



Pharmacognostic and preliminary phytochemical evaluation of the leaves of *Cucurbita Pepo*

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Abstract

Medicinal plants continue to play a significant role in traditional and modern healthcare systems due to their diverse bioactive constituents. *Cucurbita Pepo* (Cucurbitaceae) is widely used in folk medicine for its nutritional and therapeutic benefits. The present study was undertaken to evaluate the pharmacognostic characteristics and preliminary phytochemical profile of *Cucurbita Pepo* leaves. Pharmacognostic evaluation included organoleptic, microscopic, and physical parameters to establish diagnostic features of the plant material. The dried leaf powder was subjected to successive solvent extraction using petroleum ether, chloroform, ethyl acetate, methanol, and water. Preliminary phytochemical screening of the extracts was performed to detect the presence of major phytoconstituents. The results revealed characteristic microscopic features such as epidermal cells, stomata, xylem vessels, fibers, starch grains, and calcium oxalate crystals, which aid in authentication of the drug. Phytochemical analysis indicated the presence of carbohydrates, proteins, alkaloids, flavonoids, phenolic compounds, tannins, saponins, and glycosides in various extracts. These findings provide useful information for the identification, standardization, and quality control of *Cucurbita Pepo* leaves and support their potential medicinal applications.

Keywords: *Cucurbita Pepo*, pharmacognosy, phytochemistry

Introduction

Medicinal plants have long served as a valuable source of therapeutic agents and continue to contribute significantly to drug discovery and healthcare systems worldwide. Among these, members of the family Cucurbitaceae are well recognized for their nutritional value as well as their medicinal potential. *Cucurbita Pepo*, commonly known as pumpkin or squash, is a widely cultivated plant that has been traditionally used in various cultures for the management of several ailments [1].

Cucurbita Pepo is an annual trailing or climbing herb characterized by broad leaves, yellow flowers, and edible fruits and seeds. Different parts of the plant, including leaves, fruits, and seeds, are utilized in traditional medicine due to their diverse biological activities. The plant is reported to possess antioxidant, anti-inflammatory, analgesic, antidiabetic, and anthelmintic properties, which are attributed to the presence of bioactive phytochemicals [2].

Phytochemical investigations of *Cucurbita Pepo* have revealed the presence of carbohydrates, proteins, amino acids, flavonoids, phenolic compounds, alkaloids, tannins, saponins, and terpenoids [3]. These constituents are known to contribute to the pharmacological actions of the plant and enhance its therapeutic relevance. The leaves, in particular, have gained attention for their medicinal importance and potential use in herbal formulations.

Despite its extensive traditional use, systematic pharmacognostic evaluation of *Cucurbita Pepo* is essential to ensure proper identification, standardization, and quality control of the plant material. Pharmacognostic studies involving organoleptic, microscopic, and physical parameters provide reliable diagnostic features that help prevent adulteration and ensure consistency in herbal preparations. Therefore, the present study aims to establish detailed pharmacognostic standards and preliminary phytochemical characteristics of *Cucurbita Pepo*, thereby supporting its safe and effective use in herbal medicine.

Materials and Methods

Chemicals

All chemicals and solvents used in the study were of analytical grade. Petroleum ether, chloroform, ethyl acetate, methanol, and distilled water were used for extraction procedures. Reagents required for preliminary phytochemical analysis were prepared freshly using standard laboratory methods. All chemicals were procured from reliable commercial suppliers and were used without further purification.

Procurement of Plant Material

Fresh leaves of *Cucurbita Pepo* were collected from a cultivated source during the healthy growing stage of the plant. The collected plant material was authenticated by Dr. E. Narsimha Murthy, Specimen Accession No: ENM-100131 based on macroscopic and taxonomic characteristics. A voucher specimen was prepared and preserved in the institutional herbarium for future reference. The leaves were washed thoroughly with water to remove adhering dirt and foreign matter, shade-dried at room temperature, and then pulverized to obtain a coarse powder. The powdered material was stored in a well-closed container until further use [4].

