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A pharmacognostic and preliminary phytochemical study of *Epipremnum aureum* (Linden & Andre) G. S. bunting

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Abstract

Epipremnum aureum (Linden & Andre) G.S. Bunting (Family Araceae) commonly known as Money plant is a vigorously growing liana. It is a common indoor plant generally used for ornamental purposes. It comprises numerous cultivars bearing leaves with white, yellow, or light green variegation. In the present study a pharmacognostic evaluation of the plant species was undertaken. In addition to the macroscopic and microscopic characterizations; evaluation of physicochemical characteristics; preliminary phytochemical parameters and HPTLC fingerprint analysis of the plant methanolic extracts has been carried out. The thin layer chromatographic analysis and the preliminary phytochemical studies of the hot and cold methanolic extracts of the leaves showed the presence of various phytochemical constituents such as alkaloids, tannins, flavonoids, triterpenoids, and saponins. These observations were further confirmed by HPTLC fingerprint analysis. The findings reported in this study can be said to be the first report on the pharmacognosy of E. aureum and will prove of immense use for the correct identification of the plant species which is of key importance in establishing pharmacopeial standards.

Keywords: Epipremnum aureum (Linden & Andre) G.S. Bunting, HPTLC fingerprint Pharmacognosy, Physicochemical analysis, Phytochemistry

Introduction

India has an ancient heritage of traditional medicine which is based on various systems such as Ayurveda, Siddha, Unani and Homeopathy. The evaluation of these drugs is primarily based on phytochemical, pharmacological and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure maintenance of the quality of herbal medicine which largely contributes to its safety and efficacy [1]. A literature search revealed lack of systematic pharmacognostic data for the plant *Epipremnum aureum* (Linden & Andre) G.S. Bunting.

The aim of the present study was to evaluate the pharmacognostic and pharmacological characteristics of this plant species. For this purpose a microscopic and macroscopic study accompanied by preliminary phytochemical; physicochemical evaluation which included thin layer chromatography analysis and HPTLC fingerprint analysis was undertaken.

E. aureum (Family Araceae) is commonly known as the Money plant. It is a monocot that grows vigorously and rapidly covering a wide area. The plant can reach varying heights or it can scramble on the ground. When grown on the ground and if unrestricted in the wild, this liana can grow up on trunks of huge trees by attaching its aerial roots to their surfaces, reaching 10-20 meters. It is a popular indoor house plant with numerous cultivars bearing leaves with white, yellow, or light green variegation.

Taxonomical classification

The taxonomical classification of the plant is as per the system of Bentham and Hooker. [2].

Table 1: Taxonomical classification of *E.aureum*

Kingdom	*Plantae*
Division	*Spermatophyta*
Subdivision	*Angiospermae*
Class	*Monocotyledonae*
Series	*Nudiflorae*
Family	*Araceae*
Genus	*Epipremnum*
Species	*aureum*
Botanical name	*Epipremnum aureum*(Linden& Andre) G.S. Bunting

Morphology of plant

E. aureum is a climber climbing by means of aerial roots which hook over tree branches. This evergreen plant comprises of simple, alternate, entire and heart-shaped leaves. The leaf surface

is waxy. Leaves are small, generally varying between 8-20 cm in length, but if they are grown along the ground or under favorable conditions they are longer and big in size. The leaves are beautifully variegated with white, cream, yellow and various shades of green in different cultivars. Colors, variegation and size of foliage are extremely variable, changing according to the lighting conditions and other cultural factors.

Some of the earlier studies have reported that foliage plants, including golden pothos (*E. aureum*) can purify the air of various atmospheric chemicals, including formaldehyde. [3-4]. According to another study by NASA on the use of common indoor plants for indoor air purification, Golden Pothos is one of the top 3 plants besides Philodendron and Spider Plant that have been labeled the most effective in removing formaldehyde. [5]. The plant has been found to be effective in removing benzene and carbon monoxide too. [6].

The present study can be said to be the first attempt to establish pharmacognostic; phytochemical; macroscopic and microscopic characteristics of *E. aureum* so as to standardize the plant material.

Material and Methods

Plant material collection and authentication

Fresh plant material was procured from the Maharashtra Nature Park, Mahim in Mumbai and was identified and authenticated at the BLATTER HERBARIUM, St. Xavier's College, Mumbai (Voucher specimen accession no. 78393). Leaves were collected and washed under running tap water thrice and later air dried in shade. Dried leaves were powdered and packed in an air tight container. Dried leaf powder was used for physicochemical studies; hot and cold methanolic extract preparation. The methanolic extracts were further used for preliminary phytochemical analysis and HPTLC fingerprint analysis.

Macroscopic and Microscopic studies

Pharmacognostical evaluation was carried out by taking free hand sections of the fresh leaf, stem, and root. The sections were cleared with chloral hydrate solution and then stained with phloroglucinol and HCl or 1% safranin and mounted in glycerin. These sections of the specimens were microscopically examined, recorded and photodocumented with MOTIC photomicroscope provided with MOTIC IMAGE PLUS 2.0 software.

Extraction of plant material

Air dried leaves were processed for hot methanolic extraction using a Soxhlet apparatus. For the cold methanolic extract, the air dried leaves were subjected to agitation with methanol for 12 hr on a rotary shaker at the ambient temperature. Extracts were filtered and evaporated to dryness and the percentage yields were calculated.

