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Alteration in secondary metabolites in *Lasioptera* sp. (Diptera, Cecidomyiidae) induced galled and ungalled leaves of *Glycosmis pentaphylla* (Retz.) DC.

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ABSTRACT: *Glycosmis pentaphylla* (Retz.) DC. (Rutaceae) is an ethnobotanically important medicinal plant. On this host, cylindrical- green epiphyllous galls are induced on the leaves by *Lasioptera* sp. (Diptera, Cecidomyiidae). In the present study, the changes in concentration of major secondary metabolites during the development of insect-induced plant galls in *G. pentaphylla* analysed and the results show that gall tissues contained significantly higher concentrations of tannins, phenols, and alkaloids, but lower concentrations of flavonoids, compared with ungalled leaves. These gall-associated alterations in secondary metabolite profiles may represent host defense responses initiated by gall induction and reflect the complex biochemical interactions between the gall inducer *Lasioptera* sp. and the host plant.
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KEY WORDS: Gall inducer, phenol, flavonoids, tannin, alkaloids

INTRODUCTION

Insect-induced galls represent a highly specialized form of plant-insect interaction in which gall-inducing insects manipulate host plant tissues to form novel structures that provide nutrition and protection for the developing inducer (Raman *et al*., 2005). Gall formation is associated with localized physiological stress in host plants and involves extensive cellular hypertrophy and hyperplasia, leading to the differentiation of specialized tissue zones such as nutritive and mechanical zones (Mishra *et al*., 2020). Gall inducing insects are known to modulate host defense signaling pathways through the production or manipulation of phytohormones such as salicylic acid, jasmonic acid and, ethylene, thereby influencing the synthesis and accumulation of secondary metabolites (Bennet and Wallsgrove, 1994; Erb *et al*., 2012). The production of plant secondary metabolites in galled tissues is strongly dependent on the specific insect-plant interaction (Dicke, 1999). Among these metabolites, phenolic compounds and alkaloids are particularly important due to their defensive roles against herbivores, phytophagous insects and pathogens (Cornell and Hawkins, 2003; Lattanzio, 2013). Phenolic compounds, including phenols, flavonoids and tannins constitute one of the largest and widely distributed groups of plant secondary metabolites and are frequently reported to accumulate in higher concentrations in gall tissues, especially in the outer mechanical layers (Abrahamson *et al.,* 1991; Bronner, 1992; Cuevas- Reyes *et al.,* 2004; Mishra *et al.,* 2020). In contrast, inner nutritive tissues often

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contain reduced levels of phenolics, which may facilitate larval feeding by minimizing chemical deterrence (Ferreira *et al.,* 2017; Tataroglu *et al.,* 2023). Alkaloids and terpenoids have also been reported to function as chemical defenses in galls, acting as feeding deterrents and contributing to protection against natural enemies (Amorim *et al*., 2017; Kuster *et al.,* 2019).

Glycosmis pentaphylla (Retz.) DC. (Rutaceae) is an ethnobotanically important shrub or small tree widely distributed across south and southeast Asia and Australia. The species is well known for its medicinal properties (Babu and Nair, 2019; Babu and Radhamany, 2021). Leaves of *G. pentaphylla* attacked by *Lasioptera* sp. (Diptera, Cecidomyiidae) inducing galls. The present study investigates the variation in phenols, flavonoids, tannins, and alkaloids between galled and ungalled leaves of *G. pentaphylla*, with the aim of elucidating the biochemical consequences of gall.

MATERIALS AND METHODS

Cecidomyiid *induced g*alls along with the leaves and ungalled leaves (Fig 1), were collected from the 3rd to 6th phytomeres below the shoot apex to ensure comparable leaf and gall developmental stages (Motta *et al.*, 2005) from Janakikkad (11.64253° N; 75.78353° E) during 2021-2022. The galls (excluding all leafy part) were washed thoroughly, dissected for removing insects and its remnants like exuviae, shade dried and powdered in a mixer and used for the solvent extraction. For the sample preparation, the dried samples of the galls and ungalled leaves (both 15g) were extracted separately with methanol (99%) using a Soxhlet apparatus (15 cycles) and the extracts was evaporated to dryness to obtain the crude extracts of the galls and the ungalled leaves.

