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Pharmacognostic Evaluation and HPTLC Profiling of Callisia Fragrans (Lindl.) Woodson – A Traditionally Used Medicinal Plant

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ABSTRACT: Callisia fragrans (Lindl.) Woodson, a perennial plant of the family Commelinaceae, has long been valued in traditional medicine for its anti-inflammatory, antimicrobial, and wound-healing properties. Despite its ethno medicinal significance, scientific validation and standardization data remain limited. The present study aimed to evaluate the pharmacognostic characteristics of Callisia fragrans through physicochemical analysis, preliminary phytochemical screening, and High Performance Thin Layer Chromatography (HPTLC) fingerprinting. Physicochemical parameters, including, total ash, acid-insoluble ash, water-soluble extractives, and Alcohol soluble extractive values, were determined to establish baseline standards for quality control. Preliminary phytochemical analysis revealed the presence of key secondary metabolites such as flavonoids, phenols, alkaloids, glycosides, quinones, coumarins, sugars, and saponins, which are known for various biological activities. HPTLC profiling of the plant extract further confirmed the presence of these phytoconstituents, with distinct chromatographic bands observed under UV and visible light, indicating a complex phytochemical composition. These findings not only support the therapeutic potential of Callisia fragrans but also provide a reliable framework for its identification, standardization, and potential pharmaceutical applications. This study offers essential pharmacognostic data that may contribute to the development of quality herbal formulations and further pharmacological investigations involving Callisia fragrans.

KEYWORDS: Callisia fragrans, Pharmacognostic, Phytochemical screening, HPTLC, Drug standardization.

I. INTRODUCTION

Plants are vital to daily life, providing food, clean air, and a variety of crucial ecosystem functions. With an estimated 100,000 natural compounds [(1,2)] many of which remain undiscovered, although around 5,000 phytochemicals have been individually identified[(3,4)]. These include alkaloids, tannins, saponins, flavonoids, terpenoids, carotenoids, glycosides, and phenolic compounds-secondary metabolic products that contribute to plant growth, reproduction, and defence mechanisms. They also play roles in photosynthesis and serve as antioxidants, helping prevent chronic diseases in humans [(5)]. Consequently, natural herbal therapy continues to gain popularity over synthetic pharmaceuticals[(6,7,8).]

Callisia fragrans (Lindl.) Woodson is an herbaceous plant of the Commelinaceae family, which includes over 500 species. Native to tropical America, particularly Mexico, it thrives in fertile, moist soils with partial shade, grows in a rosette form, reaching about 0.25 meters in height and 1 meter in spread [(9)]. Its thick, trailing stems bear large, spirally arranged, green or purplish leaves. The plant produces white, fragrant flowers in dense clusters, usually blooming in late winter or early spring. It propagates through seeds or cuttings. It is widely cultivated for both ornamental and medicinal purposes and is known by various names including basket plant, inch plant, and domestic ginseng[(10)]. In traditional medicine systems, particularly in Eastern Europe, Russia, and parts of Asia, C. fragrans is valued for its antibacterial, antiviral, anti-inflammatory, and antioxidant properties. The leaves are used externally to treat burns, skin diseases, and joint pain, and internally to support immune function, digestion, and cholesterol regulation. Folk remedies often involve

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ethanol-based infusions or decoctions of the plant parts[11,12]. Compounds such as flavonoids, sterols, and vitamins are believed to contribute to its medicinal effects[13].

Despite its widespread traditional use and promising pharmacological potential, Callisia fragrans remains understudied scientifically. There is a lack of comprehensive pharmacognostic profiles, making it difficult to ensure authenticity, quality, purity, and safety in commercial herbal preparations. Pharmacognostic standardization, including morphological, anatomical, phytochemical, and chromatographic studies, is essential to establish quality control parameters and prevent adulteration or misidentification—especially important in herbal drug development and regulatory compliance[14].

