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Determination of Phytochemical Composition of *D. falcata*, parasitizing *S. simentia*

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ABSTRACT

The present study was undertaken to evaluate the phytochemical composition, total phenolic content (TPC), and total flavonoid content (TFC) of leaf extracts of *Dendrophthoe falcata* and *Senna siamea* using different organic solvents. Leaf powders were sequentially extracted with n-hexane, petroleum ether, toluene, chloroform, ethyl acetate, acetone, and methanol, and the extraction yield was determined. Among the extracts, *D. falcata* exhibited higher extraction yields than *S. siamea*, with ethyl acetate (2.29%), acetone (1.99%), and methanol (1.78%) producing the highest yields. In contrast, methanol (1.28%), acetone (1.23%), and chloroform (1.11%) extracts of *S. siamea* showed comparatively higher recovery. Preliminary phytochemical screening revealed the presence of carbohydrates, reducing sugars, monosaccharides, pentose sugars, proteins, and amino acids in all extracts of both plant species. Qualitative analysis of secondary metabolites demonstrated the occurrence of phenols, flavonoids, tannins, alkaloids, glycosides, phytosterols, terpenoids, saponins, and phlobatannins, with solvent-dependent variations. Ethyl acetate and methanol extracts of *D. falcata* exhibited positive responses for almost all secondary metabolites, whereas methanol and acetone extracts of *S. siamea* showed the broadest phytochemical profile. Quantitative estimation indicated that *D. falcata* possessed higher total phenolic and flavonoid contents than *S. siamea*. The highest TPC was observed in the ethyl acetate extract of *D. falcata* (96.1%), followed by methanol (92.8%) and acetone (88.6%), while methanol extract of *S. siamea* showed the highest TPC (89.5%). Similarly, the highest TFC was recorded in the ethyl acetate extract of *D. falcata* (94.8%), followed by methanol (90.4%) and acetone (88.1%). In *S. siamea*, methanol (93.5%) and acetone (89.3%) extracts exhibited the highest flavonoid contents.

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1. INTRODUCTION:

Plants have long served as vital sources of therapeutic agents for human health, forming the foundation of traditional medicine systems such as Ayurveda, Siddha, Unani, and Traditional Chinese Medicine (Fabricant and Farnsworth, 2001). These medicinal properties are largely due to phytochemicals, which are secondary metabolites synthesized by plants in addition to their primary metabolic products. While primary metabolites, such as carbohydrates, proteins, and lipids, are essential for growth and development, secondary metabolites perform adaptive functions aiding in defense against pathogens, herbivores, and environmental stress (Harborne, 1998). Phytochemicals exhibit a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects

(Balunas and Kinghorn, 2005). Their structural diversity and bioactivity have made them key contributors to modern drug discovery notable examples include morphine (*Papaver somniferum*), quinine (*Cinchona* spp.), artemisinin (*Artemisia annua*), paclitaxel (*Taxus brevifolia*), and salicylic acid (from *Salix* species)

Plants have been a rich source of bioactive compounds for centuries, serving as the foundation for traditional medicine systems and modern pharmaceutical discoveries (Fabricant & Farnsworth, 2001). These bioactive molecules, broadly referred to as *phytochemicals*, are secondary metabolites produced by plants to aid in growth, reproduction, and defense against environmental stresses, pathogens, and herbivores (Harborne, 1998). Unlike primary metabolites, which are essential for basic physiological processes, secondary metabolites possess unique structural diversity and are often responsible for the plant's therapeutic and pharmacological properties (Balunas and Kinghorn, 2005).

Phytochemical analysis plays a critical role in identifying, characterizing, and quantifying these compounds, thereby providing insights into their potential biological activities (Thupurani et al., 2013; Murali Krishna Thupurani et al., 2013a; Murali Krishna Thupurani et al., 2013b; Gorripati et al., 2018; Thupurani Murali Krishna, 2018; Racha Srikanth et al., 2020; Gujjari Sreehitha Pratap et al., 2021; Challa Surekha et al., 2022; Srilekha Konakanchi et al., 2023; Praveen Kumar 2024; Ganta Prashanthi, 2024; Ganta Prashanthi, 2025). Common classes of phytochemicals include alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, and glycosides, each contributing to a wide range of bioactivities such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects (Pietta, 2000). The qualitative and quantitative profiling of these compounds not only enhances our understanding of plant chemistry but also forms the basis for bioassay-guided isolation and drug development (Cowan, 1999).

