

Research Article

Preliminary studies of antioxidant and anti-inflammatory activities in methanol extracts of mistletoe (*Dendrophthoe falcata*) in guava (*Psidium guajava*)

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ABSTRACT

Mistletoe's antioxidants and anti-inflammatory properties, attributed to bioactive compounds, make it a potential natural remedy for oxidative stress and inflammation-related ailments. This study is focused on evaluating the antioxidant and anti-inflammatory potential of mistletoe (*Dendrophthoe falcata*) grown on guava (*Psidium guajava*). Cold extraction with methanol was used to maximize the extraction of heat-sensitive bioactive compounds. Samples were collected from three guava trees hosting *Dendrophthoe falcata* mistletoe, including guava (S₁L, S₂L, S₃L) and mistletoe leaves (S₁M, S₂M, S₃M). The mistletoe's antioxidant activity was evaluated through total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ferric-reducing power analysis. The mistletoe leaf sample (S₃M) from tree 03 displayed the highest TPC (169.46 ± 2.06 GAE mg/g) and TFC (46.16 ± 1.15 CE mg/g). S₃M also exhibited the lowest IC₅₀ value (0.091 ± 0.001 mg/mL) in the DPPH test, indicating strong radical scavenging activity. The FRAP assay yielded a value of 0.523 ± 0.010 mg/g BHT equivalent for S₃M. Positive correlations were observed between TPC, TFC, and antioxidant activities. Additionally, the mistletoe leaf samples (S₃M) demonstrated significant anti-inflammatory effects in the heat-induced hemolysis assay (IC₅₀ = 488.302 ± 23.407 µg/mL) and egg albumin denaturation assay (IC₅₀ = 311.582 ± 12.404 µg/mL), suggesting potential anti-inflammatory properties. The host leaf sample from host tree 03 displayed higher antioxidant activity (TPC: 239.06 ± 2.45 mg/g, TFC: 65.03 ± 1.65 mg/g, IC₅₀ for DPPH: 0.086 ± 0.004 mg/mL, FRAP: 0.565 ± 0.013 mg/g BHT equivalents) and anti-inflammatory activity (IC₅₀ for heat-induced hemolysis: 466.889 ± 23.417 µg/mL, IC₅₀ for egg albumin denaturation: 120.758 ± 19.190 µg/mL). Despite sample variations, mistletoe's antioxidant and anti-inflammatory properties were evaluated without hindrance. In conclusion, methanol extracts of mistletoe exhibit promising antioxidant and anti-inflammatory activity, require further research in this area.

Keywords: Guava, mistletoe, antioxidant activity, anti-inflammatory activity, methanol extract, cold extraction

Abbreviations: S₁L – Leaves from host 01, S₂L – Leaves from host 02, S₃L – Leaves from host 03, S₁M – Mistletoe leaves from host 01, S₂M – Mistletoe leaves from host 02, S₃M – Mistletoe leaves from host 03, DPPH – α, α-diphenyl-β-picrylhydrazyl, DMSO – Dimethyl sulfoxide, BHT- Butylated hydroxytoluene, GAE – Gallic acid equivalents, n – number of replicates, TPC – Total Phenolic Content, TFC – Total Flavonoid Content, RBC – Red blood cells, RT – Room temperature

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INTRODUCTION

Mistletoe is a parasitic plant with unique adaptations that allows it to grow on various host trees. It has been used in traditional medicine for centuries and is currently being studied for potential therapeutic properties. However, the safety and effectiveness of mistletoe extracts are still debated, requiring further research. Mistletoe is ecologically important, providing habitat and food for many animals. It also holds cultural and mythological significance, with a rich history in rituals and folklore. Despite its long history, mistletoe remains enigmatic, and more research is needed to understand its ecological role, physiological mechanisms, and cultural importance. Mistletoe is a traditional and alternative medicine often used in cancer treatment. It may offer potential benefits for cancer patients, such as improving quality of life and boosting immune function. Scientific research supports its use, but further studies are required for a better understanding of mistletoe's therapeutic effects (Hajto *et al.*, 1999).

