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Anatomical Profiling and Phytochemical Analysis of *Christia Vespertilionis* (L.F.) Bakh. F.

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Abstract

Christia vespertilionis (L. f.) Bakh. f., (Family Fabaceae), commonly known as 'Red Butterfly Wing', is currently getting attention as an important, unexploited medicinal plant with anti-malarial and anti-cancer properties. The identification of raw drug is mostly based on morphological features and there is a possibility to select adulterants instead of correct plant species. Therefore, studies were done on the macroscopic and microscopic characters of leaf, petiole, stem and root of *Christia vespertilionis*. The micro-anatomy of leaf surface was studied by performing SEM. Preliminary phytochemical screening was carried out to study the phytoconstituents present in the plant by using methanolic leaf extract. An attempt was also made to separate the components present in the leaf by HPTLC analysis. The phytochemical analysis showed the presence of alkaloids, flavonoids, glycoside, tannin, diterpenes, coumarin and quinine. Morphological and anatomical features which are characteristic to the plant, such as butterfly wing-like leaf, acuminate apex, hooked trichome, anamocytic stomata etc. will be useful for the correct identification of the plant and the phytochemical analysis will be helpful in the standardization and quality assurance of the plant.

Keywords
Christia vespertilionis, HPTLC, Macroscopic, Microscopic, Phytochemical analysis,SEM.

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INTRODUCTION

Christia vespertilionis (L.f.) Bakh.f. belongs to the family Fabaceae. It is a widespread species in tropical Southeast Asia and is native to Cambodia, Indonesia, China, Thailand and Vietnam. It has been introduced into many other countries. Hence, thisspecies is

assessed as Least Concern (LC) according to the IUCN Red List of Threatened Species.[1]It is rendered important due to its medicinal properties in treating various ailments such as snake bites, tuberculosis, bronchitis, inflamed tonsils, cold, muscle weakness, poor blood circulation, scabies and bone fractures.[2,3,4,5,6]Butterfly tea refers to decoction of *Christia vespertilionis* leaves that has been consumed by cancer patients in Malaysia and gained a wide range of popularity due to its potential to be used as an alternative to modern medicine.[3,7]

The methanolic and aqueous-methanol (1:4 v/v) extract of roots, leaves and stems of *C. vespertilionis* were evaluated for anti-plasmodial activity, of which theaqueous-methanolic stem extract was the most active (IC_{50} 7.5 µg/mL) against *Plasmodium falciparum* NF-54. [8]

A study of *C. vespertilionis* extracts showed antiproliferative and proapoptotic effects in all MTC (human medullary thyroid carcinoma) and SI-NET (human small intestinal neuroendocrine tumor) cell lines, whereby high growth inhibition was observed by treatment with the ethyl acetate-extracts (CV-45) in tumor cell lines. [9,10] There are many constituents present the plant extract which contributes to its different properties. One such study revealed a chlorophyll derivative, pheophorbid-a in the plant extracts that exhibited high anti-proliferative activity in MTC cells. [11]

In the present work, the morphological, microscopical and phytochemical studies of different parts of *Christia vespertilionis* (L. f.) Bakh. f. has been carried out which will be useful for proper identification and authentication of crude drug.

MATERIALS AND METHODS

Christia vespertilionis (L. f.) Bakh.f. plant parts (leaf, stem, petiole and root) were collected. The plant was identified and authenticated from Dept. of Botany, CMS College, Kottayam, Kerala, S.India. The leaves were separated and dried under shade, pulverised using mechanical grinder and stored in an air-tight container for further use.

Anatomical study

Anatomical features were studied by taking transverse section of leaf, petiole, stem and root of *Christia vespertilionis,* which were stained using safranine and mounted in glycerine, and observed under microscope. The micro-anatomy of thelower surface of leaf was studied by SEM (Scanning Electron Microscope).

