Quantification of bioactive compounds of major medicinal plants from Bangladesh

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Abstract

The medicinal and nutritional benefits of various plant leaves are a rich source of phytochemicals, which play vital roles in providing better solutions for other diseases. In this study, quantified the levels of bioactive compounds in the leaves of twenty-four medicinal plant species collected in Bangladesh from the National Botanic Garden, Dhaka, Bangladesh, based on visual coloration and documented medicinal uses in traditional and local Bangladeshi medicine. . Bioactive compounds, including total phenol content, flavonoids, β–carotene, anthocyanins, and lycopene levels, were quantified and evaluated. The T80 UV/Vis Spectrophotometer (PG Instruments, United Kingdom) was used to estimate and assess the levels of these bioactive compounds, a method that is simple, rapid, and cost-effective, where fresh leaf extracts were analyzed in triplicate at specific wavelengths, and data were subjected to one-way ANOVA followed by Duncan's Multiple Range Test ($P \leq 0.05$). *Ipomoea batatas* (Sweet potato), *Amaranthus gangeticus* (Red spinach), *Cordyline fruticosa* (Ti plant), *Tradescantia spathacea* (Boatlily), *Tradescantia zebrina* (Wandering dude) showed the maximum level of β – carotene content (153 mg/100g), lycopene content (109 mg/100g), flavonoid content (810 mg/100g RE), anthocyanin content (267 mg/100g) and phenolic content 615 mg GAE/100g respectively. Pearson correlation analysis identified extremely strong positive relationships between β-carotene and lycopene ($r = 0.9264$) and between anthocyanins and total phenolic content ($r = 0.9712$), indicating common metabolic regulation. These selected plants could be considered a new natural source of bioactive compounds and could open future applications in the food, chemistry, and pharmaceutical industries. This study identified novel insights into the bioactive compositions of tropical medicinal plants.

Keywords: Bioactive compounds; Medicinal plant; Phytochemical profiling; Flavonoids; Antioxidant.

1. **Introduction**

Numerous medicinal properties have been attributed to natural plants. In contemporary times, medicinal plants are used to formulate pharmaceuticals, healthcare products, foods, chemicals, and cosmetics. These plants are rich sources of vitamins, minerals, and phytochemicals, which support our bodies and help combat various microbial and other ailments. Across the globe, indigenous peoples use various herbs and plants for their diverse medicinal properties. As an example, teas from the plant, also known as harval tea, are well-known nowadays for their antioxidant content and benefits, including phenolics and fat-soluble vitamins, which play a significant role in human physiology. Besides vitamins and minerals, phenolics also show potential to support the body, functioning as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators against various pathogens and diseases [1-3].

Various bioactive compounds, also known as phytochemicals, such as flavonoids, carotenoids, and phenolic compounds, are available along with tannins, phytoestrogens, phytosterols, and glucosinolates, and have significant medicinal importance, including tumor-inhibitory and anticarcinogenic potential [4, 5]. Nowadays, these bioactive compounds, as secondary metabolites in plants, are of significant importance, and a new era of research is underway for their anticarcinogenic, antitumor, antioxidant, and wound-healing effects.

The modern techniques used to identify and isolate these important bioactive compounds from various medicinal plants are intricate. While different animals, such as Guinea pigs and rats, are often used to assess the antioxidant and therapeutic activities of these compounds, these findings will be utilized in humans [5, 6]. Consequently, using phytochemicals for therapeutic and antioxidant purposes requires identifying them, then extracting and refining them into drugs with minimal or no side effects, akin to antibiotics [7].

Inhibiting mutagenic reactions in Deoxyribonucleic acid (DNA) and inactivating carcinogens represent a new approach to preventing various cancers and are a noble strategy that utilizes anticarcinogens and antioxidants. Enzymatic inactivation, scavenging carcinogens, and inhibition of reactive oxygen species are notable mechanisms utilized for this purpose [8].

Nowadays, in modern medicine, most of the anticarcinogens are extracted and purified from the traditional plants that were clinically used to treat different diseases worldwide, such as flavopiridol, epipodophyllotoxin, betulinic acids, combretastatin, camptothecin, silvestrol, taxols, vinblastine, vincristine, irinotecan, and topotecan, which have shown promising results in various studies [9].

Ellagic and gallic acids, like phenolic compounds, can interrupt tumor cell spread and possess DNA-binding properties which effectively interfere with carcinogenic cell development [10]. Different plant parts such as fruits, shoots, leaves, and roots, such as *Amaranthus gangeticus* and *Tradescantia zebrina*, reveal phenolic compounds which have significant anticarcinogenic potential [7].

There are very few studies which quantified the bioactive composition of plant leaves [11-14]. Comprehensive characterization, functionalization, and utilization of bioactive compounds from plant leaf-type studies are very few; studies which use spectrophotometric methods as a preliminary, practical approach for the initial screening, identification, and quantification of major bioactive compounds with significant effects in plant leaves are very few. Plant leaves have complex,

heterogeneous matrices that lead to variable levels of pigments, phenolics, and other phytochemicals. For the precise identification and quantification of the individual bioactive compounds, we can use high-performance liquid chromatography (HPLC) or mass spectrometry, but these techniques are expensive and time-consuming, require specialized solvents, and are not easily scalable for large sample sets. On the other hand, UV-Vis spectrophotometry is very simple, super fast, cost-efficient, and widely available in routine laboratories, ensuring replication of experiments. These experiments also enable simultaneous processing of multiple leaf samples, making them ideal for comparative screening across numerous species—such as those in this study, which includes 24 plants. Again, spectrophotometric assays for the Folin–Ciocalteu assay for total phenolics content, the aluminum chloride assay for flavonoid content, and specific and fixed wavelength measurements for carotenoids are well-established, replicable, reproducible, and have been widely validated for leaf tissues [4, 15, 16]. For this reason, these methods are highly efficient as a first-line tool to identify promising leaf sources of bioactive compounds before more targeted, expensive analyses are undertaken.

