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Climate-Driven Variations in Secondary Metabolites and Antioxidant Capacity of the Medicinal Plant *Globularia alypum* L.

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Abstract: This study explored the variability of secondary metabolites and antioxidant activity in samples of Globularia alypum collected from six different sites in Tunisia. We prepared ethanol extracts from dried plant material and measured total phenolic content (TPC), total flavonoid content (TFC), condensed tannins (CT), and antioxidant activity using standard colorimetric methods. Our statistical analysis showed significant differences among the samples for all the parameters we measured. The antioxidant activity ranged from 80.84% to 91.22%, with the Hafouz site boasting the highest TPC at 0.3965 mg GAE/g DM, which correlates with its strong antioxidant potential. Tannins were found in low quantities and seemed to play a limited role in antioxidant capacity. Interestingly, the flavonoid content varied, with Seliana having the highest TFC, even though its antioxidant activity was lower. We confirmed a strong positive correlation between TPC and antioxidant activity.

Keywords: *Globularia alypum; Total phenolic content; Flavonoids; Condensed tannins; Antioxidant activity; Environmental factors; Variability;*

1. Introduction

Natural plants play a crucial role in human health and have long been recognized as an important source of bioactive compounds with diverse therapeutic applications, including antibacterial, anti-inflammatory, antioxidant, anticancer, and antimicrobial activities. These pharmacological properties are largely attributed to the presence of secondary metabolites such as polyphenols, flavonoids, tannins, alkaloids, and terpenoids, which are known to exert protective effects against oxidative stress and various pathological conditions (Cowan, 1999; Pandey and Rizvi, 2009; Newman and Cragg, 2016; Diass *et al*., 2021; Ouahabi *et al*., 2023; Merzouki *et al*., 2025). *Globularia alypum* L., commonly known as shrubby globularia, is a perennial herb that thrives in the Mediterranean region, especially in Tunisia. Traditionally, this plant has been used in local medicine for its therapeutic benefits, which include anti-inflammatory, antioxidant, and antimicrobial properties. Phytochemical analyses have uncovered a range of bioactive compounds in *G. alypum*, particularly polyphenols, flavonoids, and tannins (khlifi *et al*., 2011; Hajji *et al*., 2018; Nouir *et al*., 2023). These compounds are well-known for their antioxidant properties, which are essential in reducing oxidative stress, a factor linked to the development of various chronic diseases. For example, a study by Chograni *et al.* (2013) found that the leaves and flowers of Tunisian *G. alypum* exhibited significant antioxidant activity and high levels of phenolic and flavonoid.

Further research by Ouffai *et al.* (2020) evaluated the antioxidant and antihemolytic activities of aqueous extracts from Algerian *G. alypum*. The study found that the decoction extract had the highest total phenolic content and demonstrated potent antioxidant properties, as evidenced by DPPH radical scavenging and FRAP assays.

In vivo studies have also highlighted the therapeutic potential of *G. alypum*. Djellouli *et al.* (2021) investigated the effects of an aqueous extract of *G. alypum* in hypercholesterolemic rats. The results indicated that the extract significantly reduced lipid peroxidation and enhanced antioxidant enzyme activities across various tissues, suggesting its potential to manage hypercholesterolemia and associated oxidative stress.

These studies underscore the pharmacological significance of *Globularia alypum*, particularly its antioxidant properties attributed to its rich polyphenolic composition (Friscic *et al.*, 2018). This positions *G. alypum* as a promising candidate for the development of natural antioxidant agents, warranting further exploration and validation of its therapeutic applications.

The objective of this study is to evaluate the effect of climatic conditions on the production of secondary metabolites and antioxidant activity in *Globularia alypum*, to better understand the impact of climate change on this plant resource with high pharmacological potential. This analysis will determine how environmental variations influence the plant's biochemical quality and therapeutic properties, thereby contributing to the conservation and sustainable exploitation of this species amid current climate challenges.