Phytochemical analysis

The preliminary phytochemical analysis for the powdered leaf of *Christia vespertilionis* was carried on the methanolic extract of the leaf which was obtained by soxhlet extraction (Hot extraction technique).The qualitative analysis of secondary metabolites such as alkaloids (Mayer's test, Dragendroff's test), flavonoids (Alkaline reagent test, Lead acetate test), carbohydrates (Benedict's test, Fehling's test),Protein (Xanthoproteic test, Ninhydrin test) glycoside, tannin ($FeCl_3$ test, Bromine water test), phenol ($FeCl_3$ test), saponins (Froth test), anthraquinones (Brontrager's test), phytosterol(Salkwoski's test), resin, diterpene (Copper acetate test), coumarin, quinine etc. were carried out using standard methods (Harborne, 1998).[12]

HPTLC (High performance Liquid Chromatography) was performed for the leaf extract. The leaf extract was applied to TLC plates of size 10 x 10 cm which were pre-coated with Silica gel 60 F_{254} (Merck 1.05554.0007) for the detection. The extracts were applied as bands with a CAMAG Automatic TLC Sampler III (Linomat). Separations were performed in CAMAG twin trough chambers previously saturated by the solvents for 30 minutes at the room temperature. Solvent system selected as the better mobile phase for the separation of components for TLC is selected as the mobile phase. The solvent system selected for analysis was Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5). After that the plates were taken out of the chamber and dried in air. The air-dried plates were observed under UV light. The air-dried plates were scanned under UV light at 254nm and 366nm with the help of HPTLC scanner. The fingerprint developed by this method are number of peaks, $R_f$value and area percentage of the sample.

RESULTS AND DISCUSSION

1. Morphological study

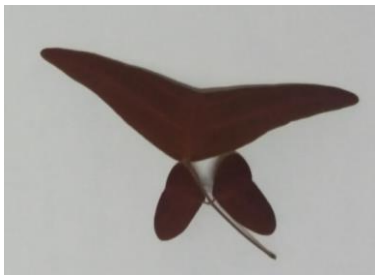
Christia vespertilionis (L. f.) Bakh.f. is a non-climbing perennial herb (60-120cm) which has unique butterfly-winged leaves. It is commonly known as 'Red Butterfly Wing' and also 'Green Mariposa'. The juvenile leaves have a purple tint, whereas mature leaves are dark green with pale green stripes along prominent

veins.They have small flowers. The racemes are simple andterminal. (Figure 1)

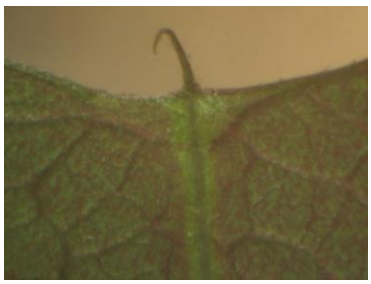
Leaves

Leaves are trifoliate with long petiole and stipulate. It shows alternate phyllotaxy. The whole leaf surface has a hairy nature. The leaflets are often broader than long. The leaf is butterfly-winged shape with entire margin, acuminate apex, with terminal leaflet blade rhombic and slightly cordate base and reticulate venation. The mid rib is prominent. (Figure: 2)

Figure 1: *Christia vespertilionis*



a. Entire Leaf



b. Apex of leaf

c. Mid vein of Leaf

d. Base of leaf

Figure 2 : Morphology of leaf of *C. vespertilionis*

Petiole

The petiole is thin, grooved and hairy. It is greenish with red tinge. (Figure: 3)