This study only focuses on the identification and quantification of the major bioactive compounds present in the traditional medicinal plants highly available in Bangladesh, which were further utilized in different extraction systems such as alkaline or enzymatic extraction, including sample preparation, yield calculation, and characterization, and non-comparable results, which have been identified as potential sources of error affecting the accuracy of findings. These identifications of bioactive compounds highlighted the medicinal value and use of these traditional plants and their potential health benefits, introducing a new dimension to research and industrial utilization.

2. Materials and Methods

2.1 Location of the plant samples

A total of, 24 plants were randomly selected and collected from the National Botanic Garden (23°48′N, 90°21′E) in Mirpur, Dhaka, Bangladesh, during the summer season (March-April) in 2023; their names are listed in **Table S1**. The national garden is maintained by the government organization of the People's Republic of Bangladesh, where plants are maintained using standard horticultural practices without chemical fertilizers. The National Botanic Garden is a managed vast horticultural collection rather than an experimental field or laboratory; for this reason, all the plants, trees, and shrubs were maintained under routine garden management practices without any specific pesticide or intentional chemical stress treatments. No species-specific records of soil properties, irrigation schedules, or nutrient management were utilized because the plants were not cultivated; rather, they were collected as part of a controlled agronomic library and provided experimental samples for the researcher not only in Bangladesh but also worldwide. For this reason, no specific cultivation records were available for any individual species, as plants were grown fully in natural conditions in open beds with regular irrigation. In this study, All plants are selected on the basis of medicinal use locally in Bangladesh, and availability in the whole country as mature, healthy plants free from visible symptoms of any disease, insect infestation, mechanical injury, or physiological disorders. For each species in this study, plant parts (leaves) were collected from three to five individual plants. The leaves were of comparable size and developmental stage at the same phenological stage. The leaves were fully expanded and mature, and were harvested from the middle

canopy to ensure that all samples represented the same physiological maturity stage, which reduces variation associated with leaf age and development. The leaves were collected between 8:00 and 10:00 AM to minimize daylight variation in metabolite content. After collection, the leafy parts of each plant were thoroughly washed and rinsed multiple times with tap water, and then cut into smaller pieces to obtain a uniform composite sample.

2.1.1 **Sample preparation and basis of expression**

All collected leaves were washed, cut into small pieces, and homogenized fresh without drying. Bioactive compound contents are expressed as mg per 100 g of fresh weight. Fresh samples were used to avoid degradation of heat-labile compounds such as anthocyanins, lycopene, and β-carotene that can occur during drying, and to reflect the actual bioactive composition as consumed or initially extracted in traditional preparations.

2.2 Extraction of β-carotene

Five grams of plant leaves were combined with 100 ml of methanol, stirred for 24 hours at 25ºC and 150 rpm, and then filtered using Whatman filter paper no. 4. The methanolic extract was collected, and 100 mg of the extract was subsequently mixed with a combination of 10 mL acetone-hexane (4:6). After vigorously shaking the mixture for one minute, it was filtered again using Whatman filter paper no. 4 [17]. A T80 UV/Vis Spectrophotometer (PG Instruments, United Kingdom) was used to measure absorbance. The absorbance of carotenoid content was measured at 453 nm, 505 nm, and 663 nm, and the carotenoid content was calculated using the following equation (1) and expressed as mg β-carotene equivalents per 100 g fresh weight.

$$\text{Carotenoids Content } \left({}^{mg}/_{100g}\right) = 0.216 \times \text{A663} - 0.304 \times \text{A505} + 0.452 \times \text{A453} \quad (1)$$

2.3 Extraction of lycopene

Calorimetric determination of lycopene from plant leaves by extraction with hexane, ethanol, and acetone, followed by absorbance measurement at 503 nm. 1 g of plant leaves was collected and soaked in 80 mL of a hexane-ethanol-acetone mixture (2:1:1) in a flask. Then, we vortexed the solution for 10 minutes and added 10 mL of water. The new solution was vortexed for ten minutes [15]. Lycopene absorbance was measured at 503 nm and expressed as mg per 100 g fresh weight.

2.4 Extraction of flavonoids

Flavonoid extract was prepared using methanol: 10 mg of plant leaves were soaked in 10 mL of methanol, and 1 mL was collected in a test tube. 5 mL of water and 0.1 mL of potassium acetate (CH_3CO_2K) were added to the test tube, which was then left to rest for 5 minutes. Then, 3 mL of aluminum chloride ($AlCl_3$) was added again for 30 minutes at room temperature [4]. QC samples for the determination of accuracy at six concentrations (0, 10, 20, 30, 40, and 50 µg/mL) were also prepared by serial dilutions of the freshly prepared Rutin stock solution and used within 24 h. Flavonoid absorbances were measured at 415 nm, using equation 2 to determine TFC, where Y is the

absorbance of the sample, and X is the total flavonoid content in mg/100g Rutin equivalents (RE) per 100g fresh weight.

$$Y = 0.0059 \times X + 0.0527 - - - - - - - - - - (2)$$

$$R^2 = 0.981$$

2.5 Extraction of anthocyanin

1 g of plant samples and 40 mL of 50% ethanol are placed in a flask at pH 3.0. Then it was kept at a warm temperature (50 °C) for 1 hour. Then, we filtered the solution, collected a 1 mL aliquot in a test tube, and added 4 mL of methanol [4]. Anthocyanin absorbance was measured at 530 nm and calculated using the following equation. Anthocyanin content was expressed as mg cyanidin-3-glucoside equivalents per 100 g fresh weight.