This study has been focused on mistletoe species; *Dendrophthoe falcata* which is a sizable, bushy evergreen stem hemiparasite that relies on penetrating roots called haustoria to obtain nutrients from host trees. This epiphytic parasite is widely distributed across various host plants worldwide and poses significant threats to economically cultivated plants. It can be found on numerous host plants throughout India, particularly tropical trees such as guava, mango trees and occasionally on select timber-yielding trees. Recent reports indicate that *D. falcata* has expanded its host range and can now be observed growing on a variety of tree species (Subhashini *et al.*, 2019). *D. falcata*, also referred to as *Loranthus longiflorus* Desr., is a perennial climbing woody plant that thrives as a parasitic species. It is native to tropical areas, particularly in countries such as Australia, Bangladesh, China, India, Malaysia, Myanmar, Sri Lanka, and Thailand (Nickrent and Vidal-Russell, 2007). *Loranthus* species are serious parasites of a large variety of economic plants, both angiosperms and gymnosperms. At present *Loranthus spp.*, are destructive to economic plants in many parts of the world. *D. falcata* is a large bushy parasitic plant that grows on a variety of host plants in deciduous forests throughout India. The global distribution of *Loranthaceae* was categorized into seven geographic

regions: Australia, New Zealand, Asia, Africa, South America, Malaysia west of Wallace's line, and Malaysia east of Wallace's line (New Guinea). In India and Bangladesh, *Dendrophthoe falcata* is also referred to as "Farolla" and is utilized for treating asthma, wounds, ulcers, and pulmonary tuberculosis. It is also integrated into traditional medicines as an aphrodisiac, diuretic, astringent, and narcotic (Rafe *et al.*, 2018). In Sri Lanka, it is known as "Pilla" and is used in their ethnomedicinal system to treat cancerous tumors (Karunaratne and Uduwela, 2020). In Ayurveda, *Dendrophthoe falcata* is recognized as "Vanda" or "Bandaka" and is employed to address conditions such as diarrhea, swelling, renal calculi, and epilepsy (Shahare and Bodele, 2019).

Psidium guajava, commonly known as guava, is an important fruit crop and medicinal plant in tropical and subtropical countries. It has been widely used as fruit and in traditional medicine worldwide. The plant contains various phyto-constituents, particularly phenolic, flavonoid, carotenoid, terpenoid, and triterpene, which have shown beneficial biological activities. The extracts and metabolites of *P. guajava*, especially from its leaves and fruits, have been shown to possess pharmacological activities, including antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities (Gutiérrez *et al.*, 2008). Guava leaves also possess antioxidant properties attributed to the polyphenols they contain. Guava leaves contain various plant chemicals, including acids, sugars, oils, flavonoids, tannins, and terpenes. This rich composition makes guava leaves a promising candidate for various therapeutic applications (Joshep and Mini priya, 2011).

Antioxidant and anti-inflammatory compounds are vital for overall well-being and health. They neutralize harmful free radicals, reducing the risk of various health issues like aging, cardiovascular diseases, and cancer. The study on mistletoe's (*Dendrophthoe falcata*) antioxidant and anti-inflammatory properties is crucial due to its potential therapeutic applications in areas like cancer treatment, cardiovascular health, and immune system modulation (Patil *et al.*, 2011). Understanding mistletoe's bioactive compounds can aid in the development of new interventions for human health improvement. The main

objective of this study was to extract methanol from mistletoe leaves and host leaves samples from each guava host and assess their potential health benefits. This research aims to determine the total phenolic and total flavonoid content of the methanol extracts to understand their antioxidant properties. Additionally, the study will evaluate the antioxidant activity through DPPH radical scavenging and ferric reducing ability tests. Furthermore, the anti-inflammatory potential will be investigated using heat-induced hemolysis of RBC and egg albumin denaturation assay. By comparing the antioxidant and anti-inflammatory activity of the methanol extracts from mistletoe leaves and host leaves, this research seeks to shed light on their potential therapeutic applications and contribute to the development of new interventions for improving human health.

MATERIALS AND METHODS

Raw materials and sampling

Three guava (*Psidium guajava*) plants with infestation of mistletoe (*Dendrophthoe falcata*) were selected for the study from the following places in December 2022.