Fig.3: Petiole

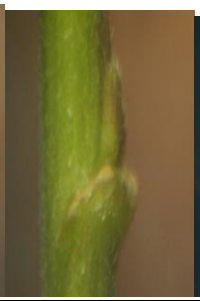


Fig.4: Stem

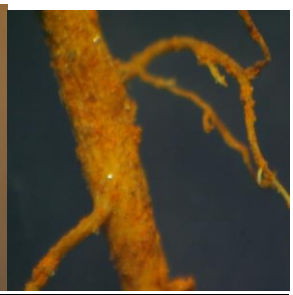


Fig.5: Root

Stem

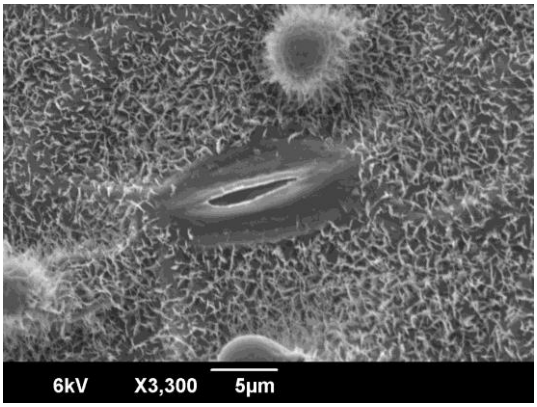
The stem is green in colour, cylindrical and hairy. The stem is herbaceous, unbranched, shows clear differentiation into nodes and internodes. (Figure: 4)

Root

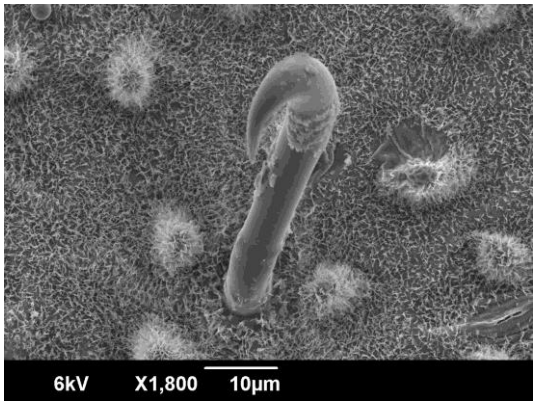
Mature root is dark yellow in colour. The tap root is branched, cylindrical and with ridges and furrows. Small lateral root hairs are also present. (Figure: 5)

2. Micro-anatomy of abaxialsleaf

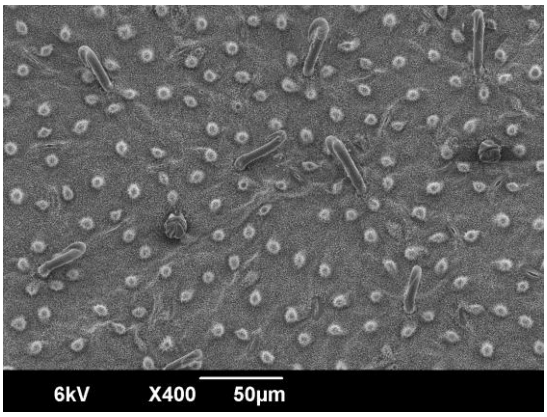
Micromorphological studies of the abaxialleaf surface pattern were done by using Scanning Electron Microscope (SEM). The leaf has clearly defined epidermal cells with stomata and hooked trichomes as well as straight unicellular hairs. The hairs are sparsely arranged. (Figure: 6)



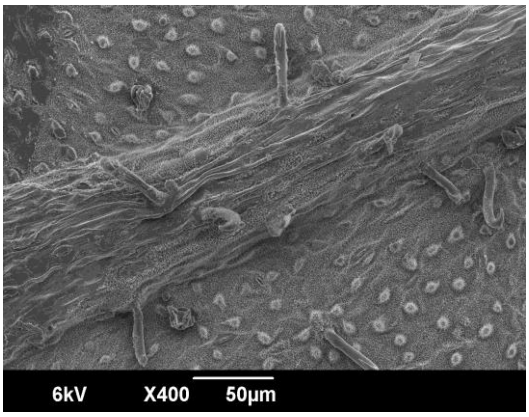
a. Stomata



b. Hooked trichome



c.Abaxial surface



d. Midvein

Figure6: SEM analysis of abaxial leaf surface (a, b, c and d)