$$Anthocyanin\ Content\ (mg/100g) = \frac{Absorbance \times Molecular\ Weight\ of\ cyanidin-3-glucoside\ (449.2) \times Dilution\ Factor}{Molar\ Absorptivity \times Sample\ Weight} \times 100 - - - - - - - - - - (3)$$

2.6 Extraction of total phenol

25% ethanol was used in this method to extract total phenol from plant leaves. One gram of plant leaves was soaked in 20 mL of 25% ethanol for 15 minutes. Then, we filtered the solution through a Whatman No. 2 filter paper. Then, we collected a 1mL sample from the filtrate, mixed it with 5 mL Na_2CO_3 and 1mL Folin reagent, and transferred it to a volumetric flask. Then it was kept at room temperature for 1 hour [4, 16, 18]. Quality control (QC) samples to assess accuracy at five concentrations (10, 20, 30, 50, and 60 PPM) were also prepared by serial dilution of the freshly prepared gallic acid stock solution, which was used within 24 h. Phenolic content was quantified at 765 nm using spectrophotometry (T80 UV/Vis Spectrophotometer, PG Instruments, United Kingdom) against absorbance values against a calibration curve of gallic acid and expressed as milligrams of gallic acid equivalents per 100 g fresh weight (mg GAE/100 g fresh weight). Equation 2 was used to calculate the total phenolic content (TPC), where Y is the absorbance of the sample and X is the total phenolic content, expressed as mg GAE/100 g fresh weight.

$$Y = 0.0019 \times X + 0.2066 - - - - - - - - - - (4)$$

$$R^2 = 0.995$$

2.7 Statistical Analysis

All measurements were carried out in triplicate for each sample, and averages and standard deviations were calculated from these triplicate measurements. Data were analyzed using statistical software R (Windows version 4.3.3). A single-factor analysis of variance was conducted in this study. Duncan Multiple Range Test (DMRT) was utilized for significant differences, where differences were considered significant at $P \leq 0.05$.

3. Results and Discussion

3.1β-Carotene content

Fig 1 & Table S2 represent the concentrations of extracted β-Carotene from plant parts, revealing significant amounts in the respective species, where *Ipomoea batatas* showed the maximum concentration at 153.21 mg/100 g, and *Ajuga reptans* at 138.67 mg/100 g, indicating high β-carotene content present in the leaves. In addition to *Ipomoea batatas* and *Ajuga reptans*, the leaves of Lawsonia inermis and Euphorbia cotinifolia also contain significant amounts of β-carotene, comparable to those in Amaranthus gangeticus, establishing them as excellent sources of this carotenoid. Maintaining a healthy life, it is important to consume a minimum of 15-45 mg of β-Carotene daily, supported by different previous nutritional studies [19]. In Bangladesh, *Ipomoea batatas* and *Amaranthus gangeticus* leaves are traditionally used in food preparations such as steaming and infusion with oil, whereas *Euphorbia cotinifolia* is highly toxic, and *Lawsonia inermis* is prohibited for direct consumption. For pharmaceutical use, all species, including toxic ones, can be processed under controlled environmental conditions into standardized extracts, powders, coated or encapsulated formulations. Also, though it is uncommon in Bangladesh, young shoots of *Ajuga reptans* can be utilized in salads in Brazil [20].

β-carotene, which is commonly present alongside water-soluble and fat-soluble vitamins, is abundant in plant leaves and has been reported to inhibit various cancers [20]. The antioxidant capacity of plant leaves varies with their content of metabolites such as vitamins, carotenoids such as β-carotene and lycopene, and other pigments [21].

Carotenoids, which are precursors to vitamin A, are typically derived from flowering plants and herbs and serve as colorants and bioactive compounds; they are very common in tropical plant species, as evidenced by previous studies [22]. Comparable levels of β-carotene have been reported in medicinal plants [11-13]. Still, certain plant leaves, such as wine palm (*Mauritia vinifera*), are promising sources of β-carotene[23].

3.2 Determination of lycopene

There are different types of carotenoids, such as lycopene and β-carotene, with lycopene having a higher capacity to reduce singlet oxygen than β-carotene, showing nearly twice the value [24]. Lycopene plays a crucial role in defense against carcinogenic diseases by protecting DNA from free radical attack, protecting lipid particles, low-density lipoproteins, and proteins [25].

From the **Fig 2 & Table S2**, leaves of *Amaranthus gangeticus* exhibited the highest concentrations of lycopene, which is 109.44 mg/100g, and *Tradescantia zebrina* leaves at 84.11 mg/100g. Furthermore, leaves from *Tradescantia zebrina, Ipomoea batatas,* and *Lawsonia inermis* are significant sources of lycopene [26, 27].

3.3 Flavonoids determination

Fig 3 & Table S2 present the quantification of flavonoid content, showing that both Cordyline fruticosa varieties exhibit the highest levels of flavonoids, with *Cordyline fruticosa* at 810.46 mg/100 g RE, *Cordyline rubra* at 502.13 mg/100 g RE, and *Plectranthus scutellarioides* at 352.18 mg/100 g

RE. The flavonoid content of these plant leaves falls within a range similar to that reported for medicinal plants in various previous studies [28-31]. However, the sources of plant leaf samples vary with environmental conditions and soil composition, making direct comparisons challenging.

