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Evaluating Seasonal Variations in Total Phenol and Flavonoid Retention in Stem Powder of hemiparasite *Dendropthoe falcata* var pubescens (Hook.f)

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ABSTRACT

The hemiparasite *Dendropthoe falcata* var pubescens, belonging to family Loranthaceae, is known as "vanda" in the Indian Ayurvedic system. Due to the abundance of phenols and flavonoids in the entire plant, it is significant from an ethno-botanical perspective. Widely utilized in traditional medicine, the plant is reputed for its therapeutic properties, including cooling, bitter, astringent, aphrodisiac, narcotic, and diuretic effects. Environmental factors such as temperature, photoperiod, and seasonal changes substantially influence the phytochemical profile of the plant. This study was undertaken to evaluate the impact of seasonal variations on the total phenol and flavonoid content in the dried stem powder of *D. falcata*. Conducted over two consecutive years, the investigation focused on three seasons: rainy, winter, and summer. Statistical analysis using one-way ANOVA revealed significant seasonal variations ($p \leq 0.01$) in the phytochemical composition of the dried stem powder. Thus, the optimal sampling is necessary for utilization of plants in traditional medicine and phyto-pharmaceutical applications.

INTRODUCTION

Plants are a reservoir of bioactive compounds, and their biochemical composition is profoundly influenced by environmental factors, making them dynamic systems of nutrient and metabolite diversity (Katiyar *et al.*, 2012). The hemiparasite *Dendrophthoe falcata* var. *pubescens* of the family Loranthaceae, commonly known as "Vanda" in the Indian Ayurvedic system, stands out for its therapeutic potential. This plant is rich in phenolic compounds and flavonoids, which contribute to its traditional medicinal applications, including cooling, astringent, aphrodisiac, and diuretic properties. However, the phytochemical profile of plants like *D. falcata* is not static; it is significantly influenced by climatic factors such as temperature, photoperiod, and seasonal variations (Alekutty *et al.*, 1993). Additionally, they vary depending on the host plant they infest (Calvin and Wilson, 2009).

Seasonal changes, marked by variations in sunlight exposure, temperature, and rainfall, affect the plant's metabolic processes, directly influencing the synthesis of primary and secondary metabolites (Gouvea *et al.*, 2012). Photosynthesis, respiration, and nutrient absorption are key metabolic activities regulated by the duration of sunlight exposure, ultimately shaping the biochemical makeup of the plant (Tattini *et al.*, 2000). Similarly, temperature variations impact soil microbial activity and plant-microbe interactions, which are crucial for nutrient assimilation and metabolite production (Sankhalkar and Vernekar, 2016). During hotter months, increased evaporation and water loss may hinder nutrient uptake, whereas rainy seasons can enhance soil nutrient availability through the deposition of atmospheric particles and rainwater's mildly acidic nature (Sankhalkar and Vernekar, 2016). These environmental dynamics collectively contribute to the variability in phytochemical composition across seasons.

Studies have consistently demonstrated that seasonal factors affect the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in plants. For instance, Sankhalkar and Vernekar (2016) observed that climatic factors significantly alter the phytochemical profile of plants. Similarly, research by Czigle et al. (2019) on *Ginkgo biloba* leaves revealed higher phenolic content in autumn, while flavonoid concentrations peaked during the rainy season. They reported 1.01 and 1.09 fold higher moisture content (approximately 62.40%) during rainy seasons as compared to spring and autumn respectively. The protein content was found to be 1.14 and 1.56 fold greater in autumn. Similarly the carbohydrate content was 1.16 and 1.28 fold higher in autumn as compared to rainy and spring seasons respectively. The trace elements like sodium and potassium were also present in higher concentration during autumn. In addition they reported that the total phenolic content was comparatively higher in autumn, while total flavonoid content was slightly higher in rainy season. This may be due to the temperature sensitivity of flavonoids. Such observations highlight the intricate interplay between environmental conditions and plant biochemistry. Seasonal changes not only influence the phytochemical content but also alter the biological activities of plants. This phenomenon is evident from studies like those of Osadebe et al. (2009), which demonstrated seasonal variations in the anti-diabetic potency of *Viscum album*. Higher concentrations of

flavonoids and essential oils during the rainy season correlated with enhanced anti-diabetic activity, emphasizing the role of specific phytochemicals in therapeutic applications. Similarly, antimicrobial potential and other pharmacological properties have shown seasonal dependencies (Osadebe et al., 2008).