Dendrophthoe falcata (*D. falcate*) (L.f.) Ettingsh., commonly called the Indian Mistletoe, is a hemiparasitic plant belonging to the family Loranthaceae. It attaches to the branches of host trees using haustoria to extract water and nutrients while performing photosynthesis. Traditionally, *D. falcata* has been used for treating wounds, ulcers, skin infections, asthma, menstrual disorders, and certain cancers. Phytochemical investigations of *D. falcata* have reported the presence of flavonoids, tannins, terpenoids, phenolics, saponins, and glycosides, many of which are associated with

antioxidant, antimicrobial, and anti-inflammatory properties.

Senna siamea (Lam.) H.S. Irwin & Barneby, known as the Kassod Tree, is a leguminous species valued for timber, ornamental use, and medicinal properties. It contains anthraquinones, flavonoids, and phenolic compounds known for antimicrobial, hepatoprotective, and antioxidant activities. When *D. falcata* parasitizes *S. siamea*, the phytochemical profile of the mistletoe may be influenced by secondary metabolites translocated from the host. Such host-parasite interactions can alter the concentration and diversity of active compounds, thereby affecting the plant's therapeutic potential.

Despite the extensive ethnomedicinal use of *D. falcata*, limited studies have explored its phytochemical composition when growing specifically on *Senna simentia* (*S. simentia*). Understanding this profile is critical for establishing chemical-bioactivity correlations for antioxidant, antimicrobial, and anti-inflammatory effects, identifying compounds with potential pharmacological value, adding to the ethnopharmacological database on host-specific mistletoe chemistry.

In this paper, the extraction and phytochemical composition of *D. falcata*, parasitizing *S. simentia* H.S. Irwin & Barneby, along with its host plant, are explored through systematic qualitative and quantitative assays.

MATERIALS AND METHODS:

Solvents and Standards:

All the solvents (Ethyl acetate, Acetone, toluene and Methanol) are purchased from Merck chemicals India. The reference drugs (Phenolic acids) used in the study was purchased from Sigma-Aldrich. All other chemicals purchased are HPLC grade.

Reagents:

The reagents include *Barfoed*, *Hager*, *Bial*, *Mayer*, *Wagner*, *Molisch*, *Burchard*, *Benedict solution*, *Selwinoff*, *Dragendorff*, *Fehling's solution A and B*, *Folin-Ciocalteu*, were purchased from Sigma-Aldrich.

Collection and Authentication of Plant Material:

The Leaves of *D. falcata* and *S. simentia* were collected from the RTC colony, chengicherla cross, Peerzadiguda, Hyderabad, Telangana 500098. Dr. Mustafa, taxonomist, Professor, Department of Botany, Kakatiya University, Warangal performed the authenticity.

Extraction:

The leaves of *D. falcate* and *S. simentia* are washed

in distilled water to remove the dust and other accumulated dirt. The leaves were cleaned in the lab with an absorbent cloth and allowed to air dry in the shade. The leaves were ground to a fine powder using a mechanical grinder. Each powder (250g) was extracted using n-Hexane, Petroleum ether, toluene, Chloroform, ethyl acetate, acetone, and methanol. The solvents are evaporated using rotating evaporators at the required boiling point temperature. The yield of the crude extract was determined. For upcoming research, all of the unprocessed extracts are retained in storage at -20 °C.

Preliminary Phytochemical Analysis:

The Leaf crude extracts of both plants were separately dissolved using 5% DMSO and used for determination of different phytochemicals present in leaf crude extracts. The tests are carried out by the standard methods described by Harbone (1973).

Screening for Carbohydrates:

Molisch's Assay:

Add 1 or 2 drops of alpha naphthalene solution to 2ml of leaf crude extracts, and shake the tubes for 2 to 3 minutes. Gently pour 1ml of concentrated H₂SO₄ through the walls of the tube. At the intersection of two distinct layers, look for the creation of a thick violet colour. This suggests that there are carbohydrates in the sample.

Benedict's Assay:

Add around 2 ml of leaf crude extracts to 2 ml of freshly prepared Benedict's solution. The tubes were kept in water bath for about 10 minutes. The presence of reducing sugars in the sample was detected by looking for a colour shift to yellow, green or red.

Fehling's Assay for Reducing Sugars:

When leaf crude extracts are mixed with 1ml of Fehling's A and B solutions and incubated in a water bath for 5 to 10 minutes, if a brick-red precipitate appears; it indicates presence of reducing sugars in samples.

Screening of Monosaccharides:

Barfoed's Assay:

To 2 ml of leaf crude extracts, add 2 ml of Barfoed's reagent and incubate using water bath for about 3-5 min. The formation of red color confirms as positive result for monosaccharides.

Screening for Pentose Sugars:

Bial's Assay:

To 2ml of leaf crude extracts, about 4 ml of Bial's reagent was added and is incubated at room temperature using water bath for about 8-10 min. If blue-green color appears, it confirms positive result

for the pentose sugars.

