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Growth Stage-Dependent Polyphenol Profiles and Antioxidant Properties in Medicinal Plants of Iraqi Kurdistan

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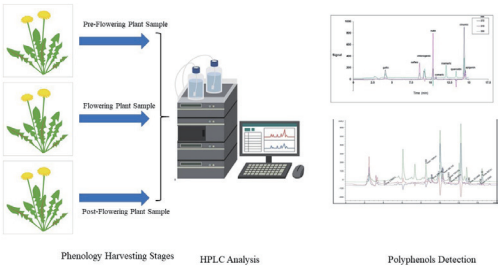
Abstract: Medicinal plants, rich in polyphenolic compounds, play a crucial role in traditional and modern medicine due to their antioxidant, anti-inflammatory, and disease-preventing properties. This study investigated the polyphenol content and antioxidant activity across various growth stages (pre-flowering, flowering, and post-flowering) of four medicinal plants: *Primula auriculata* L., *Stachys vulgaris* L., *Verbascum spp*., and *Ajuga oblongata* L., cultivated in the Soran region, Erbil Province, Iraqi Kurdistan. Using high-performance liquid chromatography (HPLC), nine types of polyphenols were identified and quantified, including gallic, caffeic, and chlorogenic acids. The results showed significant variations in polyphenol concentrations across different plant species and growth stages. For example, *Primula* exhibited the highest levels of gallic acid (41.91 mg/kg) during pre-flowering, while *Ajuga* showed peak chlorogenic acid content (827.62 mg/kg) post-flowering. Antioxidant activity was highest in *Ajuga* during flowering (75.33%) and lowest during post-flowering. Overall, polyphenol content was positively correlated with antioxidant activity, highlighting the importance of growth stage in optimizing the medicinal benefits of these plants. These findings contribute to the ethnobotanical knowledge of Kurdish medicinal plants and offer insights into the optimal harvest times for maximizing bioactive compounds.



Key words: medicinal plants, wild plants, natural products, ethnobotany, harvest time optimization

1 Introduction

Globally, it is estimated that 35,000 to 70,000 plant species are used medicinally, with 6,500 species found in Asia. Kurdistan, particularly Southern Kurdistan, is known for its rich biodiversity[1)]. However, the traditional uses of medicinal plants in this region remain under-researched and poorly documented. Thus, the study of Kurdish ethnobotany may be crucial for understanding local plant uses. This research is the first study, and therefore, the objective of this investigation was to document the traditional uses of Kurdish medicinal plants in Soran, Erbil Province, Iraqi Kurdistan[1, 2)]. Medicinal plants and plant-derived medicine are widely used in traditional cultures worldwide, and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals. As more and more natural remedies are being commercialized, there is a need for a user-friendly reference guide to the plants and their products[3)]. Medicinal lants contain many polyphenolic compounds, which makes them a good source of polyphenol compounds. In addition, medicinal lants have sundry biological impacts, such as anti-inflammatory and antioxidant properties, and they can also play a significant role in preventing many diseases[4)]. Also, researchers explore the antioxidant features of polyphenols for dietary purposes. Plenty of these compounds are found in our diet, and their role is to hinder different diseases attached to oxidative stress, like cancer, cardiovascular and neurodegenerative diseases[5)]. Some polyphenol-rich herbs which belong to the

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Lamiaceae family are used as spices and as source of essential oils. However, many traditional medicinal lants can be used in animal healthcare[6, 7]. Polyphenols contain more than one phenolic hydroxy group and a large family of naturally occurring organic compounds characterized by multiple phenol units[8]. They are abundant in plants, and structurally diverse polyphenols improve digestion and enhance brain health and protect the body against various diseases such as type 2 diabetes and heart disease[9]. Polyphenols are structurally highly different compounds but essential for various functions in plants, responsible for organoleptic and nutritional properties of plant-derived foods, and useful for numerous practical applications[10].