Normally, samples of Cordyline species exhibited significantly higher flavonoid levels ($P < 0.05$) than other plant leaves. For instance, 17.1±2.8 mg RE/100 g d.b. for *Tradescantia zebrina* and 10.6 ± 4.0 mg RE/100 g d.b. for *Tradescantia pallida* (Malaysia), values which surpass those found in this study [14]. Similarly, 3.93 ± 0.008 mg quercetin/100 g d.b. for *Tectona grandis* and 27.2 mg RE/100 g d.b. for Prunus cerasifera, values close to those reported in several studies [32, 33]. Again, another study reported 6.92 mg RE/100 g d.b. for *Oxalis corniculata*, while 14.32 mg quercetin/g for *Basella alba*, both of which are notably lower than the levels observed here [34, 35].

3.4 Determination of anthocyanins

Anthocyanins, vibrant pigments responsible for the blue, red, and purple hues in leaves and fruits, are notably abundant in various berries [36]. Among the species examined, *Tradescantia spathacea* exhibited the highest level of anthocyanins at 267.18 mg/100 g, followed by *Oxalis triangularis* at 161.67 mg/100 g and *Alternanthera dentata* at 151mg/100 g (Fig. **4 & Table S2**). With their diverse colors and potential health benefits, anthocyanins are used in dietary supplements, nutraceuticals, and pharmaceutical products.

The total anthocyanin contents observed in the leaves of *Tradescantia spathacea, Oxalis triangularis,* and *Alternanthera dentata* were lower than those reported for various vegetable leaves. For instance, several studies have reported that purple cabbage leaves contain 629 mg/100 g d.b., red lettuce varieties contain 100 to 300 mg/100 g d.b., and purple leaves of Perilla frutescens contain 42.33 mg/100 g d.b. [37-39].

3.5 Total phenolic determination

Previous studies have categorized the phenolic content of plant leaves into three groups: low, medium, and high. Generally, plants with phenol content below 500 mg GAE/100 g are classified as having low concentrations of phenolic compounds, those ranging from 500 to 2500 mg GAE/100 g fall into the medium concentration category, and those above 2500 mg GAE/100 g are categorized as having high concentrations of phenolic compounds [40].

Tradescantia zebrina exhibited the highest level of phenolic compounds among the plants examined, followed by *Lawsonia inermis, Plectranthus scutellarioides,* and *Acalypha wilkesiana* (**Fig 5 & Table S2**). The leaves of these plants are high in phenolic compounds, making them excellent sources. Except for *Caladium Bombshell*, the leaves of the other examined plants can be categorized as having a medium level of phenolic compounds and still represent good sources of these compounds.

Notably, higher levels of phenolic compounds were found in Tectona grandis, Tradescantia zebrina, and Tradescantia pallida (Malaysia) than in this study [14, 33]. However, the levels reported for *Prunus cerasifera* were comparable to those obtained in several studies [32]. Previous studies reported lower levels in *Oxalis corniculata* and *Basella alba* [34, 35]. These discrepancies may be

attributed to various factors affecting the properties of bioactive compounds in plant leaves, such as cultivation methods, climate, plant varieties, and the time of year of harvest [41].

3.6 Correlation Analysis

Pearson correlation analysis was performed in Prism to examine the relationships among the phytochemical components (β-carotene, lycopene, flavonoids, anthocyanins, and total phenolic content) in the plant species under investigation (**Fig 6**). For the correlation coefficient (r) and 95% confidence interval (CI) of the coefficient, a metric was employed to determine how related every two variables are. The correlation coefficient ranges from -1 to +1, where a value close to +1 indicates a strong positive relationship, while a value close to -1 indicates a strong negative relationship.

In this study, several pairs of variables showed extremely strong positive correlations, showing that the concentrations of the pairs can change in proportion to each other. For instance, the correlation between β-carotene and lycopene was very positive (r = 0.9264) and had a 95% CI of 0.8141 to 0.9264 because of their similar biosynthetic pathway as carotenoids. In addition, flavonoids and total phenolic content showed moderately strong positive correlations as expected due to the fact that flavonoids are a subset of total phenolic content.

Furthermore, several pairs exhibited strong positive correlations, indicating that the concentrations of these phytochemicals tended to increase together. In particular, anthocyanins and total phenolic content showed a very strong positive correlation (r = 0.9712), suggesting that plant species with higher anthocyanin concentrations also possessed higher total phenolic contents.

All correlations of most of the studied pairs of variables appeared very weak or even zero correlation, meaning no significant correlation exists; for instance, anthocyanins and flavonoids had very weak correlation (r = 0.01494, 95% CI: 0.02131 to 0.9919). Confidence intervals (95%) for the coefficient of correlation indicate the reliability of the estimates: wide confidence intervals (0.8141 to 0.9264) indicate greater uncertainty in the correlation estimate, whereas narrow confidence intervals (0.5491 to 0.9977) indicate a strong estimate. It is worth noting that correlations between variables whose confidence intervals include zero were not statistically significant.

This information on the studied correlations has an important value regarding different metabolic pathways of the studied bioactive compounds because, for instance, a positive correlation of β-carotene and lycopene suggests common regulation, as carotenoids are theoretically controlled by the same genetic and environmental factors, while a lack of correlation between anthocyanins and flavonoids may be interpreted as their separate control. Again, due to resource allocation at the biosynthetic level, the strong positive correlation observed between anthocyanins and total phenolic content suggests that these phytochemicals accumulate concurrently in the studied ornamental plant species. This finding is biologically plausible because anthocyanins are a subclass of phenolic compounds and share common biosynthetic pathways. Therefore, increased anthocyanin accumulation is often associated with increased total phenolic content.

