Seasonal variation of verbascoside as a principal phenolic compound linked with antioxidant potentials of Clerodendrum glandulosum Lindl. leaves

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ABSTRACT
This study reports the influence of seasonality on the accumulation of verbascoside as a principal phenolic compound in Clerodendrum glandulosum Lindl. leaves along with possible alteration of antioxidant potentials. Leaves were collected during winter (December 2018), spring (February 2019), summer (May 2019), monsoon (July 2019), autumn (October 2019), and extracted with 95% aqueous methanol by cold maceration. The total phenolic content and antioxidant capacities (DPPH, ABTS and FRAP) were estimated by spectrophotometric technique, and verbascoside content was estimated by HPLC-PDA. Results indicate that the leaves collected during summer and winter both exhibited the highest total phenolic content verbascoside accumulation and antioxidant potentials which are significantly different (p < 0.05) than other seasons. Correlation studies further demonstrated that the total polyphenol and verbascoside contents were directly proportional to the antioxidant potentials. Thus, the study concludes that winter and summer are the best seasons for collecting leaves from this plant to obtain maximum antioxidant potential.
1. Introduction

Phenolic compounds are the major class of phytochemicals present in the plant kingdom which are widely acclaimed for diverse biological activities (Rasouli et al. 2017). Biosynthesis of phenolic metabolites in the plants are primarily reliant on their genetic make-up including genome size and expression of essential genes involved in biosynthetic pathways (Sharma et al. 2019). However, the accumulation and concentration of many phytochemicals may also vary with seasonal changes, geographical location, growth stages of the plants and environmental stresses (Ribeiro et al. 2020). Since phenolic compounds exhibit strong antioxidant properties, the absence or partial reduction of the compounds due to seasonal changes directly influence the therapeutic potential of the plants (Yao et al. 2016).

Clerodendrum glandulsum Lindl. (Family: Lamiaceae) is a perennial species endemic to the North-Eastern region of India. It is an evergreen flowering shrub of approximately 1.5-3 m height, containing heart-shaped, simple leaves and bears white tube-shaped flowers (Figure S1) (Deori et al. 2013). Traditionally the leaves are used in metabolic syndromes like diabetes and hypertension. C. glandulosum leaf contains an array of phenolics (verbascoside, martynoside), steroids (colebrin A-E), terpenoids (maslinic acid, oleanoleic acid). Verbascoside, the principal phenolic compound identified in C. glandulosum (Deb et al. 2021) as well in several other species (Bardakci et al. 2019; Frezza et al. 2019; Garro et al. 2020; Gharari et al. 2020; Gökömen et al. 2020; Khentoul et al. 2020) across the world, is an acclaimed antioxidant.

However, the impact of seasonal changes on the variation of verbascoside accumulation in C. glandulosum leaves and its influence on the overall antioxidant capacity has not been reported yet. Therefore, in this study the effect of seasonal changes in the accumulation of verbascoside, as major phenolic compound was investigated and correlated with the total antioxidative potentials of the leaves of C. glandulosum.

2. Results and discussion

2.1. Effect of seasonal changes on total phenolic content and verbascoside content

Accumulative dynamics of total phenolic content (TPC) in the C. glandulosum leaves were presented in Figure S2. Spectrophotometric analysis depicted that, TPC in the leaves collected during winter (280.88 ± 3.93 mg GAE g⁻¹) and summer (273.94 ± 4.88 mg GAE g⁻¹) was found higher than the other seasons. On the contrary, the lowest phenolic content (241.44 ± 7.08 mg GAE g⁻¹) was found in the leaves collected during the post-monsoon or autumn.

HPLC analysis of the extracts exhibited the presence of verbascoside as the principal compound in C. glandulosum (Figure S3). Similar to TPC, the major phenolic compound verbascoside was also detected in high concentrations during winter (224.31 ± 4.24 mg g⁻¹) and summer (197.01 ± 4.09) respectively (Figure S2). The least concentration of verbascoside was estimated in monsoon and autumn. No significant difference (p < 0.05) in the concentration of verbascoside was observed in the leaves collected during spring (186.44 ± 5.15 mg g⁻¹) and summer (197.01 ± 4.09 mg g⁻¹).
which contains slightly lower concentration of verbascoside compared to winter season. Following the overall estimated content, a direct relationship between the accumulation of total phenolic and verbascoside during different seasons were established according to the following order: winter $>$ summer $>$ spring $>$ monsoon $>$ autumn.