Determination of physicochemical properties

Physicochemical properties such as the percentage of total ash, acid insoluble ash, water soluble ash, alcohol soluble and water soluble extractive values and the moisture content were determined as per the standard procedures mentioned by Khandelwal [7]. and Kokate. [8].

Preliminary phytochemical screening

The hot and cold methanolic extracts were subjected to various chemical tests as per the standard procedure for qualitative phytochemical analysis. [9].

Screening of Phytoconstituents by Thin layer chromatography

The hot and cold methanolic extracts were subjected to TLC for detection of different phytochemical constituents as per methods described by Wagner. [10]. The TLC analysis of the plant methanolic extracts was undertaken under the following conditions:

Adsorbent: Precoated silica gel 60 F 254 plates

Samples: Hot and cold methanolic extracts of *E. aureum* (50 mg/ml in methanol).

Table 2: Solvent systems and spray reagents for derivatization of TLC

No.	Phytochemical Constituent	Solvent system	Derivatization and Visualization
1.	Flavonoids	Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:1:1:2.6)	Anisaldehyde sulfuric acid reagent followed by heating at 110^0C for 10 min
2.	Saponins	Chloroform: Glacial acetic acid: Methanol: Water (6.4:3.2:1.2:0.8)	Anisaldehyde sulfuric acid reagent followed by heating at 110^0C for 10 min
3.	Tannins	Toluene: Acetone: Formic acid (6:6:1)	Ferric chloride reagent
4.	Triterpenoids	Chloroform: Glacial acetic acid: Methanol: Water (6.4:3.2:1.2:0.8)	Anisaldehyde sulfuric acid reagent followed by heating at 110^0C for 10 min

HPTLC fingerprint of plant methanolic extract

HPTLC fingerprint analysis of hot and cold methanolic extracts of *E. aureum* was performed using CAMAG Linomat 5 instrument. The chromatograms were scanned by CAMAG TLC scanner 4 and the R_f values analyzed using CAMAG WinCATS planar chromatography manager software. The TLC plates were later derivatized and photodocumented using CAMAG Visualizer & CAM.

Adsorbent: Precoated silica gel 60 F 254 plates

Samples: Hot and cold methanolic extracts of *E. aureum* (10 mg/ml and 50 mg/ml in methanol)

Table 3: Solvent system and spray reagent for derivatization for HPTLC

No.	Solvent system (Mobile Phase)	Derivatization and Visualization
1.	Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:1:1:2.6)	Anisaldehyde sulfuric acid reagent followed by heating at 110^0C for 10 min

UV spectra analysis of plant extract

For the evaluation of UV spectra, solutions of final concentration of 100 ppm of hot and cold methanolic extracts of the leaves of *E. aureum* were scanned in the range of 190nm to 500nm and absorbance of different peaks were analyzed on a Perkin Elmer UV–Visible spectrophotometer Lamda 25 using UV WINLAB Software.

FTIR spectra analysis of plant crude extract

For determination of IR spectra, KBr pellets were loaded with 10µL of hot and cold methanolic extract (10 mg/10 ml in methanol) and IR spectra were recorded using a Jasco FT / IR–460Plus Fourier Transform Infrared Spectrophotometer. The IR spectra were later analyzed using Spectra Manager FT/IR–460/A0716608461 Software.

Results

Macroscopic and Microscopic studies

Transverse section of root

Phellem is indistinct and is made up of 3-4 layers of compactly arranged suberised cells. Phellogen comprises a few layers of compactly arranged thin walled cells. Cortex made up of 10 or more layers of parenchyma with intercellular spaces. Some cells show the presence of raphides and tannins. Endodermis is one layered. Inner to the endodermis is the single layered pericycle in which tangentially elongated more or less rectangular shaped cells are compactly arranged. Vascular elements are centrally arranged, comprising 8 or more groups of xylem, alternating with patches of phloem. Xylem exarch, pith absent. Inner to the metaxylem, sclernchymatous cells are interspersed with a few patches of parenchyma. (Plate 1; Figure. C, D and E)

Transverse section of stem

Epidermis ridged, comprising compactly arranged parenchymatous cells, followed by a few layers of hypodermal chlorenchyma. Cortex many layered, parenchymatous, some cells show presence of raphides. The cortex is characterized by the presence of vascular bundles more or less in a ring. Vascular bundles are closed, conjoint and endarch. Phloem of thin walled cells capped with a 3-4 layered sclerenchymatous tissue. Inner to the multilayered cortex is a single layered endodermis followed by the pericycle. Pith is large, parenchymatous, with scattered vascular bundles similar to those present in the cortex. (Plate 1; Figure. F)

Transverse section of leaf passing through midrib

The upper epidermis is covered with a cuticle. Epidermal cell are radially elongated and compactly arranged in a monolayer. It is followed by 2 -3 layers of chlorenchyma cells. Below the chlorenchyma cells there are compactly arranged polygonal parenchymatous cells filled with raphides. Few cells show the presence of tannins. Lower epidermis is similar to upper epidermis. Three vascular bundles are closer to the upper epidermis whereas the remaining four vascular bundles are at the centre. Each vascular bundle shows xylem towards upper epidermis and phloem towards lower epidermis. These vascular bundles are covered by sclerenchymatous patches (Plate 1; Figure. G).