Primula auriculata belongs to the section Auricula within the genus *Primula*[11]. *Primula auriculata* can be distinguished by its specific morphological traits, which include non-scariose and longer bracts, and fragrant leaves that turn yellowish or brownish when pressed[12]and also thrives in high mountain environments, which are characterized by specific ecological conditions that have influenced its evolutionary path[12]. *Stachys vulgaris*, a member of the *Lamiaceae* family, has been associated with various medicinal properties, including antioxidant, anti-inflammatory, analgesic, antibacterial, and cytotoxic effects[13]. The plant's extracts and essential oils are rich in non-volatile and volatile compounds, contributing to its extensive spectrum of therapeutic properties. Studies have shown that *Stachys* species, including *Stachys vulgaris*, have potential applications in medicine, cosmetics, dietary supplements, and the food industry[13]. *Verbascum*, commonly known as mullein, is a genus of flowering plants in the *Scrophulariaceae* family. This genus includes a variety of species, some of which are used for their medicinal properties, while others are notable for their ecological roles and hybridization[14]. Environmental factors significantly influence the growth and essential oil composition of *Verbascum* species. A study on *Verbascum songaricum* in Iran found that factors such as annual rainfall, altitude, and soil properties affect essential oil yield. Semi-arid cold climates with higher precipitation and fertile soil were identified as optimal for essential oil production[15]. *Ajuga oblongata* is a species within the genus *Ajuga*, which belongs to the *Lamiaceae* family. This genus comprises over 300 species of herbaceous flowering plants distributed across temperate regions of Asia, Europe, Australia, North America, and Africa[16]. The genus is known for its diverse phytochemical profile, including neo-clerodane diterpenoids, phytoecdysteroids, flavonoids, and iridoid glycosides, which contribute to its wide range of biological activities[17].

Despite the predominant focus on antioxidant activities, polyphenols have also been implicated in other roles independent of their antioxidant property like antibacterial, anti-inflammatory, and modulation of ion transport[18]. Polyphenols are popularly known as antioxidants and are primarily found in dietary supplies. One of the mechanisms by which polyphenols exert their actions is by regulating the protein kinase C(PKC), a central kinase in intracellular signal transduction[19].

In this case study, we will give more consideration to working on four types of medicinal plants. This case study aims to determine what the polyphenols are like before harvesting, during harvesting, and after harvesting and determine the rate of medicinal plants, whether they are high before harvesting, during, or after harvesting.

2 Material and Methods

2.1 Collect plant samples

Specimens of several medicinal plants(*Stachys vulgaris* L., *Primula auriculata* L., *Verbascum spp*., and *Ajuga oblongata* L.)were cultivated in the educational garden of Soran University, located in Soran, within the Erbil province of the Iraqi Kurdistan region, at an altitude of 700 meters above sea level. Plant samples(leaves and flowers) were randomly collected during three different phenology growth stages: pre-flowering, flowering, and post-flowering. The harvest time for each of the four plants was not the same. Each stage involved three replicates of plant specimens. The wild medicinal plant samples were shade-dried for 21 days to preserve their properties.

2.2 Polyphenols

2.2.1 Extraction of polyphenols

To extract polyphenols, 2 grams of the powdered plant sample were mixed with 4 mL of methanol containing 1% acetic acid. The extraction was enhanced using ultrasonic waves for 20 minutes. After centrifugation and filtration, the supernatant was subjected to high-performance liquid chromatography(HPLC)for identification and quantification of phenolic acids. Phenolic acids were identified and quantified by comparing their retention times and UV-Vis spectra with those of commercial standards(Fig. 1). The HPLC analysis was performed using an Agilent 1100 series system(Agilent Technologies, USA)equipped with a diode array detector(DAD), set at 250, 272, and 310 nm. Separation was achieved on a C18 ZORBAX Eclipse XDB column (4.6 mm×250 mm, 5 μm particle size)obtained from Dr. Maisch GmbH, Germany. Commercial standards of phenolic acids including[e.g., gallic acid, caffeic acid, ferulic acid, etc.]were purchased from Sigma-Aldrich(USA), and standard calibration curves were constructed for quantification. Data were analyzed using ChemStation software.

2.2.2 Total antioxidant

Total antioxidant was measured according to the method of Brand Williams et al.(1995)[20]by DPPH standard.

