

Pharmacognostic and Phytochemical Evaluation of the Bulbs of *Hippeastrum puniceum* (Lam.) Voss.

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ABSTRACT

The plant *Hippeastrum puniceum* is a widely seen ornamental plant. The fresh bulbs of the plant were used traditionally for healing wounds, curing tumours and piles. As till date no relevant scientific studies had been conducted on this plant. So the present study was conducted to scientifically validate the pharmacognostic and physicochemical parameters. The microscopic evaluation of the bulb shows the presence of starch grains, mucilage cells and xylem fibers. Physicochemical parameters like ash values, extractive values, crude fibre content and fluorescence analysis were also established. Preliminary phytochemical screening of various extracts showed the presence of alkaloids, carbohydrates, flavonoids, tannins, saponins, terpenoids, proteins and amino acids. Total phenolic and flavonoid content of various bulb extracts were determined by Folin Cio-Calteau and Aluminium chloride colorimetric method respectively. The choloform extract showed highest amount of phenolics and the highest amount of flavonoid was present in ethyl acetate extract.

Keywords: *Hippeastrum puniceum*,

INTRODUCTION

Plant materials are used throughout the world as home remedies, OTC drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the world drug market¹. However, a key obstacle in the acceptance of the alternative medicines is the lack of documentation and stringent quality control. Thus there is a real need of standardisation and quality control of herbal drugs. Despite the modern techniques, identification of plant drug by pharmacognostic study is still more reliable². These studies help in identification and standardization of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine. Phytochemical screening is an effective tool in identification of the different active chemical constituents present in a plant extract by treatment with various chemical reagents Thus the present pharmacognostic and phytochemical study is undertaken to establish certain botanical and chemical standards for the identification and quality assurance of the plant *Hippeastrum puniceum*.

It is a bulbous perennial plant, native to South America. It is grown as an ornamental plant worldwide as it bears orange coloured highly attractive flowers. It is seasonal shrub. It sets flowers in summer season, the aerial parts die out and the plant remains dormant for many months. The plant belongs to family Amaryllidaceae and the major common feature of this family is the presence of powdered and observed under microscope for the identification of special features.^{4,5} (Figure 3&4)

underground bulbs. The alkaloid present in *Hippeastrum puniceum* showed insecticidal activity³. The bulbs of the plant were traditionally used in curing piles, tumours and various inflammatory disorders like asthma. Some of tribal community used the bulbs in healing wounds, sores and swellings. The present study includes macroscopic and microscopic evaluation, physicochemical analysis and phytochemical analysis of various extracts. Relevance of the present study increases as there are very less scientific research carried out on the various aspects of this plant.

MATERIALS AND METHODS

Collection and authentication of the plant: Plant used in the present study, *Hippeastrum puniceum* (Lam.) Voss was collected from Ettumanoor, Kottayam, Kerala, authenticated by Dr.Jomy Augustine, St. Thomas College, Pala. The voucher specimen of the plant (No: 2252) was deposited in Department of Pharmacognosy, University College of Pharmacy, Cheruvandoor, Ettumanoor, Kottayam.(Figure 1)

Pharmacognostic studies: The morphological studies were performed to determine the colour, shape, size, odour and taste of the plant specimen (Figure 2). Microscopic evaluation of the bulbs of *Hippeastrum puniceum* was conducted and the photographs of transverse section were taken. For powder microscopic study the fresh bulbs of the plant was shade dried,

Physicochemical evaluation: Physicochemical parameters like various ash values (Total ash, acid insoluble ash and