3. Anatomical studies

T.S. of Leaf

The transverse section of leaf shows adaxial and abaxial epidermis with cubical-shaped epidermal cells. The epidermal cells on both sides show cuticle. The epidermis consists of unicellular hooked-trichomes. In the midrib region, upper epidermis is followed by hypodermis made of Collen chymatous cells. It is followed by ground tissue in the mibrib region, which surrounds the vascular elements. The mesophyll cells consist of palisade cells and spongy cells filled with chlorophyll and interrupted by vascular cells. Palisade cells are unilayered. Midrib shows the presence of collateral vascular bundles. It consists of scleren chymatous bundle cap. (Figure: 7, 8)

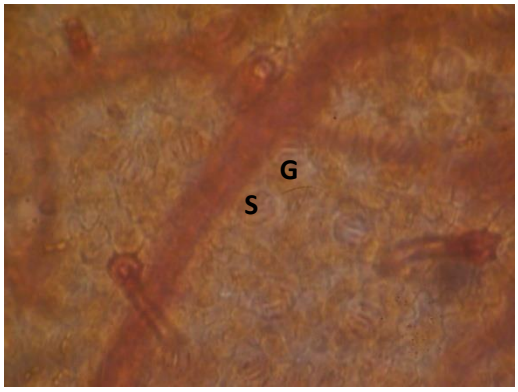


Fig 7. Anamocytic stomata (G-Guard cells; S- Subsidiary cells)

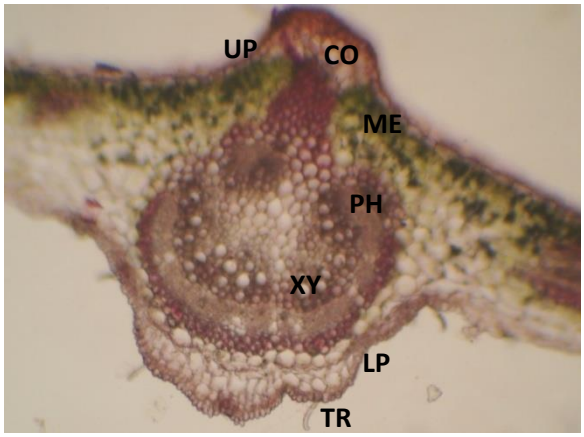


Fig 8. Anatomy of leaf (UP-Upper epidermis, CO-Collenchyma, ME-Mesophyll, PH-Phloem, XY-Xylem, LP-Lower Epidermis,TR-Trichome)

T,S. of Petiole

The transverse section of petiole is triangular in outline. The single layered epidermis is composed of cubical-shaped cells with unicellular hooked-trichomes. The cortical region is filled with chlorenchymatous cells. It is followed by vascular bundles which consist of xylem and phloem with sclerenchymatous cap. The xylem and sclerennchyma cap form a continuous ring. Parenchymatous pith is present at the centre. The section of petiole also shows 2 secondary bundles placed laterally above the primary bundles. (Figure :9)

T.S. of Stem

Transverse section of stem is circular in outline. The epidermis is single layered and composed of cubical-shaped cells with unicellular hooked-trichomes occasionally. Just below the epidermis, the cortex is filled with 4-5 layers of chlorenchyma cells. The cortex is followed by vascular bundles which forms a continuous ring. It consists of phloem elements just above the xylem and capped by fibre of sclerenchyma. The vascular bundle is open, collateral and endarch. The pith is filled with parenchymatous cells. (Figure: 10)

T. L. S. of stem

The transverse longitudinal section (TLS) of stem showed that, inter-vessel pitting is alternate, the sieve tubes with compound sieve plate, companion cells, sclereids and phloem parenchyma. (Figure: 11)

R. L. S. of stem

The radial section (RLS) showed the vessels and tracheids; vessels with spiral and annular thickening; Xylem consists of fibres, parenchyma, tracheids, axial and ray parenchyma cells. (Figure: 12)

T. S. of Root

Transverse section of root is circular in outline. The root shows secondary thickening with periderm formation. It includes phellogen, phellum and phelloderm. These cells are rectangular. The cork cambium or phellum divides to form phellogen towards the outer side and phelloderm towards the inner side. The phellogen is brown coloured dead cells.