Screening for Hexose Sugars:

Cobalt Chloride Assay:

2ml of Cobalt Chloride solution should be combined with approximately 3ml of leaf crude extract, both should be submerged in water for 1-2 minutes. Next, add 3 or 4 drops of NaOH. Observe for colour change as the lower layer turns purplish while the upper layer turns greenish-blue. This formation indicates the existence of glucose, fructose, or a mixture of these molecules.

Selwinoff's Assay:

To 1ml of leaf crude extracts of *E. Paniculata*, 5–6 ml of Selwinoff's reagent is added. For 2-3 minutes, the tubes were kept in the water bath at room temperature. The samples were examined for the development of a red colour. This shows that the ketoses are present in the sample.

Tollen's Phluroglucinol Assay:

To 2ml of leaf crude extracts, add 4ml of 0.5% Phluroglucinol and 2.5ml of conc.HCl are combined. The tubes were heated and watched to see if a yellow or red tint developed. This demonstrates that hexose carbohydrates, such as galactose are present in the samples.

Screening for Proteins:

Biuret Assay:

Approximately, 3ml of leaf crude extract is taken and 2ml of NaOH is added. Then add 2-3 drops of 1% CuSO₄. The tubes were observed for colour change to pink or violet. This suggests that proteins are present in the sample.

Xanthoproteic Assay:

Add 1ml of conc.H₂SO₄ to 3ml of leaf crude extracts, and watch for white precipitate that becomes yellow as it boils. Furthermore, adding 1ml of NH₄OH will turn this yellow precipitate to orange. This suggests that the samples include proteins containing tryptophan and tyrosine residues.

Test for Aminoacids:

Ninhydrin Assay:

About 2-3 drops of 5% v/w Lead Acetate are combined with 3ml of leaf crude extracts and the mixture is left in the water bath for 10 minutes. Observe the tubes whether they become blue or purple. This indicates that the test samples contain amino acids.

Detection of Secondary Metabolites:

Creening for Phenols:

A few drops of FeCl₃ were added to the leaf crude extracts of in order to conduct the phenol detection

test. The presence of phenols is indicated by observing intense colour.

Screening for Tannins:

Potassium Dichromate Test:

To 1ml of each of leaf crude extracts from, add 1% potassium dichromate and were incubated in water for 2-3 minutes. Tannins are present because of the thick precipitate.

NaCl Test:

Combine 1ml of leaf crude extracts with 1ml of distilled water. After 2-3 minutes at room temperature, add a pinch of NaCl and check for precipitation. The presence of tannins is indicated by precipitation.

Screening for Glycosides:

Baljet's Assay:

2ml of sodium picrate are incubated with 2ml of leaf crude extracts. The presence of glycosides is indicated by the bright orange colour.

Legal's Assay:

2 ml of leaf crude extracts are mixed with 1ml of pyridine and add few drops of Sodium Nitroprusside solution. The samples were observed for pink to red color, which confirms glycosides in the tested extracts.

Screening for Phytosterols:

Liebermann's Assay:

The leaf crude extracts of were solubilized in conc. HCl. Few drops of aqueous sodium nitrate are added along the side walls of the tube. The reaction is observed for color change from red to blue which is a positive result for phytosterols in the extracts.

Liebermann-Burchard's Assay:

2ml of leaf crude extracts were dissolved in chloroform and add 2 ml of Liebermann Burchard's reagent and incubate at room temperature. The tubes were observed for the development of green color which indicates for the phytosterols presence.

Screening for Flavonoids:

Lkaline Reagent Assay:

To 2ml of leaf crude extracts, few drops of 1 N NaOH is added and incubated at room temperature. It is observed for disappearance of color from yellow to colorless. This indicates the presence of flavonoids.

Lead Acetate Assay:

To 2ml of leaf crude extracts, few drops of Lead Acetate solution is added and observed for yellow color precipitation it indicates presence of flavonoids in the crude extracts.

Screening for Alkaloids:

Mayer's Assay:

1ml of leaf crude extracts was mixed with 1ml of Mayer's reagent. The samples are maintained at room temperature and are observed for reddish-brown precipitate, which confirms the presence of alkaloids in the crude extracts.

Wagner's Assay:

1ml of leaf crude extracts are mixed with 1ml of Wagner's reagent. The samples are maintained at room temperature and observed for reddish-brown precipitate, which confirms the alkaloids in the crude extracts.

Screening for Terpenoids:

Salkowski Test:

About 1ml of leaf crude extracts were mixed with 2 ml of chloroform and 5 ml of conc. H₂SO₄. The samples are observed for reddish-brown color at interface indicates the presence of terpenoids in the tested crude extracts.