The present study aims to perform a comprehensive phytochemical, and HPTLC investigation of Callisia fragrans. Preliminary phytochemical screening for bioactive constituents, Physicochemical parameter evaluation and HPTLC fingerprint profiling for standardization. Rigorous scientific validation will ensure their safe and effective integration into modern medicine.

II. MATERIALS AND METHODS

Collection and Authentication of Plant

The fresh plant material of Callisia fragrans was collected from various regions within Thrissur district, Kerala, including Parappur, Thalore, Ponnukara, and Mannuthy, during the month of March 2024 (Figure -1). The collected specimens were botanically identified and authenticated by a Botanist at Sitaram Ayurveda Pvt. Limited, Thrissur, Kerala. A voucher specimen was prepared and deposited in the herbarium of QC Department of Sitaram Ayurveda Pvt. Limited under the reference number SAPL/QC/112 for future reference.

Figure -1 Callisia fragrans habit

Preparation of plant material

The freshly collected plant parts of Callisia fragrans were thoroughly rinsed 2–4 times with running tap water, followed by a final wash with distilled water to remove adhering soil, dust, and other contaminants. The cleaned materials were then shade-dried at room temperature for 4–5 weeks to ensure complete removal of moisture. After drying, selected plant parts were chopped into small pieces using sterilized scissors, while others were powdered using a Mixer grinder to obtain a fine powder. The processed samples were stored in clean, air-tight containers and kept in a cool, dry place until further use for further analyses.

Physicochemical Analysis

Physicochemical parameters were determined as per standard procedures prescribed in the Ayurvedic Pharmacopoeia of India and WHO guidelines on quality control of herbal materials[15,16].

Total Ash Value: Accurately weighed 3gm of the ground drug in a silica crucible which is previously dried and weighed. Incinerate at a temperature not exceeding 600°C until free from carbon, cool in a desiccator for 30 minutes and weigh without delay, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 600°C. Calculated the percentage of ash with respect to the air-dried sample. Check and reduce

Acid Insoluble Ash: Boil the ash obtained in above for 5 minutes with 25ml of dil. HCl, collected the insoluble matter in a gooch crucible on an ash less filter paper, washed with hot water and ignite to constant weight. The percentage of acid insoluble ash with reference to the air-dried drug is calculated.

Alcohol Soluble Extractives: Macerate 5 gm of the coarsely powdered drug with 100 ml of alcohol of the specified strength in a stoppered flask for 24 hrs, shaking frequently during 6 hrs and allow to stand for 18 hrs. Filter precautions to be taken to prevent the loss of solvent. Evaporate 25 ml of the filtrate to dryness in tarred flat bottomed shallow dish and dry at 105°C to constant weight and weigh. Calculate the percentage of alcohol soluble extractive with reference to the dried drug.

Water Soluble Extractives: Macerate 5gm of the coarsely powdered drug with 100ml of chloroform water in a stoppered flask for 24 hours shaking

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frequently during 6 hours and allow to stand for 18 hrs. Filter rapidly, taking precautions against loss of solvent. Evaporate 25ml of the filtrate to dryness in tarred flat bottomed shallow dish and dry at 105°C to constant weight and weigh. Calculate the percentage of water soluble extractive with reference to the dried drug.

Preliminary Phytochemical Screening

Successive extraction of powdered Callisia fragrans was carried out using solvents of increasing polarity: Chloroform, Acetone, Methanol and Water using Soxhlet apparatus[17].

The obtained extracts were subjected to qualitative tests for the presence of Carbohydrates (Molisch's test, Benedict's test, Fehling's test), Ketoses (Seliwanoff's test), Proteins (Biuret's test), Starch (Iodine (KI) test), Quinones (Concentrated H_2 SO_4 test), Flavonoids (Alkaline reagent test), Phenols (Ferric chloride test), Saponins (Foam test), Alkaloids (Wagner's reagent test) Tannins (Ferric chloride test), Coumarins (NaOH test), Glycosides (Liebermann's test, Keller–Kiliani test, Salkowski's test forTerpenoids and steroids)[18].