Transverse section of leaf passing through lamina

Upper epidermis comprises of tabular cells that are compactly arranged and covered with cuticle. Mesophyll is differentiated into a two layered palisade tissue. It is followed by 5 – 6 layers of spongy cells and obliquely cut vascular bundle at frequent intervals. Calcium oxalate crystals are also observed. Upper epidermis shows presence of paracytic stomata. Lower epidermis is single layered cells similar to upper epidermis. (Plate 1; Figure. H and I)

Physicochemical properties

The ash values, extractive values, moisture content are represented in Table 4.

Table 4: Physicochemical parameters of *E. aureum*

No.	Description of general characteristics	Result
1	% Yield of Hot methanolic extraction	2.05 %
2	% Yield of Cold methanolic extraction	2.15 %
3	Total ash value **(% w/w)**	Not more than 17.85%
4	Water soluble ash value **(% w/w)**	Not more than 7.41%
5	Acid insoluble ash value **(% w/w)**	Not more than 4.13%
6	90% Ethanol extractive value **(% w/w)**	9.41 %
7	water extractive values **(% w/w)**	7.04%
8	Loss on drying (% w/ w)	8.16 %

Preliminary phytochemical screening

Preliminary phytochemical studies of the hot and cold methanolic extracts showed presence of reducing sugars, flavonoids, alkaloids, tannins and phenolic compounds (Table 5).

Table 5: Preliminary phytochemical analysis of *E. aureum*

Sr. No.	Test	Expected Result	Inference	Hot Methanolic extract	Cold Methanolic extract
1	**Test for Reducing sugars**				
1.1	Molish's test	Violet ring at junction of two liquids	Reducing sugars present	+	+
1.2	Fehling's test	Yellow precipitate turns red	Reducing sugars present	+	+
2	**Test for starch using 20% tannic acid**	Presence of precipitate	Starch present	-	-
3	**Test for proteins**				
3.1	Biuret test	Pink or violet color	Proteins present	-	-
3.2	5% Lead acetate	White precipitate	Proteins present	+	+
4	**Test for steroids**				
4.1	Salkowski reaction	Chloroform layer appears red and acid layer shows green fluorescence	Steroids present	-	-
4.2	Liebermann-Buchard reaction	Chloroform layer appears red and acid layer shows green fluorescence	Steroids present	-	-
5	**Test for tannins and phenolic compounds**				
5.1	5% $Fecl_3$ solution	Deep blue-black color	Tannins and phenolic compounds present	+	+
5.2	Lead acetate solution	White precipitate	Tannins and phenolic compounds present	+	+
6	**Test for alkaloids**				
6.1	Dragendroff's test	Orange brown precipitate	Alkaloids present	+	+
6.2	Mayer's test	Presence of precipitate	Alkaloids present	+	+
7	**Test for Cardiac glycosides**				
7.1	Keller-Killiani test	Reddish brown color appears at junction of two liquids and upper layer appears bluish green	Deoxy sugars & Cardiac glycosides present	-	-
8	**Test for flavonoids**				
8.1	Shinoda test	Pink color formed	Flavonoids present	-	-
8.2	Lead acetate solution	Yellow precipitate	Flavonoids present	+	+

Key: +:- Phytoconstituent detected; -:- Phytoconstituent not detected

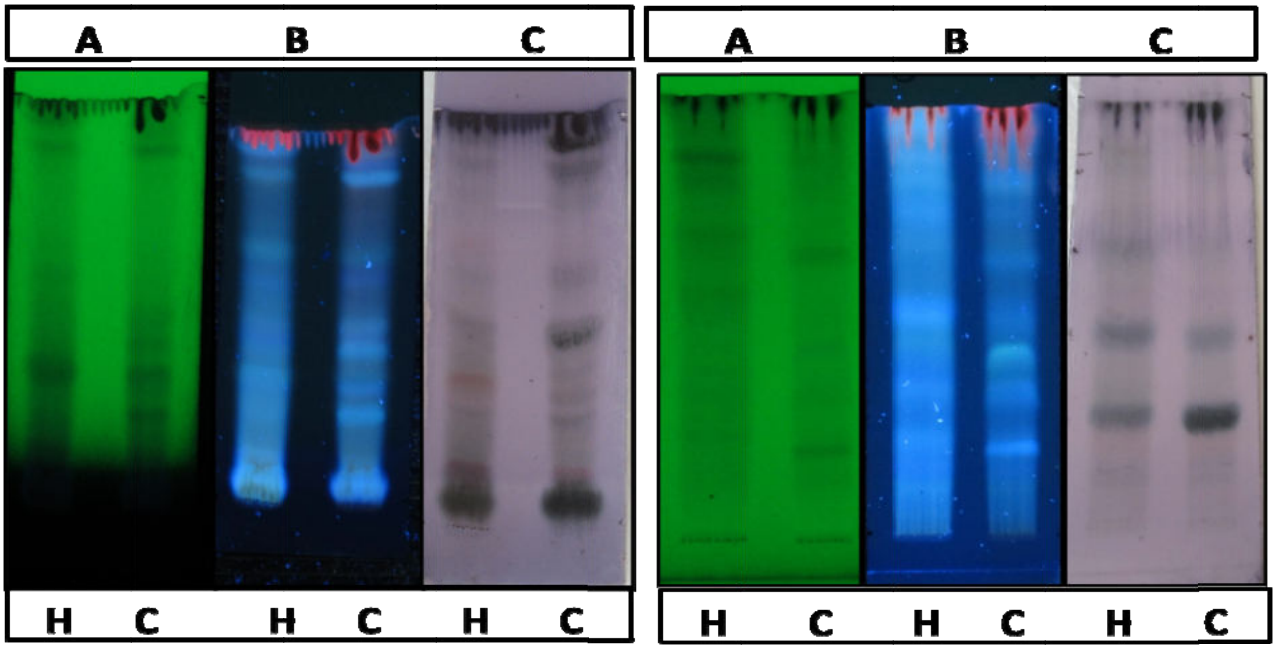

Figure 1: TLC of hot and cold methanolic extracts of the leaves of *E. aureum* for detection of flavonoids. KEY: - H: Hot methanolic extract; C: Cold methanolic extract A: - TLC plate with extracts at 254 nm; B: - TLC plate with extracts at 366 nm; C: TLC plate with extracts after derivatization with anisaldehyde sulfuric acid reagent followed by heating at 110^0C for 10 min