Phelloderm is living cells forming 3-4 layers. The stelar portion consists of xylem and phloem developed enormously so that the cells in between the stele and periderm got crushed. Xylem fibres are highly lignified and surrounded externally by phloem. Medullary rays are present. (Figure: 13)

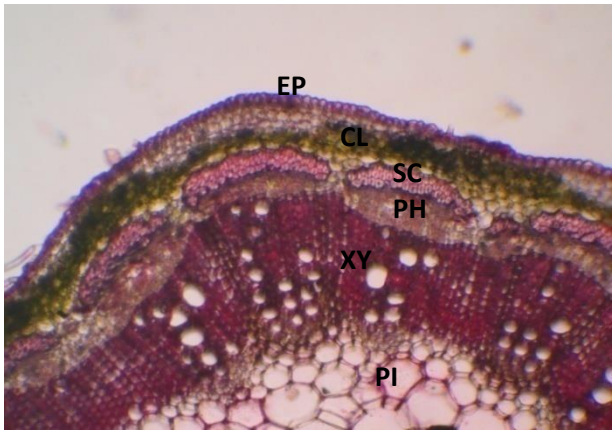


Fig 9. Anatomy of stem (EP- Epidermis, CL-Chlorenchyma, SC-Sclerenchymatous cap, PH-Phloem, XY-Xylem, PI-Pith)

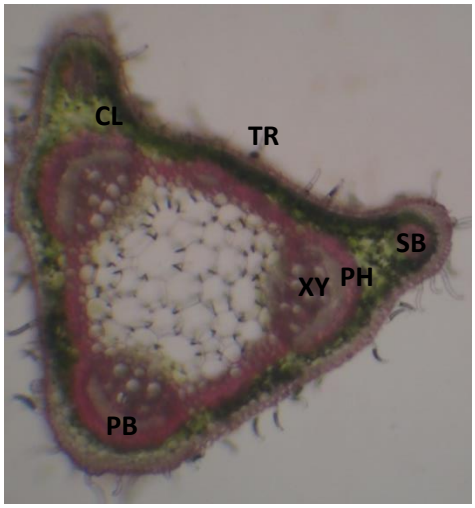


Fig 10. Anatomy of petiole (TR-Trichome, CL-Chlorenchyma, SB- Secondary bundles, PH-Phloem, XY-Xylem, PB-Primary bundles)

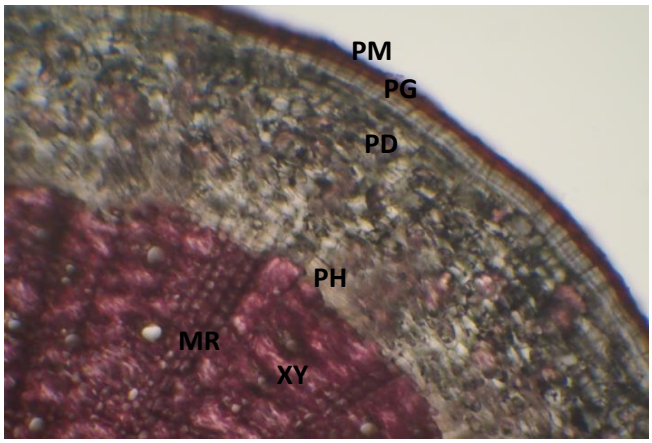


Fig 11. Anatomy of root (PM- Phellum, PG-Phellogen, PD-Phelloderm, PH- Phloem, MR- Medullary rays, XY-Xylem)