162, Magamma, Homagama, Sri Lanka (6.8173° N, 79.9943° E)

University of Kelaniya, Dalugama, Sri Lanka (6.9754° N, 79.9156° E)

Magamma East, Homagama, Sri Lanka (6°49'27.7"N 80°00'02.9"E)

Preparation of plant extracts

The raw leaves from each guava host plant and mistletoe (2.70 g) were taken into conical flasks separately, and methanol (50.0 mL) was added to each flask. Covered flasks with aluminum foils were placed on the shaker and allowed to stand for 48 hours in the dark. After 48 hours, methanol extracts (the supernatant) were poured into petri dishes and kept in the dark until they dried (3-5 days). The powdered form of the extracts was obtained.

Determination of total phenolic content (TPC)

TPC in the methanol extracts of each mistletoe and host plant samples were determined according to the folin-

ciocalteau method with slight modifications (Sato *et al.*, 1996). Gallic acid was used as the standard phenolic compound. Samples (125 µL) was mixed with folin-ciocalteau reagent (10% v/v, 625 µL). Sodium carbonate was added eight minutes later (7.5% w/v, 500 µL). Subsequently, the shaken mixture was allowed to stand for one hour at room temperature, and the absorbance was measured at 760 nm with respect to a blank. The total phenolic content was expressed as mg of gallic acid equivalent (GAE) per gram of extract.

Determination of total flavonoid content (TFC)

TFC in the methanol extract of each mistletoe and plant sample was determined according to the AlCl₃ colorimetric method with slight modifications (Atanassova, 2011). Sample extract (175 µL) was added to DI water and NaNO₂ solution (5% w/v, 53 µL). After incubating for 6 minutes at room temperature, AlCl₃ (10% w/v, 53 µL) was added, and after another 6 minutes, NaOH (4% w/v, 700 µL) was added, followed by the addition of deionized water (70 µL). The absorbance was measured at 500 nm with respect to the blank. The total flavonoid content of the samples was expressed as catechin equivalents.

Determination of radical scavenging activity of DPPH

The assay was carried out in a flat bottom 96-well microtiter plate according to the method described by Chatatikun and Chiabchalard (2013) with slight modifications (Chatatikun and Chiabchalard, 2013). Sample (160 µL) was mixed with methanolic DPPH solution (0.25 mM, 40 µL). The reaction mixture for 15 minutes at room temperature under dark conditions. The absorbance was measured at 517 nm with respect to a blank. The % inhibition was calculated using the following equation,

$$\% \text{ Scavenging activity} = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100$$

Determination of Fe³⁺ reducing ability (FRAP assay)

FRAP was carried out according to a method described in Senevirathne and Kotuwegedara (2009) with slight modifications (Senevirathne and Kotuwegedara, 2009). Sample (10 µL) was mixed with Sodium phosphate buffer (25 µL) and potassium ferricyanide (1% w/v, 25 µL). The mixture was incubated at 45°C for 20 minutes.



Figure 1: a) Identification of the attachment site (indicated by an arrow) of the mistletoe (*Dendrophthoe falcata*) to its host (the circled area). b) Enlarged view of the mistletoe attachment site to the host as depicted in image a. c) Depiction of mistletoe growth (*Dendrophthoe falcata*) on a host guava tree (*Psidium guajava*). d) Visual representation of mistletoe leaves (*Dendrophthoe falcata*) intertwined with the leaves of the host guava tree (*Psidium guajava*). Both mistletoe and host leaves can be observed. e) Illustration showing the separated mistletoe leaves (*Dendrophthoe falcata*). f) Illustration showing the guava (*Psidium guajava*) host leaves that have been taken off the tree

trichloroacetic acid (10% v/v, 25 μ L) was added to the reaction mixture and diluted with DI water (85 μ L). A freshly prepared ferric chloride solution (0.1% w/v, 17 μ L) was added to each sample at the end. Another incubation was carried out for 10 minutes. The absorbance was measured at 700 nm respects to a blank. The ferric reducing power of each sample were expressed as BHT equivalents.