2. Methodology

2.1 Plant material

Samples of *Globularia alypum* (**Figure 1**) were collected in December from six Tunisian sites. The plant was identified by Professor Abdelhamid Khaldi, a botanist, and the specimen was deposited in the INRGREF herbarium under the reference GA-2024. Samples were dried, ground into a fine powder, and stored until further analysis.

Figure 1. Photo of *Globularia alypum*

2.2 *Extract preparation*

Extracts were prepared from *G. alypum* samples using ethanol. 5 g of plant dried sample were socked in 50ml ethanol. The mixture was agitated for 24 hours at 200rpm and then filtered, dried and weighted.

2.3 *Free radical scavenging activity*

The effect of the different extracts on DPPH radical was studied, employing the method described by Brand-Williams *et al.*, (1995). Briefly, 5 ml of DPPH solution (0.004%, in Methanol) was incubated with varying concentrations of the extracts (0.1–0.8 mg/L). The reaction mixture was shaken well and incubated for 30 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A_{\text{Sample (517 nm)}}}{A_{\text{Control (517 nm)}}}\right] \text{x } 100$$

2.4. *Total phenols content (TPC)*

Total phenols were determined by Folin Ciocalteu reagent (Singleton and Rossi, 1965). A dilute extract of each sample extract (0.03-0.5 g/L) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (500 µl, 1:10 diluted with distilled water) and aqueous Na2CO3 (2 ml, 2%). The mixtures were allowed to stand for 30 min and the total phenols were determined by colorimetry at 755 nm. The standard curve was prepared using 0, 0.03, 0.06, 0.12, 0.25, 0.5 g/L solutions of gallic acid in water. Total phenol values are expressed in terms of gallic acid equivalent (EGA mg/g of DM), which is a common reference compound.

2.5. *Total flavonoids content (TFC)*

The total flavonoid content of *G. alypum* extracts was determined by the aluminum chloride colorimetric method (Quettier Deleu *et al.,* 2000). 1 ml of diluted sample was mixed with 1 ml of 2% aluminum chloride methanolic solution. The mixture was allowed to stand for 15 min, and absorbance was measured at 430 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per mL of extract (mg RE/g DM).

2.6. *Determination of condensed tannins (CT)*

The method described by Broadhurst and Jones (1978) was used to determine the total condensed tannin content in *G. alypum* plants. 0.5 ml of the extract was mixed with 3 ml of vanillin (4% in methanol) and 1.5 ml of Hydrochloric acid. After incubation for 15 min at 20◦C in the dark, the absorbance was read at 500 nm. The condensed tannin content was calculated from a calibration curve prepared with a solution of catechin (30 ppm). The results were expressed in mg of catechin equivalent per mL of juice (mg CE/g DM).

2.7. *Statistical analysis*

The statistical analysis was done with the GLM procedure (General Linear Models) of the SAS (9.0) program. Correlations were performed by SPSS.20 program.

3. Results and Discussion

Results of total phenol, flavonoids and tannins and antioxidant activity of *G. alypum* from different Tunisian sites are summarized in **Table 1**. Statistical analysis showed significant variability between the samples under study, for all the considered parameters. The antioxidant activity ranged from 80.84% in Seliana to 91.22% in Sousse. Hafouz and Kasserine exhibited DPPH values of 89.20% and 90.57%, respectively. The highest TPC was observed in Hafouz (0.3965 mg EGA/g DM), supporting its superior antioxidant potential, while Seliana exhibited the lowest TPC (0.1775 EGA mg/g DM). Tannins were present in minimal quantities across most regions. Seliana had the highest CT (0.122 mg CE/g DM), while other regions, such as Sbikha and Sousse, displayed negligible levels (0.005 mg CE/g DM and 0.001 mg CE/g DM, respectively). This indicates that tannins may not play a dominant role in the antioxidant activity under investigation. The highest level of TFC was recorded in Seliana (2.108 mg RE/g DM), followed by Hafouz (1.186 mg RE/g DM). Sousse, despite its strong antioxidant activity, displayed the lowest flavonoid content (0.966 mg RE/g DM), suggesting the participation of other phytochemicals such as phenols in determining antioxidant properties.