Figure 2: TLC of hot and cold methanolic extracts of the leaves of *E. aureum* for detection of triterpenoids. KEY: - H: Hot methanolic extract; C: Cold methanolic extract. A: - TLC plate with extracts at 254 nm; B: - TLC plate with extracts at 366 nm; C: - TLC plate with extracts after derivatization with anisaldehyde sulfuric acid reagent followed by heating at 110^0C for 10 min

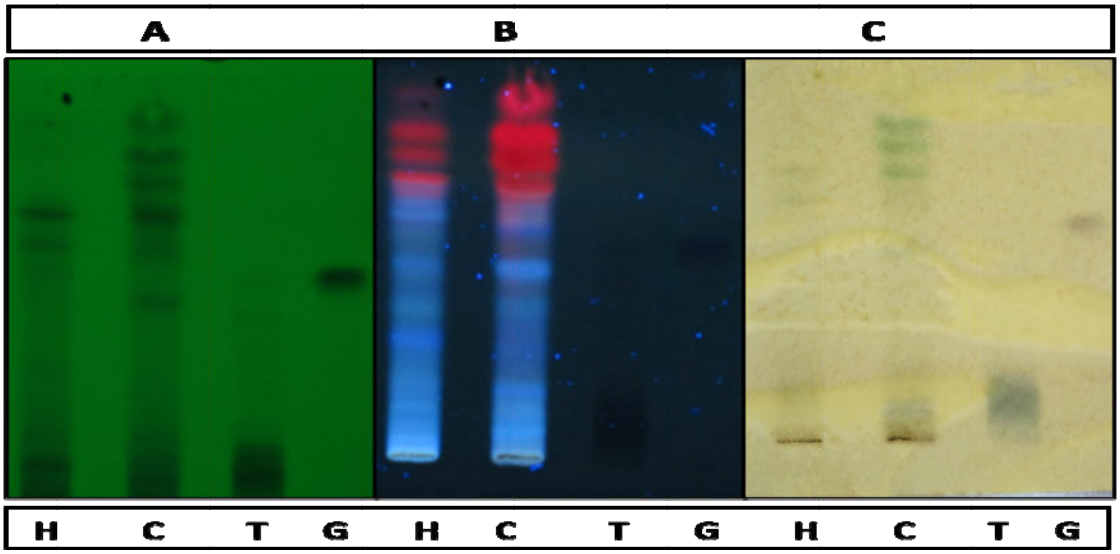

Figure 3: TLC of hot and cold methanolic extract of leaves of E. aureum for detection of tannins KEY: - H: Hot methanolic extract; C: Cold methanolic extract; T: Standard Tannic acid; G: Standard Gallic acid; .A: -TLC plate with extracts at 254 nm; B: - TLC plate with extracts at 366 nm; C: -TLC plate with extracts after derivatization with ferric chloride reagent

HPTLC Fingerprinting

Results of HPTLC fingerprint analysis are as per figure 4, 5 and 6 whereas R_f analysis results are as per Tables 6 and 7.

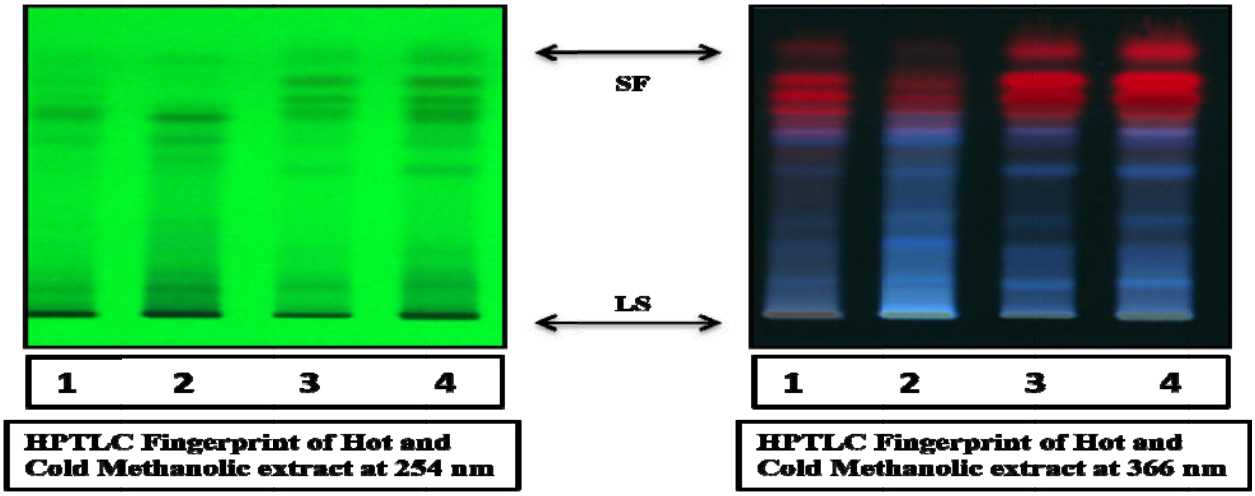

Figure4: HPTLC Fingerprint analysis of hot and cold methanolic extracts of leaves of E. aureum. Key: Track 1: Hot methanolic extract (10 mg/ml); Track 2: Hot methanolic extract (50 mg/ml); Track 3: cold methanolic extract (10 mg/ml); Track 4: cold methanolic extract (50 mg/ml); LS: Loading site; SF: Solvent front