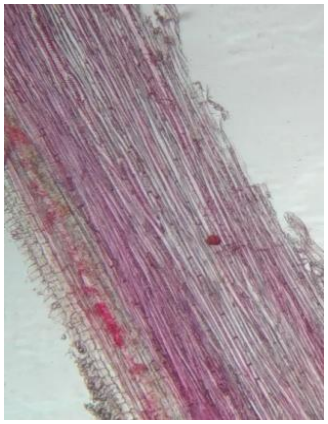


Fig 12. TLS of stem

Fig 13. RLS of stem

4. Phytochemical analysis

Phytochemical screening is an important method for analysing the presence of various bioactive compounds present in the plant which is responsible for its medicinal properties. Qualitative analysis of the methanolic leaf extract of *Christia vespertilionis*was carried out, which is represented in Table 1. They showed the presence of alkaloids, flavonoids, proteins, glycoside, tannin, diterpenes, coumarin and quinines.

Table 1: Phytochemical analysis of the methanolic leaf extract of *Christia vespertilionis.*

Sl. No.	Constituents	Methanolic extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Carbohydrates	-
4.	Proteins	+
5.	Amino acids	-
6.	Glycoside	+
7.	Tannin	+
8.	Phenol	-
9.	Saponins	-
10.	Anthraquinone	-
11.	Phytosterol	-
12.	Resin	-
13.	Diterpene	+
14.	Coumarin	+
15.	Quinine	+

'+' sign indicates the presence and '-' sign indicates the absence

Table 2: Phytochemical profiling of *Christia vespertilionis* by using HPTLC showing peaks with Rf and area with area % at 254 nm

Peak No.	R_f value	Area (AU)	%Area (AU)
1	0.09	262.3	1.15
2	0.26	384.0	1.68
3	040	2238.7	9.79
4	0.45	2288.3	10.01
5	0.51	1535.2	6.72
6	0.60	3349.4	14.65
7	0.68	1547.6	6.77
8	0.75	4291.7	18.78
9	0.80	5532.5	24.20
10	0.87	1428.5	6.25

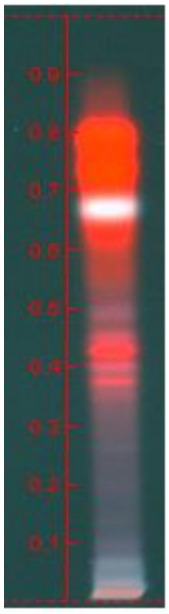
Table 3: Phytochemical profiling of *Christia vespertilionis* by using HPTLC showing peaks with Rf and area with area % at 366 nm

Peak No.	R_f value	Area (AU)	%Area (AU)
1	0.08	683.7	1.56
2	040	2510.1	5.72
3	0.45	3953.3	9.01
4	0.51	1707.7	3.89
5	0.58	1987.0	4.53
6	0.68	8523.2	19.42
7	0.75	11744.9	26.76
8	0.79	12777.0	29.11