Pharmacognostic Evaluation of *Cucurbita Pepo*

Organoleptic Evaluation

Organoleptic evaluation is a sensory analysis method that assesses a product's quality through human senses such as appearance, color, texture, aroma, and taste [5]. This approach is vital for determining freshness, signs of deterioration, and overall quality. By closely examining these sensory attributes, it ensures that the product aligns with quality standards and meets consumer expectations.

Microscopic Evaluation

Microscopic evaluation of the powdered drug is an essential pharmacognostic tool for authentication and quality

assessment. The powdered material consists of fragments of various cellular components arranged as identifiable tissues. Detailed microscopic examination enables the study of diagnostic characters and surface constants, including stomatal number, stomatal index, and palisade ratio, which are of significant taxonomic value [6]. These parameters aid in confirming the identity of the powdered drug and in detecting possible adulteration. In the present study, the powdered leaf material of *Cucurbita Pepo* was analyzed microscopically, with or without staining, to observe characteristic structural features of diagnostic importance.

Powder Analysis of Leaf (Qualitative and Quantitative analysis)

Powder analysis of the leaf was carried out by placing a small quantity of the finely powdered sample on a clean microscopic slide. The powder was treated with 1% phloroglucinol solution and a drop of concentrated hydrochloric acid, mounted in glycerol, then covered with a cover slip and examined under a microscope at suitable magnifications. Various diagnostic characters such as vascular elements, fibers, trichomes, calcium oxalate crystals, and starch grains were carefully observed and recorded using standard microscopic techniques [7-11]. Staining reactions were employed wherever necessary to enhance the visibility of specific cellular components, aiding in the identification and authentication of the powdered leaf drug. Microscopic examination revealed the presence of lignified fibers and xylem vessels, which appeared pink to reddish in color due to the phloroglucinol-HCl reaction. Stone cells were observed as thick-walled structures showing pinkish-red coloration, confirming lignification. Starch grains appeared colorless to faintly bluish, while calcium oxalate crystals were seen as bright, refractile bodies. Trichomes were also observed as elongated or multicellular outgrowths, contributing to the diagnostic features of the powdered leaf. These microscopic characteristics are useful for the identification and authentication of the powdered leaf drug.

Determination of Stomatal Index

The stomatal index was determined to evaluate the proportion of stomata present on the leaf epidermis. A thin epidermal peel was carefully prepared and mounted on a clean glass slide using a suitable mounting medium. The preparation was examined under a microscope at appropriate magnification, and the number of stomata as well as the number of epidermal cells present within a defined microscopic field were counted. The stomatal index was then calculated using the standard formula, which expresses the ratio of the number of stomata to the total number of epidermal cells and stomata, multiplied by 100. This parameter serves as an important diagnostic and taxonomic constant for the identification and quality assessment of the leaf drug.

Physical Evaluation

Physical standardization of the leaf material was conducted in accordance with established pharmacognostical guidelines by determining moisture content, crude fiber, and ash parameters including total ash, acid-insoluble ash, and water-soluble ash. Extractive values were evaluated using solvents of increasing polarity, namely petroleum ether, chloroform, alcohol, and water, to characterize the solubility

behaviour and relative abundance of phytochemical constituents [12]. Ash values were employed to assess the extent of inorganic matter and possible contamination with extraneous materials, while extractive values provided an indirect measure of the concentration and chemical diversity of extractable secondary metabolites. All determinations were performed in triplicate, and the data were expressed as mean \pm standard deviation. Percentage values were calculated on a weight-by-weight basis with reference to air-dried plant material.