Determination of anti-inflammatory activity by heat-induced hemolysis of RBC

The anti-inflammatory activity of the methanol extract of each mistletoe and plant sample was determined according to the heat-induced hemolysis with slight modifications (Hettihewa, 2021). Sample (1.0 mL) was mixed with the RBC suspension (100 μ L). The reaction mixture was mixed well using a vortex machine and incubated at 56°C for 30 minutes. After the incubation, they were allowed to come to the RT. The tubes were centrifuged at 4°C for 15 minutes. This resulted supernatants (200 μ L) were loaded into a microtiter plate, and absorbance values were measured at 560 nm. The same procedure was followed for the o-acetylsalicylic acid standard series. The experiment was carried out in triplicates and the percent. Inhibition of hemolysis was calculated according to the following equation.

$$\text{Inhibition (\%)} \text{ of hemolysis} = \left(\frac{V_c - V_t}{V_c} \right) \times 100;$$

Where, V_c = Absorbance of negative control, V_t = Absorbance of test sample

Determination of the anti-inflammatory activity by egg albumin denaturation assay

The egg solution (100 μ L) was mixed with the phosphate buffer (pH 6.3, 900 μ L) and plant extract of each sample (300 μ L). The reaction mixtures were heated in a water bath at 37°C for 15 minutes and at 70°C for 5 minutes. The tubes were cooled to RT, and the absorbance values were measured at 660 nm (J A B N, 2017). Respects to a blank. Acetyl salicylic acid was used as the positive control. Per cent inhibition was calculated using the equation given below:

$$\% \text{ inhibition of denaturation} = \left(1 - \frac{D}{C} \right) \times 100$$

Where, D – Absorbance of the test sample, C – Absorbance of the negative control

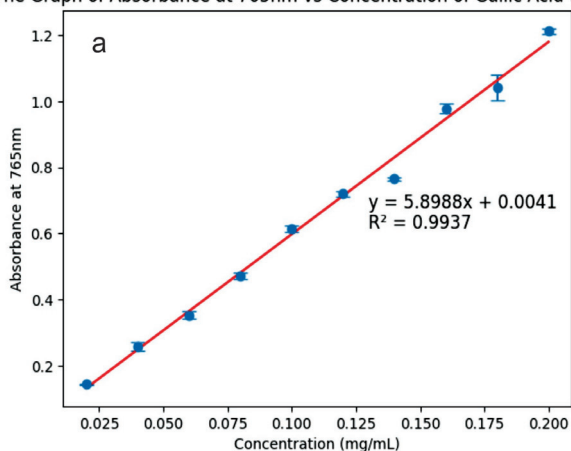
RESULTS AND DISCUSSION

The study is about determining the presence of antioxidant and anti-inflammatory activities in the mistletoe (*Dendrophthoe falcata*), which has been grown on the guava (*Psidium guajava*). Host leaves were tested to identify whether there is an influence of those properties of guava host leaves towards the properties of mistletoe leaves. Since most of the bioactive compounds responsible for the antioxidant and anti-inflammatory properties are heat sensitive, the cold extraction method involving lower temperatures was used for the extraction process to obtain a higher yield (Rodrigues *et al.*, 2015). Methanol was the solvent used due to its higher-yielding capacity toward the bioactive compounds (Truong *et al.*, 2019). Testing one host with mistletoe to detect the presence of specific activities will be meaningless, and it will lead to incorrect conclusions. Therefore, three guava host plants (S_1L , S_2L , S_3L) from the same variety (*Psidium guajava*) with the same array of mistletoe (S_1M , S_2M , S_3M) (*Dendrophthoe falcata*) were obtained from three different locations.

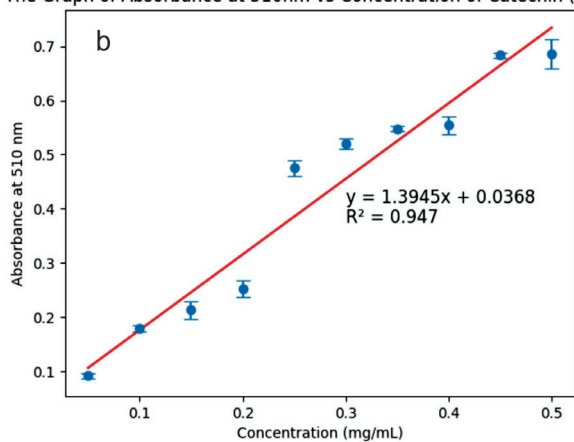
Total phenolic content (TPC) of the mistletoe and guava host leaf samples were determined as Gallic Acid Equivalents (GAE) using the gallic acid standard curve ($y=5.8995x+0.0041$) ($R^2=0.9937$) in Figure 2a. The TPC of each sample has been presented in Table 1. According to Table 1, guava host leaf samples had shown the higher TPC value than the mistletoe leaf samples. Mistletoe leaves obtained from host 03; S_3M had a higher TPC value (169.46 ± 2.06 mg/g) than other mistletoe samples while guava leaves obtained from host 02; S_2L (263.13 ± 1.04 mg/g) had a higher TPC value than other guava leaf samples. A higher total phenolic content (TPC) value in a sample implies that the sample contains a larger quantity of phenolic compounds. This suggests that the sample may have stronger antioxidant properties and may be considered to have a superior phenolic content in terms of quality.

