RESEARCH PAPER

Investigation on Pharmacognostical Characteristics, Phytochemical Profiling and Anti-amoebic Potential of *Epipremnum aureum* Leaves

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Abstract
Epipremnum aureum, also called as money plant or pothos, is a perennial evergreen ornamental plant of the family Araceae. It is widely planted and is known for its attractive heart-shaped foliage and for its climbing and trailing habit. The leaves were collected, authenticated, and subjected to organoleptic, microscopical, physicochemical, extraction, and phytochemical investigations. Physicochemical evaluation showed the presence of water and alcohol soluble constituents and satisfactory quality characteristics. Extraction with solvents of different polarity resulted in different yields of the respective extracts, and the aqueous extract had a relatively high extractive yield. The preliminary phytochemical screening showed the presence of some important classes of secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, saponins, steroids/triterpenoids, and carbohydrates in different extracts. These phytoconstituents were also confirmed by thin layer chromatographic analysis. A biological analysis of *Entamoeba histolytica* HM-1: IMSS revealed concentration-dependent antiamoebic action. The flavonoid fraction (79.5 ± 1.1%; IC_{50} 38.9 µg/mL) and alkaloid fraction (64.0 ± 1.4%; IC_{50} 64.4 µg/mL) were the next most active fractions, after the ethanolic extract, which produced 91.3 ± 0.8% inhibition with an IC_{50} of 24.7 µg/mL. Overall, the study shows that *E. aureum* leaves have promising in-vitro antiamoebic activity, thus provided a comprehensive pharmacognostic and preliminary phytochemical profile of *E. aureum* leaves. Pharmacognostic and phytochemical characterization of *E. aureum* leaves revealed characteristic profiles which is of great importance to be used as quality-control parameters. The results are also useful for future studies on its bioactive compounds and medicinal uses.

Keywords: *Perennial, Foliage, Pharmacognostic, Phytochemical, Extraction, Bioactive compounds.*
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1. INTRODUCTION

Nature provides number of sources as drugs for curing several aliments which include plants, animals and various microorganisms. There are huge number of drugs developed from natural sources, among them 80% of drugs come directly or indirectly from plant sources. From the olden days the plants are used in therapeutic purpose but during that time there was no proper evidence regarding causes of illnesses and which plant could be utilized as a cure, everything was known based on experience only. Later the knowledge of usage of specific medicinal plants for treatment of certain diseases led to remarkable increase in the usage of medicinal plants. *Epipremnum aureum* is a perennial, evergreen decorative plant that is a member of the Araceae family [1]. It is native to Southeast Asia and New Guinea and is commonly grown indoors in India under the name "money plant" or " pothos" [2]. The plant has glossy, dark-green, heart-shaped leaves with a smooth, waxy surface and a distinctive climbing or trailing habit [3]. Cultivar and environmental factors can affect leaf size and color [4], which can range from green to yellow, cream, and white. The plant can climb tree trunks because of its long, flexible branches and aerial rootlets. Its stems can also spread out on the ground to provide cover. It can grow to a significant length under favourable conditions and is easily propagated through stem cuttings [5].

The existence of various secondary metabolites-naturally occurring bioactive substances found in various plant parts, such as leaves, roots and stems largely responsible for their medicinal potential. Phytochemical studies of *Epipremnum aureum* have shown that the plant contains a variety of important bioactive compounds, including flavonoids, alkaloids, glycosides, phenolic compounds, saponins, terpenoids, and steroids [6]. These phytoconstituents might account for the various pharmacological effects reported for the plant including anti-inflammatory activity, antioxidant activity, antibacterial activity, antiviral activity, antitermite activity, adaptogenic activity, and other pharmacological activities. The biological potential of *E. aureum* is quite wide, but comparatively less information exists on its antiprotozoal properties. Hence, the aim of the present study was to evaluate the phytochemical profile of *E. aureum* leaves in petroleum ether, chloroform, ethanol and aqueous extracts. The study additionally sought to evaluate the possible antiprotozoal activity. This investigation may give some preliminary evidence for the therapeutic potential of *E. aureum* and could

be helpful in continuing investigation of the biologically active constituents of the plant.

