex of flavonolignans and triterpenoids, validating the traditional use of its flower heads for liver ailments (**Figure 4**) (Mahmoud and Selim, 2025).

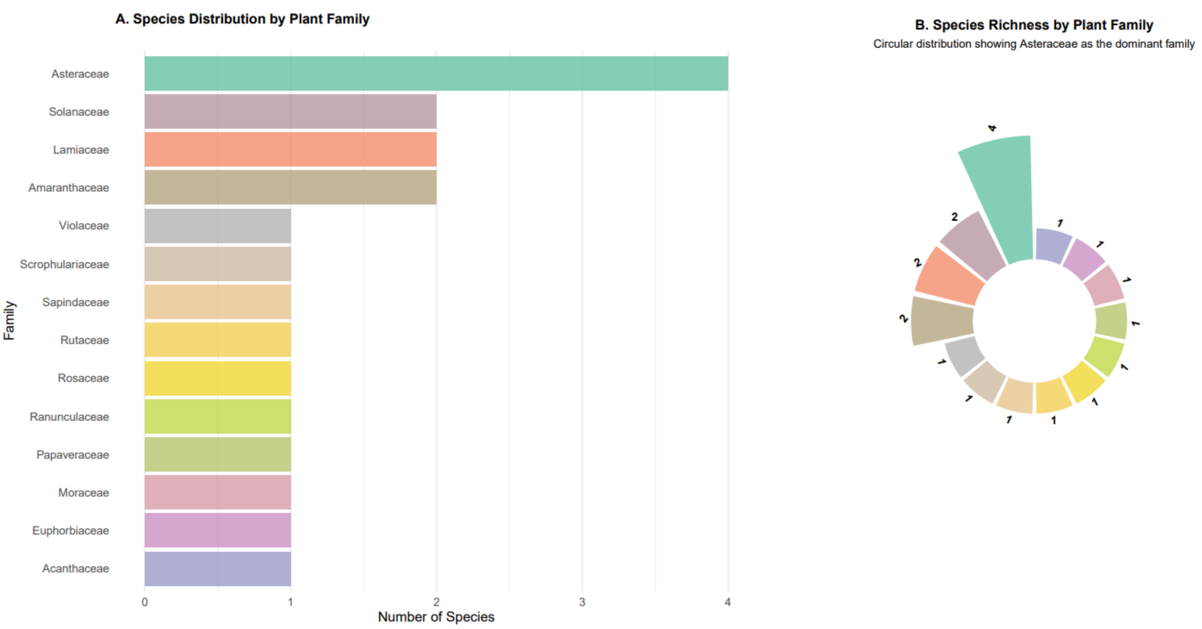


**Figure 4: Comparative analysis of plant family distribution, species distribution by plant families (A), and species richness by plant families (Asteraceae dominant family).**

## Conclusion

In conclusion, the presence of a wide range of bioactive secondary metabolites is confirmed by the thorough phytochemical investigation of these 20 chosen medicinal plants, including alkaloids, flavonoids, tannins, saponins, terpenoids, and triterpenoids. The distinct and often potent profiles of species such as *Ficus carica*, *Vitex negundo,* and *Withania somnifera* give a scientific foundation for their respected position in conventional medicine, confirming their application and emphasizing them as excellent options for upcoming drug development studies. Since these chemicals are known to control fungal infections, their identification is crucial for agricultural sustainability in addition to human therapies. As a result, these therapeutic plants can be turned into potent biopesticides, providing a long-term substitute for artificial fungicides. Their dual potential highlights their vital significance as sources of innovative medicinal agents and as essential elements of environmentally friendly integrated pest management systems, eventually promoting agricultural security and global health.

## Author(s), Editor(s) and Publisher's declarations

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### Contribution of authors

Research superior(s): SMS. Conceptualization and designing the study: WK. Conduction of experiment: ZUI, AU. Instrumentation and analysis: SGA.

### Permissions and ethical compliance

This study does not involve human/animal subjects, and thus no ethical approval is required.

### Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

### Supplementary material

No supplementary material is included with this manuscript.

### Conflict of interest

The authors declare no conflict of interest.

### Availability of primary data and materials

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### Authors' consent

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It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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