3.7 Practical implications and beneficiaries

This study provides a detailed, reliable dataset on the bioactive compound profiles of 24 medicinal plant species commonly found in Bangladesh, which is expected to benefit several groups of participants in the research groups. First, researchers can use this dataset as a baseline for further investigations into phytochemicals across different extraction methods, characterization, bioactivity evaluation, compound isolation, and the development of nutraceuticals, functional foods, and pharmaceutical products. The identification of the plant species with different concentrations of bioactive compounds may also serve as promising candidates for advanced analytical and chemical studies using metabolomic and chromatographic techniques.

Next, the food, pharmaceutical, and nutraceutical industries may use these findings to identify industrially and economically valuable plant species as potential natural sources of antioxidants, phenolic compounds, flavonoids, anthocyanins, β-carotene, and lycopene, which may facilitate the extraction and development of natural ingredients, functional food products, dietary supplements, and plant-derived therapeutic innovative formulations.

Again, government organizations such as policymakers, different public and private agricultural extension agencies, and local and international botanical conservation authorities may use the results to prioritize the conservation, sustainable utilization, and cultivation of medicinal plant species with high bioactive compound potential. These findings also support future strategies aimed at promoting value-added utilization of indigenous plant species and strengthening the medicinal plant sector.

At last, this study can contribute to sustainable development awareness, encouraged by the use of locally grown and available medicinal plants as renewable natural resources to produce high-value bioactive compounds that are highly effective for the treatment of chronic diseases. This contribution not only potentially reduces the dependence on synthetic medicine and additives but also promotes environmentally sustainable resource utilization and creates new economic opportunities through the development of plant-based health products.

4. Conclusions

Different plant parts contain different pigments, such as β–carotene, lycopene, anthocyanins, and flavonoids, which encourage perceptions of using these plant species, with significant amounts of bioactive compounds and different macro and micro nutrients, particularly anthocyanins, flavonoids, and phenolic compounds. This study provides valuable evidence confirming the nutritional properties of medicinal plant leaves, particularly the broad range of properties of the identified pigments and compounds, which are not only a rich source of bioactive compounds but also can be utilized as low-cost, renewable, non-renewable resource components for the pharmaceutical industry, thereby providing positive economic and environmental impacts. From a traditional preparation perspective, *Ipomoea batatas* and *Amaranthus gangeticus* leaves are locally processed through light steaming or infusion in Bangladesh, making them accessible sources of bioactive compounds and vitamins for local populations. For pharmaceutical and nutraceutical applications, all studied species, including Tradescantia zebrina, Cordyline fruticosa, Lawsonia inermis, and even the toxic Euphorbia cotinifolia, can be processed into standardized extracts, powders, or encapsulated formulations through controlled chemical, physical, or mechanical extraction methods. For pharmaceutical and nutraceutical applications, *Tradescantia zebrina, Cordyline fruticosa, Lawsonia inermis*, and the

toxic leaves of *Euphorbia cotinifolia* can be processed by chemical, physical, and mechanical extraction under controlled conditions. It is clear from researchers' studies that bioactive compounds play a significant role in protecting against many diseases; hence, there is a greater scope than just providing medicines.

5. Limitation

This study did not conduct any specific cultivation because, in Bangladesh, there are no specific cultivations for these plants other than *Amaranthus gangeticus* and *Ipomoea batatas.* So, the plant materials were collected from a managed botanical garden rather than a controlled cultivation experiment. There are no specific agronomic variables for these species, and there are no specific cultivation parameters, such as soil nutrient composition, a precise irrigation schedule, and fertilizer history, present for individual plant species, so the factors could not be standardized. Although these factors can influence bioactive profiles, future studies will be based on these studies, in which controlled cultivation experiments will be conducted to isolate genotypes from environmental effects on bioactive compound accumulation.

List of Abbreviations:

- ANOVA : Analysis of Variance
- DMRT: Duncan Multiple Range Tests
- DNA: Deoxyribonucleic acid
- GAE: Gallic acid equivalent
- HPLC: High-Performance Liquid Chromatography
- QC: Quality control
- RE: Rutin equivalent
- TPC – Total Phenolic Content
- TFC: Total Flavonoid Content
- $AlCl_3$ – Aluminum chloride
- CI – Confidence Interval
- d.b. – Dry basis
- Na_2CO_3 – Sodium carbonate
- PPM – Parts Per Million
- rpm - Revolutions per minute
- r – Correlation coefficient
- UV/VIS (or U/VIS) – Ultraviolet–Visible (Spectrophotometer)

Authors' Contribution:

T.K.: Methodology, Investigation, Formal Analysis, Data Curation, Writing – Original Draft Preparation.

M.A.: Conceptualization, Validation, Software, Resources, Writing – Review & Editing, Visualization, Supervision, Project Administration.

All authors have read and agreed to the published version of the manuscript.

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Supplementary Material
The supplementary material contains data supporting the findings of this study. Supplementary Tables S1–S2 provide experimental data and supporting information.

Ethical Complaince:
The National Botanic Garden in Bangladesh is open to all. Still, it does not need permission/approval or a legal procedure for the collection of a small number of samples. Also, no clinical trial was done for the study.

Availability of Data and Materials:
Data will be made available on request by mail with the corresponding author.