Screening of Saponins:

Froth Assay:

The leaf crude extracts of are mixed with 20ml of distilled water and vigorously shaken for 15 minutes. The appearance of a 1 cm layer of froth confirms the presence of Saponins in the crude extracts.

Foam Test:

Approximately 1ml of leaf crude extracts are vigorously shaken with 2ml of distilled water. If foam produced persists for more than 10 minutes, it indicates presence of Saponins in the tested crude extracts.

Detection of Phlobatannins:

2ml of leaf crude extracts are mixed with 1% aqu.HCl and are observed for red precipitate. It indicates presence of phlobatannins in the tested crude extracts.

Quantification of Total Phenolic Content (TPC):

The Folin-Ciocalteu reagent was used to measure the leaf extract's Total Phenolic Content (TPC) (Mc Donald et al., 2001). 350 µl of 1N Na₂CO₃ and 500 µl of diluted Folin-Ciocalteu reagent were combined with 1 mL (250 mg/mL of crude extract) of the leaf extracts. For around 25 to 35 minutes, the sample combination is kept at room temperature. At 760 nm, the absorbance is measured. To compare the outcomes, standard tannic acid is utilized as a reference. Three duplicates of the experiment were conducted. Tannic acid equivalent (TAE) (mg/g) is the unit of measurement used to represent the TPC.

Quantification of Total Flavonoid Content (TFC):

The Chang et al. (2002) technique was used to assess the leaf crude extracts for their Total Flavonoid Content. Briefly describing the procedure, 0.6 ml of crude extracts from *E. Paniculata* leaves are incubated for 1 hour with 0.6 ml of a 2% Aluminum Chloride solution. The findings were compared using the standard Catechin solution as a reference. The absorbance was measured in a UV-Vis spectrophotometer at 420 nm. In leaf crude extracts, the Total Flavonoid Content is expressed as mg Catechin (CE)/g of dried plant material. Three duplicates of the experiment were conducted.

RESULTS:**Yield Percentage of Crude Extracts:**

The crude extracts of leaf were collected by evaporation of solvents using rotary evaporation. The yield percentage of extract was calculated using the formula shown below in the box. The total weight of the dry leaf used for extraction is 250g. Comparing to *S. simentia*, the *D. falcata* yielded high crude extract. Ethyl acetate followed by Methanol and Acetone resulted significant yield. Whereas, the high yield from *S. simentia* resulted from the Methanol and Acetone followed by chloroform. The result represented in table 1.0 and 1.1

$$\% \text{ of Yield} = \frac{\text{Weight of dry extract}}{\text{Weight of dry plant powder}} \times 100$$

Table 1.0 Yield Percentage of the *D. falcata* leaf crude extract after solvent evaporation

	Extract	Yield (g)	Yield (%)
<i>D. falcata</i>	n-hexane	0.84	0.33
	Petroleum ether	0.61	0.24
	Toluene	1.33	0.53
	Chloroform	2.09	0.83
	Ethyl acetate	5.74	2.29
	Acetone	4.98	1.99
	Methanol	4.45	1.78

Table 1.1 Yield Percentage of the *S. simentia* leaf crude extract after solvent evaporation

	Extract	Yield (g)	Yield (%)
<i>S. simentia</i>	n-hexane	0.55	0.22
	Petroleum ether	0.39	0.15

Table 1.2 Preliminary Phytochemical analysis from the leaf crude extracts of the *D. falcata*

Phyto constituents	Hexane	Petroleum ether	Toluene	Chloroform	Ethyl acetate	Acetone	Methanol
Benedicts test	+	+	+	+	+	+	+
Molisch test	+	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+	+
Monosaccharide's							
Barfoed test	+	+	+	+	+	+	+
Pentose sugars							
Bials test	+	+	+	+	+	+	+
Selwinoff's test	+	+	+	+	+	+	+
Tollen phloro-glucinol test	+	+	+	+	+	+	+

	Toluene	0.81	0.32
	Chloroform	2.79	1.11
	Ethyl acetate	1.21	0.48
	Acetone	3.08	1.23
	Methanol	3.22	1.28

Detection of Organic Constituents:

The preliminary screening of phytochemicals from the *D. falcata* and *S. simentia* crude extracts of leaf results in the presence of monosaccharides, pentose sugars and amino acids. The results were represented in table 1.2 and 1.3.