Flavonoids are natural compounds found in plants that possess strong antioxidant properties. The total flavonoid content (TFC) of plants is strongly correlated with their antioxidant activity, making it an important parameter for evaluating their potential health benefits. TFC of each mistletoe leaf and guava leaf sample was obtained from the catechin standard curve ($y=1.3945x+0.0368$, $R^2=0.947$)

The Graph of Absorbance at 765nm vs Concentration of Gallic Acid (mg/mL)



The Graph of Absorbance at 510nm vs Concentration of Catechin (µg/mL)



The Graph of Absorbance at 700 nm vs Concentration(mg/mL)

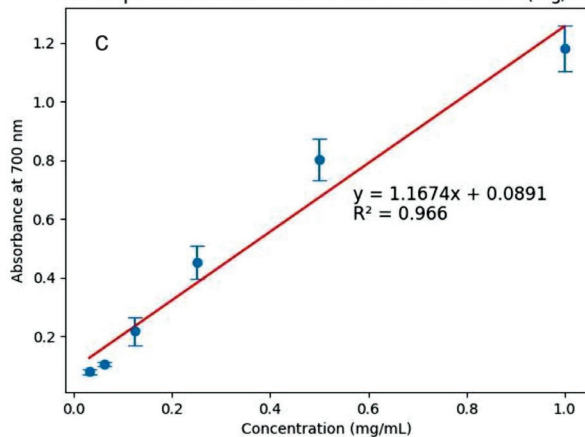


Figure 2: a) The standard curve of gallic acid for the determination of Total Phenolic Content (TPC), b) The standard curve of Catechin for the determination of Total Flavonoid Content (TFC), c) The standard curve of BHT for the determination of antioxidant activity through FRAP assay

(Figure 2b). Table 2 presents the TFC value of the samples as catechin equivalents(mg/g). Table 2 revealed that the total flavonoid content (TFC) was found to be higher in guava host leaf samples as compared to the mistletoe leaf samples. Among the mistletoe samples, mistletoe leaves collected from host 03; S₃M, had a higher TFC value of 46.16 ± 1.15 mg/g compared to other mistletoe leaf samples. On the other hand, among the guava leaf samples, the TFC value was found to be the highest in leaves obtained from host 02; S₂L, with a TFC value of 68.86 ± 0.76 mg/g compared to other guava leaf samples. A higher TFC value indicated that the sample being tested contains a greater number of flavonoids. This may suggest that the sample has a greater potential for antioxidants, and it may also indicate that the sample is of higher quality in terms of its flavonoid content.

The sensitivity of the sample towards the DPPH radical scavenging activity is determined by the IC₅₀ value of that sample. If the IC₅₀ value is low, that means the radical

Table 1: Total phenolic content in the samples

Sample	Total phenolic content (TPC) as GAE/(mg/g)
S ₁ M	142.90 ± 1.19 ^d
S ₁ L	175.79 ± 8.21 ^c
S ₂ M	96.35 ± 4.90 ^e
S ₂ L	263.13 ± 1.04 ^a
S ₃ M	169.46 ± 2.06 ^c
S ₃ L	239.06 ± 2.45 ^b

Each data point represents the mean ± SD (n=3). Different Means that do not share a letter are significantly different. Letters indicate a significant difference (p≤0.05).