Consent for Publication:
Not applicable

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Figures:

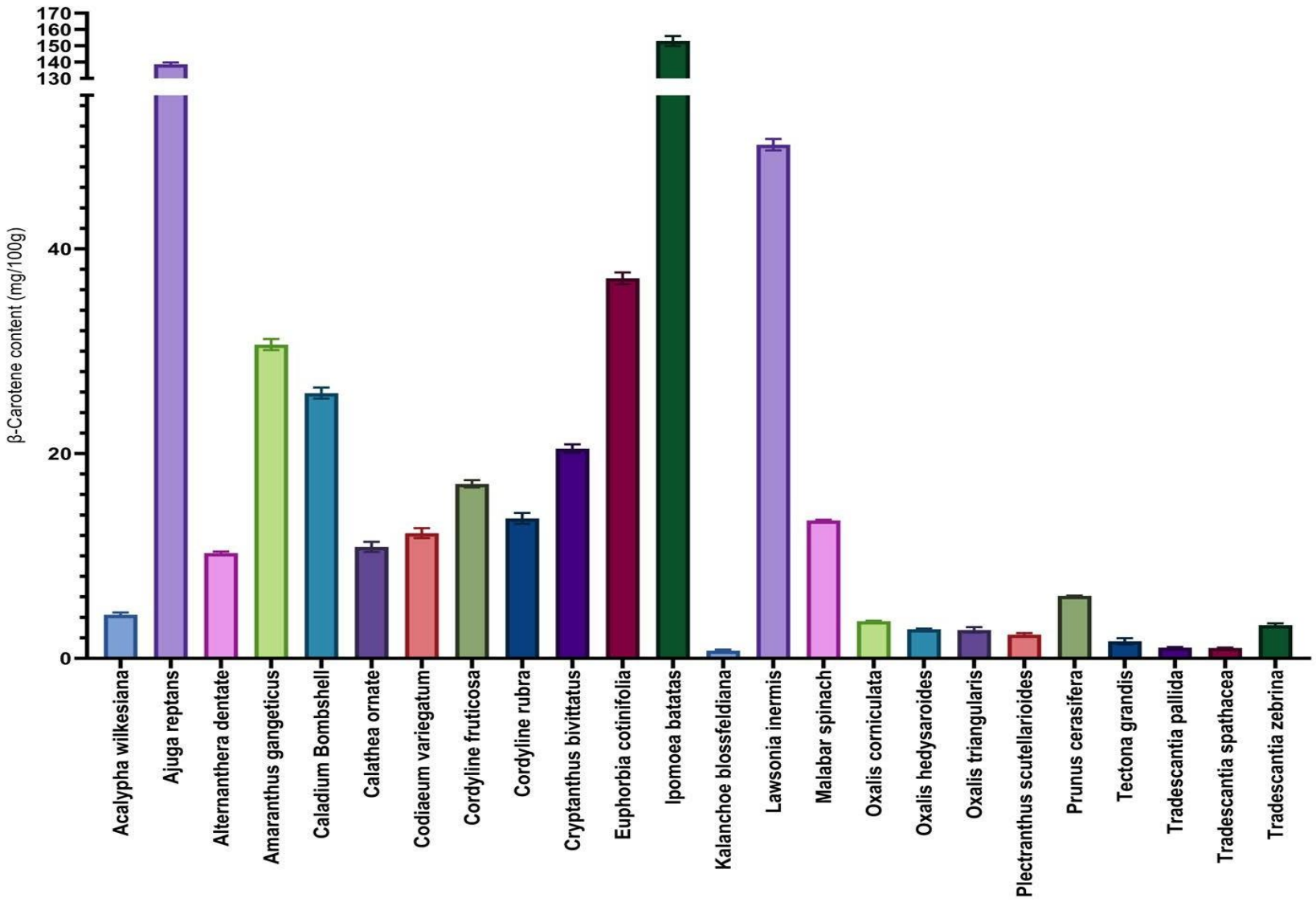

Fig 1. Quantitative distribution of five major bioactive compounds in the leaves of 24 medicinal plant species from Bangladesh. (A) β-carotene content (mg/100 g); (B) lycopene content (mg/100 g); (C) total flavonoid content (mg rutin equivalents/100 g); (D) total anthocyanin content (mg/100 g); (E) total phenolic content (mg GAE/100 g). Data are presented as mean ± standard deviation (n = 3). Bars with different letters indicate statistically significant differences at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Fig. 2 leaves of *Amaranthus gangeticus* exhibited the highest concentrations of lycopene, which is 109.44 mg/100g, and *Tradescantia zebrina* leaves at 84.11 mg/100g.

Fig. 3. Present the quantification of flavonoid content, showing that both Cordyline fruticosa varieties exhibit the highest levels of flavonoids, with *Cordyline fruticosa* at 810.46 mg/100 g RE, *Cordyline rubra* at 502.13 mg/100 g RE, and *Plectranthus scutellarioides* at 352.18 mg/100 g RE.