2. MATERIALs AND METHODs

2.1. Collection and Authentication of Plant Material

Epipremnum aureum fresh leaves were gathered at the Uttar Pradesh University of Medical Sciences (UPUMS), New Campus in Saifai, Etawah, Uttar Pradesh, India. To get rid of dust and other sticking contaminants, the gathered plant material was thoroughly cleaned under running tap water. A herbarium specimen was then created for botanical verification and identification. Prof. (Dr.) Rajvir Singh, Head of the Department of Botany at K.K.P.G. College in Etawah, Uttar Pradesh, verified the plant material.

2.2. Organoleptic Evaluation

Standard pharmacognostic techniques were used to evaluate the plant material's organoleptic characteristics. The sample's color and shape were visually inspected with the unaided eye. The plant material was softly crushed between the fingers and smelled to determine its odor, and the tip of the tongue was used to determine its taste.

2.3. Microscopical Study

Standard pharmacognostic procedures were used to evaluate the fresh leaves under a microscope. In order to eliminate chlorophyll and other interfering substances, free-hand transverse sections of the leaves were produced and treated with potassium hydroxide (KOH). The sections were then mounted in glycerin on glass slides after being stained with safranin to improve the visibility of cellular and tissue features. To investigate the anatomical features of the leaf, the produced slices were inspected under a light microscope with a 10x objective lens.

2.3.1. Powder Microscopy

2.3.1.1. Determination of Particle size

The calibration factor was determined by first calibrating the eyepiece micrometer with the help of a stage micrometer. A small quantity of the powdered drug was weighed accurately and placed on a clean glass slide. The slide was then placed on the stage of the microscope and observed under a suitable magnification to identify the fibers present in the powdered drug. The size of the individual fibers was determined with the help of a calibrated eyepiece micrometer. The number of divisions of the eyepiece micrometer covered by each fiber was noted carefully. The actual size of the fibers was determined by multiplying the number of divisions of the eyepiece micrometer by the appropriate calibration factor.

2.3.1.2. Determination of Fiber Length and Width

The calibration factor was obtained first by calibrating the eyepiece micrometer using a stage micrometer. A small amount of the powdered drug was weighed out and carefully placed on a clean glass slide. The slide was prepared and then placed under a microscope to view. Length and width of fibers were measured with a calibrated eyepiece micrometer. The number of divisions of the eyepiece micrometer that corresponded to the length and width of each fiber was recorded. The number of eyepiece divisions occupied by these fibers was then multiplied by the appropriate calibration factor to give the actual dimensions.

2.4. Determination of Foreign Matter

A thin coating of about 50 g of the material was evenly distributed on a spotless surface after being precisely weighed. Using a 6x-10x magnifying lens or visual inspection with the unaided eye, the sample was closely inspected for the presence of extraneous elements. When necessary, a suitable sieve was employed to make it easier to separate and identify foreign material unique to the herbal material.

2.5. Determination of Moisture Content

After precisely weighing 5 g of the leaf sample, it was put into a china dish that had already been weighed. The sample was dried at 105°C in a hot air oven until it reached a consistent weight. Drying was carried out until there was no more than a 5 mg discrepancy between two consecutive measurements. The weight loss in relation to the original weight of the air-dried sample was then used to calculate the percentage moisture content, which was then reported as % (w/w).

2.6. Extractive value

2.6.1. Ethanol-soluble extractive value

In a closed container, 100 ml of ethanol was used to macerate 5 g of the air-dried powdered crude medication after it had been precisely weighed. To aid in extraction, the mixture was shaken sporadically for the first six hours. After that, it was left alone for the entire 24-hour maceration period. The mixture was filtered once the extraction process was finished, and the filtrate was carefully evaporated to dryness over a water bath using an evaporating dish. A hot air oven was used to further dry the residue until its weight remained consistent. Based on the weight of the air-dried crude drug, the ethanol-soluble extractive value was then computed as a percentage (% w/w).

2.6.2. Water soluble extractive value

In a closed container, 100 ml of distilled water was used to macerate 5 g of the air-dried powdered crude drug after it was precisely weighed. To ensure effective extraction, the mixture was shook periodically for the first six hours before being left to stand for a full twenty-four hours. Following the maceration process, the extract was filtered, and the filtrate was carefully dried in an evaporating dish over a water bath. After that, the residue was dried in a hot air oven until it reached a consistent weight. After that, the water-soluble extractive value was calculated and given as the air-dried crude drug's percentage (% w/w).