Table1. Total phenol, flavonoid and condensed tannins content and antioxidant activity of *G. alypum* from different Tunisian sites

Locality	TPC (mg EGA /g DM)	CT (mg CE/g DM)	TFC (mg RE/g DM)	Antioxydant activity DPPH (%)
Seliana	0.1775±0.01	0.122±0.02	2.108±0.01	80.84±0.15
Sbikha	0.1865±0.02	0.005±0.01	1.069±0.02	88.29±0.22
Hafouz	0.3965±0.01	0.038±0.00	1.186±0.01	89.20±0.10
Kasserine	0.25±0.03	0.003±0.01	1.028±0.00	90.57±0.30
Sousse	0.303±0.01	0.001±0.00	0.966±0.01	91.22±0.05

Several studies have shown that *G. alypum* is a rich source of phenolic compounds, flavonoids and condensed tannins, which contribute significantly to its antioxidant activity (Chograni *et al.*, 2013; Ouffai *et al.*, 2020; Nouir *et al.*, 2023). The total phenolic compound (TPC) content in this plant varies depending on the extraction conditions and the origin of the samples, but generally remains high, conferring a strong antioxidant capacity (Khantouche *et al.*, 2015; Asraoui *et al*., 2021). Flavonoids, an important subgroup of polyphenols, are also present in significant quantities in G. alypum, playing a key role in neutralising free radicals. Although the condensed tannin (CT) content is lower than that of total polyphenols and flavonoids, their contribution to overall antioxidant activity remains significant (Bourassen *et al.*, 2025). Collectively, the diversity and abundance of these phytochemicals support the traditional medicinal use of *G. alypum* and its continued investigation as a source of natural antioxidant agents.

Phytochemical investigations of *G. alypum* L. have revealed a diverse array of secondary metabolites that underpin its biological activities. Qualitative and quantitative analyses reported by Taghzouti *et al.* (2016) demonstrated the presence of major phytochemical groups, including polyphenols, flavonoids, tannins, terpenoids, saponins, coumarins, and cardiac glycosides in different plant organs, with methanolic extracts showing the highest concentrations. More detailed profiling has identified individual phenolic acids such as gallic acid, chlorogenic acid, caffeic acid, and ferulic acid, along with flavonoid compounds including quercetin, kaempferol, luteolin, apigenin, and their glycosylated derivatives. In addition, iridoids and seco-iridoid glycosides, which are characteristic of the Globulariaceae family and known for their anti-inflammatory and antioxidant properties, have also been detected. Recent work by El-Mernissi *et al.* (2023) further confirmed the richness of *G. alypum* in phenolic and flavonoid compounds and demonstrated a strong correlation between these phytochemicals and antioxidant activity assessed by DPPH, ABTS, and FRAP assays. The chemical diversity observed in *G. alypum* highlights its potential as a valuable source of natural bioactive compounds for pharmaceutical, nutraceutical, and functional food applications.

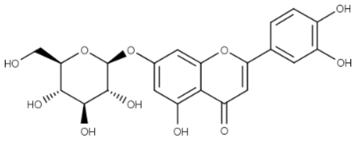
The observed differences underscore the potential impact of environmental factors on phenolic biosynthesis. Several studies reported the influence of both genetic and environmental conditions on the amount of these secondary metabolites. They demonstrated that, in addition to genetic factors, exogenous factors (edaphic and abiotic) are also involved in the qualitative and quantitative variation of phenolic compounds. Indeed, the work of Tavakoli *et al.,* (2022) has shown that extrinsic factors are involved in modulating the biosynthesis of these molecules and in the variation of their antioxidant properties. Previous studies have shown that abiotic stress factors, such as salinity, light and water deficit, can improve the synthesis of phenolic compounds in response to oxidative stress caused by the formation of reactive oxygen species in these hostile environments (Uranishi *et al.*, 2024; Zargoosh *et al.*, 20219) .

