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Research Article

Phytochemical, Physico Chemical and Elemental Analysis of Leaves and Stem of *Pothos scandens* Linn.

Joyamma Varkey[1], Seema S Nair[2*]

[1]*Department of Pharmacy, Government T.D. Medical, College Alappuzha, Kerala*
[2]*Department of Pharmacy, Government Medical College, Thiruvananthapuram, Kerala*

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ABSTRACT

Pothos scandens is an important medicinal aroid belonging to the family Araceae, used by the Kanikkars for various ailments. The main objective of this work is to explore *Pothos scandens L*. to identify various phytoconstituents, which can be used in various disease conditions, study physicochemical parameters and analyse the elemental composition of leaves and stem. The defatted leaf and stem powder were subjected to soxhlet extraction using ethanol and chloroform. Preliminary phytochemical screening of the extracts showed the presence of rich variety of phytoconstituents namely alkaloids, tannins, flavonoids, phenols, carbohydrates, xanthoproteins, coumarins etc. The crude extracts were evaluated for the determination of total phenolic content (TPC), total flavonoid content (TFC) and total alkaloid content (TAC). The ethanolic extract of stem powder showed highest values for TPC (98.48 ± 2.31 mg of GAE/g of extract) and TFC (259. 95 ± 2.7 mg of QE/g of extract). TAC was found to be highest in ethanolic extract of leaf powder (67.51± 2.85 mg of AE/gm of extract). A detailed physicochemical investigation of shade dried leaf and stem powders were carried out. The physicochemical constants evaluated were different ash values (total ash, acid insoluble ash, water soluble ash and sulphated ash), extractive values (alcohol soluble extractive value and water soluble extractive value) and loss on drying. The shade dried leaf and stem powders were subjected to elemental analysis which revealed the presence of calcium, magnesium, phosphate, sulphate, iron and chloride.

Keywords: *Pothos scandens*, phytoconstituents, soxhlet extraction, phytochemical analysis, total phenolic content, total flavonoid content, total alkaloid content, physicochemical constants, elemental analysis.

INTRODUCTION

Human beings have used plants to treat various diseases since prehistoric ages. The human use of plants as medicines may be traced back at least 60,000 years with the help of fossil studies[1,2]. If there are no plants on earth, the survival of human beings on earth would become very difficult. Nowadays a large number of new plant-derived drugs came into use. Nature has gifted our country with a large number of precious medicinal plants; therefore, India has often been referred to as the Medicinal Garden of the world. Uses of plants for curing diseases have also been mentioned in Indian Vedas[3].

This work is focused on the exploration of *Pothos scandens L*. to identify various phytoconstituents, which can be used in various disease conditions, study physicochemical parameters and analyse the elemental composition of leaves and stem. Pothos scandens is an important medicinal aroid belonging to the family Araceae, used by the Kanikkars for various ailments and mentioned in CRC World Dictionary of Medicinal and Poisonous Plants[4]. Literature reviews revealed that different parts of the plant can be used to promote healing of abscesses, for curing convulsions and epilepsy, to treat asthma, diarrhoea, cancer, small pox, muscle catches, sprains and bone fracture[5-11].

Phytoconstituents are a group of biologically significant chemicals occur naturally in plants. These are the secondary metabolites of plants which are responsible for the various pharmacological activities such as antioxidant activity, anti-inflammatory activity, antimicrobial activity, cytotoxic activity, analgesic activity, anticancer activity etc. Alkaloids, glycosides, phenolic compounds, steroids, coumarins etc. are the important phytoconstituents which are distributed in different plant parts such as leaves, roots, stems, fruits and flowers[12].

Plants contain different inorganic nutrients which are essential for growth, development and proper functioning of human body. Ingestion of these inorganic compounds in excess or limited amount can cause various health issues. Calcium, Magnesium, Potassium, Sodium, Phosphorous, iron, nitrogen, zinc etc. are the important inorganic

Table 1: Percentage yield of soxhlet extraction.