2.7. Determination of Swelling Index

A 25 ml glass-stoppered measuring cylinder was filled with precisely weighed amounts of the

powdered plant material, followed by the addition of 25 ml of distilled water (or another appropriate swelling agent). Over the course of an hour, the contents were completely combined by vigorous shaking at 10-minute intervals. After completion of the test, the ultimate volume occupied by the inflated plant material, including the mucilage, was recorded. The average swelling index was calculated and expressed in relation to one gram of the plant material.

2.8. Determination of Foaming Index

By precisely weighing 1 g of the coarsely ground plant material and boiling it with 100 ml of distilled water. Following cooling, the mixture was filtered and the filtrate's volume was set to 100 ml. Ten stoppered test tubes were filled with aliquots (1-10 ml) of the filtrate, and distilled water was added to each tube to bring the volume up to 10 ml. Before the foam height was measured, the tubes were aggressively shaken for 15 seconds and left to stand for 15 minutes [7].

2.9. Successive Extraction

Soxhlet apparatus was used to extract the dried leaves after they had been roughly pulverized and put into a filter paper thimble. Solvents with progressively higher polarity were used for successive extraction. At first, fats, waxes, lipids, and chlorophyll were removed using petroleum ether. After that, the marc was extracted using ethanol, chloroform, and water in that order. For additional analysis, each extract was gathered independently.

2.10. Phytochemical screening

Qualitative chemical assays were used to screen the obtained extracts for the presence of different phytoconstituents [8].

Table 1. Screening tests for identification of phytoconstituents

Sr. no.	Identification Tests	Procedure	Observations
Test for Carbohydrates			
1.	Molisch's test	After adding two drops of alcoholic α-naphthol solution to 2 ml of the filtrate, 1 ml of concentrated sulfuric acid was gently applied along the test tube wall. The mixture was allowed to stand after cooling in an ice-water bath.	The presence of carbohydrate is indicated by the creation of a violet ring at the junction.
2.	Fehling's test	To the filtrate, equal amounts of Fehling's solutions A and B were added and brought to a boil.	Red precipitate development is a sign that reducing sugars are present.
Test for Amino acids			
1.	Million's test	1ml of Millon's reagent was added to 1ml of the sample, heated, and then 1% sodium nitrate was added.	The presence of amino acids is indicated by a red colour.
Test for Alkaloids			
1.	Dragendorff's test	1-2ml of Dragendorff's reagent was added to the filtrate.	Reddish-brown precipitate confirm the presence of alkaloid.
2.	Hager's test	1-2ml of Hager's reagent was added to the filtrate.	Yellow precipitate indicates the presence of alkaloids.
3.	Mayer's test	1-2ml of Mayer's reagent was added to the filtrate.	Alkaloids are confirmed by a white or cream precipitate.
Test for Flavonoids			
1.	Shinoda test	Magnesium turnings and hydrochloric acid were added to the extract, and then 5 mL of 95% ethanol was added.	Crimson red colour signifies the presence of flavonoids.

2.	Alkaline reagent test	A 10% sodium hydroxide solution was added to the extract.	The presence of flavonoids is indicated by the formation of intense yellow colour.
3.	Zn test	To the extract, zinc dust and hydrochloric acid was added.	The presence of flavonoids is confirmed by the color red.
Test for Glycosides			
1.	Test A Test B	5ml of sulfuric acid were added to the extract, boiled in a water bath, and then filtered. After neutralizing the filtrate with a 5% sodium hydroxide solution, 0.1 mL of each of Fehling's solutions A and B were added. The mixture was heated in a water bath for 2-3 minutes. A red precipitate was seen to form, and its intensity was compared with that seen in Test B. The extract was combined with 5ml of water, heated, and then diluted with an equivalent amount of water. Following the addition of Fehling's solutions A and B (0.1 mL each), the mixture was heated for 2-3 minutes in a water bath. The red precipitate that was produced was examined and compared with the results from Test A.	Glycosides are present when there is more precipitate in Test A than in Test B.
Test for Tannins			
1.	Ferric chloride test	After dissolving the extract in distilled water, few drops of 5% ferric chloride solution was added.	Green, blue, or violet colour indicates the confirmation of phenolic compounds.
Test for Saponins			
1.	Foam test	After diluting the extract with distilled water, it was agitated for 15 minutes in a test tube.	The presence of saponins is indicated by the development of a stable foam layer that lasts for ten minutes.
Test for Phytosterol and Triterpenoids			
1.	Salkowski test	After adding a few drops of strong sulfuric	Triterpenoids are indicated by a golden yellow