Fig. 1: *Hippeastrum puniceum*



Fig. 2: Morphology of bulb

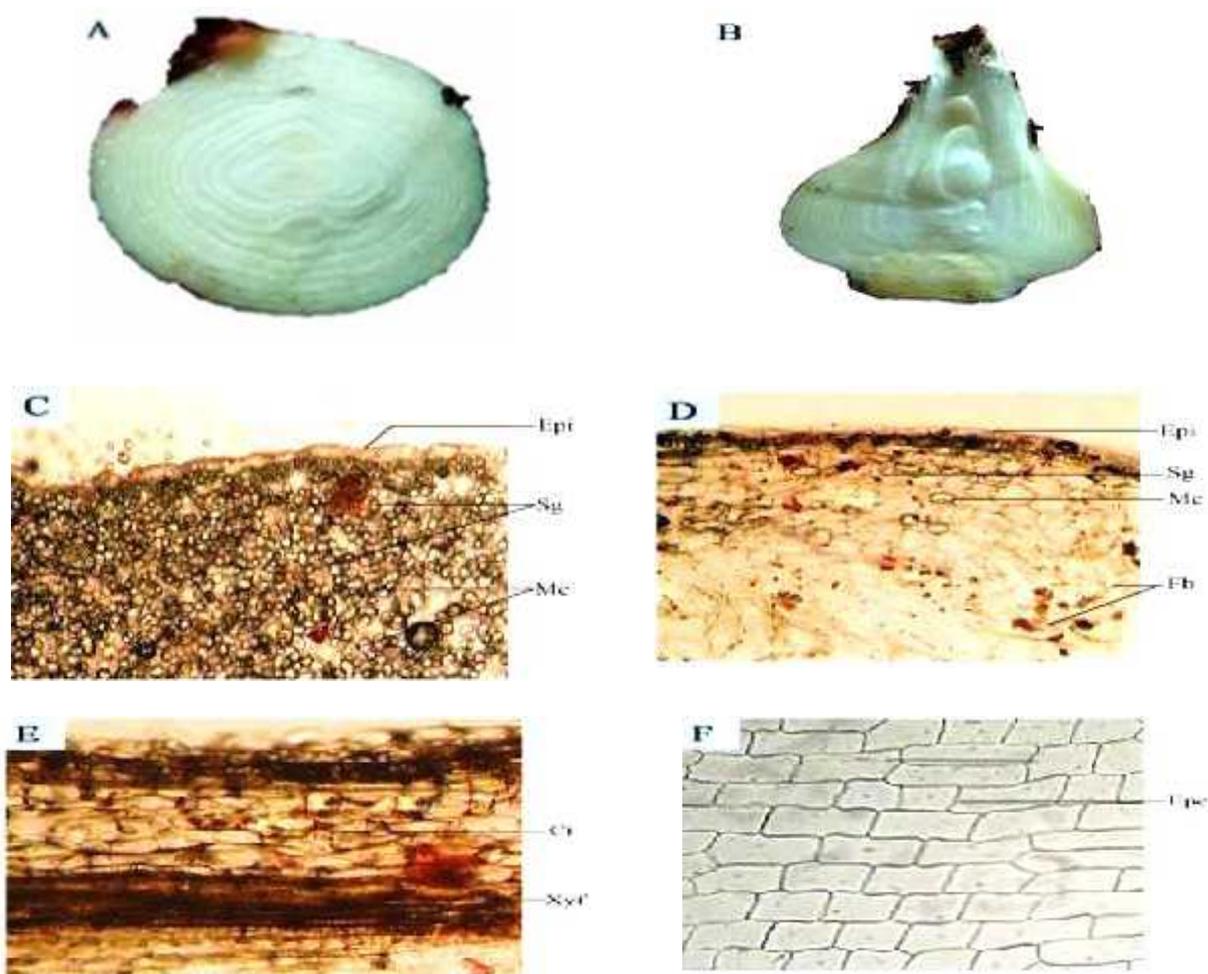


Fig. 3: A. T.S of the bulb. B. L.S of the bulb. C. T.S of the single layer(10x). D: Outer portion enlarged (40x). E inner portion showing xylem fibres (40x). F : Outer peel of the bulb(40x). Epi- epidermis Sg: Starch grains, Mc-Mucilage cells, Ct- Cortex, Xyf- Xylem fibres, Fb- fibres, Epc- Epidermal cell

water soluble ash), extractive values (alcohol soluble and water soluble extractives), loss on drying and determination of crude fibre content were determined in percentage dry weight basis.⁶