Quantitative analysis of total phenol, flavonoids, tannin, and alkaloids in galls and ungalled leaves was done by standard methods.

The total phenolic content was determined using slightly modified version of Folin and Ciocalteu reagent method described by Makkar (2003). The sample and standard reading were made using spectrophotometer at 725nm against the reagent blank. The stock standard solution was made by dissolved 10mg of gallic acid in methanol and made up to 10ml in a standard flask. Then 0.05ml of phenol extract of sample and control sample was pipetted into a series of test tubes, the contents in the all test tubes were made up to 1ml with distilled water and performed the analyses in triplicates. Another test tube marked as 'B' with 1ml of distilled water serve as blank. Then 0.5ml of Folin-Ciocalteu reagent (1 N) was added to each test tubes including the blank, and allowed to stand for 5 min at 30-32°C. After that, 2.5ml of sodium carbonate (5%) was added to all test tubes, incubated for 40 min in dark at room temperature. Measured the absorbance of the blue colour developed against the reagent blank at 725nm using spectrophotometer. The data was used to estimate the total phenol content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

The flavonoids estimation was carried out by slightly modified version of aluminium chloride (Zhishen *et al*., 1999). This method is based on the formation of the flavonoids aluminium complex, which has an absorptivity maximum at 510nm. The stock standard solution was made by dissolved 20mg of Rutin in methanol and made up to 20ml in a standard flask. Then added 0.5ml of extract of the sample and control into series of test tubes. Performed the analyses in triplicates. Made up all contents of all test tubes to 1ml with distilled water. Another test tube marked as 'B' with 1 ml of distilled water serve as blank. Then 0.15ml of 5% Sodium nitrite was added to all test tubes and incubated for 5 min at 30-32°C. After that 0.15ml of 10 % Aluminium chloride was added to all the test tubes and incubated for 6 min at room temperature. Then 2ml of 4% Sodium hydroxide was added to all the test tubes and made up to 5ml using distilled water. Allowed to stand for 15 min at 30-32°C and measured the absorbance of pink colour developed against the reagent blank at 510nm using spectrophotometer. The standard graph was plotted by concentration of Rutin on X - axis and respective absorbance on Y- axis. Calculated the amount of flavonoids in the samples and expressed as mg

Rutin equivalents/g sample.

Total tannin was estimated by standard methods of Bray and Thorpe (1954). The stock standard solution was made by dissolved 10mg of Tannic acid in methanol and made up to 10ml in a standard flask. One ml of the sample was mixed with 5ml of freshly prepared vanillin hydrochloride reagent and incubated at room temperature for 20 minutes. Absorbance was read at 510nm against reagent blank.

The total alkaloids were done by slightly modified version of Harborne (1973). The sample each at 0.25g was taken in 5ml beaker, added 10ml of acetic acid (10%) in ethanol and allowed to stand for 4 h. Then it was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle overnight and the precipitate was centrifuged for 15 min at 3000 rpm and 30-32°C. Collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

RESULTS AND DISCUSSION

The present study demonstrates significant alterations in secondary metabolite profiles between galled and ungalled leaves of *G. pentaphylla*. Galled tissues exhibited significantly higher concentrations of total phenolics, tannins and alkaloids, whereas total flavonoid content was significantly reduced compared to ungalled leaves.