Part used	Solvent	%yield
Leaf	Chloroform	5
	Ethanol	6
Stem	Chloroform	3
	Ethanol	3

**Author for Correspondence:seema.s.nair@gmail.com*

Table 2: Qualitative analysis of phytochemicals in leaves and stems of *Pothos scandens.*

Test	Chloroform extract		Ethanol extract	
	Leaf	Stem	Leaf	Stem
Alkaloid	+	+	+	+
Anthraquinone	-	-	-	-
Coumarin	+	+	+	+
Flavonoid	+	+	+	+
Glycoside	-	-	-	-
Phenol	+	+	+	+
Reducing sugar	-	-	+	+
Saponin	+	-	+	-
Tannin	+	+	+	+
Xanthoprotein	+	+	+	+

+ Present, - absent

Table 3: Total phenolic contents in the leaf and stem extracts of *Pothos scandens* expressed in terms of gallic acid equivalent (mg of GAE/g of extract)

Sample	Absorbance		
20 µg/ml gallic acid	0.836		
40 µg/ml gallic acid	1.02	Total phenolic content (mg of GAE/g of extract)	Mean (mg of GAE/g of extract) ± SE
60 µg/ml gallic acid	1.4		
80 µg/ml gallic acid	1.5		
100 µg/ml gallic acid	1.68		
	0.898	24.185	
Ethanolic extract of leaf powder (1mg/ml)	0.98	31.78	29.31±2.56
	0.982	31.96	
	1.75	103.07	
Ethanolic extract of stem powder (1mg/ml)	1.671	95.76	98.48 ± 2.31
	1.68	96.60	
	0.654	1.59	
Chloroform extract of leaf powder (1mg/ml)	0.64	0.296	1.16 ± 0.43
	0.654	1.59	
	0.738	9.37	
Chloroform extract of stem powder (1mg/ml)	0.726	8.26	7.58 ± 1.27
	0.692	5.11	

constituents present in plants which are essential to lead a healthy life. They also play important roles in enzyme reaction and other metabolic processes[13].

The purity and quality of crude drugs can be evaluated by using various physicochemical parameters such as ash values, extractive values, loss on drying etc.[14].

Ash Values – Ash value is a criterion to judge the purity of crude drugs. Different ash values such as total ash, acid insoluble ash, water-soluble ash and sulphated ash are used for detecting low-grade products, exhausted products, the presence of sandy and earthy matter etc. in crude drugs.

Extractive Values – The active chemical constituents present in crude drugs can be determined by using extractive values such as water-soluble extractive value and alcohol soluble extractive value. Extractive values are also helpful in the identification of adulterants.

Loss on Drying – Presence of moisture in a crude drug can lead to its deterioration due to the growth of microbes or activation of certain enzymes. Loss on drying is a measure of moisture content in plants.

MATERIALS AND METHODS

Identification and collection of plant material

The plant *Pothos scandens L* was identified and authenticated by Dr. G. Valsaladevi, Curator, Department of Botany, University of Kerala, Kariavattom and the specimen of Pothos scandens L.was deposited in the Herbarium of Department of Botany, University of Kerala, Kariavattom with voucher No. KUBH-6029.

Fresh plants of *Pothos scandens L.* were collected from Tropical Botanic Garden and Research Institute, Palode, Thiruvanthapuram District (Kerala) during the month of October. It was washed thoroughly with tap water, the leaves and stem are cut into small pieces and air dried under shade at room temperature for two weeks. The shade dried plant parts were powdered into coarse powder.

Extraction of plant material

About 100g of the dried leaf powder of *Pothos scandens* was defatted with petroleum ether (60-80°C) by hot continuous extraction in a soxhlet apparatus for 12 h[15]. The defatted powder material was extracted with 300ml chloroform for 48 h in soxhlet apparatus. The extract obtained was filtered and made solvent free by using rotary evaporator and the resulting semisolid mass was dried and calculated the yield.