The results highlighted a strong correlation between TPC and antioxidant activity (**Table 2**). This finding is consistent with the central role of phenolics as primary antioxidative agents. Regions such as Hafouz and Sousse, characterized by higher TPC and antioxidant activity, could serve as optimal sources for the extraction of bioactive compounds with potential applications in nutraceuticals, pharmaceuticals, and functional foods. However, the low tannin contribution and the moderate flavonoid content in some samples suggest that further investigation into individual phenolic compounds and their interactions is warranted to fully elucidate the biochemical mechanisms underlying these observations. Several studies have also confirmed a positive correlation between the content of phenolic compounds, particularly flavonoids, and antioxidant activity, highlighting the importance of these compounds in the therapeutic potential of *G. alypum* (Bourassen *et al*., 2025). These results confirm the pharmacological interest of this Mediterranean plant, particularly for its use in the formulation of natural antioxidant products.

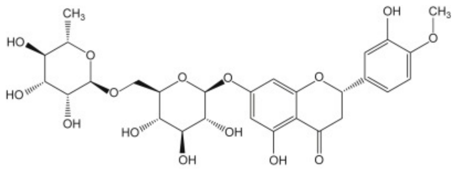
Table2. Correlation table between Phenolic Compounds (TPC, CT, TFC) and Antioxidant Activity (DPPH %)

	TPC	**CT**	**TFC**	**Antioxydant activity (DPPH %)**
TPC	1	0.337	-0.909	0.849
CT	0.337	1	-0.881	0.040
TFC	-0.909	-0.881	1	-0.812
Antioxydant activity (DPPH %)	0.849	0.040	-0.812	1

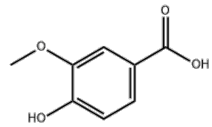
Survey literature indicates that *G. alypum* ethanol extract is rich in bioactive secondary metabolites, primarily phenolic compounds, flavonoids, and iridoids, which contribute to high antioxidant, antibacterial, and anti-inflammatory activities (Taghzouti *et al.,* 2016; El Mernissi *et al.,* 2023). The determined compounds include luteolin-7-O-glucoside, luteolin-4'-O-glucoside, hesperidin, vanillic acid, and ferulic acid, along with strong polyphenolic and flavonoid profiles which contribute to the medicinal properties (Es-Safi *et al.,* 2005; Friščić *et al.,* 2022; Nouir *et al.,* 2023b; Asraoui *et al.,* 2025). The presence of cyclic rings and various functional groups containing O, N… as well as double bonds forming V or U letters facilitates the comprehension of their pharmaceutical profile (Toxicity, Drug-score, bioavailability, and their mechanism of action) as summarized in this organigram of POM Theory proposed by Prof Taibi Ben Hadda and his collaborators (Figure 2).