The seasonal influence extends to various plant parts, each showing optimal bioactive compound concentrations in specific seasons. For instance, barks are often harvested in spring, while flowers and leaves yield the optimum amounts of essential oils during winter (Szakiel et al., 2011). Understanding these variations is essential for optimizing the collection and utilization of plant materials for medicinal and industrial purposes.

Given the dynamic influence environmental factors have on the phyto-constituent, this study was undertaken to assess whether phenols and flavonoids are retained to differing extent in the stems of *Dendrophthoe falcata*,during different seasons.

MATERIALS AND METHODS

Collection and identification of *Dendrophthoe falcata* L.f) var. pubescens (Hook. f)

The hemi-parasitic plant of *Dendrophthoe falcata* was collected along with its flowers from the region in and around the Yeoor hills, Thane city. This study area was selected due to its dense vegetation. The plant was identified based on the references from Flora of the Presidency of Bombay (Cooke, 1958) and Flora of Maharashtra (Almeida, 1996; 2003).

Selection of host plants

The host plants were selected based on three primary criteria during the survey

(i) Prevalence: Native host plants commonly infested by the hemiparasite were prioritized.

(ii) Importance: The medicinal value of the host plants.

(iii) Morphological compatibility: Hosts with stem diameters comparable to the hemiparasite were chosen to ensure healthy growth and consistent sampling throughout the study period.

Based on these criteria, the selected host plants were *Mangifera indica* (Mango), *Melia azedarach* (Neem), and *Ficus religiosa* (Peepal).

Sample collection and processing

Fresh plant samples of the hemiparasite *D. falcata* var. *pubescens* growing on three different hosts i.e., *Mangifera indica* (HM), *Melia azedarach* (HN) and *Ficus religiosa* (HP) were collected in three different seasons (summer, rainy and winter) for two consecutive years (2016-2017 and 2017-2018). The stems of the hemiparasite *D. falcata* (L. f) var. *pubescens* growing on these hosts (i.e., HM, HN and HP) and the host plant themselves were dried in shade, powdered and stored in air-tight bags at room temperature in the dark.

Extraction of phytochemicals

The phytochemicals in samples were extracted using (50ml of 80%) solvents like methanol, petroleum ether, ethyl acetate, and distilled water in a Soxhlet apparatus. The recovered extract was concentrated in a rotary evaporator and used for estimation of Total Polyphenol Content (TPC) and Total Flavonoid Content (TFC). The concentration of plant extract used for estimation of TPC and TFC was 1mg/ml.

Estimation of Total Phenol Content

The Folin- Ciocalteau (FC) Spectrophotometer method (Singleton and Rossi, 1965) was slightly modified as suggested by Lobo et al., (2011) to determine TPC. One mL of the plant extracts were mixed with 1 mL of phenolic FC reagent in the ratio of 1: 1 and 10 mL of 8% w/v saturated sodium carbonate solution prepared in distilled water was added after 3 mins incubation. The total volume was adjusted to 20 ml. This was followed by thorough mixing and incubation in dark for 45 mins. The sample absorbance of was measured at 765nm using a spectrophotometer against the reagent blank. The TPC of extract was expressed in terms of gallic acid equivalent per gram of dry plant material (GAE/g dry plant). All reactions were carried out in triplicate. It was calculated from the calibration curve of gallic acid with linear equation y = 0.003x + 0.005, r2 = 0.998.