2.2.3 Total phenolic content

Total phenolics content was determined by the Folin

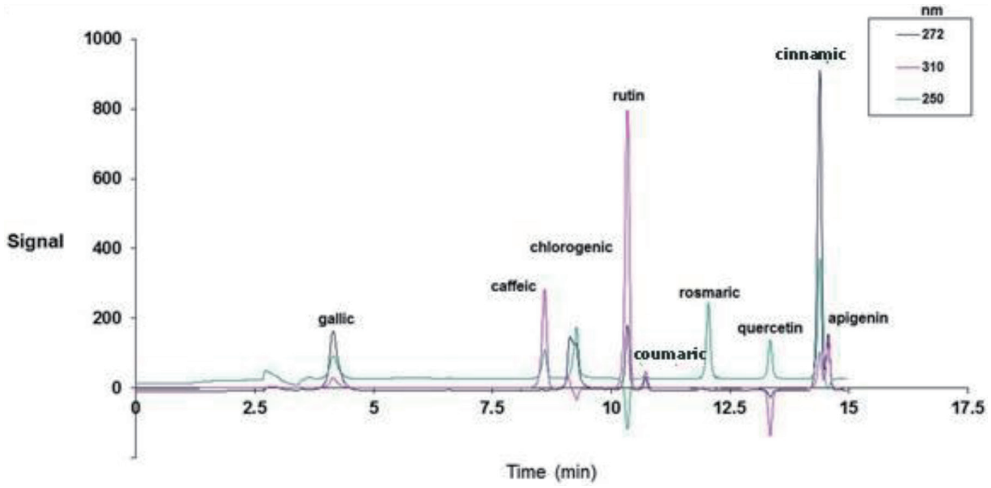

Fig. 1 Chromatographic Profile of Phenolic and Flavonoid Compounds. This figure represents the HPLC chromatographic separation of phenolic and flavonoid compounds identified in the study. Peaks corresponding to specific compounds such as gallic acid, caffeic acid, chlorogenic acid, and others are labeled according to their retention time in minutes. The different colors in the graph indicate the wavelengths (nm) at which these compounds were detected, illustrating their unique absorbance properties.

Cicalteau method as described by Singleton et al. (1999)[21], with minor modifications, according to phenols' colorimetric oxidation/reduction reaction. Polyphenol extraction was carried out by 10 mL 85% methanol added to 1 g fine powder of floret. Next, to 250 µL of extract, 250 µL of sterile distilled water was added, and then 2.5 mL of Folin-Cicalteau reagent and 2 mL of 7.5% sodium carbonate were added. The absorbance of samples was measured at 765 nm by spectrophotometer (PG Instruments T80 UV, UK). Gallic acid was used for the calibration curve. Results were expressed as mg Gallic Acid Equivalent/100 g fresh weight.

2.3 Statistical analysis

All data were analyzed by SAS software (SAS 9.4). One-way ANOVA was carried out, and significant differences between groups were measured by Tukey's multiple range test at $p < 0.05$.

3 Results and Discussion

The results presented in Table 1 outline the polyphenol content in four medicinal plants at different growth stages, showing notable variability in the concentrations of nine types of polyphenols.

Gallic Acid: The highest concentration of gallic acid was recorded during the pre-flowering stage of *Primula* (41.91 mg/kg), while the lowest was found in post-flowering Verbascum (2.53 mg/kg). In *Ajuga* and *Stachys*, the gallic acid levels increased after flowering, reaching 24.16 mg/kg and 23.05 mg/kg, respectively. However, *Verbascum* showed its peak value (17.08 mg/kg) during the flowering stage.

Caffeic Acid: The highest caffeic acid content was detected in pre-flowering *Stachys* (450.6 mg/kg), while the lowest was found in post-flowering *Primula* (1.56 mg/kg). *Ajuga* showed an increase in caffeic acid concentration after flowering (67 mg/kg) compared to pre-flowering and flowering stages (37 and 54.73 mg/kg, respectively). In contrast, *Verbascum* exhibited a decline in caffeic acid content in the post-flowering stage (64.07 mg/kg), following higher levels during the pre-flowering (180.31 mg/kg) and flowering stages (103.63 mg/kg).