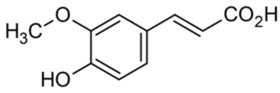
luteolin-7-O-glucoside



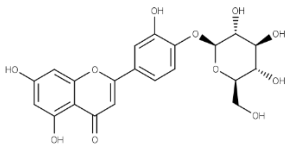
hesperidin



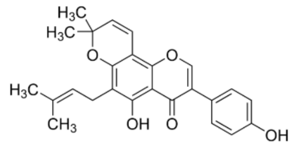
vanillic acid



ferulic acid



luteolin-4'-O-glucoside



asperuloside

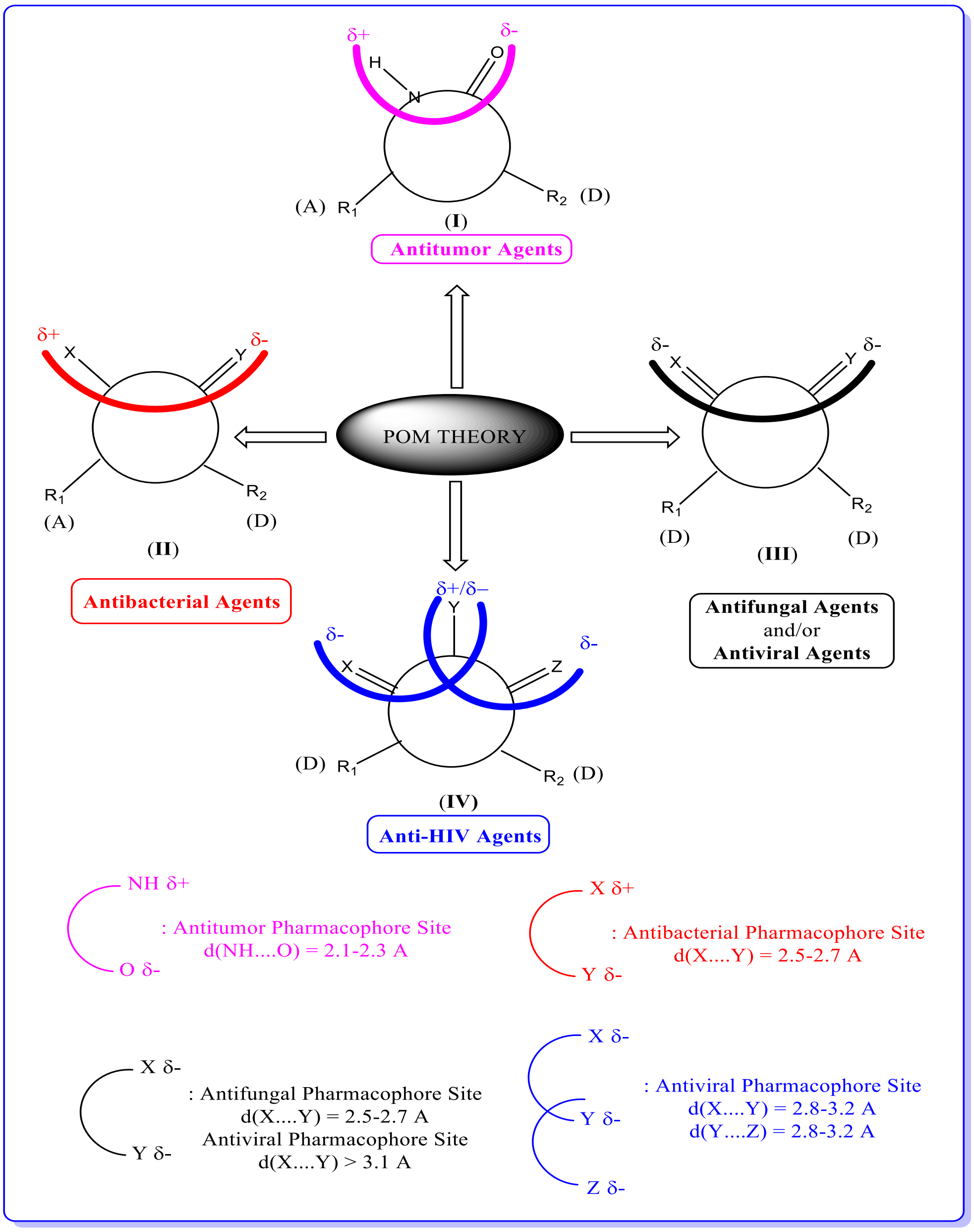


Figure 2. POM Theory Organigram's showing the principal pharmacophore sites, as antibacterial (), antifungal, antiviral, and antitumor (Bechlem et *al*., 2020; Grib *et al*., 2020; Kawsar et *al*., 2022; Kadda *et al.,* 2026)

Conclusion

This study highlighted significant variability in the secondary metabolites and antioxidant activity of *Globularia alypum* across the different Tunisian locations studied. The total phenolic compound content, which is particularly high in certain sites, appears to be the main factor contributing to the high antioxidant capacity observed. The low content of condensed tannins suggests that these compounds play a minor role in overall antioxidant activity, while the disparity in flavonoids highlights the importance of other phenolic compounds in these mechanisms. Furthermore, the variations observed underscore the significant influence of environmental factors on the biosynthesis of bioactive compounds. These results reinforce the potential of *G. alypum* as a natural source of antioxidants for pharmaceutical and nutraceutical applications, while highlighting the need for further studies to deepen our understanding of the biochemical interactions involved. Finally, it is essential to take into account the effects of climate change on this plant resource in order to ensure its preservation and sustainable use.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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