Sample Preparation

Dried plant material of Callisia fragrans was extracted using methanol and chloroform. The resulting extracts (CF-ME and CF-CH, respectively) were concentrated and filtered. Approximately 1 g of each extract was dissolved in 10 mL of methanol, sonicated for 15 minutes, and filtered through Whatman No. 1 filter paper.

Chromatographic condition and development.

HPTLC analysis was performed using a CAMAG HPTLC system equipped with a Linomat IV applicator and TLC Scanner 3. The stationary phase consisted of pre-coated silica gel 60 F254 HPTLC plates (E. Merck) with a plate size of 5.0 × 10.0 cm. A sample volume of 5 µL was applied as 8 mm bands using an automatic CAMAG ATS 4 applicator with nitrogen as the spray gas [19,20].

Chromatographic development was carried out in a Twin Trough Chamber (20 × 10 cm) using a mobile phase consisting of Toluene and Ethyl acetate in a ratio of 7:3. The solvent front was allowed to migrate to a distance of 80 mm, followed by drying in a hot air oven at 60°C for 5 minutes. Detection was conducted using a CAMAG TLC Scanner 3 at a wavelength of 550 nm in absorbance/remission mode with a data resolution of 100 µm/step.

Visualization of the developed plates was performed under UV light at 254 nm and 366 nm, as well as post-derivatization using anisaldehyde sulphuric acid reagent, observed under white light. The entire system was controlled using winCATS Planar Chromatography Manager software (Version 1.4.4).

III. RESULTS AND DISCUSSION:

Physicochemical Parameters

Physicochemical analysis was performed to establish standard values useful in quality control and raw material authentication. The results are summarized in Table 1**.**

Table 1: Physicochemical Parameters of Callisia fragrans

Sl. No	Parameters	Observed values
1.	Total Ash	16.15 ± 0.22
2.	Acid Insoluble Ash	1.78 ± 0.08
3.	Alcohol Soluble Extractive	7.33 ± 0.31
4.	Water Soluble Extractive	24.35 ± 0.11

Note: Values are expressed as mean ± SD (n=3)

The physicochemical parameters serve as essential quality control indicators for the authentication and standardization of herbal raw materials. The results obtained for Callisia fragrans show consistent values across triplicate readings, as indicated by the low standard deviations.

The total ash value of 16.15 ± 0.22% represents the total amount of inorganic residue remaining after incineration, including both physiological and non-physiological ash. This relatively high ash content suggests the presence of inherent mineral constituents or a degree of extraneous matter, such as soil or sand.The acid insoluble ash was found to be 1.78 ± 0.08%, which specifically reflects the amount of silica, particularly from contamination like sand or siliceous earth. A value within acceptable limits indicates minimal contamination, supporting the quality of the collected material.The alcohol soluble extractive value was 7.33 ± 0.31%,

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suggesting the presence of moderately polar constituents such as phenolics, glycosides, flavonoids, and some alkaloids. This value is useful for estimating the yield of bioactive components soluble in ethanol. The water soluble extractive value was significantly higher at 24.35 ± 0.11%, indicating a strong presence of polar compounds like sugars, tannins, saponins, glycosides, and some organic acids. The higher value also reflects the potential effectiveness of aqueous preparations of Callisia fragrans in traditional use.

Preliminary Phytochemical Screening

The phytochemical analysis of various extracts revealed the presence of a broad spectrum of secondary metabolites (Table 2), which are known to contribute to the plant's therapeutic properties.

The phytochemical screening of Callisia fragrans extracts in various solvents revealed diverse secondary metabolites with solvent-dependent solubility.