Detection of Secondary Metabolites:

The qualitative analysis conducted to detect the different types of secondary metabolites of *D. falcata* and *S. simentia* leaf crude extracts revealed to possess certain secondary metabolites. Among the *D. falcata* different crude extracts Ethyl acetate, Methanol crude extracts resulted in positive response for all the tests of secondary metabolite conducted. On the other hand, Acetone crude extract was also given positive sign for all secondary metabolite evaluation except for Terpenoids. On the other hand, n-hexane, petroleum ether, Toluene, chloroform crude extracts resulted negative response for Phytosterols, phlobatannins, Saponins, Terpenoids and Glycosides. Whereas, the test conducted for Alkaloids, Flavonoids, Tannins and Phenols were resulted in positive. Results are represented in table 1.4. In contract, the *S. simentia* leaf crude extracts, showed positive response for the tests conducted for alkaloids and flavonoids. Among the different crude extracts of *S. simentia*, Methanol and Acetone resulted positive response for all the tests conducted. On the other hand, the remaining crude extracts were showed positive response for tests conducted for alkaloids, Flavonoids, Phenols, Terpenoids and Saponins and the test conducted for Phytosterols, phlobatannins Glycosides and Tannins were resulted in negative. Results are represented in table 1.5. The Symbol “+” in indicates the positive response by the extract towards the corresponding phytochemical test and the Symbol “-“ in indicates the negative response by the extract towards the corresponding phytochemical test. Distilled water as a blank for the test of secondary metabolites and reagents are used as blank for proteins and carbohydrates.

Cobalt chloride test	+	+	+	+	+	+	+
Xanthoprotein test	+	+	+	+	+	+	+
Biuret test	+	+	+	+	+	+	+
Amino acids							
Ninhydrin test	+	+	+	+	+	+	+
“+” indicates for positive response for the corresponding phytochemical test							

Table 1.3 Preliminary Phytochemical analysis from the leaf crude extracts of the *S. simentia*

Phyto constituents	Hexane	Petroleum ether	Toluene	Chloroform	Ethyl acetate	Acetone	Methanol
Benedicts test	+	+	+	+	+	+	+
Molisch test	+	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+	+
Monosaccharide's							
Barfoed test	+	+	+	+	+	+	+
Pentose sugars							
Bials test	+	+	+	+	+	+	+
Selwinoff's test	+	+	+	+	+	+	+
Tollen phloro-glucinol test	+	+	+	+	+	+	+
Cobalt chloride test	+	+	+	+	+	+	+
Xanthoprotein test	+	+	+	+	+	+	+
Biuret test	+	+	+	+	+	+	+
Amino acids							
Ninhydrin test	+	+	+	+	+	+	+
“+” indicates for positive response for the corresponding phytochemical test							

Table 1.4 Preliminary determination of secondary metabolites from the leaf crude extracts of the *D. falcata*

Secondary metabolites	Hexane	Petroleum ether	Toluene	Chloroform	Ethyl acetate	Acetone	Methanol
Phenols	+	+	+	+	+	+	+
Glycosides	-	-	-	-	+	+	+
Tannins	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Phytosterols	-	-	-	-	+	+	+
Terpenoids	-	-	-	-	+	-	+
Alkaloids	+	+	+	+	+	+	+
Saponins	-	-	-	-	+	+	+
Phlobatannins	-	-	-	-	+	+	+
“+” indicates for positive response and “-” indicates negative response for the corresponding secondary metabolite test							

Table 1.5 Preliminary determination of secondary metabolites from the leaf crude extracts of the *S. simentia*

Secondary metabolites	Hexane	Petroleum ether	Toluene	Chloroform	Ethyl acetate	Acetone	Methanol
Phenols	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	+	+
Tannins	-	-	-	-	-	+	+
Flavonoids	+	+	+	+	+	+	+
Phytosterols	-	-	-	-	-	+	+
Terpenoids	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-
“+” indicates for positive response and “-” indicates negative response for the corresponding secondary metabolite test							

Total Phenolic Content (TPC):

As per Table 1.6 and Fig 1.0 and Fig 1.1, the total phenolic content (TPC) of *D. falcata* leaf crude extracts was found to be higher than that of *S. simentia* extracts. In *D. falcata*, the ethyl acetate extract (96.1%) exhibited the highest TPC, followed by methanol (92.8%) and acetone (88.6%). Lower TPC values were observed in extracts obtained using n-hexane (32.1%), petroleum ether (38.3%), toluene (35.5%), and chloroform (46.2%).

In contrast, the leaf crude extracts of *S. simentia* demonstrated slightly lower TPC values compared with *D. falcata*. Among these, the methanol extract (89.5%) recorded the highest TPC, followed by acetone (85.3%) and chloroform (73.2%). The lowest values were obtained with n-hexane (26.8%), petroleum ether (31.9%), and toluene (22.5%), ethyl acetate (41.6%). The TPC values of both plants were compared against standard tannic acid (98.9%).

$$TPC = C = c V/m$$

Where, C = total phenolic content mg TAE/g dry extract, c = concentration of tannic acid obtained

from calibration curve in mg/ml, V = volume of extract in ml, m = mass of extract in gram.