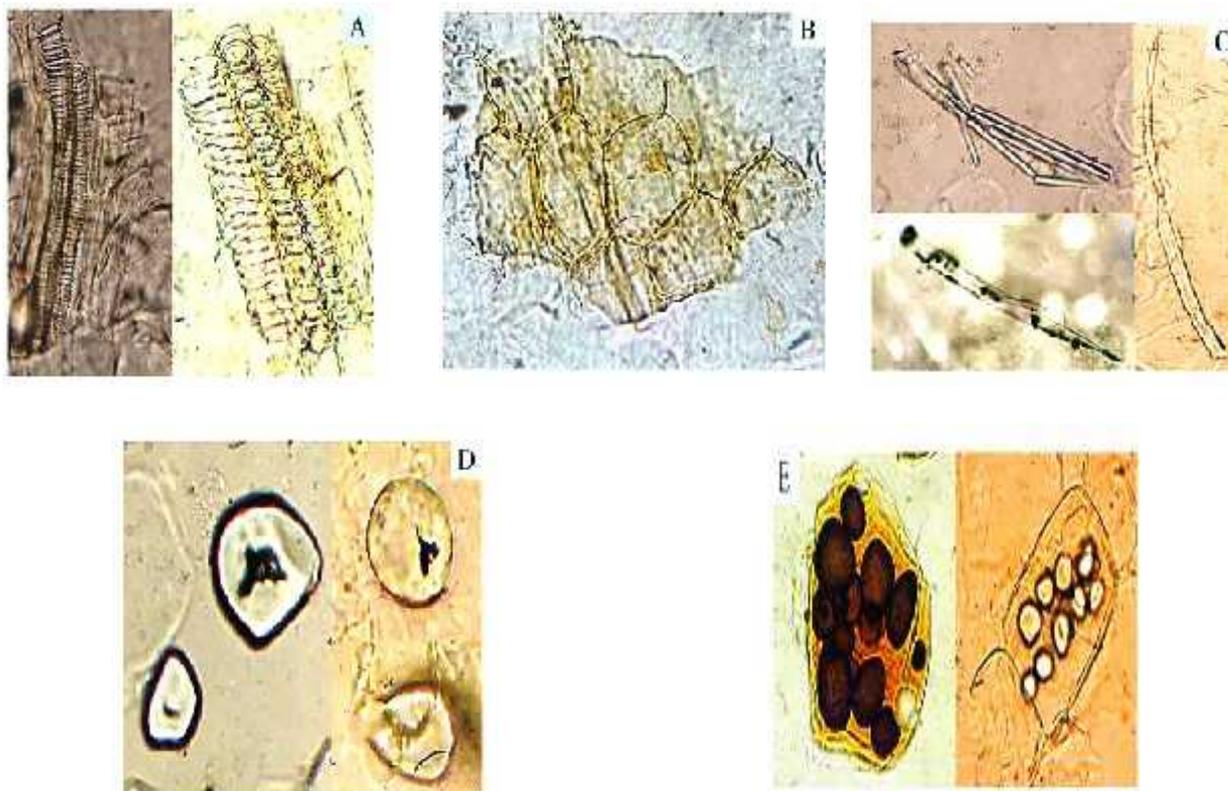


Fig. 4: Powder microscopy of Bulb: A-Xylem vessels with spiral and anular thickening, B- Parenchyma cells in surface view, C- Fibres, D-Mucilage cells, E- Parenchyma cells with starch grains.

Table 1: Determination of physicochemical parameters

Parameters studied	Dry weight basis (% w/w)
Loss on drying	9.18 ± 0.04
Total ash	7.42 ± 0.05
Acid insoluble ash	2.12 ± 0.06
Water insoluble ash	3.32 ± 0.03
Alcohol soluble extractives	3.12 ± 0.03
Water soluble extractives	26.13 ± 0.02
Crude fibre content	9.45

Flourescence analysis: The dried powder of *H. punicum* bulbs were observed under visible and UV light after treatment with various chemical reagents. The fluorescence of the compounds present in the extract can be analysed.^{7,8}

Extraction: Fresh bulbs of optimum size were shade dried and coarsely powdered. Extractions of the powdered drug were carried out by Soxhlation using ethanol as solvent, thus total ethanolic extract were obtained. This total ethanolic extract was further fractionated using various solvents in increasing order of their polarity such as Petroleum ether, Chloroform, Ethyl acetate. The remaining extract (i.e. after fractionating with Ethyl acetate) is considered as alcoholic extract.⁹

Preliminary phytochemical screening: All the prepared extracts were subjected to preliminary phytochemical screening using various chemical reagents.¹⁰

Estimation of total phenolic content: 10 mg of the extracts were weighed, dissolved in methanol and made up to 10 ml with methanol. 1ml was pipetted out from each extract solution and 5ml of Folin Cio -Calteau

reagent was added. After 5 minutes, 4ml of 7.5% Sodium carbonate solution was added and incubated at room temperature for 2 hours. Then, absorbances were measured at 750nm and the values obtained were plotted against concentration. Gallic acid was used as standard.¹¹

Estimation of total flavonoid content (Aluminium chloride colorimetric method): 10mg of the sample extracts were weighed, dissolved in methanol and made up to 10ml with methanol. 1ml was pipetted out from each dissolved sample, add 4ml of water followed by 0.3ml of Sodium nitrite was added. After 5minutes, 0.3ml of 10 % Aluminium chloride solution was added and at the sixth minute 2ml of 1M Sodium hydroxide was added. Mixed well and absorbance was measured at 510nm and the values were interpreted in the standard graph of Rutin to get the milligram equivalents of Rutin.¹²

RESULT AND DISCUSSION

Macroscopy: Large white coloured bulbs, 6-10cm across which are rather like onion in appearance, bitter in taste having no specific odour.(Figure 2).