The total phenolic content in galls and ungalled leaves was quantified using a standard curve of Gallic acid ($y = 0.008x+0.012$, $R^2 = 0.998$). Gall tissues exhibited a significantly higher phenolic content (9.45 ± 1.30 mg/g GA) than ungalled leaves (6.12 ± 1.25 mg/g GA). A paired-samples t-test confirmed that the difference was not statistically significant, $p > 0.05$. The elevated phenolic concentration in gall tissues is consistent with previous studies establishing increased accumulation of phenolic compounds in insect-induced galls (Abrahamson *et al*., 1991; Hartley, 1998; Connor *et al*., 2012; Hall *et al.,* 2017). In cecidomyiid-induced galls, phenolic compounds are often concentrated in mechanically protective tissues, where they may deter herbivores and natural enemies (Mishra and Patni, 2008; Mishra *et al*., 2020). The increased phenolic levels observed in *G. pentaphylla* therefore suggests defensive response mediated by the gall inducer.

Total flavonoid content was estimated using a rutin standard curve ($y = 0.0015x+0.079$, $R^2 = 0.996$). Gall tissues contained significantly lower flavonoid levels (9.15 ± 1.67 mg/g R) compared to ungalled leaves (17.13 ± 2.40 mg/g R). The difference was statistically significant ($p < 0.05$). The reduction in flavonoid concentration within gall tissues contrasts with the general trend of increased phenolic accumulation in galls. Flavonoids can exert toxic or inhibitory effects on insect development, particularly during early larval stages (Guimarães *et al*., 2021). Suppression of flavonoid synthesis within gall tissues may therefore represent a strategic manipulation by the gall inducer to create a favourable nutritive environment. Similar patterns have been reported in gall-inducing systems where inner gall tissues are characterized by reduced defensive compounds, facilitating larval feeding (Abrahamson *et al.,* 1991; Hartley and Lawton, 1992), while Motta *et al.* (2005) did not detect any flavonoids or flavones in galls.

Tannin content was quantified using a tannic acid standard curve ($y = 0.007x + 0.0018$, $R^2 = 0.98$). Galled tissues showed a significantly higher tannin concentration (22.73 ± 0.46 mg/g T) compared to the ungalled leaves (6.06 ± 1.09 mg/g T). Statistical analysis indicated a highly significant difference between the two tissue types ($p < 0.05$). High tannin accumulation in galls has been widely documented and is often associated with defensive functions (Abrahamson *et al*., 1991; Connor *et al*., 2012). Tannins are known to reduce tissue palatability and inhibit digestion in herbivores, thereby protecting gall tissues from external feeders and natural enemies (Hartley, 1998). In cecidomyiid-induced galls, tannins are frequently localized in outer cortical and sclerenchymatous tissues (Mishra and Patni, 2008, Mishra *et al*., 2020). This significantly

Fig. 1 *Glycosmis pentaphylla* (Retz.) DC. leaves - **A**: ungalled, **B**: induced galls by the *Lasioptera* sp.

higher tannin concentration observed in *G. pentaphylla* galls supports the hypothesis that tannin accumulation contributes to gall defense while maintaining a relatively less defended inner nutritive zone.

Alkaloid concentration was substantially higher in galled tissues (120 ± 0.001 mg g^{-1}) compared to ungalled leaves (30 ± 0.015 mg g^{-1}). This difference was statistically significant ($p < 0.05$). Alkaloids are well-known defensive compounds their accumulation in galls has been previously reported to enhance chemical defense for gall inducers (Mani, 1964). Feeding activity of young gall-inducing insects has been shown to influence host biochemical pathways, leading to elevated alkaloid concentrations (Kuster *et al.,* 2020). In *G. pentaphylla*, alkaloids are naturally abundant in leaves (Babu and Radhamany, 2021).

In conclusion, the results indicate that gall induction in *G. pentaphylla* leads to selective increase in concentration of phenolics, tannins, and alkaloids, accompanied by a reduction in flavonoids. Such patterns support the nutritional and defensive compartmentalization hypotheses, wherein gall inducers manipulate host metabolism to create a protective outer gall structure while maintaining a chemically suitable environment for larval development (Abrahamson *et al.,* 1991; Hartley and Lawton, 1992; Isaias *et al.,* 2014; Ferreira *et al.,* 2017). These findings highlight the complex biochemical reprogramming associated with insect-induced gall formation and its implications for plant defense and insect survival.

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