Table 1.6 Total Phenolic content determination from the leaf crude extracts of the *D. falcata* and *S. simentia*

Crude Extract	<i>D. falcata</i> TPC (%)	<i>S. simentia</i> TPC (%)	Tannic Acid (%)
n-Hexane	32.1	26.8	98.9
Petroleum ether	38.3	31.9	
Toluene	35.5	22.5	
Chloroform	46.2	73.2	
Acetone	88.6	85.3	
Methanol	92.8	89.5	
Ethyl acetate	96.1	41.6	

Total Flavonoid Content (TFC):

The total flavonoid content (TFC) analysis revealed that both *D. falcata* and *S. simentia* leaf crude extracts contained significant amounts of flavonoids, with *D. falcata* exhibiting comparatively higher levels. In *D. falcata*, the ethyl acetate extract (94.8%) demonstrated the highest TFC, followed by methanol (90.4%) and acetone (88.1%). Lower TFC values were observed in n-hexane (51.2%), petroleum ether (48.5%), toluene (33.9%), and chloroform (41.5%) extracts. *S. simentia*, TFC values were slightly lower than those of *D. falcata*. The highest TFC was observed in methanol (93.5%) and acetone (89.3%), followed by chloroform (79.5%). Lower values were recorded for n-hexane (21.6%), petroleum ether (24.2%), and toluene (37.7%), ethyl acetate (42.7%).

All values were compared with the standard catechin (96.0% at 250 mg/mL). The results are represented in Table 1.7 Fig. 1.2 A and B.

$$TFC = C = c \cdot V/m$$

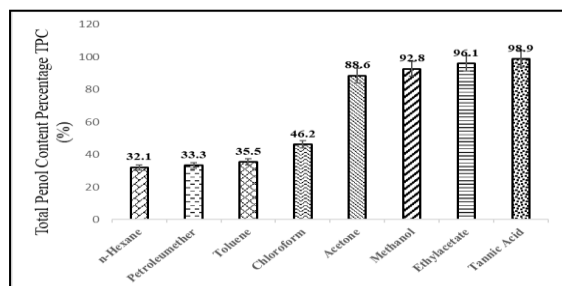


Fig. 1.0 Total Phenolic content determination from the leaf crude extracts of the *D. falcata*

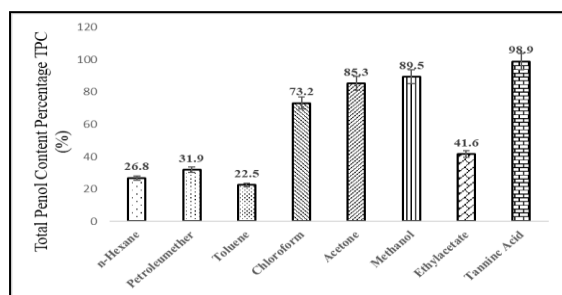


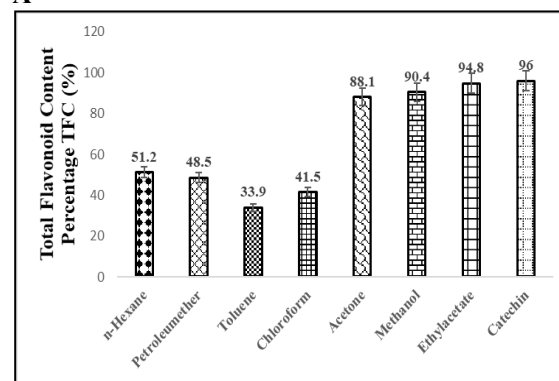
Fig. 1.1 Total Phenolic content determination from the leaf crude extracts of the *S. simentia*

Where, C = Total Flavonoid Content mg CTE/g dry extract, c = concentration of Catechin obtained from calibration curve in mg/ml, V = volume of extract in ml, m = mass of extract in gram

Table 1.7 Total Flavonoid content determination from the leaf crude extracts of the *D. falcata* and *S. simentia*

Solvent	<i>D. falcata</i> TFC (%)	<i>S. simentia</i> TFC (%)	Catechin (%)
n-Hexane	51.2	21.6	96.0
Petroleum ether	48.5	24.2	
Toluene	33.9	37.7	
Chloroform	41.5	79.5	
Acetone	88.1	89.3	
Methanol	90.4	93.5	
Ethyl acetate	94.8	42.7	