Table 2: Total flavonoid content in the samples

Sample	Total flavonoid content (TFC) as catechin equivalents/(mg/g)
S ₁ M	45.01 ± 2.13 ^c
S ₁ L	53.56 ± 1.33 ^b
S ₂ M	18.58 ± 1.08 ^d
S ₂ L	68.86 ± 0.76 ^a
S ₃ M	46.16 ± 1.15 ^c
S ₃ L	65.03 ± 1.65 ^a

Each data point represents the mean ± SD (n=3). Different Means that do not share a letter are significantly different. Letters indicate a significant difference (p ≤0.05).

scavenging occurs in a lower concentration which marks a significant antioxidant potential in the sample. Table 3 shows that the DPPH radical scavenging activity was found to be higher in guava host leaf samples as compared to the mistletoe leaf samples due to their lower IC_{50} values. Among the mistletoe samples, mistletoe leaves collected from host 03; S_3M had the lowest IC_{50} value (0.091 ± 0.001 mg/mL) compared to other mistletoe leaf samples. On the other hand, among the guava leaf samples, the IC_{50} value was the lowest in leaves obtained from host 02; S_2L , with the IC_{50} value of 0.059 ± 0.004 mg/mL compared to other guava leaf samples. In simpler terms, the results in Table 3 indicate that the guava host leaf samples had better DPPH radical scavenging activity compared to the mistletoe leaf samples and that the mistletoe leaves from host 03; S_3M and guava leaves from host 02; S_2L had the highest DPPH radical scavenging activity among their respective sample groups.

FRAP assay indicates the ferric reducing potential of a certain sample. Higher ferric-reducing capability is frequently thought to be a sign of greater antioxidant potential. Ferric-reducing ability was expressed as BHT equivalents(mg/g) and it was obtained from the linear regression equation of the calibration curve ($Y=1.1674X+0.0891$, $R^2= 0.966$) (Figure 2c). As the results mentioned in Table 4, all the host leaf samples (*Psidium guajava*) had a higher value for the ferric-reducing ability than the mistletoe (*Dendrophthoe falcata*), samples with respect to BHT equivalents which implies a higher antioxidant potential for the host leaf samples (*Psidium guajava*).

Table 3: The IC_{50} values of the samples by DPPH assay

Sample	IC_{50} value(mg/mL)
BHT standard	0.081 ± 0.002^e
S_1M	0.107 ± 0.004^b
S_1L	0.082 ± 0.004^e
S_2M	0.129 ± 0.08^a
S_2L	0.059 ± 0.004^d
S_3M	$0.091 \pm 0.001^{b,c}$
S_3L	0.086 ± 0.004^e

Each data point represents the mean \pm SD (n=3). Different Means that do not share a letter are significantly different. Letters indicate a significant difference ($p \leq 0.05$).

Table 4: Ferric Reducing Ability as BHT equivalents in the samples

Sample	Ferric reducing ability as BHT equivalents (mg/g)
S_1M	0.319 ± 0.017^d
S_1L	0.433 ± 0.012^c
S_2M	0.243 ± 0.008^e
S_2L	0.525 ± 0.013^b
S_3M	0.523 ± 0.010^b
S_3L	0.565 ± 0.013^a

Each data point represents the mean \pm SD (n=3). Different Means that do not share a letter are significantly different. Letters indicate a significant difference ($p \leq 0.05$).

At the consideration of correlation studies, it can help to understand the strength and direction of the relationship between variables. In this case, it is engaging in understanding how the presence of phenolic and flavonoid compounds in a sample relates to its antioxidant activity. A strong positive correlation between TPC and TFC with antioxidant activity would indicate that the higher the TPC and TFC values in a sample, with the higher antioxidant activity. This information can help determine the potential health benefits of natural products and aid in developing new antioxidant-rich products. Table 5 shows the Pearson correlation coefficients for TPC, TFC and antioxidant activity (DPPH radical scavenging and ferric-reducing activity). These coefficients measure the strength of the relationship between variables and can range from -1.00 to +1.00. A coefficient of 0 indicates no connection between the variables. Coefficients closer to +1 or -1 indicate a strong positive or negative correlation, respectively. According to Fidrianny, if Pearson correlation coefficient value[®] is in the range of $0.60 \leq r \leq 0.97$, it indicates a positive and strong correlation, and if it is $-0.60 \leq r \leq -0.97$, it indicates a negative and strong correlation. According to the results in Table 5 it indicates that there is a strong positive correlation between TPC and TFC with antioxidant activities. Therefore, based on these findings, it can be

Table 5: Pearson's correlation coefficients among TPC, TFC, and antioxidant activities

Sample	$1/IC_{50}$ (DPPH radical scavenging activity)
TPC	0.842
TFC	0.888

concluded that TPC and TFC are closely related to antioxidant activities.