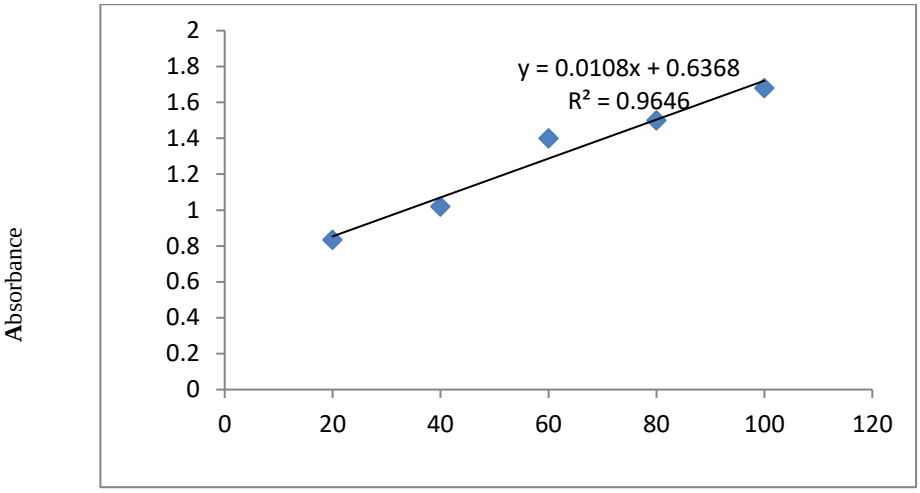

Figure 1: Calibration curve of Standard gallic acid for total phenolic content.

Table 4: Total flavonoid contents in the leaf and stem extracts of *Pothos scandens* expressed in terms of Quercetin equivalent (mg of QE/g of extract).

Sample	Absorbance	Total flavonoid content (mg of QE/g of extract)	Mean (mg of QE/g of extract) ± SE
20 µg/ml quercetin	0.083		
40 µg/ml quercetin	0.182		
60 µg/ml quercetin	0.308		
80 µg/ml quercetin	0.385		
100 µg/ml quercetin	0.484		
Ethanolic extract of leaf powder (1mg/ml)	0.716	145.82	
	0.716	145.82	147.42± 1.6
	0.74	150.62	
Ethanolic extract of stem powder (1mg/ml)	1.3	262.62	
	1.3	262.62	259. 95 ± 2.7
	1.26	254.62	
Chloroform extract of leaf powder (1mg/ml)	0.294	61.42	
	0.242	51.02	54.49 ± 3.4
	0.242	51.02	
Chloroform extract of stem powder (1mg/ml)	1.03	208.62	
	1.012	205.02	206.75 ± 1
	1.02	206.62	

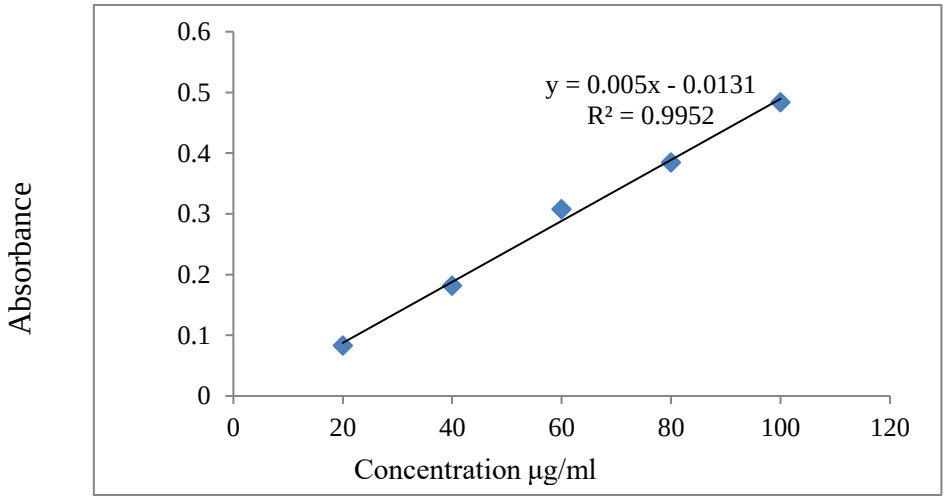

Figure 2: Calibration curve of standard Quercetin for total flavonoid content.

Ethanol extract of leaf powder was also prepared following the same method as above; using another 100gm.Chloroform and ethanol extracts of stem powder were also prepared in the same manner.