A



B

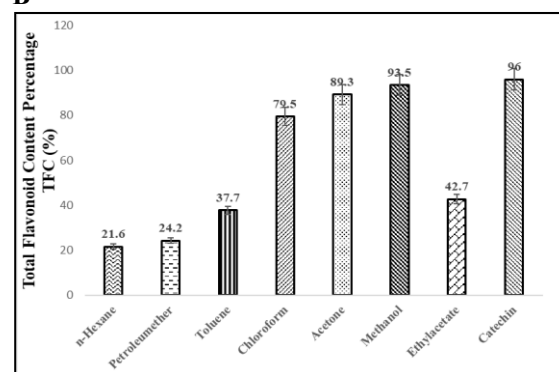


Fig. 1.2 Total Flavonoid Content (TFC); A- leaf crude extracts of *D. falcata*; B- Leaf crude extracts of *S. simentia*. The results are compared with standard Catechin

3.11 DISCUSSION:

The present study shows that *Dendrophthoe falcata* yielded a greater amount of crude extract from 250 g of dried leaf material than *Senna simentia* under identical extraction conditions (rotary evaporation). Solvents of intermediate polarity ethyl acetate, methanol and acetone produced the highest yields for *D. falcata*, whereas methanol and acetone yielded the most for *S. simentia* (Tables 1.0 and 1.1). This trend is consistent with many phytochemical surveys in which polar to mid-polar solvents extract

a larger fraction of polar secondary metabolites (phenolics, flavonoids, glycosides), whereas nonpolar solvents (n-hexane, petroleum ether, toluene) preferentially extract lipophilic compounds and generally give lower overall yields (Pattanayak and Mazumder, 2010).

Preliminary qualitative screening detected monosaccharides, pentose sugars and amino acids in both plants (Tables 1.4–1.5). Secondary metabolite screening indicated that ethyl acetate and methanol extracts of *D. falcata* showed consistent positive reactions across multiple tests (alkaloids, flavonoids, tannins/phenols, saponins, etc.), whereas nonpolar fractions (n-hexane, petroleum ether, toluene, chloroform) were negative for several polar classes but positive for alkaloids, flavonoids, tannins and phenols. *S. simentia* extracts also returned strong positive reactions for alkaloids and flavonoids; its methanol and acetone extracts showed the broadest suite of positive tests. These patterns align with previously published phytochemical analyses for both taxa, which report abundant phenolics and flavonoids in polar extracts (Kong et al., 2023).

Quantitative TPC and TFC results demonstrate that *D. falcata* leaf extracts possess higher overall phenolic and flavonoid content than *S. simentia* under the tested solvent series (Tables 1.6 and 1.7; Figs. 1.0, 1.1, 1.2 A and 1.2 B). Specifically, ethyl acetate, methanol and acetone extracts of *D. falcata* showed the highest TPC (96.1%, 92.8%, 88.6%) and TFC (94.8%, 90.4%, 88.1%). For *S. simentia*, methanol and acetone fractions were the richest in phenolics and flavonoids (TFC: 93.5% and 89.3%, respectively). The proximate dominance of mid-polar solvents indicates a predominance of mid- to high-polarity phenolic and flavonoid classes (flavonols, flavones, phenolic acids) in both species. Higher TPC/TFC often correlates with stronger in-vitro antioxidant and multiple pharmacological activities; therefore, the elevated values in *D. falcata* suggest substantial bioactivity potential (Scialert, 2012; Chengala). Phenolic compounds and flavonoids are widely recognized for their broad spectrum of biological activities, mediated through multiple biochemical and molecular mechanisms. Their antioxidant properties are attributed to free radical scavenging, stabilization of reactive intermediates, and metal ion chelation, which collectively protect biomolecules from oxidative damage (Procházková et al., 2011). They exert anti-inflammatory effects by modulating key signaling pathways, including NF- κ B and MAPK, suppressing the expression of pro-inflammatory mediators such as COX-2 and iNOS, and reducing the secretion of cytokines like TNF- α , IL-1 β , and IL-6. Their antimicrobial actions involve disruption of

microbial cell membranes, inhibition of critical enzymes, chelation of essential metal ions, and potentiation of antibiotic activity (Daglia, 2012; Kumaraswamy gullapelli et al., 2014; Budarapu Neelamma et al., 2016; Ramya Sucharitha et al., 2016; Amarnath Velidandi et al., 2022; Nagavelli Ramu et al., 2023; Nagavelli Ramu et al., 2024). In the context of diabetes management, they demonstrate significant inhibitory activity against carbohydrate-hydrolyzing enzymes such as α -amylase and α -glucosidase, along with protective effects on pancreatic β -cells (Tadera et al., 2006). Furthermore, their anticancer and cytotoxic properties are mediated through apoptosis induction, cell cycle arrest, suppression of angiogenesis, and inhibition of metastatic progression (Khan et al., 2019; Ramos, 2008). Considering these diverse mechanisms, coupled with the high total phenolic content (TPC) and total flavonoid content (TFC) observed in ethyl acetate, methanol, and acetone fractions, these extracts represent promising candidates for bioassay-guided fractionation to isolate potent bioactive constituents.