Chlorogenic Acid: The highest chlorogenic acid content was observed in post-flowering *Ajuga* (827.62 mg/kg), whereas the lowest was in post-flowering *Primula* (2.8 mg/kg). In *Stachys*, chlorogenic acid levels increased throughout the growing season, with the highest concentration in the post-flowering stage (420.68 mg/kg), compared to the pre-flowering (360.72 mg/kg) and flowering stages (232.79 mg/kg). Conversely, *Verbascum* saw a decrease in chlorogenic acid after flowering (52 mg/kg), compared to the pre-flowering and flowering stages (130.33 and 148.63 mg/kg, respectively).

Rutin: The highest rutin concentration was found in post-flowering *Verbascum* (120.38 mg/kg), with the lowest in post-flowering *Primula* (3.3 mg/kg). In *Ajuga*, rutin content increased post-flowering (18.58 mg/kg) compared to the pre-flowering and flowering stages (6.8 and 4.34 mg/kg, respectively). In *Stachys*, however, rutin levels decreased in the post-flowering stage (16.17 mg/kg) compared to earlier stages (47.37 mg/kg in pre-flowering and 55.26 mg/kg in flowering).

Coumaric Acid: The highest coumaric acid concentration

Table 1 Phenolic and flavonoid compound contents in different plants at various harvesting stages.

Plants	Harvesting time	Gallic acid	Caffeic acid	Chlorogenic acid	Rutin	Coumaric acid	Rosmarinic acid	Quercetin	Cinnamic acid	Apigenin
Primula	Pre- Flowering	41.91 ± 22.3^{a}	12.77 ± 2.3^{fg}	236.99 ± 9.4^{c}	70.27 ± 4.3^{b}	1043.26 ± 34^{b}	32.94 ± 2.4^{cd}	23.61 ± 4.3^{f}	6.28 ± 4.3^{d}	1.37 ± 0.06^{g}
	Flowering	8.15 ± 1.4^{d}	170.27 ± 10^{c}	534.73 ± 23^{b}	54.22 ± 3.4^{c}	1477.92 ± 42^{a}	10.49 ± 1.3^{f}	32.89 ± 5.3^{e}	7.13 ± 1.2^{cd}	62 ± 7.3^{cd}
	Post- Flowering	4.33 ± 0.7^{fg}	1.56 ± 0.1^{f}	2.8 ± 0.3^{i}	3.3 ± 0.2^{g}	115.7 ± 6.7^{gh}	3.6 ± 0.5^{g}	2.86 ± 0.3^{h}	0.19 ± 0.05^{f}	2.5 ± 0.2^{fg}
Ajuga	Pre-Flowering	6.77 ± 1.1^{de}	37 ± 4.3^{ef}	149.56 ± 11^{fg}	6.86 ± 1^{g}	274.09 ± 8.7^{ef}	35.39 ± 2.1^{c}	88.12 ± 6.4^{a}	22.44 ± 3.7^{b}	12.29 ± 0.9^{efg}
	Flowering	5.95 ± 0.8^{def}	54.73 ± 4.2^{e}	184.36 ± 12.2^{f}	4.34 ± 0.8^{g}	55.13 ± 4.4^{h}	83.69 ± 4.3^{a}	22.45 ± 2.1^{f}	8.32 ± 2.4^{c}	15.25 ± 2.8^{ef}
	Post-Flowering	24.16 ± 3.65^{b}	67 ± 5.2^{e}	827.62 ± 26^{a}	18.58 ± 2.4^{f}	437.47 ± 14^{d}	29.6 ± 1.9^{cd}	66.66 ± 5.4^{b}	8.56 ± 2^{c}	62.18 ± 8.4^{cd}
Stachys	Pre-Flowering	6.39 ± 1.3^{def}	450.68 ± 15.3^{a}	360.72 ± 7^{d}	47.37 ± 5.5^{d}	379.55 ± 23^{de}	26.37 ± 2.3^{e}	54.99 ± 7.5^{c}	3.5 ± 0.3^{e}	472.63 ± 36^{a}
	Flowering	5.79 ± 1.54^{ef}	255.85 ± 12.3^{b}	232.79 ± 8.7^{e}	55.26 ± 3.4^{c}	220.84 ± 10.5^{fg}	12.86 ± 1.2^{f}	35.37 ± 4.3^{e}	7.29 ± 0.7^{cd}	64.66 ± 4.4^{cd}
	Post-Flowering	23.05 ± 3.6^{b}	67.19 ± 4.3^{e}	420.68 ± 24^{c}	16.17 ± 1.2^{f}	166.93 ± 8.6^{fg}	13.13 ± 2.1^{f}	39.69 ± 3.7^{d}	8.35 ± 1.1^{c}	145.13 ± 9.7^{b}
Verbascum	Pre-Flowering	1.42 ± 0.2^{h}	180.31 ± 4.9^{c}	130.33 ± 8.9^{g}	21.39 ± 2.2^{f}	22.47 ± 1.4^{h}	5.28 ± 0.5^{g}	12.95 ± 3.2^{g}	3.65 ± 0.6^{e}	133.39 ± 5.4^{b}
	Flowering	17.08 ± 3.2^{c}	103.68 ± 3.4^{d}	148.63 ± 4.7^{fg}	39.12 ± 5.4^{c}	910.8 ± 27^{c}	10.14 ± 1.3^{f}	14.98 ± 2.6^{g}	31.81 ± 5.4^{a}	23.28 ± 3.1^{e}
	Post-Flowering	2.53 ± 0.1^{gh}	64.07 ± 3.2^{e}	52 ± 4.5^{h}	120.38 ± 8^{a}	450 ± 7.9^{d}	51.91 ± 7.9^{b}	23.45 ± 3.7^{f}	22.28 ± 3.9^{b}	66.41 ± 8.6^{c}