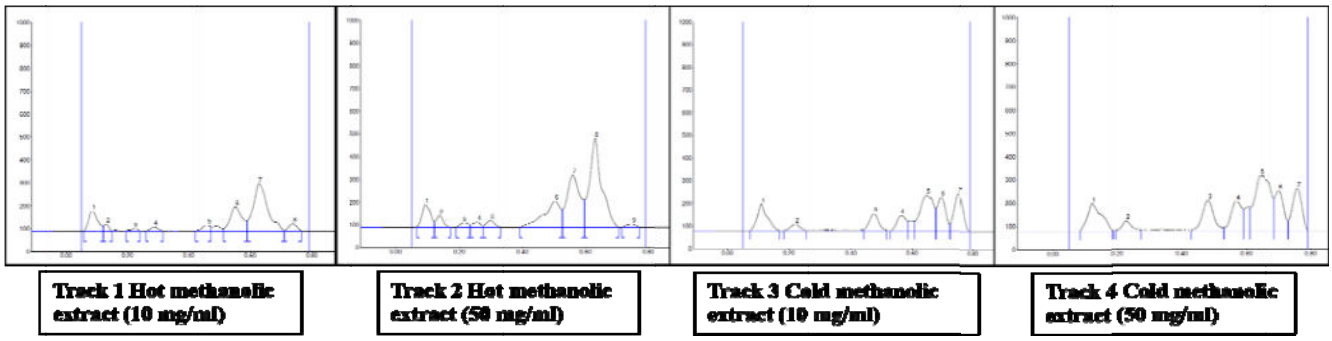

Figure 5: HPTLC Fingerprint analysis of hot and cold methanolic extracts showing peaks at 254 nm. Key: Track 1: Hot methanolic extract (10 mg/ml); Track 2: Hot methanolic extract (50 mg/ml); Track 3: cold methanolic extract (10 mg/ml); Track 4: cold methanolic extract (50 mg/ml)

Table 6: HPTLC fingerprint of hot and cold methanolic extracts of leaves of *E. aureum* with R_f analysis at 254 nm

Hot methanolic extract (10mg/ml)			Hot methanolic extract (50mg/ml)			Cold methanolic extract (10mg/ml)			Cold methanolic extract (50mg/ml)		
Peak	Max R_f	% Area	Peak	Max R_f	% Area	Peak	Max R_f	% Area	Peak	Max R_f	% Area
1	0.09	13.37	1	0.09	7.10	1	0.11	15.21	1	0.12	13.92
2	0.13	2.51	2	0.14	3.22	2	0.22	3.65	2	0.23	3.20
3	0.22	1.48	3	0.21	1.44	3	0.48	9.34	3	0.48	12.28
4	0.29	2.40	4	0.26	1.53	4	0.57	8.30	4	0.57	11.58
5	0.46	3.51	5	0.30	2.11	5	0.65	28.21	5	0.65	31.65
6	0.55	21.78	6	0.50	16.34	6	0.70	16.07	6	0.70	12.78
7	0.63	50.46	7	0.56	26.02	7	0.76	19.22	7	0.76	14.59
8	0.74	4.48	8	0.63	41.10						