Where, “y” = the absorbance of the plant extract sample and “x” = the concentration of gallic acid in ‘mg/mL’ calculated from the graph.

Estimation of Total Flavonoid Content

The aluminium chloride method (Kumthekar, 2014) was used for evaluating the TFC of the plant extract. A 10% aluminium chloride solution (0.1 ml) was combined with 1 ml of plant extracts. To the resultant combination, 0.1 mL of 1M potassium acetate was added and the total volume was brought to 10ml by adding 8.8 ml of distilled water. The tubes' contents were left to react at room temperature for half an hour. The test solution and standard absorbance was measured against the reagent blank using spectrophotometer at 510 nm. The TFC of each plant extract was expressed in terms of quercetin equivalent per gram of dry sample. All reactions were carried out in triplicate. It was calculated from the calibration curve of Quercetin with the linear equation y=0.008 x + 0.015, r2 = 0.999.

Where, “y” = the absorbance of the plant extract sample and “x” = the concentration of quercetin in mg/mL calculated from the graph.

Evaluation of seasonal variation in phenol and flavonoid content of *D. falcata*

The samples were evaluated for changes in total phenol and flavonoid content during different seasons (summer, rainy and winter). The samples were assessed twice in a year at an interval of six months.

Statistical analysis

The results were represented as mean ± S.E.M. The data analysis was done using Analysis of Variance (ANOVA) and Student t test with P value ≤ 0.001 and 0.01 set as significant.

RESULTS

The study revealed variations in TPC and TFC of *D. falcata* sampled from all three host plants during different seasons. The changes noted in TPC and TFC in different samples collected in different seasons are represented in Figures 1 and 2 respectively. The TPC in the stem of *D. falcata* growing on *M. indica* (DfM) was highest during summer, followed by rainy and winter seasons, in both years. In the first year, the TPC ranged from 9.08 mg/g to 18.13 mg/g, while in the second year, it ranged from 9.14 mg/g to 17.9 mg/g. A decrease of 1.26% in TPC was observed during the summer of the second year, whereas increase of 6.9% and 0.6% were observed during the rainy and winter seasons, respectively. The differences were statistically significant ($p \leq 0.01$), with F values of 1859 (Year 1) and 8693 (Year 2). Seasonal variation in TPC between years was also significant for the rainy season (F = 504.88), while it was less pronounced for winter (F = 0.082) and summer (F = 16.35).

For the hemiparasite growing on *M. azedarach* (DfN), the TPC ranged from 10.12 mg/g to 14.43 mg/g in the first year and from 9.65 mg/g to 14.70 mg/g in the second year. The highest TPC was recorded in summer, followed by rainy and winter seasons. An increase of 1.87% was noted during summer, while decrease of 17.11% and 4.64% were observed during the rainy and winter seasons, respectively. These observations were statistically significant ($p \leq 0.01$), with F values of 6230 (Year 1) and 20179 (Year 2). Seasonal variations in TPC between years were significant, with F values of 93.14 for summer, 93.3 for winter, and 6885 for the rainy season.

In case of the hemiparasite growing on *F. religiosa* (DfP), the TPC ranged from 11.26 mg/g to 12.20 mg/g in the first year and from 10.48 mg/g to 15.76 mg/g in the second year. The highest TPC was recorded during winter, followed by summer and rainy seasons. Between the two years, increase of 5.89% and 12.73% were observed during summer and winter, respectively, while a 3.58% decrease was noted during the rainy season. These variations were statistically significant ($p \leq 0.01$), with F values of 117 (Year 1) and 306 (Year 2).