The means in each column followed by similar letter(s) are not significantly different using Tukey's multiple test ($p < 0.05$).

was in the flowering stage of *Primula* (1477.9 mg/kg), while the lowest was in pre-flowering *Verbascum* (22.47 mg/kg). In *Ajuga*, coumaric acid levels increased significantly post-flowering (437.47 mg/kg), compared to the pre-flowering (274.09 mg/kg) and flowering stages (55 mg/kg). However, in *Stachys*, coumaric acid decreased post-flowering (166.93 mg/kg), compared to pre-flowering (379.55 mg/kg) and flowering stages (220.84 mg/kg).

Rosmarinic Acid: The highest concentration of rosmarinic acid was in the flowering stage of *Ajuga* (83.69 mg/kg), while the lowest was in post-flowering *Primula* (3.6 mg/kg).

Quercetin: The highest quercetin content was observed in pre-flowering *Ajuga* (88.12 mg/kg), and the lowest in post-flowering *Primula* (2.86 mg/kg). In *Ajuga* and *Stachys*, quercetin levels increased post-flowering but declined during flowering (from 88.12 mg/kg in pre-flowering to 22.45 mg/kg in flowering).

Cinnamic Acid: The highest cinnamic acid content was found in flowering *Verbascum* (31.81 mg/kg), while the lowest was in post-flowering *Primula* (0.19 mg/kg).

Apigenin: The highest apigenin content was observed in pre-flowering *Stachys* (472.63 mg/kg), and the lowest in pre-flowering *Primula* (1.37 mg/kg). The apigenin content increased in *Ajuga*, *Stachys*, and *Verbascum* post-flowering, while in *Primula*, it decreased after flowering.

3.1 Antioxidant capacity

Figure 2 illustrates the antioxidant activity rates of four medicinal plants across various growth stages. In *Primula*, the peak antioxidant activity of 72% is observed during the flowering stage, whereas the lowest activity, 29%, occurs during the pre-flowering stage. For *Ajuga*, the highest antioxidant activity of 75.33% is recorded during the flowering stage, with the lowest activity of 13.5% noted post-flowering. In *Stachys*, the maximum antioxidant activity of 61.6% is observed during the pre-flowering stage, while the lowest, 45%, is seen post-flowering. Lastly, in *Verbascum*, the highest antioxidant activity of 17.23% is detected during the post-flowering stage, and the lowest, 11.46%, is found during the flowering stage.