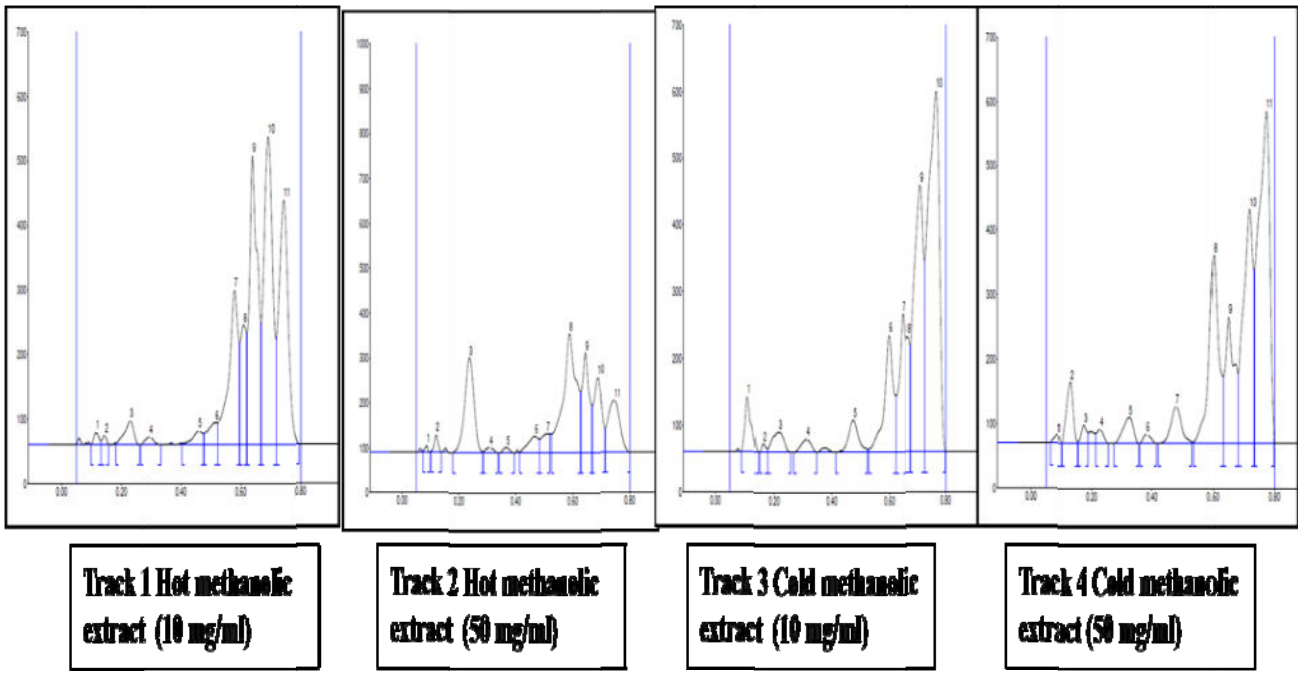

Figure 6: HPTLC Fingerprint analysis of hot and cold methanolic extracts showing peaks at 366 nm Key: Track 1: Hot methanolic extract (10 mg/ml); Track 2: Hot methanolic extract (50 mg/ml); Track 3: cold methanolic extract (10 mg/ml); Track 4: cold methanolic extract (50 mg/ml);

Table 7: HPTLC fingerprint of hot and cold methanolic extracts of leaves of *E. aureum* with R_f analysis at 366 nm

Hot methanolic extract (10mg/ml)			Hot methanolic extract (50mg/ml)			Cold methanolic extract (10mg/ml)			Cold methanolic extract (50mg/ml)		
Peak	Max R_f	% Area	Peak	Max R_f	% Area	Peak	Max R_f	% Area	Peak	Max R_f	% Area
1	0.12	0.66	1	0.09	0.42	1	0.11	3.26	1	0.09	0.45
2	0.15	0.42	2	0.12	1.43	2	0.16	0.38	2	0.13	3.99
3	0.23	2.24	3	0.24	17.98	3	0.22	2.34	3	0.17	1.00
4	0.30	0.68	4	0.31	0.95	4	0.31	1.30	4	0.23	0.97
5	0.46	1.43	5	0.37	0.81	5	0.48	3.22	5	0.32	2.63
6	0.52	2.11	6	0.47	3.77	6	0.60	10.61	6	0.38	0.71
7	0.58	14.57	7	0.51	3.32	7	0.65	8.72	7	0.48	3.91
8	0.61	6.70	8	0.59	32.31	8	0.67	4.58	8	0.60	16.74
9	0.64	23.11	9	0.65	14.27	9	0.71	25.71	9	0.65	10.91
10	0.69	26.30	10	0.69	12.29	10	0.77	39.88	10	0.72	22.11
11	0.74	21.79	11	0.75	12.44				11	0.77	36.59

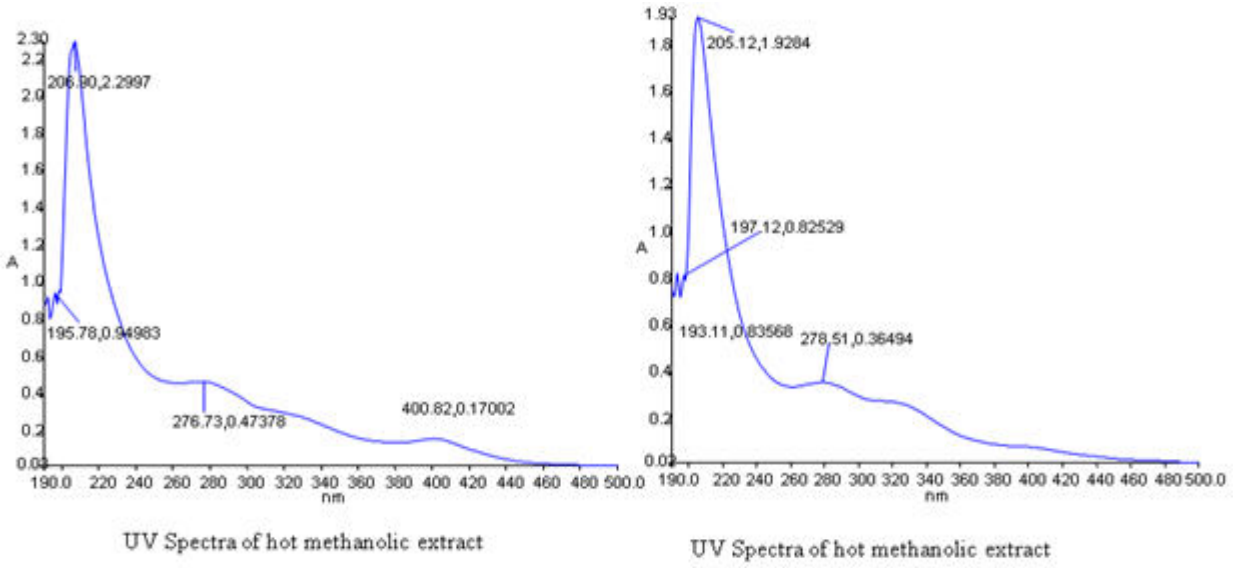

Figure 7: UV spectra of hot and cold mthanolic extracts of *E. aureum*

Table 8: UV Spectra of hot and cold methanolic extracts of *E. aureum*

UV spectra Hot methanolic extract		UV spectra Cold methanolic extract	
Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance
400.97 nm	1.6993	278.66 nm	0.3639
276.58 nm	0.4737	205.12 nm	1.924
206.54 nm	2.3010	196.68 nm	0.8483
195.48 nm	0.9325	193.34 nm	0.7791