5. HPTLC analysis

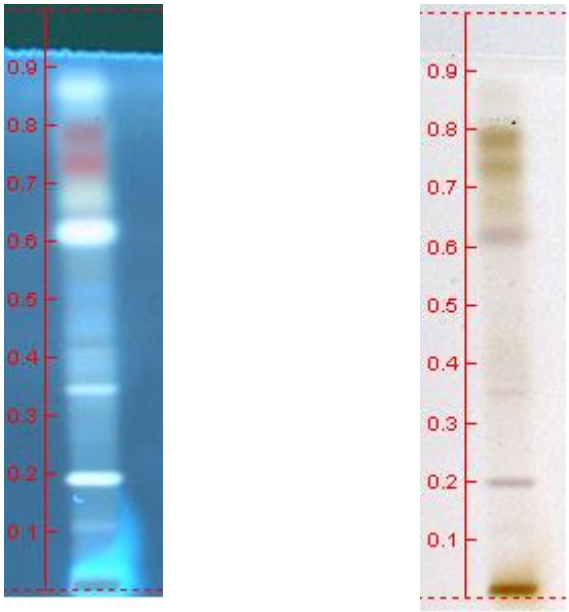
The best results of HPTLC analysis of methanolic leaf extract of *Christia vespertilionis* were obtained in solvent system Toluene: Ethyl acetate: Formic acid: Methanol in the ratio 7:5:1:0.5. TLC plate of methanolic leaf extract scanned at 254 nm wavelength signified the existence of 10 phytoconstituents and the R_f values ranged from 0.09 to 0.87. Out of 10 components, the components with R_{fmax} values 0.45, 0.60, 0.75and0.80 were found to be more predominant as the percentage area was 10.01%, 14.65%, 18.78 and 24.20% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 10.00 %. The total peaks present in HPTLC profile of *C. vespertilionis*is 10 with total area of 22,858. 2 (AU) (Figure :14 & 15; Table 2).

AT 254nm
(a)



AT 366nm
(b)

AT WHITE LIGHT
(c)



(d)DERIVATIZATION AT 366nm **(e)DERIVATIZATION AT WHITE LIGHT**

Figure 14: HPTLC profile of *Christia vespertilionis* before (a, b & c) and after (d & e) derivatization by using HPTLC analysis

TLC plate of methanolic leaf extract scanned at 366 nm wavelength signified the existence of nine phytoconstituents and the Rf values ranged from 0.08 to 0.79. The total peaks present in HPTLC profile of *C. vespertilionis* is nine with total area of 43886.9 (AU) (Figure: 14 & 16; Table 3).

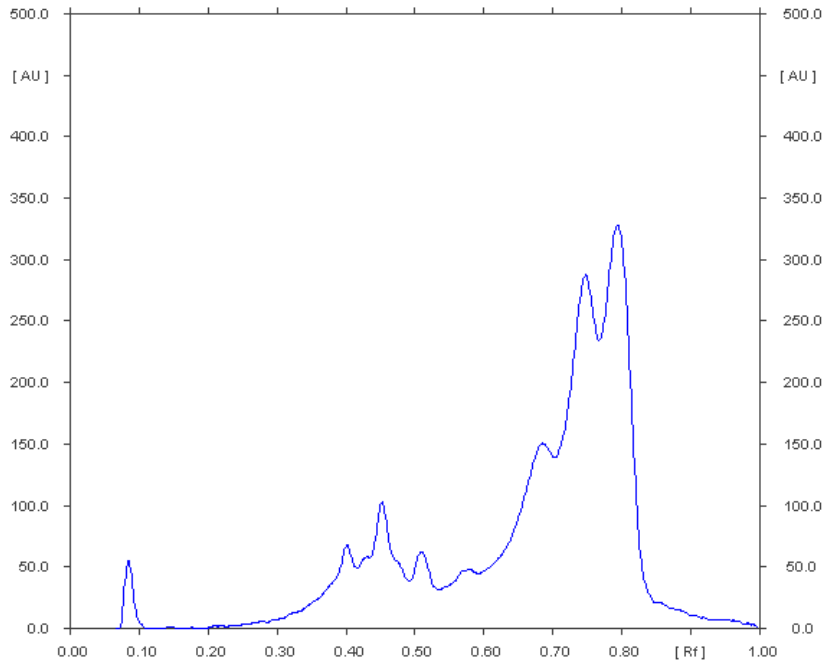


Figure 15: An overview graph of *Christia vespertilionis* sample at 254 nm before derivatization by using HPTLC analysis