Table 5: Total alkaloid content in the leaf and stem extracts of *Pothos scandens* expressed in terms of atropine equivalent (mg of AE/g of extract).

Sample	Absorbance		
20 µg/ml atropine	0.059		
40 µg/ml atropine	0.199	Total alkaloid content (mg of AE/gm of extract)	Mean (mg of AE/gm of extract) ± SE
60 µg/ml atropine	0.294		
80 µg/ml atropine	0.383		
100 µg/ml atropine	0.444		
	0.3	64.67	
Ethanolic extract of leaf powder	0.341	73.21	67.51± 2.85
	0.3	64.67	
	0.284	61.33	
Ethanolic extract of stem powder	0.3	64.67	62.86 ± 0.97
	0.29	62.58	
	0.024	7.17	
Chloroform extract of leaf powder	0.02	6.33	6.47± 0.37
	0.018	5.92	
	0.01	4.25	
Chloroform extract of stem powder	0.013	4.875	4.875 ± 0.36
	0.016	5.5	

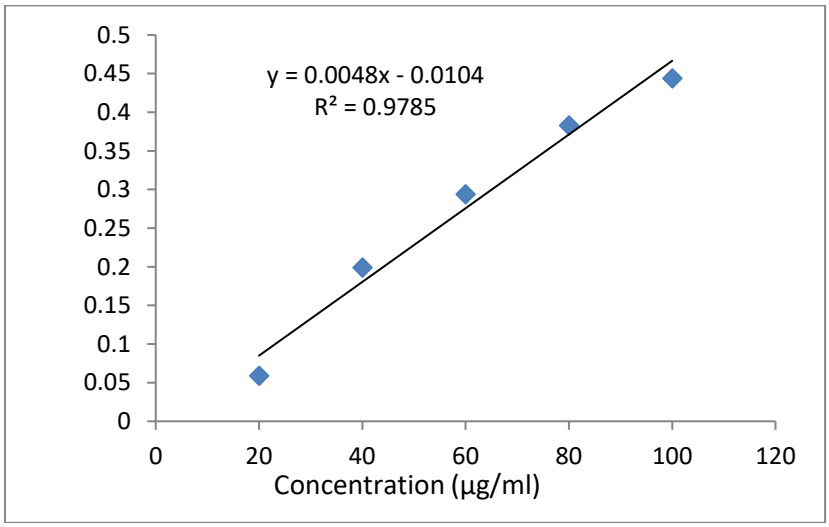

Figure 3: Calibration curve of standard atropine for total alkaloid content.

The dried extracts were then stored in refrigerator until being used up.

Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out as per the standard procedures[16,17], to identify the secondary metabolites present in the chloroform and ethanol extracts of leaves and stem of *Pothos scandens.*

Quantitative estimation of phyto constituents[18,19]

Determination of total phenolic content by Folin Ciocalteu method

The total phenolic content in plant extracts was determined by using Folin Ciocalteu method.

Reagents required

Dilute Folin Ciocalteu reagent with equal volume of distilled water, 20%sodium carbonate in water, Gallic acid

Procedure

Prepared 1 mg/ml solution of each extract. Mixed 1ml of each sample solution with 0.25 ml of Folin Ciocalteu reagent and 1.25 ml of 20% sodium carbonate solution. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described. Incubated the reaction mixtures for 40 minutes at room temperature. After the reaction period the absorbance of standard and test solutions were measured against the reagent blank at 725 nm.

The total phenolic content can be calculated from the calibration curve as mg of GAE/gm of extract by using the following formula.

$$\boldsymbol{T = \frac{CV}{M}}$$

Where T=total content of phenolic compounds (milligram per gram of plant extract), C= the concentration of gallic acid established from the calibration curve (milligram per millilitre), M= the gram weight of plant extract.