Estimation of Crude Fibre (Acid Detergent Fibre, ADF)

The acid detergent fibre content of the leaf material was determined to quantify the cellulose and lignified components resistant to acid detergent treatment. Accurately weighed powdered leaf sample 2g was subjected to reflux digestion with 50ml acid detergent solution containing acetyl trimethyl ammonium bromide in standardized (1litre) sulphuric acid. The mixture was heated initially under vigorous conditions and subsequently under controlled reflux for a fixed duration. After digestion, the residue was transferred to a pre-weighed sintered crucible and thoroughly washed with hot distilled water until complete removal of detergent residues. The residue was further rinsed with 60 ml acetone to facilitate dehydration, dried in a hot air oven at 105 °C to constant weight, cooled in a desiccator, and weighed. The remaining acid-insoluble fraction represented the ADF content, which was calculated and expressed as a percentage of the air-dried sample.

Moisture Content (loss on drying)

The moisture content of the leaf material was determined by the loss on drying method. Accurately weighed fresh leaf sample of *Cucurbita Pepo* (10 g) was placed in a pre-weighed evaporating dish and dried in a hot air oven maintained at 105 °C. The sample was weighed at regular intervals until a constant weight was achieved, indicating complete removal of moisture. Drying was continued until the difference between two successive weighing's did not exceed the specified limit (0.25%). The dried sample was allowed to cool in a desiccator to prevent moisture absorption before final weighing. The percentage moisture content was calculated with reference to the initial air-dried sample weight.

Determination of Total Ash

Total ash content of the powdered leaf drug was determined to evaluate the amount of inorganic residue remaining after incineration. Accurately weighed sample (2g) was placed in a previously ignited and tarred silica crucible and gradually incinerated in a muffle furnace at a temperature not exceeding 450 °C until complete combustion of organic matter was achieved. The crucible was allowed to cool in a desiccator and then weighed. The process of incineration was repeated until a constant weight was obtained. The percentage of total ash was calculated with reference to the air-dried sample.

Determination of Acid-Insoluble Ash

The acid-insoluble ash value was determined to estimate the presence of siliceous matter in the leaf drug. The total ash obtained was treated with 25ml dilute hydrochloric acid and gently boiled for a specified duration. The insoluble residue was separated by filtration through an ashless filter paper

and washed thoroughly with hot distilled water to remove soluble salts. The filter paper containing the residue was ignited in a pre-weighed crucible until a constant weight was obtained. The weight of the remaining residue was recorded, and the percentage of acid-insoluble ash was calculated with reference to the air-dried sample.

Determination of Water-Soluble Ash

Water-soluble ash was determined to assess the amount of inorganic matter soluble in water. The total ash was boiled with 25ml distilled water for a fixed period, and the insoluble portion was collected on an ashless filter paper. The residue was washed with hot distilled water, ignited in a tarred crucible, cooled in a desiccator, and weighed. The difference between the total ash and the weight of the insoluble residue represented the water-soluble ash content, which was calculated as a percentage of the air-dried sample.

Determination of Alcohol-Soluble Extractive Value

The alcohol-soluble extractive value was determined to estimate the quantity of constituents soluble in alcohol. Accurately weighed powdered leaf material (5g) was macerated with 100 ml of 95% ethanol in a closed container for 24 hours, with frequent shaking during the initial hours to ensure proper extraction. The mixture was then filtered, and a measured volume of the filtrate was evaporated to dryness in a tarred evaporating dish. The residue was dried at 105 °C to constant weight, cooled in a desiccator, and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried sample.

Determination of Water-Soluble Extractive Value

The alcohol-soluble extractive value was determined to estimate the quantity of constituents soluble in alcohol. Accurately weighed powdered leaf material (5 g) was macerated with 100 ml of 95% ethanol in a closed container for 24 hours, with frequent shaking during the initial hours to ensure proper extraction. The mixture was then filtered, and a measured volume of the filtrate was evaporated to dryness in a tarred evaporating dish. The residue was dried at 105 °C to constant weight, cooled in a desiccator, and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried sample.

Determination of Chloroform-Soluble Extractive Value

The alcohol-soluble extractive value was determined to estimate the quantity of constituents soluble in alcohol. Accurately weighed powdered leaf material (5g) was macerated with 100 ml of 95% ethanol in a closed container for 24 hours, with frequent shaking during the initial hours to ensure proper extraction. The mixture was then filtered, and a measured volume of the filtrate was evaporated to dryness in a tarred evaporating dish. The residue was dried at 105 °C to constant weight, cooled in a desiccator, and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried sample.