		acid, the extract was shaken and allowed to stand.	color, whereas steroids are indicated by a red lower layer.

2.11. Screening of Phytoconstituents by Thin layer chromatography (TLC)

In accordance with Wagner's approach, the extracts were examined using thin-layer chromatography (TLC) to identify the presence of different phytoconstituents. The following parameters were used for the TLC analysis of the *E. aureum* extracts:
Adsorbent: silica gel G coated plates.
Sample preparation: few mg of extract dissolve in ethanol.
Visualization: iodine chamber [9].

Table 2. Solvent system for identification of phytoconstituents

Sr. no.	Solvent system	Phytocontituents
1.	n- Butanol: Glacial acetic acid: Water (4:1:1)	Carbohydrate
2.	Benzene: Chloroform: Ethanol (4:2:1)	Alkaloid
3.	Chloroform: Ethyl acetate (60:40)	Flavonoid
4.	Ethyl acetate: Methanol: Water (81:11:8)	Glycoside
5.	Chloroform: Ethyl acetate: Methanol (5:3:2)	Tannin
6.	Chloroform: Methanol: Water (70:30:4)	Saponin
7.	Toluene: Ethyl acetate (7:3)	Steroid
8.	Toluene: Chloroform: Ethanol (40:40:10)	Terpenoid

2.12. Preparation of ethanolic extract

The authenticated leaves were thoroughly washed with fresh water and then shade dried for 5 days after which they were coarsely powdered with mechanical grinding. 20 g of the powdered crude drug was subjected to cold maceration with 90% ethanol for 48 hours. On completion of extraction, the extract was filtered, concentrated on water bath and stored in a desiccator for future use.

2.13. Preparation of alkaloidal extract

- The alkaloids were obtained by the method of hot percolation in a Soxhlet apparatus at 30° C. Initially 15 g of powdered crude drug was weighed and packed in the filter paper thimble and then placed inside the Soxhlet extractor.
- The crude drug was defatted with n-hexane for 24 h. The plant material was removed after defatting and air dried for 30 mins to remove residual solvent.
- The dried defatted powder was treated with 12 ml of ammonia solution, with constant monitoring of the pH. The basified material was spread on filter paper and allowed to dry again.
- A fresh thimble was prepared and the dried basified drug was returned to the Soxhlet apparatus. Extraction was performed with chloroform as the solvent.
- Chloroform was used as extraction solvent and extraction was done for 24h to get alkaloid rich extract. The chloroform extract was then transferred to a separating funnel and extracted three times with 25 ml of acidic water (25 ml of water containing 1 ml of hydrochloric acid) by gentle shaking. The acidic treatment converted the alkaloids into their water-soluble salt form, facilitating their transfer into the aqueous layer."
- Then the alkaloid-containing acidic aqueous layer was re-extracted three times with 25 ml of chloroform to convert the alkaloids back into their free-base form and transfer them into the organic layer. The extraction was repeated until complete recovery of alkaloids in the chloroform phase was reached.
- Finally, the chloroform layer was dried over a water bath to obtain the crude alkaloid extract and calculate the percentage yield obtained.