According to the results presented in Table 6 with the IC_{50} values obtained from the heat-induced hemolysis of RBC for the determination of anti-inflammatory potential of the samples, host leaf samples had shown lower IC_{50} values than the mistletoe leaf sample. Within the samples of mistletoe, the leaves collected from host 03 (S_3M) had the lowest IC_{50} value ($488.302 \pm 23.407 \mu\text{g/mL}$) compared to other mistletoe leaves. Whereas, among the samples of guava leaves, the leaves collected from host 03 (S_3L) showed the lowest IC_{50} value ($0.059 \pm 0.004 \mu\text{g/mL}$) compared to other guava leaf samples. A lower IC_{50} value indicates the higher potential for the inhibition of inflammation.

Egg albumin denaturation assay was the other method used for the analysis of anti-inflammatory activity of the sample. At the consideration of the results in Table 7, it showed that host leaf samples have greater potential in the inhibition of the denaturation of egg albumin. While sample

S_3M ($311.582 \pm 12.404 \mu\text{g/mL}$) contains the higher activity by giving lower IC_{50} than other mistletoe samples, sample S_1L ($100.249 \pm 6.312 \mu\text{g/mL}$) indicates a higher activity with lowest IC_{50} than other host leaf samples.

CONCLUSION

In conclusion, this study aimed to investigate the antioxidant and anti-inflammatory properties of mistletoe (*Dendrophthoe falcata*) parasitizing the guava plant (*Psidium guajava*), exploring the potential impact of host plant characteristics on mistletoe features. Through comprehensive analysis of phytochemical properties, it was observed that while host leaf samples exhibited higher overall antioxidant activity, mistletoe leaf samples displayed notable levels of key antioxidant parameters such as total phenolic content, total flavonoid content, DPPH radical scavenging activity, and ferric-reducing power. This indicates a significant presence of antioxidant compounds in mistletoe leaves, suggesting their potential as a natural source of antioxidants. Moreover, the study revealed considerable anti-inflammatory activity in mistletoe leaf samples as demonstrated by their performance in heat-induced hemolysis and egg albumin denaturation assays. Although host leaves exhibited superior anti-inflammatory activity overall, mistletoe leaves displayed noteworthy values in both assays, underscoring their potential as a source of anti-inflammatory compounds. Collectively, these findings underscore the promising antioxidant and anti-inflammatory attributes of mistletoe grown on guava, opening avenues for future research to identify and characterize the specific active compounds responsible for these properties. As such, this study contributes valuable insights into the potential application of mistletoe as a natural source of antioxidants and anti-inflammatory agents, highlighting its importance for further investigations in the field of natural medicine and therapeutic development.

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Table 6: The IC_{50} values of the samples for heat-induced hemolysis

Sample	IC_{50} value ($\mu\text{g/mL}$)
S_1M	634.566 ± 18.799^b
S_1L	552.699 ± 13.127^c
S_2M	736.769 ± 31.280^a
S_2L	640.506 ± 13.845^b
S_3M	488.302 ± 23.407^d
S_3L	$466.889 \pm 23.417^{d,c}$

Each data point represents the mean \pm SD (n=3). Different Means that do not share a letter are significantly different. Letters indicate a significant difference ($p \leq 0.05$).

Table 7: The IC_{50} values of the samples for egg albumin denaturation assay

Sample	IC_{50} value ($\mu\text{g/mL}$)
S_1M	391.257 ± 13.284^b
S_1L	100.249 ± 6.312^c
S_2M	435.932 ± 20.323^b
S_2L	100.896 ± 7.868^c
S_3M	$311.582 \pm 12.404^{b,c}$
S_3L	120.758 ± 19.190^c

Each data point represents the mean \pm SD (n=3). Different Means that do not share a letter are significantly different. Letters indicate a significant difference ($p \leq 0.05$).

Conflict of interest

Authors have no conflict of interest to declare.

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