Table 2: Fluorescence analysis of powdered drug

Solvents used	Ordinary light	UV light
Powder	Whitish brown	Light brown
Powder + picric acid	Yellow	Light green fluorescence
Powder + 1N HCl	Light brown	Yellowish brown fluorescence
Powder + 1N NaOH	Yellowish brown	Light green fluorescence
Powder + 50% HNO ₃	Light brown	Yellow fluorescence
Powder + FeCl ₃	Dark brown	Green fluorescence
Powder + Iodine solution	Black	No fluorescence

Table 3: Percentage yield of various extracts

Extract	Colour and consistency	Percentage yield (% w/w)
TEE	Reddish brown semisolid mass	5.18
PEE	White creamy layer	1.20
CHE	Brown semisolid mass	0.75
EAE	Light brown mass	0.79
ALE	Reddish brown semisolid mass	2.73
AE	Dark brown semisolid mass	24.35

Table 4: Preliminary phytochemical analysis of various extracts

PHYTOCONSTITUENTS	TEE	PE	CHE	EAE	ALE	AQE
Alkaloids	++	-	-	-	++	+
Carbohydrates	+	-	+	+	+	+
Glycosides	-	-	-	-	-	-
Saponin	+	-	-	-	+	+
Tannins	+	-	++	++	+	+
Flavanoids	++	-	+	++	+	-
Terpenoids	+	+	+	+	+	-
Proteins & amino acids	+	+	-	-	+	+

(++): high amount, (+) : presence, (-) : Absence

Microscopy: Transverse section of the bulb shows an outer epidermis with ridges and furrows in outline, Outer cortical region consists of starch grains, mucilage cells and fibre. Inner cortical region consists of group of xylem fibres. Powder light brown in colour, shows xylem vessels with spiral and annular thickening, fragments of parenchymatous cells with starch grains measuring 10-20 microns. Small single groups of fibres and mucilage cells are often seen. (Figure 3,4)

Physicochemical evaluation: The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. The percentage loss on drying was found to be $9.23 \pm 0.4\%$ w/w. Controlled incineration of crude drugs results in an ash residue consisting of inorganic material (metallic salts and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. The total ash, acid insoluble ash and water soluble ash values were found to be $9.18 \pm 0.04\%$ w/w, $2.12 \pm 0.06\%$ w/w and $3.23 \pm 0.03\%$ w/w respectively.

The extractive values are useful to evaluate the chemical constituents present within a crude drug and also to estimate the solubility of specific constituents in different solvents. The alcohol and water soluble extractive value was found to be $3.12 \pm 0.03\%$ w/w and $26.21 \pm 0.02\%$ w/w respectively. Determination of crude fibre content helps in distinguishing between similar drugs or to detect adulterants. Crude fibre determination helps to remove more resistant parts of plant organs which can be used for microscopic examination. Estimation of crude fibre denotes measurement of the content of cellulose, lignin and cork cell in the plant tissue. The crude fibre content was found to be 9.14% w/w. (Table 1). The fluorescence analysis of drug extract helps to identify the drug with specific fluorescent colours and also to find out the fluorescent impurities. The study of fluorescence analysis can be used as a diagnostic tool for testing adulteration.¹³

The powdered drug showed fluorescence when treated with acids and alkalis. (Table 2)

Extraction: The percentage yield of different extracts were calculated and shown in table 3.