2.14. Preparation of flavonoidal extract

- A sample of 20 g of the powdered crude drug was accurately weighed and placed in an appropriate flask.
- The powdered drug was then mixed with 300 ml of acidified water (10 drops of concentrated hydrochloric acid (HCl) was added to 300 mL of distilled water).
- The mixture was boiled on a water bath for 1 h to facilitate extraction.
- Then it was heated and cooled to room temperature, and filtered using Whatman filter paper to obtain a clear filtrate.
- The filtrate was placed in a separating funnel and extracted with 25 ml of ethyl acetate. The extraction was performed three times and the ethyl acetate layers were collected and pooled together as the ethyl acetate fraction.
- The aqueous layer was returned to the separating funnel and re-extracted with 25 ml of n-amyl alcohol. This extraction was also repeated 3 times and n-amyl alcohol layers were collected and pooled together and the n-amyl alcohol fraction was obtained.
- Fractions of ethyl acetate and n-amyl alcohol were separated and concentrated on a water bath for the removal of solvents.
- Finally, the fractions obtained were weighed and the yield of each fraction calculated [10].

2.15. Anti-amoebic activity (In Vitro)

The trophozoites of *Entamoeba histolytica,* HM-1: IMSS were cultured axenically at 37°C with a reduced oxygen environment in a modified TYI-S-

33 medium containing 10% heat-inactivated bovine serum. Trophozoites in logarithmic growth phase were harvested after chilling the culture tubes on ice for 20 min, gently dislodged and counted with the help of the Neubauer haemocytometer. The cell suspension was diluted to about 1×10^5 viable trophozoites/ml [11]. Samples A, B and C were prepared as stock solutions either in sterile distil in dimethyl sulfoxide (DMSO) depending on the solubility of the sample and then filtered through a 0.22-µm membrane. The working concentration of 6.25, 12.5, 25, 50 and 100 µg/mL was made in culture medium [12]. The end concentration of DMSO was not greater than 0.5% v/v. The trophozoites 1×10^4 were placed in each well in a sterile culture plate and treated with the test samples for 24 h at 37°C, with untreated trophozoites as negative control, corresponding solvent concentration as vehicle control, and metronidazole as positive control [13,14]. Triplicate samples were collected for each concentration, which was repeated three times. Trophozoites were detached by chilling and gently mixed and then stained with 0.4% trypan blue after the incubation period [15]. Trophozoites were counted using a haemocytometer, both viable unstained and non-viable stained. Inhibition of growth was determined as:

Growth inhibition (%) = (1−Viable trophozoites in treatment/Viable trophozoites in untreated control)×10

3. RESULTS AND OBSERVATIONS

3.1. Collection and authentication of plant material

The leaves of *Epipremnum aureum* were freshly collected from New Campus, UPUMS, Saifai, Etawah, Uttar Pradesh. The leaves were rinsed with running water to wash away dust and dried in a hot air oven. A herbarium specimen was prepared and authenticated by the Head of Department, Prof. (Dr.) Rajvir Singh, Department of Botany, K.K.P.G. College, Etawah. The authenticated leaves were then powdered using a grinder for further study.

3.2. Organoleptic evaluation:

Table 3. Representation of organoleptic properties of *E. aureum*

Sr.no.	Description	Characteristics
1.	Colour	Variegated
2.	Odour	Characteristic
3.	Taste	Bitter
4.	Shape	Heart shape
5.	Size	8-20 cm in length

3.3. Microscopy:

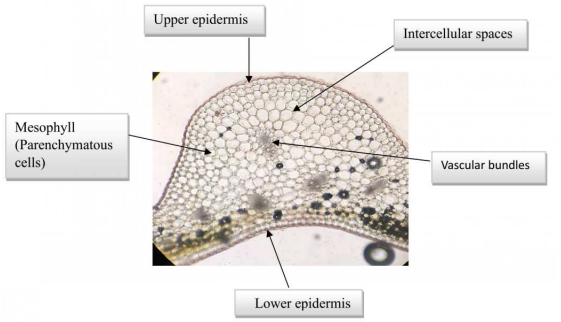


Figure 1. T. S. of leaf of *Epipremnum aureum*

3.4. Physiochemical evaluation:

Table 4. Representation of the physiochemical parameters of leaves of *E. aureum*

Sr.no.	Parameters	Observations
1.	Particle size	53µm
2.	Fiber length and width	43µm; 38 µm
3.	Foreign matter	Nil
4.	Moisture content	8 % w/w
5.	Extractive value • Water soluble • Ethanol soluble	 24 % w/w 5.6 % w/w
6.	Foaming index	Less than 1cm
7.	Swelling index	Nil