Determination of total flavonoid content

Total flavonoid content was determined by using Aluminium Chloride colorimetric method

Reagents required

Table 6: Physico chemical parameters of leaves and stem of *Pothos scandens.*

Physico chemical parameter	Mean±SE Values (%w/w) Leaf	Stem
Total ash value	14.73 ±0.13	11.08 ± 0.54
Acid insoluble ash value	1.05 ± 0.08	0.69 ±0.02
Water soluble ash value	3.33 ± 0.06	2.12 ± 0.08
Sulphated ash value	18.57±0.35	15.23 ± 0.14
Alcohol soluble extractive value	11.23±0.48	11.416±0.02
Water soluble extractive value	14.13±1.1	9.98±1.03
Loss on drying	12.87±0.49	13.93±0.18

Quercetin, 95% ethanol, 10% aqueous aluminium chloride, 1M potassium acetate.

Procedure

Prepared 1 mg/ml solution of each extract. Mixed 0.5ml of each sample solution with 1.5ml of 95% ethanol, 0.1 ml of 10% aluminium chloride solution, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated at room temperature for 30 minutes. The absorbance of test and standard solutions were determined against the reagent blank at 415 nm with a UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Determination of total alkaloid content

Reagents required

Bromocresol green solution (BCG, prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 100 ml with distilled water), phosphate buffer solution of pH 4.7 {prepared by adjusting the pH of 2 M sodium phosphate [71.6 g Na2HPO4 in 1 L distilled water] to 4.7 with 0.2 M citric acid [42.02 g citric acid in 1 L distilled water]}, standard atropine solution (prepared by dissolving 1 mg pure atropine in 10 ml distilled water.)

Procedure

Preparation of standard curve

Accurately measured 0.4, 0.6, 0.8, 1 and 1.2 ml of atropine standard solution and transferred each to different separating funnels. Then, added 5 ml pH 4.7 phosphate buffer solution and 5 ml BCG solution. Shaken well and extracted the yellow coloured complex with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to the volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

Preparation of sample solution

The plant extract (10mg) was dissolved in 2 N HCl and then filtered and washed with 10 ml chloroform. One ml of this solution was transferred to a separating funnel. The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against reagent blank prepared as above. The total alkaloid content was expressed as mg of AE/g of extract.

Physico chemical analysis

The quality and purity of the powdered drug was evaluated by using various physicochemical constants. The dried leaf and stem powder were used for the determination of physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value, and Loss on drying (moisture content).These physico chemical parameters were determined according to the standard procedures[16,20].

Elemental analysis

Ash of drug material was prepared and treated with 50% v/v HCl for 1 hour. After filtration, the filtrate was used to perform the following tests[20].

Calcium: One drop of dil. ammonium hydroxide and saturated ammonium oxalate solution was added to 10ml of the above filtrate. White precipitates of calcium oxalate, soluble in hydrochloric acid but insoluble in acetic acid, were formed.

Magnesium: White calcium oxalate precipitate was separated by filtering the above solution. The filtrate was heated and cooled. Solution of sodium phosphate in dilute ammonia solution was added. White crystalline precipitate was observed.

Sulphate: To 5ml of the test solution, 5% barium chloride solution was added. A white precipitate was formed.

Phosphate: 5ml of test solution was prepared in nitric acid and a few drops of ammonium molybdate solution were added. It was heated for about 10 minutes and left to be cooled. A yellow crystalline precipitate of ammonium phosphomolybdate was observed.

Chloride: 3 to 5ml of lead acetate solution was added to about 5 to 7ml of the filtrate. A white precipitate soluble in hot water was observed.

Nitrates: Ferrous sulphate solution was added to 5ml of the test solution. No brown colour was produced, but when sulphuric acid was added (slowly from the side of the test tube), a brown coloured ring was produced at the junction of two liquids.

Iron: Few drops of 2% potassium ferrocyanide were added to 5ml of the test solution. Dark blue coloration was observed.

Statistical analysis

All analytical determinations and measurements are repeated three times and the values are expressed as mean ±standard error (SE).

RESULTS AND DISCUSSION

Identification, collection and extraction

The plant *Pothos scandens Linn.* was identified and collected. Chloroform and ethanol extracts of coarse powder of the shade dried leaves and stem were prepared and the percentage yield was shown in Table 1

Phytochemical analysis

Preliminary phytochemical screening of chloroform

Table 7: Qualitative analysis of inorganic elements in leaves and stem of *Pothos scandens*.