Increased synthesis of phenolics has been observed in plants to prevent heat-induced oxidative damage (Sharma et al. 2019). In turn, phenolics neutralize ROS and provide heat resistance to plants by enhancing their accumulation. Similarly, stimulated production of polyphenols under cold stress has been linked to enhanced expression of essential enzymes (like phenylalanine ammonia lyase, cinnamylalcohol dehydrogenase and hydroxycinnamoyl transferase) in the biosynthetic pathway. Alike in heat stress, increased phenolic levels in plants provide protection against chilling stress (Sharma et al. 2019). Studies have also revealed that water scarcity during winter can lead to increased production of phenolic compounds (Albergaria et al., 2020; Sharma et al. 2019). Similar increment in acteoside (syn. verbascoside) content during winter has been reported (Gomes et al. 2019). In this context, our results are also in agreement with the accumulation of a high concentration of phenolic compounds, including verbascoside, during dry cold winter and warm summer.

2.2. Influence of seasonal changes on the antioxidant capacity of C. glandulosum leaves

Substantial seasonal fluctuations of antioxidant activities were observed in the leaf extracts of C. glandulosum, which are illustrated in Figure S4. The antioxidant capacity of the leaves collected during the winter season exhibited maximum antioxidant capacity in terms of scavenging DPPH ($299.25 \pm 4.24 \text{ mg AAE g}^{-1}$) and ABTS ($219.881 \pm 1.79 \text{ 4.24 mg AAE g}^{-1}$) radicals, which are higher than the leaves collected in other seasons (Figure S4). In contrast, samples collected during autumn exhibited the lowest DPPH ($231.5646 \pm 6.79 \text{ 4.24 mg AAE g}^{-1}$) and ABTS ($160.80 \pm 5.35 \text{ mg AAE g}^{-1}$) radical scavenging capacity. Similarly, leaves collected during winter ($218.71 \pm 4.81 \text{ mg AAE g}^{-1}$) and summer ($206.09 \pm 6.56 \text{ mg AAE g}^{-1}$) also demonstrated higher reducing properties in FRAP assay with no significant difference ($p < 0.05$) between the seasons (Figure S4). While, comparatively low reducing capacity of the leaf extracts collected during autumn ($148.26 \pm 8.34 \text{ mg AAE g}^{-1}$) was observed.

The antioxidant potentials of the extracts were also found to follow the similar order of TPC and verbascoside concentration in the leaves (i.e. winter $>$ summer $>$ spring $>$ monsoon $>$ autumn). However, the antioxidant capacity in winter and summer was not significantly different ($p < 0.05$) when compared with each other. Antioxidant effects of the plant extract mainly depended on the concentration of phenolic compounds as they can quench the stable free radicals (Rasouli et al. 2017). Similar to our results, other studies indicated towards the increased accumulation of antioxidant phytochemicals like phenolics and phenylpropanoids to counter heat generated ROS as well as cell collapse under cold stress (Gomes et al. 2019; Sharma et al. 2019).
2.3. Correlation between phenolic metabolite content and antioxidant capacity

Results showed a positive correlation between total phenolic content and antioxidant capacity extracts of *C. glandulosum* leaves [vs DPPH assay (*r* = 0.9776); vs ABTS assay (*r* = 0.9960); vs reducing property (*r* = 0.9997)]. Similarly, positive correlations were found between verbascoside content and antioxidant activity of the leaf extracts tested in terms of DPPH scavenging capacity (*r* = 0.8853), ABTS quenching capacity (*r* = 0.9338) and ferric reducing property (*r* = 0.9331) (Table S2 and Figure S5). This relationship between the total phenolic content and verbascoside concentration with antioxidant potentials of the extracts varying with seasonality has been depicted in Figure S6. Hence, it confirms that the presence of the total phenolics and verbascoside both are equally responsible for the antioxidant property of *C. glandulosum* leaves. This phenomenon could well be linked with the antioxidant properties of the leaves harvested during different seasons of the year.

Since, the antioxidant capacity of the extracts from different seasons is alike the trend of accumulation of total phenolics and verbascoside, hence it confirms that these effects are directly proportional to these secondary metabolites present in the leaves of *C. glandulosum*.