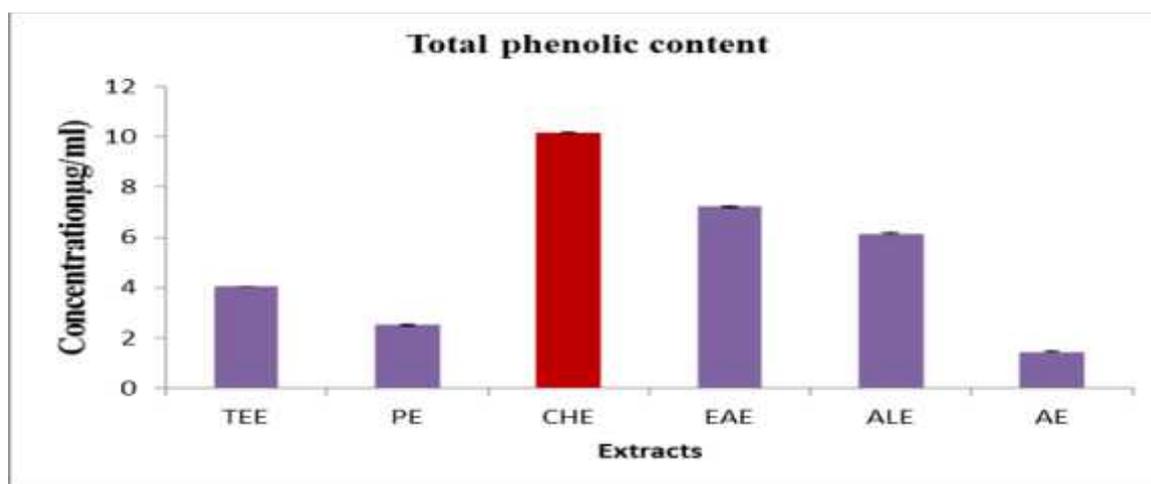


Fig. 5: Total phenolic content of various extracts

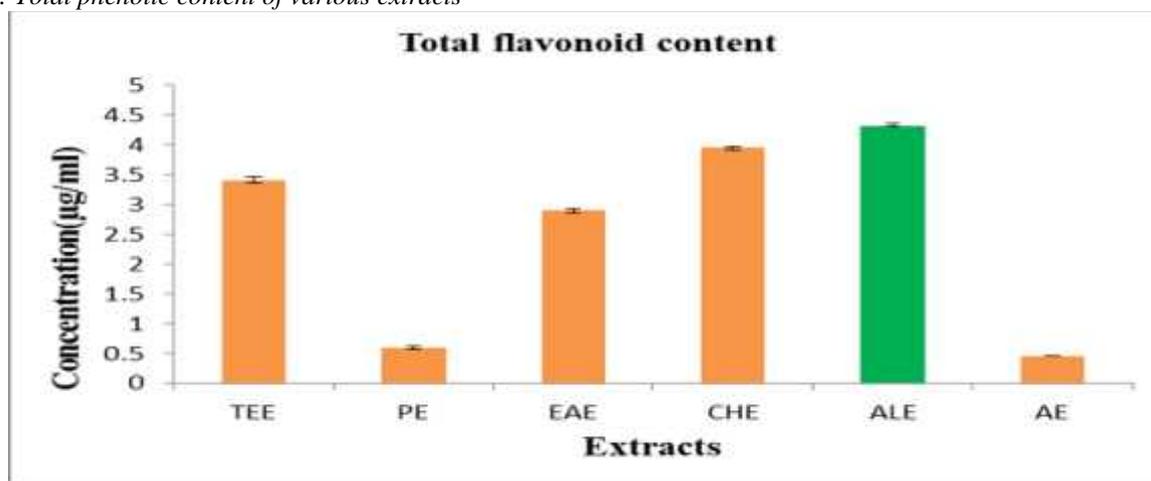


Fig. 6: Total flavonoid content of various extracts

Aqueous extract showed maximum yield followed by total ethanolic, alcoholic, petroleum ether and ethyl acetate extracts. Lowest yield was obtained from chloroform extract.

Preliminary phytochemical analysis: Total ethanolic and all other fractions except aqueous extract showed the presence of terpenoids. Presence of tannins was shown by total ethanolic, alcoholic and ethyl acetate extract. Flavonoids were present only in very trace amounts. A high amount of alkaloids were seen in total ethanolic and alcoholic extracts. Carbohydrates were present in high amounts in aqueous, alcoholic and total ethanolic extracts. Proteins and amino acids were seen in total ethanolic and alcoholic extracts. (Table 4)

Estimation of total phenolic content: The absorbance values obtained for various fractions of *H.puniceum* were recorded. From the calibration curve of Gallic acid the amount of total phenolic content in each fraction was determined and expressed in Gallic acid equivalent. (Figure 5). It was clear that the chloroform extract showed higher amount of phenolics followed by ethyl acetate and alcoholic extract. The total ethanolic extract showed fair amount of phenolics.

Estimation of total flavonoid content: The absorbance values obtained for different extracts were recorded.

From the calibration curve of Rutin the flavonoid content in each extracts were determined and expressed as Rutin

equivalents ($\mu\text{g/ml}$). Figure 6 shows that the highest amount of flavonoid was present in ethyl acetate extract, followed by alcoholic and total ethanolic extract. Aqueous extract showed negligible amount of flavonoid content.

CONCLUSION

Till date no official standards had been reported regarding the plant *Hippeastrum puniceum*. Thus the present study will be a torch light for researchers in identifying and authenticating this plant in future as this widely seen ornamental plant had not been explored much for its traditional therapeutic claim.

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