Test reagent	Observation		Remarks
	Leaf	Stem	
NH_4OH, Ammonium Oxalate	White ppt.	White ppt.	Presence of calcium
NH_3, NH_4Cl, Na_2HPO_4	White ppt.	White ppt	Presence of magnesium
Potassium ferrocyanide	Blue colour	Blue colour	Presence of Iron
5% $BaCl_2$	White ppt.	White ppt.	Presence of sulphate
HNO_3, Ammonium Molybdate	Yellow ppt.	Yellow ppt	Presence of Phosphate
Con. HCl, $AgNO_3$	White ppt.	White ppt	Presence of Chloride
Con. H_2SO_4, $FeSO_4$	No brown colour	No brown colour	Absence of nitrate

extract of leaves and stem showed the presence of alkaloid, flavonoid, phenol, tannin, xanthoprotein, and coumarin. Preliminary phytochemical screening of ethanolic extract of leaves and stem showed the presence of alkaloid, flavonoid, phenol, tannin, xanthoprotein, coumarin, glycoside, and reducing sugar. Both extracts of leaves showed the presence of saponins. The results are presented in table 2.

The Chloroform and ethanol extracts of leaves and stem of *Pothos scandens* were subjected to the quantitative estimation of total phenolic content, total flavonoid content and total alkaloid content. The results were tabulated in tables 3, 4 and 5 respectively. The calibration graphs for total phenolic content, total flavonoid content and total alkaloid content were shown in figures 1, 2 and 3 respectively.

The total phenolic content was examined using the Folin Ciocalteu reagent. The total phenolic content in the crude extracts were calculated from regression equation of calibration curve ($y=0.0108x+0.6368$, $R^2=0.9646$) and expressed as mg of gallic acid equivalent (GAE) per gram of extract. (ie mg of GAE/g of extract). (Table 3). The concentration of phenolic compounds in ethanolic extracts of leaf and stem of Pothos scandens were higher than the chloroform extracts. The highest concentration of phenolic compound was obtained in ethanolic extract of stem of Pothos scandens.

The concentration of flavonoids was determined using spectrophotometric method with Aluminium Chloride. The total flavonoid content in the crude extracts were calculated from regression equation of calibration curve ($y=0.005x-0.0131$, $R^2=0.9952$) and expressed as mg of Quercetin equivalent (QE) per gram of extract. (ie mg of QE/g of extract). (Table 4). The highest flavonoid concentration was obtained in ethanolic extract of stem of *Pothos scandens.*

he concentration of total alkaloids was determined spectrophotometrically. The total alkaloid content in the crude extracts were calculated from regression equation of calibration curve ($y=0.0048x-0.0104$, $R^2=0.9785$) and expressed as mg of atropine equivalent (AE) per gram of extract. (ie mg of AE/g of extract). (Table 5). The concentration of alkaloids in ethanolic extracts of leaf and stem of *Pothos scandens* were higher than the chloroform extracts. The highest alkaloid concentration was obtained in ethanolic extract of leaf of *Pothos scandens*.

Physico chemical analysis

The physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble extractive value, alcohol soluble extractive value and loss on drying of leaf and stem powders were determined and presented in table 6.

Elemental analysis

The elements present in the leaves and stem of *Pothos scandens* were investigated and summarized in Table 7. The elemental analysis revealed the presence of calcium, sodium, potassium, sulphate, phosphate, chloride, magnesium and iron in leaves and stem of *Pothos scandens.*

CONCLUSION

This work revealed the presence of different phytoconstituents in leaf and stem extracts of *Pothos scandens* in varying concentration. Various physico chemical parameters of leaf and stem were determined which is helpful for the proper identification of the plant and also to ensure purity and quality of the plant material. Elemental analysis showed the presence of various inorganic constituents in leaf and stem of *Pothos scandens*. Because of the presence of various bioactive constituents, the plant *Pothos scandens Linn*. can be used as a medicinal plant. Further studies are going on to isolate the bioactive constituents from the plant so as to develop potent drugs.

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