3.5. Percentage yield calculation

Table 5. Percentage yield obtained in isolated alkaloid, flavonoid and ethanolic extract of *E. aureum*

Sr.no.	Extracts	Yield (% w/w)
1.	Alkaloidal extract	0.06 % w/w
2.	Flavonoidal extract	2.15 % w/w
3.	Ethanolic extract	6.45 % w/w

Table 6. Percentage yield by successive extraction of *E. aureum*

Sr.no.	Extracts	Yield (% w/w)
1.	Petroleum ether extract	4.24 % w/w
2.	Chloroform extract	2.63 % w/w
3.	Ethanolic extract	12.42 % w/w
4.	Water extract	34.60 % w/w



Figure 2. Successive extracts of *E. aureum* leaves

3.6. Phytochemical screening:

Table 7. Represent the identification test for phytoconstituents

Sr. no.	Identification test	Observations			
Test for Carbohydrates		Pet. ether extract	Chloroform extract	Ethanolic extract	Water extract
1.	Molisch's test	– –	– –	++	++
2.	Fehling's test	– –	– –	++	++
Test for Amino Acids					
1.	Million's test	– –	– –	++	++
2.	Ninhydrin's test	– –	– –	++	++
Test for Alkaloids					
1.	Dragendorff's test	– –	++	++	++
2.	Hager's test	– –	++	++	++
3.	Mayer's test	– –	++	– –	– –
Test for Flavonoids					
1.	Shinoda test	– –	– –	++	++
2.	Alkaline reagent test	– –	– –	– –	– –
3.	Zn test	– –	– –	++	– –
Test for Glycosides					
1.Test A/Test B		– –	– –	++	++
Test for Tannins					
1.	Ferric chloride test	– –	– –	++	++
Test for Saponins					
1.	Foam test	– –	– –	– –	++
Test for Phytosterol and Triterpenoids					
1.	Salkowoski test	++	– –	– –	– –