Carbohydrates were detected by Benedict's and Fehling's tests in all extracts, while Molisch's test was negative, indicating the presence of reducing sugars but absence of polysaccharides. Ketoses, proteins, starch, and saponins were absent in all extracts. Quinones were present only in acetone and methanol extracts, suggesting partial polarity. Flavonoids and phenols were detected in all solvents, indicating their abundance and broad solubility. Alkaloids were present in chloroform, methanol, and water extracts, showing a range of polarity. Coumarins were detected across all extracts, suggesting their significant presence. Tannins were absent in all, indicating their negligible content. For glycosides, Liebermann's test was positive in chloroform and acetone, while Salkowski's test was positive in acetone, methanol, and water. Keller–Kiliani remained negative throughout. Steroids were found in acetone and methanol extracts, while terpenoids were undetected in all. Overall, methanol and acetone extracts yielded the highest variety of phytochemicals, supporting their suitability for further studies. The presence of these phytochemicals supports the traditional use of Callisia fragrans in herbal medicine for antioxidant and anti-inflammatory purposes.

Table 2: Phytochemicals Present in Different Extracts of Callisia fragrans

Phyto-constituent	Test Name	Chloroform	Acetone	Methanol	Water
Carbohydrate	Molisch'stest	-	-	-	-
	Benedict'stest	+	+	+	+
	Fehling'stest	+	+	+	+
Ketose	Seliwanoff'stest	-	-	-	-
Proteins	Biuret'stest	-	-	-	-
Starch	KI test	-	-	-	-
Quinone	H2So4 test	-	+	+	-
Flavonoid	Alkalinereagent test	+	+	+	+
Phenol	Phenolreagent test	+	+	+	+
Saponins	Foamtest	-	-	-	-
Alkaloids	Wagner's reagenttest	+	-	+	+
Tannin	FeCl3test	-	-	-	-
Coumarin	NaOH test	+	+	+	+
Glycosides	Liebermann'stest	+	+	-	-
	Keller- Kilianitest	-	-	-	-
	Salkowski'stest	-	+	+	+
Terpenoids	Testfor Terpenoids	-	-	-	-
Steroids	Test for steroids	-	+	+	-

(+ = Present; – = Absent)

HPTLC Fingerprinting

Chloroform Extract (CF-CH)

The chromatogram at 550 nm showed 12 distinct peaks with Rf values (Figure -2) ranging from 0.03 to 1.03, indicating the presence of multiple phytoconstituents. Significant peaks were observed at:Rf 0.47 (Height: 45.2 AU), 0.59

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(Height: 200.9 AU), 0.78 (Height: 136.1 AU), 0.89 (Height: 435.9 AU) and 1.03 (Height: 421.0 AU). These peaks represent major bioactive compounds, as inferred from their intensity and area under curve.

Methanolic Extract (CF-ME)

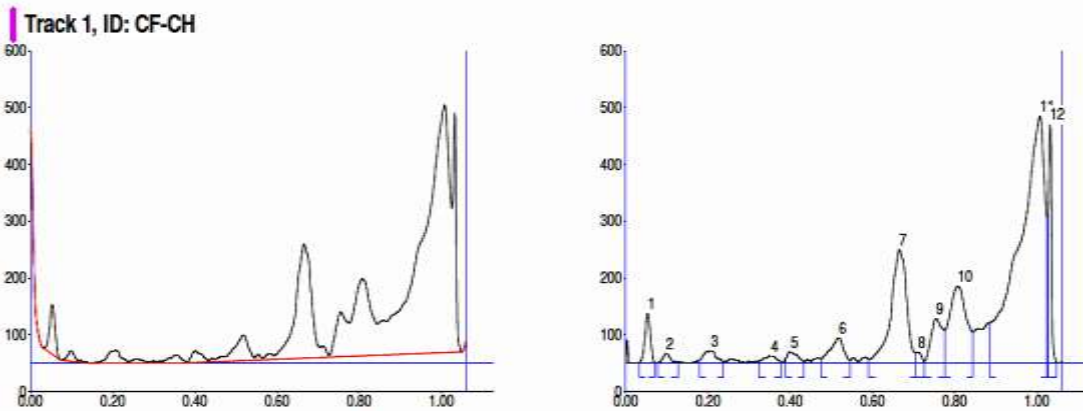
The methanol extract showed 14 peaks, indicating a rich phytochemical profile (Figure -3). Prominent peaks were recorded at Rf 0.66 (Height: 221.3 AU), 0.77 (Height: 183.0 AU), 0.91 (Height: 490.6 AU) and 1.03 (Height: 432.7 AU).