Seasonal changes in TFC were also observed across all three hosts (DfM, DfN, and DfP) over both years. In DfM and DfN, TFC was highest in summer, followed by rainy and winter seasons. In DfP, however, TFC was highest in winter, followed by summer and rainy seasons. In the second year, TFC in DfM increased by 16.45% during summer and 15.83% during the rainy season but decreased by 8.54% in winter. Conversely, TFC in DfN decreased by 2.18% during summer and 21.02% during winter but increased by 8.94% during the rainy season. For DfP, TFC decreased in all seasons, with reductions of 8.01%, 8.95%, and 11.8% during

summer, rainy, and winter seasons, respectively. These differences were statistically significant ($p \leq 0.01$).
Overall, the study revealed that phenol and flavonoid content varied significantly across seasons. The hemiparasite growing on *M. indica* and *M. azedarach* hosts exhibited the highest phenol and flavonoid levels during summer, while the hemiparasite on *F. religiosa* showed the highest levels during winter.

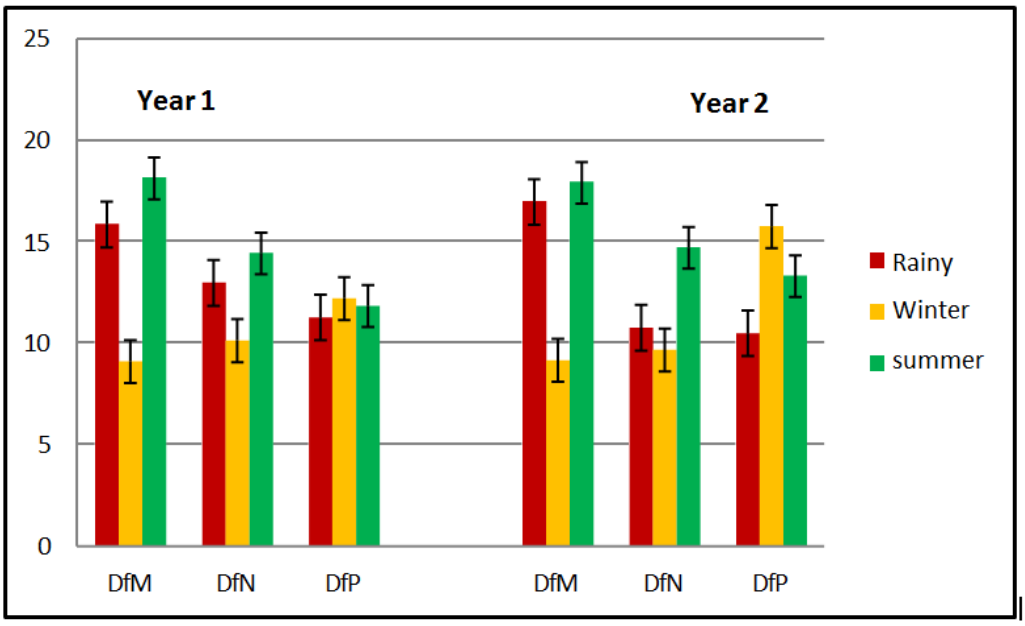

Fig. 1: Effect of Seasonal variation on the total phenol content in the stem of hemiparasite growing on three different hosts