3.6.1. Identification tests

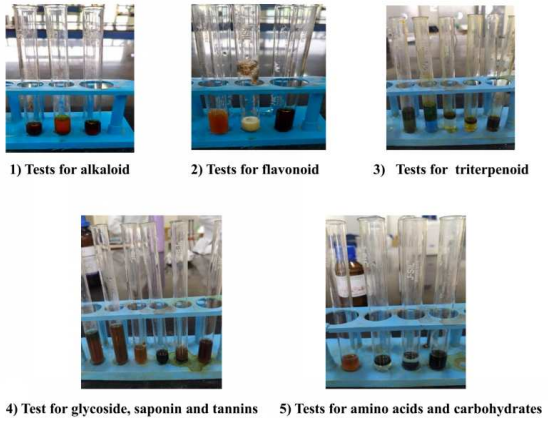


Figure 3: Identification tests

3.7. Identification of Phytoconstituents by TLC:

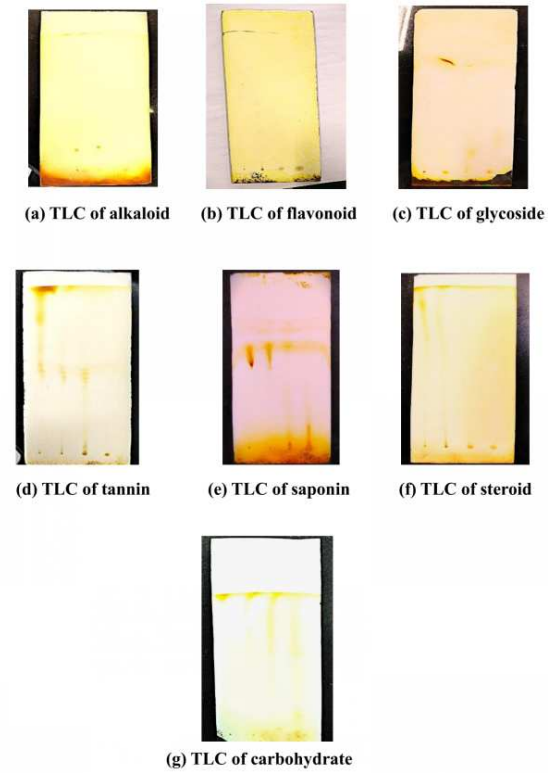


Figure 4. Images showing observed spot of phytoconstituents in *E. aureum* leaves extract : (a) TLC of alkaloid- observerd rf value : 0.15, (b) TLC of flavonoid- observerd rf values : 0.47, 0.53, (c) TLC of glycoside- observerd rf values : 0.14, 0.75, 0.93, (d) TLC of tannin- observerd rf values : 0.27, 0.34, (e) TLC of saponin- observerd rf values : 0.79, 0.81, 0.90, 0.97, (f) TLC of steroid- observerd rf values : 0.68, 0.77, 0.88, g) TLC of carbohydrate- observerd rf values : 0.63, 0.76

Anti-amoebic activity:

The ethanolic extract (Sample-A), flavonoid fraction (Sample-B), and alkaloid fraction (Sample-C) of *Epipremnum aureum* were tested for their in vitro antiamoebic efficacy against *Entamoeba histolytica* HM-1:IMSS. Concentration-dependent trophozoite growth suppression was seen in all three test samples. The ethanolic extract shows the greatest efficacy with inhibition of 91.3 ± 0.8% and an IC_{50} value of 24.7 µg/mL at 100 µg/ml concentration.

Flavonoid fraction had moderate activity with 79.5 ± 1.1% inhibition and an IC_{50} value of 38.9 µg/ml, while the alkaloid fraction showed relatively lower activity, with 64.0 ± 1.4% inhibition and an IC_{50} value of 64.4 µg/ml. The antiamoebic activity was found in the following order: ethanolic extract > flavonoidal fraction > alkaloidal fraction. The combined actions of the several phytoconstituents in the ethanolic extract may account for its somewhat higher activity.

Table 8. Evaluation of antiamoebic avtivity of ethanolic leaves extract (Sample-A) of *E.aureum*

Concentration (µg/mL)	Replicate 1 inhibition (%)	Replicate 2 inhibition (%)	Replicate 3 inhibition (%)	Mean inhibition ± SD (%)	Mean viability (%)
0	0.0	0.0	0.0	0.0 ± 0.0	100.0
6.25	12.1	13.8	12.9	12.9 ± 0.9	87.1
12.5	25.4	27.1	26.2	26.2 ± 0.9	73.8
25	48.1	50.6	49.4	49.4 ± 1.3	50.6
50	72.8	75.1	73.9	73.9 ± 1.2	26.1
100	90.6	92.1	91.3	91.3 ± 0.8	8.7

IC_{50}: 24.7 µg/mL

Table 9. Evaluation of antiamoebic avtivity of flavonoid fraction (Sample-B) of leaves of *E.aureum*

Concentration (µg/mL)	Replicate 1 inhibition (%)	Replicate 2 inhibition (%)	Replicate 3 inhibition (%)	Mean inhibition ± SD (%)	Mean viability (%)
0	0.0	0.0	0.0	0.0 ± 0.0	100.0
6.25	8.2	9.4	8.8	8.8 ± 0.6	91.2
12.5	18.5	19.8	18.9	19.1 ± 0.7	80.9
25	34.1	36.0	35.2	35.1 ± 1.0	64.9
50	55.8	58.2	56.9	57.0 ± 1.2	43.0
100	78.4	80.6	79.5	79.5 ± 1.1	20.5

IC_{50}: 38.9 µg/mL

Table 10. Evaluation of antiamoebic avtivity of alkaloid fraction (Sample-C) of leaves of *E.aureum*

Concentration (µg/mL)	Replicate 1 inhibition (%)	Replicate 2 inhibition (%)	Replicate 3 inhibition (%)	Mean inhibition ± SD (%)	Mean viability (%)
0	0.0	0.0	0.0	0.0 ± 0.0	100.0
6.25	5.1	6.3	5.7	5.7 ± 0.6	94.3
12.5	11.4	12.7	12.0	12.0 ± 0.7	88.0
25	23.5	25.2	24.3	24.3 ± 0.9	75.7
50	39.8	42.1	40.9	40.9 ± 1.2	59.1
100	62.7	65.4	64.0	64.0 ± 1.4	36.0