Determination of Ether-Soluble Extractive Value

The alcohol-soluble extractive value was determined to estimate the quantity of constituents soluble in alcohol. Accurately weighed powdered leaf material (5g) was macerated with 100 ml of 95% ethanol in a closed container for 24 hours, with frequent shaking during the initial hours to ensure proper extraction. The mixture was then filtered, and a measured volume of the filtrate was evaporated to

dryness in a tarred evaporating dish. The residue was dried at 105 °C to constant weight, cooled in a desiccator, and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried sample.

Preliminary Phytochemical Screening

Preliminary phytochemical investigation of the leaf powder was carried out to identify the major classes of secondary metabolites present in the plant material. Successive solvent extraction was performed using solvents of increasing polarity, namely petroleum ether, chloroform, ethyl acetate, methanol, and water. The extracts obtained were concentrated, weighed, and the percentage yields were calculated. Each extract was subjected to qualitative chemical tests employing standard reagents to detect the presence of various phytoconstituent groups.

The screening revealed the occurrence of carbohydrates, proteins, and amino acids, as indicated by positive reactions in respective tests. Steroidal and triterpenoid compounds were identified through characteristic colour reactions, while the presence of glycosides, including anthraquinone and saponin glycosides, was confirmed by specific diagnostic tests. Alkaloids were detected using precipitating reagents, and flavonoids were identified by colorimetric reactions. The presence of tannins and phenolic compounds was established through characteristic complex formation with suitable reagents.

The results of the phytochemical screening demonstrate the chemical diversity of the leaf material and support its potential pharmacological relevance. These findings provide a scientific basis for further isolation, characterization, and biological evaluation of the bioactive constituents present in the plant ^[13-16].

Fluorescence Analysis

Fluorescence analysis of the powdered leaves of *Cucurbita Pepo* was carried out to study the characteristic fluorescence behaviour of the plant material. A small quantity of the dried leaf powder was placed on a clean glass slide and treated separately with different chemical reagents such as sodium hydroxide, hydrochloric acid, nitric acid, sulfuric acid, ferric chloride, iodine solution, glacial acetic acid, picric acid, and methanol. The treated samples were allowed to react for a short period and then observed under visible light as well as ultraviolet light at 254 nm and 365 nm wavelengths.

The colour changes and fluorescence exhibited by the powdered drug under different conditions were carefully noted. Fluorescence characteristics varied depending on the reagent used and the wavelength of observation. These variations are attributed to the presence of different phytoconstituents that respond distinctly under ultraviolet radiation. Fluorescence analysis thus serves as a useful qualitative tool for the identification, characterization, and authentication of *Cucurbita Pepo* leaf powder and can be employed as a supportive parameter in pharmacognostic evaluation.

Results and Discussion

Pharmacognostic Evaluation

Organoleptic and Microscopic Evaluation

Organoleptic examination of the leaf drug revealed characteristic sensory attributes that are useful for preliminary identification. The leaves and leaf powder exhibited a uniform natural colour and a distinct odour typical of the plant material, indicating good freshness and

absence of visible signs of deterioration. The taste was characteristic and consistent, while the texture of the powder was fine and homogeneous, suggesting proper drying and processing. These organoleptic features provide simple yet reliable diagnostic markers for routine quality assessment.

Microscopic and micromorphological studies of the leaf powder revealed several diagnostic characters essential for authentication. The powder showed the presence of well-defined tissues such as xylem vessels, fibers, and phloem elements, confirming its vascular nature. Calcium oxalate crystals, predominantly in raphide and prismatic forms, were observed along with abundant starch grains, occurring both as simple and compound structures. Epidermal fragments with distinct stomatal arrangements were evident, and the distribution pattern of stomata supported species-specific identification. The occurrence of trichomes and lignified cells further contributed to the microscopic profile. These microscopic characteristics collectively serve as reliable parameters for identification and help in distinguishing the genuine drug from possible adulterants.