3.2 Total phenols

Figure 3 illustrates the total phenolic content extracted from four different medicinal plants, measured at three distinct time points: pre-flowering, flowering, and post-flowering. The total phenolic content was determined using High-Performance Liquid Chromatography (HPLC). The results indicate that *Primula* plants exhibit the highest total phenolic content during the flowering stage (160 mg/100 g), with the lowest content observed post-flowering (20 mg/100 g). *Ajuga* plants show the highest total phenolic content during the pre-flowering stage (30 mg/100 g), and the lowest content post-flowering (10 mg/100 g). For *Stachys*, the highest total phenolic content is recorded during the post-flowering stage (80 mg/100 g), while the lowest is also during flowering (20 mg/100 g). Finally, *Verbascum* plants have the highest total phenolic content during the flowering stage (140 mg/100 g) and the lowest post-flowering (40 mg/100 g).

4 Discussion

This study aimed to investigate the effects of harvest time and growth stage on polyphenol content and antioxidant activity in four plant species: *Primula*, *Ajuga*, *Stachys*, and *Verbascum*. Previous research has established a positive correlation between phenolic compounds and antioxidant activity in plant extracts[22, 23]. Our findings revealed that harvest time and growth stage significantly influenced polyphenol content and antioxidant activity in all four species. *Primula* and *Verbascum* demonstrated higher polyphenolic levels and antioxidant activity in post-

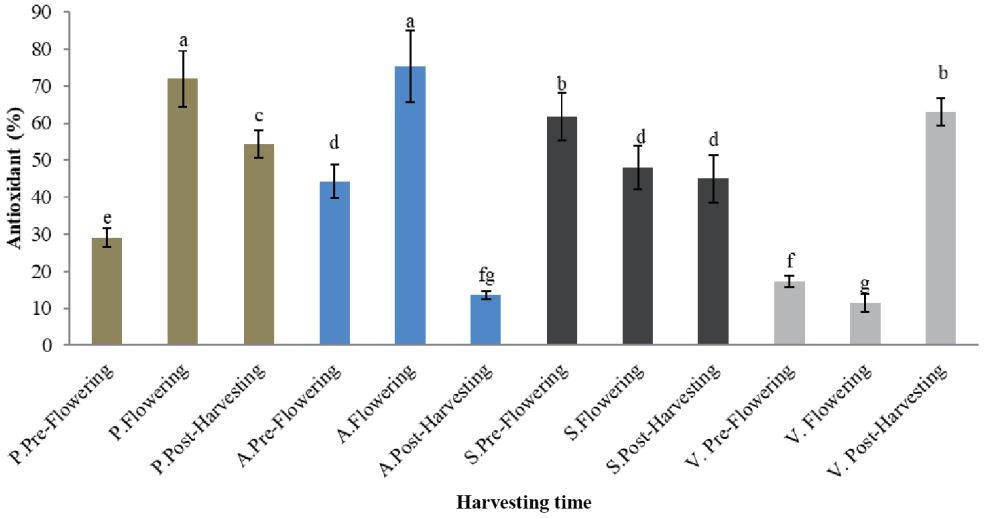

Fig. 2 Antioxidant activity of four medicinal plants at different harvesting times.

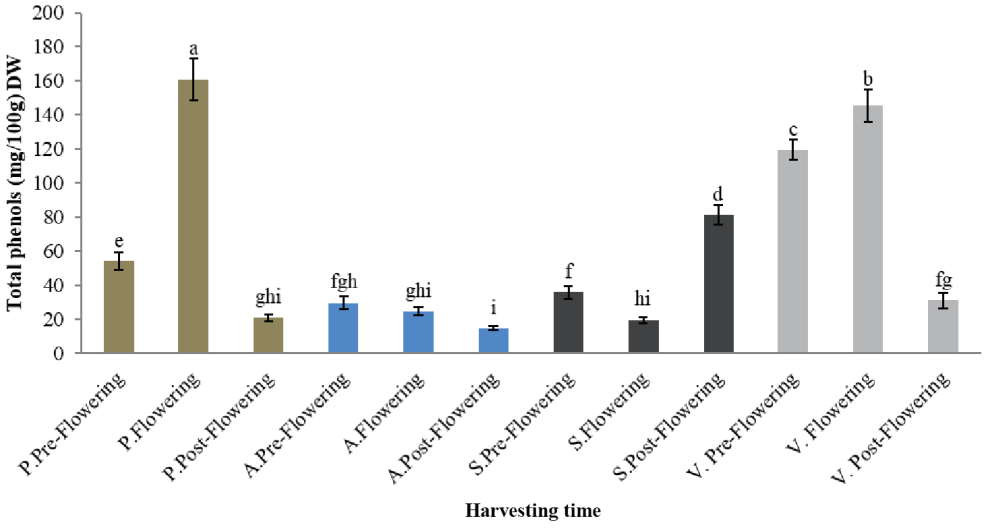

Fig. 3 Total phenols of four medicinal plants at different harvesting times.