The peak at Rf 0.91, with a maximum height of 490.6 AU and area % of 39.62, was the most dominant, suggesting a highly abundant compound.

Comparative Evaluation

Both extracts exhibited a broad range of compounds, with methanol extract showing slightly more intense and numerous peaks. This aligns with methanol's higher polarity, which likely extracts a wider spectrum of polar phyto-constituents. The profiles provide distinctive fingerprints useful for standardization and quality control of Callisia fragrans extracts.

The developed chromatographic plates of chloroform and methanol extracts at 254 nm, 366 nm, and post-derivatization under white light, along with their corresponding densitograms, are presented in Figures 4. These figures illustrate the separation profile and intensity of phytoconstituents based on Rf values and peak areas.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	0.4	0.05	88.0	5.86	0.07	0.3	1090.4	2.33	unknown *
2	0.08	1.2	0.10	18.1	1.21	0.13	1.7	300.6	0.64	unknown *
3	0.18	6.0	0.21	22.8	1.52	0.24	2.3	644.1	1.37	unknown *
4	0.33	4.0	0.35	13.3	0.88	0.38	2.2	376.1	0.80	unknown *
5	0.39	7.4	0.40	20.9	1.39	0.44	2.9	489.2	1.04	unknown *
6	0.47	8.2	0.52	45.2	3.01	0.55	4.9	1385.5	2.96	unknown *
7	0.59	6.8	0.67	200.9	13.38	0.71	18.5	7113.1	15.18	unknown *
8	0.71	18.6	0.71	20.2	1.34	0.73	3.8	238.0	0.51	unknown *
9	0.73	4.1	0.76	79.0	5.26	0.78	58.5	2034.0	4.34	unknown *
10	0.78	58.5	0.81	136.1	9.06	0.84	54.9	5251.6	11.21	unknown *
11	0.89	70.5	1.01	435.9	29.03	1.03	254.4	25035.0	53.43	unknown *
12	1.03	261.8	1.03	421.0	28.04	1.05	0.5	2900.9	6.19	unknown *

Figure -2: HPTLC profile of Callisia fragrans – Chloroform Extract

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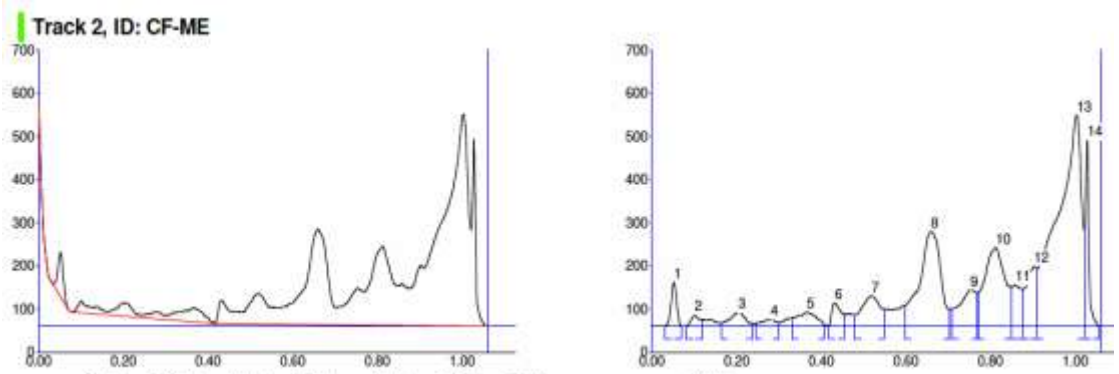