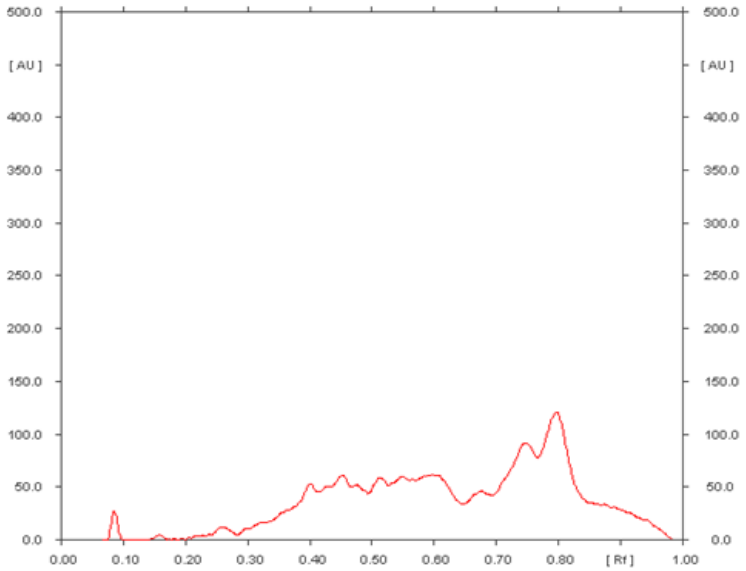


Figure 16: An overview graph of *Christia vespertilionis* sample at 366nm after derivatization by using HPTLC analysis

CONCLUSION

The present study reveals the morphological, anatomical and phytochemical evaluation of the valuable and unexploited medicinal plant, *Christia vespertilionis* (L.f.) Bakh.f (Family Fabaceae). The morphological as well as microscopic studies of any medicinal plant are vital for the identification and authentication of the plant. Morphological and microscopic evaluation serves as the primary step for establishing the identity and purity of the drug, so it should be carried out before any tests are undertaken. This will help in identifying the species and can be further used to determine the botanical identity of herbal medicine.

The present study shows that, the leaf of *Christia vespertilionis*L.f. (Bakh.f.) has butterfly-winged shape. The whole plant body has hairy nature. It has characteristic unicellular-hooked trichomes which are stiff indumenta covering leaves, petiole and stem, similar to that of *Desmodium*.[13] The leaf apex is acuminate and the base is slightly cordate[14]. The stomata are anamocytic. The epidermis is cubical-shaped with hooked trichome. The vascular bundle is collateral and contain sclerenchymatous bundle cap. The xylem and sclerenchymatous cap form a continuous ring in petiole and two secondary bundles are also seen [4]. The secondary bundles are placed laterally above the primary bundles as in the case of *Desmodium.* These characters may be considered as anatomical features for the identification of the plant.

The methanolic extract of the leaf showed presence of alkaloids, flavonoids, proteins, glycoside, tannin, diterpene, coumarin and quinine in the preliminary phytochemical tests. Various other plants from the Fabaceae also showed the presence of several phytoconstituents such as alkaloids, flavonoids and tannin, so it can be considered that these active components are found to be prevailing in this family. [15] The presence of various phytochemicals is believed to be responsible for the medicinal efficacy of the plant preparations. These phytochemicals are very helpful in the development of new drugs for the treatment of various diseases.

HPTLC profile is a good technique to detect the phytochemical variability present in medicinal plants and the HPTLC fingerprint of the leaf sample of *Christia vespertilionis* confirmed the presence of tenphyto constituents at 254 nm and eight phytoconstituents at 366nm. At 254 nm, peak number nine possess Rf value of 0.80 with the highest peak area of 24.02 %. The peak number one possess lowest Rf value, 0.09 and lowest peak area of 1.15 %. At 356 nm, peak number eight possess Rf value of 0.79 with the highest peak area of 29.11 %. The peak number one possess lowest Rf value, 0.08 and lowest peak area of 1.56 %.

Thus, the morphological, anatomical and phytochemical studies of *Christia vespertilionis* (L.f.) Bakh.f. revealed the characteristic profiling, which is important for the standardization and correct identification of the valuable medicinal plant.

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