flowering stages, while *Ajuga* exhibited optimal levels during flowering. In contrast, *Stachys* had the highest polyphenolic content in the pre-flowering stage this may be due to variation in environment condition[24]. polyphenols have traditionally been identified primarily in grasses and pastures, concerns about environmental degradation have prompted increased interest in their potential sources. Various environmental factors, including location, weather conditions, plant height, and growth stage, can influence the concentration of antioxidant compounds in plant extracts[25]. The diversity of habitats and environmental conditions across different ecosystems leads to variations in plant responses[26]. Reports on other plant species have indicated that total phenolic content (TPC) and total flavonoid content (TFC) are higher in samples harvested in October[27]. Similarly, higher levels of total phenols have been observed in *Vaccinium corymbosum* samples collected earlier in the season. Ivanauskas et al.[2] demonstrated that phenolic compounds, including flavonoids and polyphenols, peaked during the flowering stage and significantly decreased post-flowering. The study suggested that harvesting during flowering maximizes medicinal value, similar to your recommendations for Primula and Verbascum. The previous study found that the polyphenolic content and antioxidant activity were significantly higher during the flowering stage compared to other growth stages. This pattern is similar to our findings on *Primula* and *Verbascum*, where flowering was also the optimal harvest time for maximizing polyphenol content and antioxidant properties[26]. According to a study that found polyphenol content was highest during flowering and decreased during post-flowering stages, which aligns with the trends observed in your research on *Ajuga oblongata* and *Verbascum spp.*[27]. polyphenolic have various biological activi-

ties, including pharmacy and health for treating disease and good health. While many epidemiological studies have associated the consumption of polyphenols within fruits and vegetables with a decreased risk of developing several chronic diseases, intervention studies have generally not confirmed these beneficial effects[2]. This discrepancy is not fully understood but includes potential differences in dosing, interaction with the food matrix, and differences in polyphenol bioavailability; Plants collected during the exploration stage had higher amounts of polyphenolic compounds[28]. Several studies, including this study, have shown that harvesting plants during their flowering stage often yields the highest levels of polyphenolic compounds and antioxidant activities.

5 Conclusion

This study demonstrates that harvesting *Primula* and *Verbascum* during the flowering stage and *Stachys* pre-flowering optimizes polyphenol content and antioxidant activity, thereby maximizing their medicinal benefits. If the rate of polyphenols is high, the activity of antioxidants and polyphenols is higher, so according to this research, we suggest both *Primula* and *Verbascum* plants be used during flowering because most types of polyphenols have the highest rate during flowering. Moreover, *Ajuga* plants are used during flowering because most polyphenols are the highest during flowering exists, and *Stachy* plants are used before flowering because most polyphenols have the highest rate during pre-flowering.

Author Contributions

Mohammad Saadatian

Conceptualization, Methodology, and Project Administration. Mohammad Saadatian was responsible for developing the research idea, designing the study, and overseeing the overall execution of the project.

Samiaa Jamil Abdulwahid

Data Collection, Formal Analysis, and Investigation. Samiaa Jamil Abdulwahid contributed to gathering the data, performing statistical analyses, and interpreting the results of the study.

Kadhim Sedeeq Mohammed

Writing – Original Draft, Visualization, and Editing. Kadhim Sedeeq Mohammed led the writing of the first draft of the manuscript and developed figures and tables, as well as helped with reviewing and revising the paper.

Rawen Abdulhadi Abdullah

Writing – Review & Editing, Supervision, and Funding Acquisition. Rawen Abdulhadi Abdullah provided critical revisions to the manuscript, and secured the funding needed to support the project.

Acknowledgment

The authors declare there are no conflicts of interest.

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