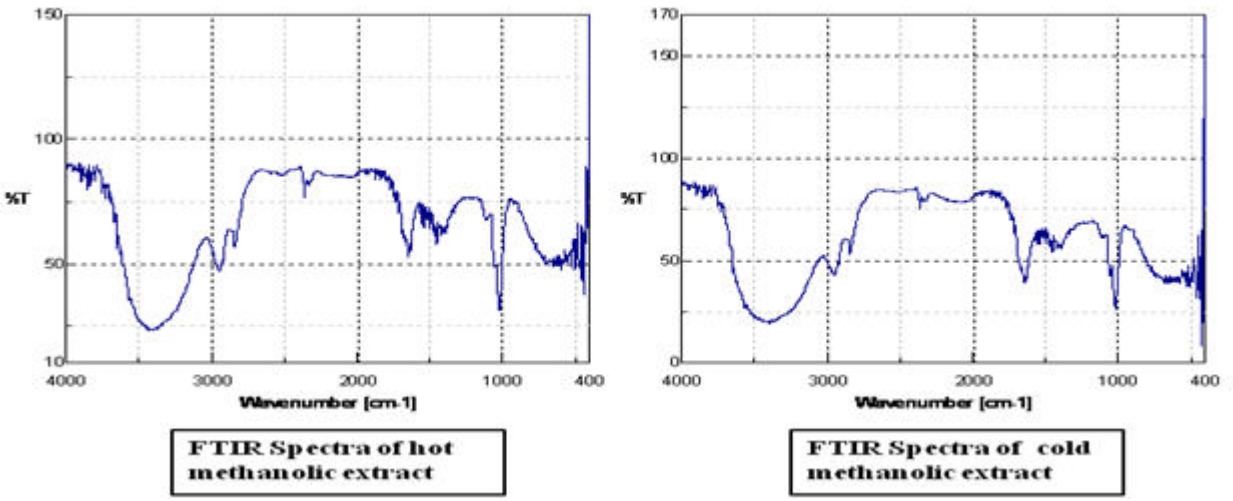

Figure 8: FTIR spectra of hot and cold mthanolic extracts of *E. aureum*

Table 9: FTIR Spectra of hot and cold methanolic extracts of *E. aureum*

FTIR Spectra Hot methanolic extract		FTIR Spectra cold methanolic extract	
Wave number (cm^{-1})	Probable representative Functionalgrouppresent	Wave number (cm^{-1})	Probable representative Functional group present
3400.79	N-H stretch (1^0, 2^0 Amines, Amides)	3402.23	N-H stretch (1^0, 2^0 Amines, Amides)
2954.41	C-H stretch (Alkanes)	2948.63	C-H stretch (Alkanes)
1644	N-H bend (Alkenes,1^0 Amines)	1739.48	C=O stretch (Carboxylic acid, Aldehydes, esters, Ketones)
1550.49	N-O Asymmetric stretch (Nitro compounds, Aromatics)	1549.52	Nitro compounds, Aromatics
1462.74	C-H bend (Alkanes, Aromatics)	1498.52	C-C stretch (Aromatics)
1107.9	C-N stretch (Aliphatic amine)	660.5	-C=C-H: C-H bend (Halogen Compounds)
		592.04	(Bromides, Alkyl halides)