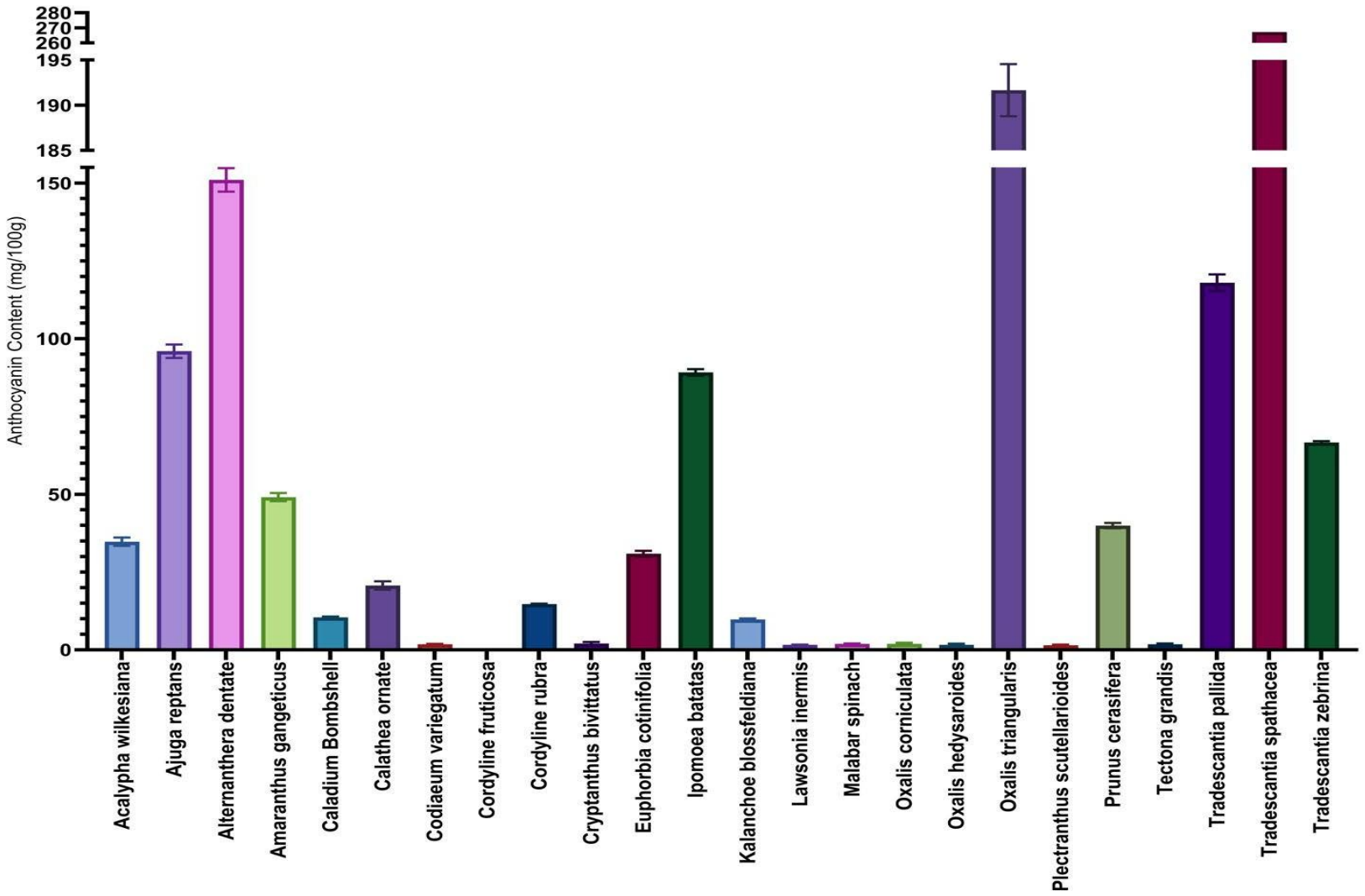

Fig. 4. Among the species examined, *Tradescantia spathacea* exhibited the highest level of anthocyanins at 267.18 mg/100 g, followed by *Oxalis triangularis* at 161.67 mg/100 g and *Alternanthera dentata* at 151mg/100 g