IC_{50}: 64.4 µg/mL

Overall activity order: Sample A > Sample B > Sample C

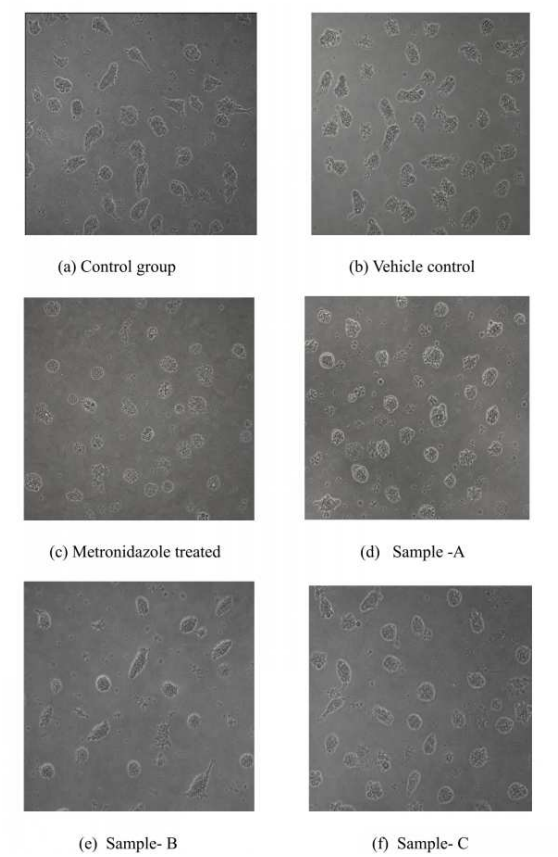


Figure 5. Images showing cell lines treatments : (a) Control group (b) Vehicle control (c) Metronidazole treated (d) Sample -A (e) Sample- B (f) Sample- C

4. CONCLUSION

The present study provided a systematic pharmacognostic, phytochemical and pharmacological evaluation of *Epipremnum aureum* leaves, establishing important parameters for their identification, authentication and standardization. The plant material authenticated and its characteristic organoleptic feature noted, which includes variegated colour, characteristic odour, heart shaped and bitter taste. Microscopical studies revealed the presence of characteristic anatomical features including upper and lower epidermis, parenchymatous mesophyll, vascular bundles, etc., which may be useful diagnostic characters for the plant. Physicochemical evaluation demonstrated a particle size of 53 µm, fibre dimensions of 43 × 38 µm, 8% moisture content and absence of foreign matter. The water-soluble extractive value (24%) was higher than the ethanol-soluble extractive value (5.6%), indicating a greater proportion of water-soluble constituents. The successive extraction yielded extracts of 4.24% petroleum ether, 2.63% chloroform, 12.42% ethanol and 34.60% water, respectively, with the highest yield obtained in the aqueous extract. On preliminary phytochemical screening, several important classes of secondary metabolites, such as alkaloids, flavonoids, glycosides, tannins, saponins and steroidal/triterpenoid constituents with

carbohydrates in selected extracts were detected. TLC analysis further supported the presence of these phytoconstituents through characteristic *Rf* values, including alkaloids (0.15), flavonoids (0.47, 0.53), glycosides (0.14, 0.75, 0.93), tannins (0.27, 0.34), saponins (0.79, 0.81, 0.90, 0.97), steroids (0.68, 0.77, 0.88) and carbohydrates (0.63, 0.76). A biological analysis of *Entamoeba histolytica HM-1:IMSS* revealed concentration-dependent antiamoebic action. The flavonoid fraction (79.5 ± 1.1%; IC_{50} 38.9 µg/mL) and alkaloid fraction (64.0 ± 1.4%; IC_{50} 64.4 µg/mL) were the next most active fractions, after the ethanolic extract, which produced 91.3 ± 0.8% inhibition with an IC_{50} of 24.7 µg/mL. Overall, the study shows that *E. aureum* leaves have promising in-vitro antiamoebic activity, thus provided a comprehensive pharmacognostic and preliminary phytochemical profile of *E. aureum* leaves along with appropriate diagnostic and quality control parameters for the standardization of the herb. The identified phytoconstituents classes also serve as a guiding point for a further isolation, characterization and biological investigation of the bioactive constituents of the plant.

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