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	0.1	0.05	103.3	5.20	0.07	0.5	1238.0	1.93	unknown *
2	0.08	0.0	0.10	27.2	1.37	0.12	14.9	475.8	0.74	unknown *
3	0.17	8.3	0.20	32.0	1.61	0.24	6.6	1113.1	1.74	unknown *
4	0.25	5.9	0.28	16.5	0.83	0.30	8.9	532.5	0.83	unknown *
5	0.33	18.9	0.37	32.5	1.64	0.41	2.8	1417.5	2.21	unknown *
6	0.42	0.6	0.43	54.2	2.73	0.46	28.1	1075.0	1.68	unknown *
7	0.48	27.7	0.52	70.8	3.57	0.55	39.2	2854.8	4.46	unknown *
8	0.60	48.7	0.66	221.3	11.15	0.70	39.3	10176.8	15.89	unknown *
9	0.71	41.8	0.75	85.2	4.29	0.77	76.8	3164.6	4.94	unknown *
10	0.77	77.0	0.81	183.0	9.22	0.85	91.7	8311.2	12.98	unknown *
11	0.85	91.7	0.86	96.1	4.84	0.88	85.6	1918.4	3.00	unknown *
12	0.88	85.9	0.90	139.9	7.04	0.91	133.7	3078.3	4.81	unknown *
13	0.91	133.7	1.00	490.6	24.71	1.02	223.4	25372.1	39.62	unknown *
14	1.02	228.6	1.03	432.7	21.79	1.06	0.5	3303.9	5.16	unknown *

Figure -3: HPTLC profile of Callisia fragrans – Methanol Extract

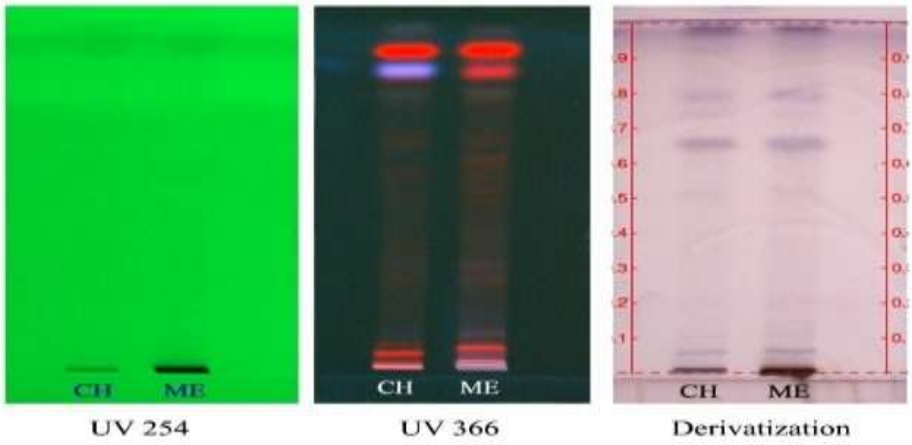

Figure -4: HPTLC Plates of Callisia fragrans – under UV 254, 366 nm and Derivatization

IV. CONCLUSION:

The present study provides a comprehensive pharmacognostic evaluation and HPTLC profiling of Callisia fragrans (Lindl.) Woodson, a traditionally used medicinal plant. The physicochemical parameters established standard values critical for raw material authentication and quality control. Preliminary phytochemical screening revealed the presence of key bioactive constituents such as flavonoids, phenols, alkaloids, coumarins, and glycosides, particularly in methanol and acetone extracts, highlighting their suitability for further phytopharmacological investigation. HPTLC fingerprinting confirmed the complex chemical composition of the plant, with methanol extract displaying a higher number and intensity of peaks compared to the chloroform extract. These distinctive chromatographic profiles provide a reliable reference for the identification and standardization of the plant material in herbal

formulations. The findings scientifically support the traditional uses of Callisia fragrans and contribute valuable baseline data for its inclusion in quality herbal drug development and future pharmacological studies.

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