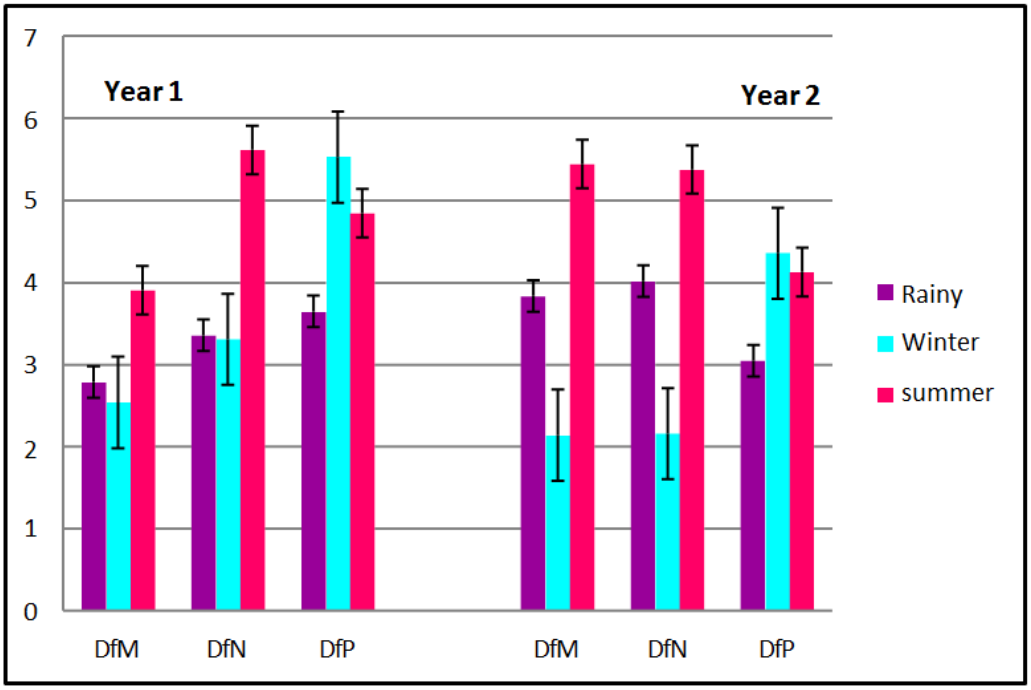

Fig. 2: Effect of Seasonal variation on total flavonoid content in the stem of hemiparasite growing on three different hosts.

DISCUSSION

This study analyzed seasonal variations in TPC and TFC in the stem of the hemiparasite *D. falcata* parasitizing three different hosts (*M. indica*, *M. azedarach* and *F. religiosa*) over two consecutive years. Observations spanned rainy, winter, and summer seasons, and statistical analysis using one-way ANOVA revealed significant seasonal changes ($p \leq 0.01$).

Plants from different families exhibit diverse metabolite profiles, often specific to their taxonomic group. The production of secondary metabolites reflects a plant's medicinal properties and defense mechanisms and is influenced by abiotic factors such as temperature, altitude, water availability, nutrients, photoperiod, and seasonal changes, as well as biotic factors like insect attacks and diseases. These factors regulate gene expression and influence metabolite synthesis (Gouvea et al., 2012). Plants tend to produce higher levels of secondary metabolites during specific life cycle stages, seasons, or in tissues requiring enhanced protection (Salminen et al., 2001).

The climatic factors also affect the diversity and concentration of micro- and macro- nutrients in plants. Although several environmental factors affect the biochemistry of plants, the main reasons for this scenario can be narrowed down to two factors. These factors are duration of sunlight exposure and temperature. The primary and secondary metabolites of plants are a result of plant metabolism. The duration of sunlight exposure affects metabolic processes like photosynthesis and respiration in plants. In turn, these factors affect the biochemical make- up of plants. The changes in temperature during different seasons also affect the soil microbial flora and therefore the plant-microbe and plant-soil interaction. This may also lead to the seasonal variations in observed biochemical profile of plants. In addition, the increased rate of evaporation and water- loss prevents the uptake of soluble nutrients from the soil. Interestingly, during the rainy seasons, it is suggested that the atmospheric harmless pollutants like ash, dust and other microscopic particles settles on the soil due to heavy rains. They act as fertilizers and might improve the biochemical profile of plants. The rainwater itself is suggested to act as a mild fertilizer due to the dissolved atmospheric content and its slightly acidic nature. Hence, the seasons greatly influence the phytochemicals (Sankhalkar and Vernekar, 2016).