Physical Evaluation

Physical evaluation parameters were assessed to establish quality standards and physicochemical stability of the leaf drug. The moisture content was found to be within acceptable limits, suggesting reduced susceptibility to microbial contamination and improved shelf life. Ash values provided insight into the inorganic composition of the drug; total ash reflected the overall mineral content, while low acid-insoluble ash values indicated minimal contamination with siliceous matter such as sand or soil. Water-soluble ash represented the fraction of inorganic constituents soluble in water, contributing to the overall purity assessment.

Crude fibre content reflected the proportion of structural

components such as cellulose and lignin, which are characteristic of leaf tissue and influence the mechanical strength and extraction behaviour of the plant material. Extractive values obtained using solvents of varying polarity demonstrated differential solubility of phytoconstituents. Higher extractive values in polar solvents suggested the predominance of polar bioactive compounds, whereas lower values in non-polar solvents indicated a limited presence of lipophilic constituents. These physical evaluation findings establish baseline quality control parameters and support the standardization and further phytochemical investigation of the leaf drug.

Table 1: Quantitative microscopy of leaf / leaf powder of *Cucurbita Pepo*

Parameter	Value
Phloem fibres	
a. Width	5-12.5 μ
b. Length	20-57.5 μ
Starch grains (diameter)	7.5-20 μ
Calcium oxalate crystals	
a. Length	15-50 μ
b. Width	5-12.5 μ
Trichomes	
a. Length	10-37.5 μ
b. Width	5-7.5 μ
Xylem Vessels	
a. Length	27.5-62.5 μ
b. Width	15-32.5 μ
Stomatal number (Lower Epidermis)	Anomocytic 255 / mm ²
Stomatal index (Lower Epidermis)	15
Palisade ratio	5.25/mm ²
Vein islet number	14/mm ²
Vein termination number	19/mm ²

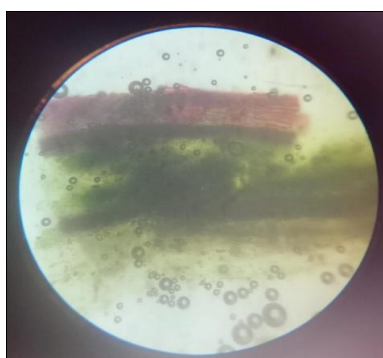


Fig 1: Phloem fibre



Fig 2: Transverse section

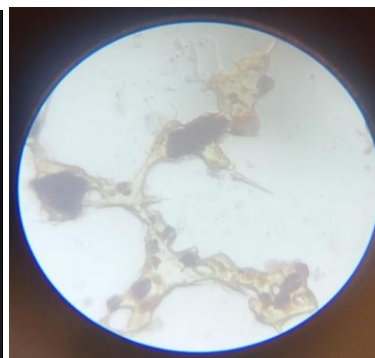


Fig 3: Calcium oxalate



Fig 4: Trichomes

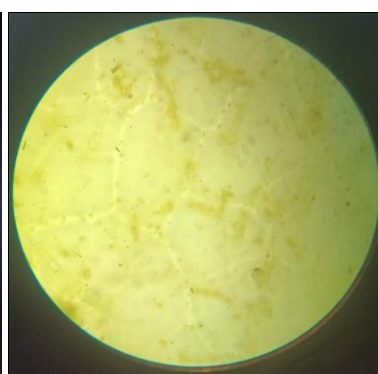


Fig 5: Stomata in lower epidermis (Anomocytic)



Fig 6: Vein islet

Table 2: Physicochemical parameters of leaf powder of *Cucurbita Pepo*

Parameter	Value % w/w
Moisture content	86
Total ash	13
Acid insoluble ash	0.05
Water soluble ash	0.1
Alcohol soluble extractive value	4.8
Water soluble extractive value	1.8
Ether soluble extractive value	7.5

Fluorescence Characteristics

Fluorescence characteristics of the powdered leaves of *Cucurbita Pepo* were examined to support the identification and standardization of the crude drug. The leaf powder, both untreated and treated with different chemical reagents, was observed under visible light and ultraviolet light at 254 nm and 365 nm wavelengths. Distinct colour changes and

fluorescence responses were noted depending on the nature of the reagent and the wavelength employed.