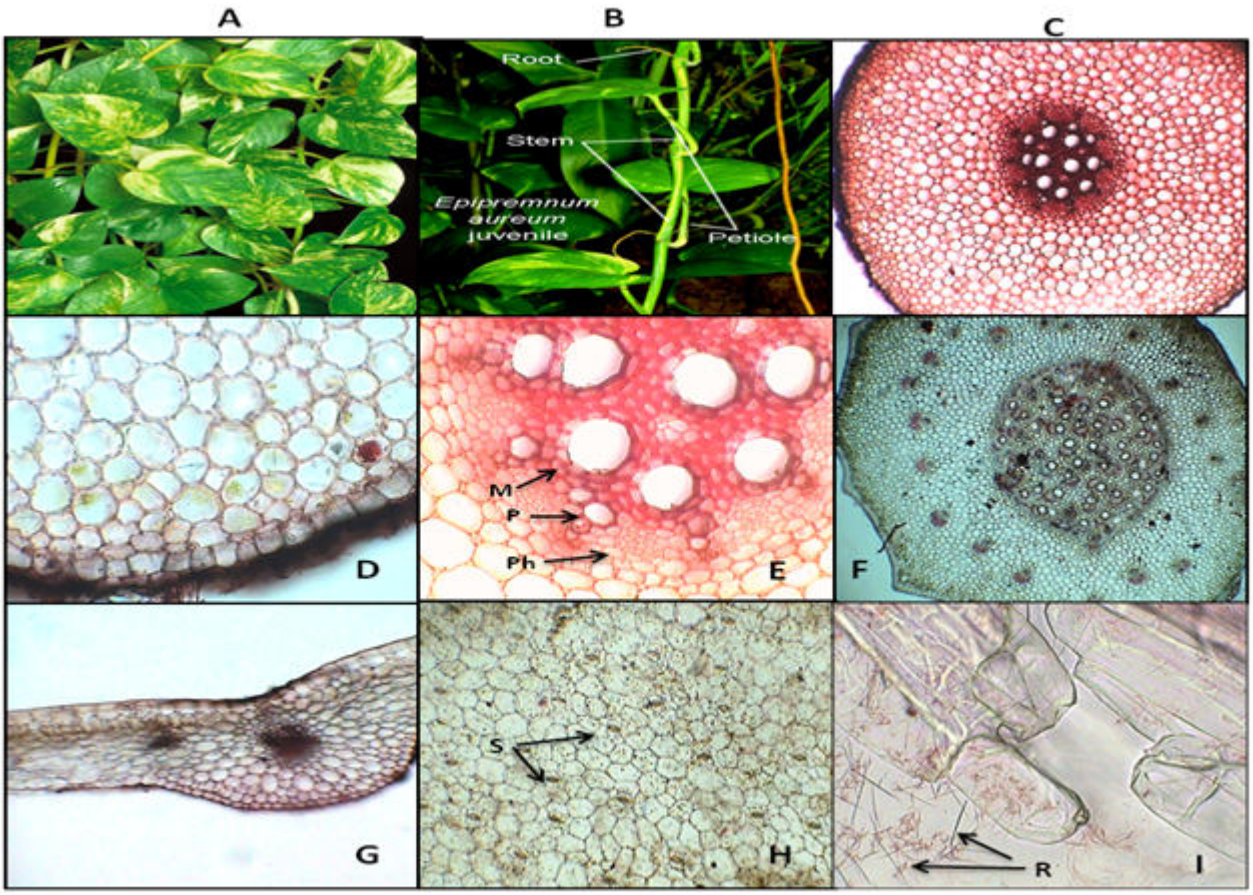

PLATE 1 Macroscopic and microscopic characteristics of the plant *E. aureum*
A & B: *Epipremnum aureum* (Linden & Andre) G.S. Bunting plant
C & D: T.S. of root
E: T. S. of root with Metaxylem (M); Protoxylem (P); and Phloem (Ph)
F: T.S. of stem
G: T.S. of leaf passing through midrib
H: Surface view of upper epidermis with stomata (S)
I: T. S. of leaf showing raphides (R)

Screening of phytoconstituents by thin layer chromatography

The hot and cold methanolic extracts showed the presence of tannins, alkaloids, flavonoids, triterpenoids, and saponins (Figures 1, 2 and 3)

HPTLC Fingerprinting

Results of HPTLC fingerprint analysis are as per figure 4, 5 and 6 whereas R_f analysis results are as per Tables 6 and 7.

UV spectra analysis of plant methanolic extracts

The hot methanolic extract exhibited peaks at 400nm, 276nm, 206nm and 195nm with an absorbance of 1.6993, 0.4737, 2.3010 and 0.9325, respectively, whereas cold methanolic extract showed peaks at 278nm, 205nm and 196nm and 193nm with absorbance of 0.3639, 1.924, 0.8483 and 0.7791, respectively, (Figure 7**,** Table 8).

FTIR spectra analysis of plant crude extract

On the basis of the peaks obtained during the qualitative IR spectra analysis of the hot and cold methanolic extracts, it can be predicted that the hot methanolic extract shows presence of Alkanes, 1^0, 2^0 Amines, Amides, Alkenes, Nitro compounds, Aromatics whereas the cold methanolic extract shows presence of Alkanes, 1^0, 2^0 Amines, Amides, Alkenes, Nitro compounds, Aromatics, carboxylic acids, aldehydes, esters, ketones and Halogen compounds (Figure 8, Table 9).

Discussion

In the present study, the plant *E. aureum* was evaluated for macroscopic and microscopic features of different parts of the plant. Physicochemical parameters showed total ash values not more than 17.85%, water soluble ash values not more than 7.41% and acid insoluble ash values not more than 4.31% and loss on drying 8.16%. The hot and cold methanolic extracts of leaves were evaluated for UV and FTIR spectra analysis. On the basis of FTIR spectra, it can be predicted that hot methanolic extract may contain alkanes, alkenes, 1^0, 2^0 amines, amides, nitro compounds, aromatic compounds and additionally carboxylic acids, aldehydes, esters, ketones and Halogen compounds in cold methanolic extract. These findings are further confirmed by preliminary phytochemical analysis of the hot and cold methanolic extracts. The hot and cold methanolic extracts showed presence of tannins, phenolic compounds, alkaloids, proteins, reducing sugars, triterpenoids and saponins. The HPTLC fingerprint analysis confirmed these findings. The detection and presence of these bioactive secondary metabolites in the plant extracts is highly indicative of pharmacological importance of the plant in terms of its medicinal uses. This aspect of its uses needs a further detailed study. In fact, such an evaluation of the plant methanolic extracts is in progress.

Conclusion

The pharmacognostic evaluation of the plant *Epipremnum aureum* (Linden & Andre) G.S. Bunting has been carried out for the first time. The HPTLC fingerprinting reported in this study can also represent an important diagnostic tool for the correct identification of the plant species and if need arises to check for adulteration of the medicinal plant material. The data reported in the present investigation will prove useful in the standardization of the drug.

Acknowledgement

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