Several studies corroborate the seasonal variation in phytochemical production. For instance, Osadebe et al. (2008, 2009) linked antibacterial and anti-diabetic activity of *Loranthus micranthus* to seasonal variations in flavonoid and alkaloid content. Kale (2010) reported maximum polyphenol levels in summer for *C. microphyllus*, while *Withania somnifera* showed higher levels in winter. Sahoo (2012) noted seasonal differences in alkaloid and polyphenol levels in multiple plants, with higher concentrations generally exhibited during summers. These variations often correlate with environmental stressors, such as light intensity, water availability, and temperature shifts (Yang et al., 2018).

Light, in particular, plays a crucial role in phenolic production. Slimestad and Verheul (2009) gave an overview on flavonoids and phenolics from fruits of tomato varieties concluding that phenolic compounds increase with rising levels of light intensity. Similarly, Ncube et al., (2011) reported highest concentration of total phenol in the leaves and bulbs of *Tulbaghia violacea*, *Drimia robusta* and *Merwilla plumbea* in spring season and ascribed increase level of phenolic compound to increase level of solar radiation. High phenolic concentrations were found to be fluctuating due to changes in temperature and dry weather conditions.

Khalid et al., (2019), reported contribution of flavonoid towards protection of delicate tissue and transfer of light energy through sensitization along with significant increase in derivatives of apigenin, luteolin, kaempferol and quercetin, and accumulation of flavonoids and other phenolic compound with increase in UV radiation. Haribal and Renwick (2001), on the other hand, insisted that the seasonal variation in flavonoid content of *Alliaria petiolata* depended on the amount of light or duration of daylight. In the samples of *Angelica keiskei*, Lee et al. (2003) observed a positive correlation between the content of flavonoid aglycones and the duration of exposure to sunshine. At the same time, higher content of flavonoids present in *Angelica keiskei* during the months of January to March showed that low temperature and high light conditions also leads to flavonoids accumulation. High and low levels of humidity, precipitation, water stress including water logging and drought are also factors affecting phytochemical composition in plants as there is osmotic shift and change in water potential. Liu et al. (2021) reported an increase in gallic acid and total flavonoid content in leaves of herbaceous peony "Taohuafeixue" and "Yangfeichuyu" under water logging due to heavy rain fall and attributed it to increase in antioxidant enzyme and content of osmotic regulator.

Choudhry et al. (2014) reported maximum amount of phenol content in *Tinospora cordifolia* in late summer due to water stress condition leading to increased production of phenolic compounds that can be attributed to the heightened activity of phenylalanine ammonia-lyase (PAL). PAL is an important enzyme involved in the formation of phenolic compounds which activates the genes required in phenylpropanoid pathway (Collakova and Della Penna, 2003). Ibrahim and Jaafar, (2011) observed that under water stress, plants used less protein for maintenance. This resulted in the availability of more phenylalanine (Phe) which, in turn, is available for the production of secondary metabolites. Deshmukh and Dhumal (2005) also reported an increase in polyphenoloxidase activity in Sorghum bicolor due to water stress conditions. Horner in the year 1990 postulated that water deficit in moderate levels helped in stimulating production of secondary metabolites. Mattila et al., (2018), while studying autumn senescence of deciduous trees viz., *Sorbus aucuparia*, *Acer platanoides*, *Betula pendula* and *Prunu spadus*, correlated synthesis of flavonoid and increase in its content to chlorophyll degradation. Hence an increase in flavonoid content during winter in the stem of hemiparasite *D. falcata* var. pubescens growing on peepal host can be corroborated with studies carried by Mattila and co-worker, as peepal is a deciduous tree which sheds its leaves in winter. Therefore, it can be suggested that the host has an impact on the phyto-chemical profile of the parasite.

CONCLUSION

This study highlights the significant influence of seasonal and host-specific factors on the phytochemical composition of *D. falcata* var. pubescens. The findings highlight the complex relationship between hemi-parasites and their hosts, shaped by environmental conditions and physiological changes. These insights contribute to understanding the adaptive strategies of parasitic plants and their potential medicinal applications.

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