The untreated powder exhibited a characteristic greenish appearance under visible light, with variations in fluorescence under ultraviolet illumination. Treatment with acids, alkalis, and organic reagents produced noticeable changes in colour and fluorescence intensity, indicating the presence of diverse phytoconstituents that respond differently to chemical interaction and ultraviolet radiation. Dark green, brownish, bluish-green, and black fluorescence patterns were observed under UV light, which are useful diagnostic indicators.

Fluorescence analysis proved to be a rapid and reliable qualitative method for detecting variations in the powdered drug and for distinguishing authentic plant material from possible adulterants. The observed fluorescence behaviour of *Cucurbita Pepo* leaf powder can therefore be used as a supportive pharmacognostic parameter in the quality control and standardization of herbal raw materials.

Table 3: Fluorescence analysis of the *Cucurbita Pepo* leaf powder

TESTS	Visible/Day Light	UV Light (254nm)	UV Light (365nm)
Powder +Picric acid	Green	Light Green	Bluish Green
Powder + NaoH	Light Green	Light Green	Black
Powder + Glacial acetic acid	Light Green	Green	Black
Powder + Fecl3	Light Green	Light Green	Black
Powder + Iodine	Yellow	Light Green	Black
Powder + HCL	Colourless/Light Green	Light Green	Black
Powder + H2So4	Dark Green	Dark Green	Black
Powder+ Nitric Acid	Brown	Dark Green	Black
Powder + Methanol	Dark Brown	Dark Green	Black
Powder + Alone	Green	Dark Green	Bluish Green

Preliminary Phytochemical Evaluation

Preliminary phytochemical evaluation of the leaf extracts revealed the presence of diverse classes of secondary metabolites, confirming the chemical complexity of the plant material. Successive solvent extraction using solvents of increasing polarity resulted in differential extraction of phytoconstituents, indicating variation in solubility profiles of bioactive compounds. This polarity-based extraction pattern provides useful insight into the chemical nature of constituents present in the crude drug.

Qualitative chemical tests demonstrated the presence of carbohydrates and reducing sugars, suggesting the occurrence of primary metabolites that may contribute to nutritional and therapeutic properties. Positive reactions for proteins and amino acids indicated the presence of nitrogenous compounds. Alkaloids were detected in selected extracts, supporting the potential pharmacological relevance of the plant. Flavonoids and phenolic compounds were prominently identified, indicating strong antioxidant potential and possible involvement in anti-inflammatory and protective activities.

The detection of glycosides, including saponin and anthraquinone types, suggests a role in membrane activity and other biological effects. Steroids and triterpenoids were also observed, which are often associated with anti-inflammatory and antimicrobial properties. Tannins were identified through characteristic reactions, reflecting their astringent nature and possible role in wound healing and antimicrobial activity.

Overall, the preliminary phytochemical evaluation confirms the presence of multiple bioactive constituents in the leaf

material. These findings provide a scientific basis for further isolation, characterization, and pharmacological investigation of individual compounds and support the traditional medicinal relevance of the plant.

Phytochemicals	Methanolic extract	Chloroform extract
Carbohydrates	+	+
Proteins	+	+
Amino acids	+	+
Glycosides	+	+
Flavonoids	+	+
Tannins	+	+
Steroid	+	-
Alkaloids	-	+

Conclusion

Preliminary phytochemical evaluation of the leaf extracts revealed the presence of diverse classes of secondary metabolites, confirming the chemical complexity of the plant material. Successive solvent extraction using solvents of increasing polarity resulted in differential extraction of phytoconstituents, indicating variation in solubility profiles of bioactive compounds. This polarity-based extraction pattern provides useful insight into the chemical nature of constituents present in the crude drug.

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