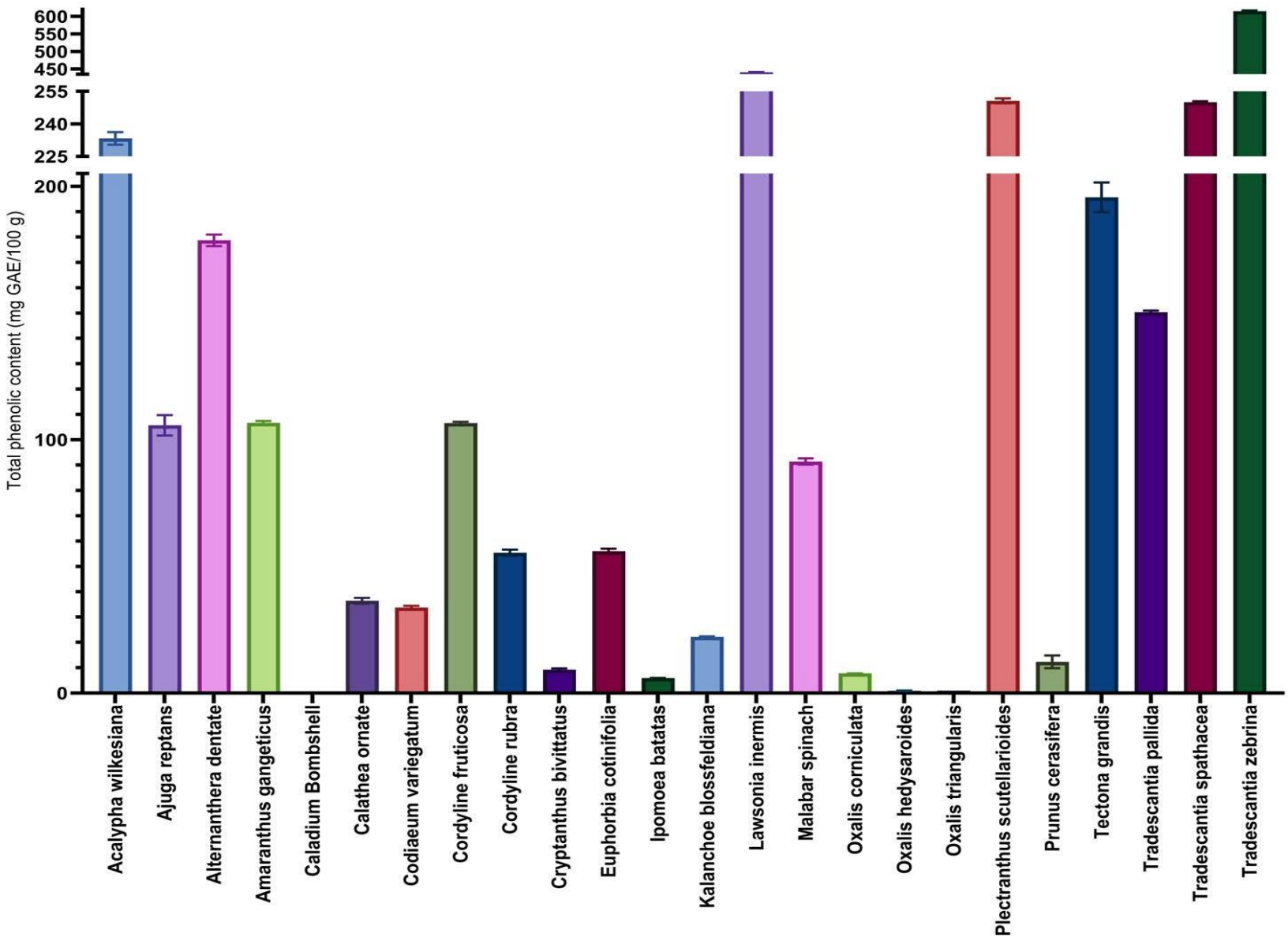

Fig. 5. *Tradescantia zebrina* exhibited the highest level of phenolic compounds among the plants examined, followed by *Lawsonia inermis, Plectranthus scutellarioides,* and *Acalypha wilkesiana*

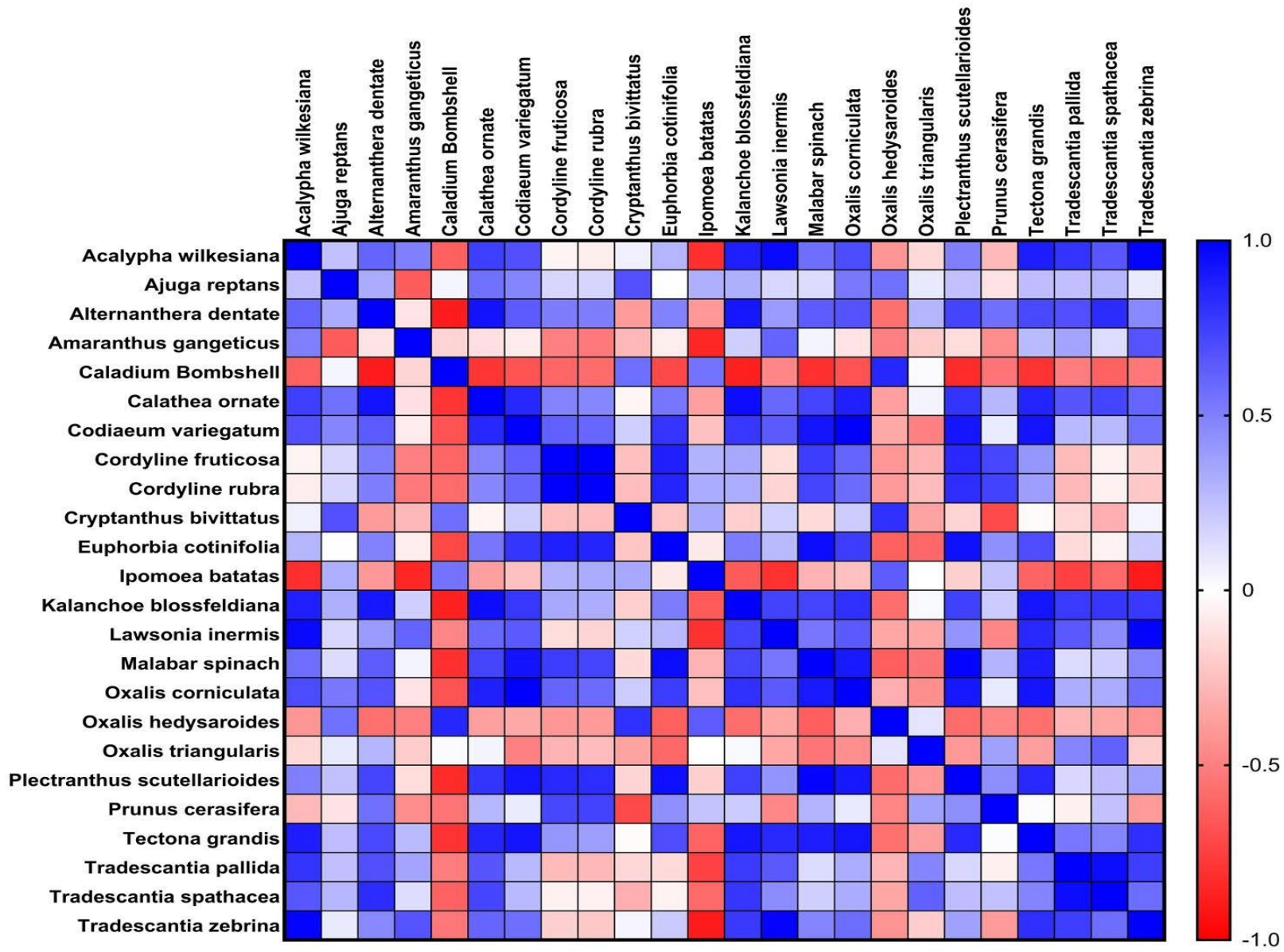

Fig 6. Pearson correlation matrix among β-carotene, lycopene, flavonoids, anthocyanins, and total phenolic content across the 24 studied medicinal plant species. The heatmap displays correlation coefficients (r) and corresponding 95% confidence intervals, with color intensity indicating the strength and direction of the linear relationships.