A number of studies on *D. falcata* report rich phenolic and flavonoid content and corresponding antioxidant, antimicrobial, wound-healing and anti-inflammatory activities (Kong et al., 2023). Recent reviews and experimental studies confirm traditional uses (wound healing, anti-inflammatory, anti-tumor) and identify candidate compounds (various flavonols, phenolic acids). Similarly, *Senna* species (including *S. siamea*) are documented for antioxidant, antimicrobial, antifungal and antidiabetic properties; these are usually associated with flavonoids, tannins and anthraquinone derivatives in the genus. Our quantitative results are consistent with those published reports and further indicate that solvent polarity strongly dictates extract composition and bioactivity.

Although plant phenolics/flavonoids are generally considered safer than many synthetic drugs, they are not devoid of risk. Reported concerns include hepatotoxicity, allergic reactions, and clinically relevant interactions with pharmaceuticals (e.g., modulation of cytochrome P450 enzymes or P-glycoprotein transporters), which can alter drug pharmacokinetics. Additionally, crude herbal preparations can vary widely in composition and may be contaminated (heavy metals, microbes) or adulterated. Conversely, some synthetic drugs (notably certain synthetic cannabinoids, opioids and designer drugs) have well-documented severe adverse effects including cardiovascular toxicity, respiratory depression, neurotoxicity, and fatal overdoses highlighting that “synthetic” does not automatically imply greater safety or risk without context. Thus, thorough toxicological profiling is

essential before therapeutic use.

3.12 CONCLUSION:

The comparative evaluation of *Dendrophthoe falcata* (L.f.) Ettingsh. parasitizing *Senna siamea* and its host plant revealed that both species are rich in bioactive phytoconstituents, particularly phenolics, flavonoids, tannins, and alkaloids. Quantitative estimations demonstrated notably high total phenolic content (TPC) and total flavonoid content (TFC) in both plants, which are strongly correlated with their observed biological activities. These secondary metabolites are known to play vital roles in mitigating oxidative stress, inhibiting microbial growth, modulating inflammatory pathways, and regulating carbohydrate metabolism. Antioxidant assays confirmed that extracts from both plants effectively neutralize free radicals, suggesting their potential as natural oxidative stress modulators. The antimicrobial screening indicated broad-spectrum inhibitory activity against Gram-positive and Gram-negative bacterial strains, with the ethyl acetate extracts of *D. falcata* and methanolic extracts of *S. siamea* showing the highest efficacy. The observed antibacterial effects are likely due to the disruption of microbial membranes, inhibition of key enzymes, and interference with quorum sensing mechanisms.

Anti-inflammatory assays revealed significant suppression of protein denaturation and stabilization of lysosomal membranes, indicating strong anti-inflammatory potential in both plants. The α -amylase inhibition assay demonstrated promising antidiabetic activity, particularly in the ethyl acetate fraction of *D. falcata* and the methanolic fraction of *S. siamea*, suggesting a potential role in managing postprandial hyperglycemia.

The study establishes that both *D. falcata* and *S. siamea* possess multifaceted pharmacological activities, with *D. falcata* showing slightly higher potency in antioxidant and antidiabetic assays, while *S. siamea* exhibits notable antimicrobial potential. These findings justify further bioassay-guided fractionation, isolation, and structural elucidation of active compounds, with a view toward developing phytopharmaceutical formulations targeting oxidative stress-related disorders, infectious diseases, inflammation, and diabetes.

CONCLUSION:

The present investigation demonstrated that both *Dendrophthoe falcata* and *Senna siamea* leaves are rich sources of phytochemically important compounds. Among the two plants studied, *D. falcata* exhibited comparatively higher extraction yields and a broader distribution of secondary metabolites, particularly in the ethyl acetate and

methanol extracts. Preliminary phytochemical screening confirmed the presence of carbohydrates, reducing sugars, amino acids, phenols, flavonoids, tannins, alkaloids, glycosides, phytosterols, terpenoids, saponins, and phlobatannins, indicating the medicinal significance of these plants.

Quantitative analysis revealed that *D. falcata* possessed significantly higher total phenolic content (TPC) and total flavonoid content (TFC) than *S. siamea*. The ethyl acetate extract of *D. falcata* showed the highest TPC (96.1%) and TFC (94.8%), while methanol and acetone extracts also demonstrated substantial levels of these bioactive compounds. In *S. siamea*, methanol and acetone extracts recorded the highest phenolic and flavonoid contents. The higher accumulation of phenolics and flavonoids in polar and semi-polar solvent extracts suggests that these solvents are more effective for recovering antioxidant-rich phytochemicals.

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CONFLICT OF INTEREST:

The authors declare that there are no conflicts of interest associated with this study.

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