3. Experimental (supplementary material)

4. Conclusion

Comparative analysis demonstrated that, in comparison to monsoon and autumn, winter and summer both seasons influence the accumulation of phenolic compounds (including verbascoside) along with high antioxidant capacity in *C. glandulosum* leaves. Phenolics and phenylpropanoid accumulation have been subjected to the abiotic stress (temperature, cold stress, humidity, rainfall etc.) experienced in different seasons. Thus, this study uncovers the effect of seasonality in the accumulation of total phenolics and verbascoside content in *C. glandulosum* and its correlation with antioxidant activity. The results may have impact in determining appropriate harvesting season for this species towards deriving medicinal benefits in turn.

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Disclosure statement

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References

Albergaria ET, Oliveira AFM, Albuquerque UP. 2020. The effect of water deficit stress on the composition of phenolic compounds in medicinal plants. S. Afr. J. Bot. 131:12–17.

Bardakci H, Türköz AE, Kırzızbekmez H. 2019. Simultaneous quantification of six phenylethanoid glycosides in some Turkish Scutellaria species by a new HPLC-DAD method. Nat Prod Res. 33(14):2116–2119.

Deb PK, Khound P, Bhattacharjee S, Choudhury P, Sarma H, Devi R, Sarkar B. 2021. Variation in chemical constituents, in-vitro bioactivity, and toxicity profile among different parts of Clerodendrum glandulosum Lindl. (C. colebrookiannum Walp.). S. Afr. J. Bot. 140:50–61.

Deori C, Roy DK, Talukdar SR, Pagag K, Sarma N. 2013. Diversity of the genus Clerodendrum Linnaeae (Lamiaceae) in Northeast India with special reference to Barnadi Wildlife Sanctuary. Assam, Pleione. 7:473–488.

Frezza C, Bianco A, Serafini M, Foddai S, Salustri M, Reverberi M, Gelardi L, Bonina A, Bonina FP. 2019. HPLC and NMR analysis of the phenyl-ethanoid glycosides pattern of Verbascum thapsus L. cultivated in the Etna area. Nat Prod Res. 33(9):1310–1316.

Garro HA, Bruna-Haupt E, Cianchino V, Malizia F, Favier S, Menacho-Márquez M, Cifuentes D, Fernández CO, Pungitore CR. 2020. Verbascoside, synthetic derivatives and other glycosides from Argentinian native plant species as potential antitumoral agents. Nat Prod Res. 10:1–6..

Gharari Z, Bagheri K, Derakhshani B, Sharafi A. 2020. HPLC-DAD-ESI/MSn analysis of phenolic components of Scutellaria araxensis, S. bornmuelleri and S. orientalis. Nat Prod Res. 12:1–6..

Gökmen A, Küsz N, Karaca N, Demirci F, Hohmann J, Kırzızbekmez H. 2020. Secondary metabolites from Verbascum bugulifolium Lam. and their bioactivities. Nat Prod Res. 11:1–5..

Gomes AF, Almeida MP, Leite MF, Schweiger S, Stuppner H, Halabalaki M, Amaral JG, David JM. 2019. Seasonal variation in the chemical composition of two chemotypes of Lippia alba. Food Chem. 273:186–193.

Khentoul H, Bensouici C, Reyes F, Albanese D, Sarri D, Ratiba M, Fadila B, Seghiri R, Boumaza O. 2020. Chemical constituents and HRESI-MS analysis of an Algerian endemic plant – Verbascum atlanticum Batt. – extracts and their antioxidant activity. Nat Prod Res. 34(20):3008–3012.

Rasouli H, Farzaei MH, Khodarahmi R. 2017. Polyphenols and their benefits: a review. Int. J. Food Prop. 20:1–1741.

Ribeiro DA, Camilo CJ, de Fátima Alves Nonato C, Rodrigues FFG, Menezes IRA, Ribeiro-Filho J, Xiao J, de Almeida Souza MM, da Costa JGM. 2020. Influence of seasonal variation on phenolic content and in vitro antioxidant activity of Secondatia floribunda A. DC. (Apocynaceae). Food Chem. 315:126277.

Sharma A, Shahzad B, Rehman A, Bhardwaj R, Landi M, Zheng B. 2019. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. Molecules. 24(13):2452.

Yao XH, Zhang ZB, Song P, Hao JY, Zhang DY, Zhang YF. 2016. Different harvest seasons modify bioactive compounds and antioxidant activities of Pyrola incarnata. Ind. Crops and Prod. 94:405–412.