



## PHARMACOGNOSTIC EVALUATION OF *HYPOESTES CANCELLATA* NEES (ACANTHACEAE)

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### ABSTRACT

*Hypoestes cancellata* is a species of the savanna belt occurring in the forests of West and Central Africa belonging to the family Acanthaceae. It is traditionally used for the treatment of diabetes, cancer and fever. The present study aims to establish some pharmacognostic features that will aid the authentication of the plant and its crude drug. The macroscopic, microscopic and physicochemical features as well as phytochemical constituents of the methanol extract of the plant were determined using standard procedures. Macroscopic features of *H. cancellata* revealed that the leaf is ovate in shape with acute apex, symmetrical base, entire margin, and smooth surface. Microscopic examination of the leaf revealed epidermal cells with wavy anticlinal walls, diacytic stomata, cystoliths and sessile glandular trichomes as well as multicellular covering trichomes. Transverse section of the leaf showed vascular bundles surrounded by the cortex while transverse section of the stem revealed epidermis followed immediately by cortex and then the vascular bundles with a wide central pit. Microscopy of the powdered plant material revealed features such as sclereids, prismatic calcium oxalate crystals and starch grains. Physicochemical evaluation showed water soluble extractive value of 28.00±0.88%, ethanol soluble extractive value of 19.00±0.67% and moisture content of 6.67±0.11%. The total ash, acid insoluble and water-soluble ash values of the powder were found to be 8.00±0.20%, 1.50±0.17% and 3.50±0.17% respectively. Preliminary phytochemical screening revealed the presence of carbohydrate, saponins, flavonoids and steroids. The results of the study can serve as a source of information and provide suitable standards for identification of *H. cancellata*.

**Keywords:** *Hypoestes cancellata*, Macroscopy, Microscopy, Physicochemical parameters, Phytochemical

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### INTRODUCTION

Medicinal plants are the oldest form of medication employed in traditional medicine globally. They have been in use for thousands of years. The knowledge of their therapeutic effects was transmitted over the centuries from generation to generation within human communities across civilisations (Marrelli, 2021). Owing to their availability, wide acceptability by many cultures and perceived safety and efficacy, medicinal plants use is ever increasing especially in developing countries (Ssenku *et al.*, 2022). Despite wide usage, herbal

medicines are associated with poor quality, often due to substitution and adulteration which can sometimes lead to significant adverse effect to the user (Jitäreanu *et al.*, 2023). Correct identification, quality assurance, and establishment of pharmacognostic standards of medicinal plants are very important parameters for their evaluation (Majid *et al.*, 2021).

The genus *Hypoestes* belonging to the family Acanthaceae consists of more than 150 species. Members of this genus have been used traditionally for treatment of

various disease conditions such as eye sores, liver, skin diseases, respiratory infections, anemia, malaria, scabies, typhoid, hypertension, and gonorrhoea (Alhaidari, 2018). *Hypoestes cancellata* is a species of the savanna belt found in grassland, wooded grassland and deciduous woodland areas that have seasonal rainfall and are prone to fire during dry-season (Darbyshire, 2015). Local information suggests that *Hypoestes cancellata* is used in the treatment of diabetes mellitus, cancer and fever.

## MATERIALS AND METHODS

### Collection, Identification and Preparation of the Plant Material

The fresh whole plant of *Hypoestes cancellata* was collected from Kudendan, Chikun Local Government Area of Kaduna State in November, 2020. The plant was authenticated at the Herbarium Section of the Department of Botany, Ahmadu Bello University Zaria with a voucher number 1033. The roots of the collected plant were washed under a running tap to remove adhering sand. The plant material was shade dried, subsequently size reduced and then stored in an air tight container. 200g of the powdered plant material was extracted in a stoppered jar by maceration using 70% methanol with occasional shaking for 72 hours.

### Organoleptic Examinations of the Fresh and Powdered Plant Material

Morphological and organoleptic features of the fresh leaf and powdered whole plant material such as shape, appearance, colour, taste and odour were examined using standard procedures (WHO, 2011).

### Microscopic Evaluation

The microscopic evaluation of epidermal surface of the leaf and transverse sections of the leaf and stem, as well as the powdered whole plant was carried out using standard

methods (Brain and Turner, 1975 and Evans, 2009). The prepared sections (upper and lower epidermises, transverse sections of leaf and stem) were cleared with 50% sodium hypochlorite solution and mounted on a microscope slides with dilute glycerol and observed under the light microscope. The photomicrographs were taken and documented. Micrometric measurements of some diagnostic features as well as their quantitative evaluation was carried out as described by Evans (2009) and WHO (2011).

### Chemo Microscopic Examination

The histochemical detection of cell wall materials and cell inclusions such as cellulose, lignin, calcium carbonate, calcium oxalate, mucilage of the powdered plant material was carried out using standard techniques as described by WHO (2011).

### Determination of Physicochemical Parameters

Physicochemical parameters such as water and alcohol extractive values, total ash, acid insoluble and water-soluble ash values and moisture content were determined according to the method described by Evans (2009) and WHO (2011).

### Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out using the methods described by Evans (2009), Sofowora (2008) and Shah and Seth (2010).

## RESULTS

### Organoleptic Evaluation of the Leaf of *H. cancellata*

The organoleptic evaluation of the leaf of *H. cancellata* revealed an ovate shaped leaf of about 5.18 × 2.73cm with acute apex, symmetrical base, entire margin, smooth surface and odourless. Table 1 shows a summary of the organoleptic findings.

**Table 1: Organoleptic Characteristics of *Hypoestes cancellata***

<b>Evaluative Features</b>	<b>Characteristic</b>
<b>Apex</b>	Acute
<b>Shape</b>	Ovate
<b>Base</b>	Symmetrical
<b>Margin</b>	Entire
<b>Venation</b>	Reticulate
<b>Surface</b>	Smooth
<b>Colour</b>	Green
<b>Taste</b>	Bland
<b>Odour</b>	Odourless
<b>Length (cm)</b>	5.18±0.13
<b>Width (cm)</b>	2.73±0.06



Plate I: *Hypoestes cancellata* in its Natural Habitat (captured on 17th November, 2020 at Kudendan, Chikun LGA, Kaduna State)

### Microscopic Features of the Leaf of *H. cancellata*

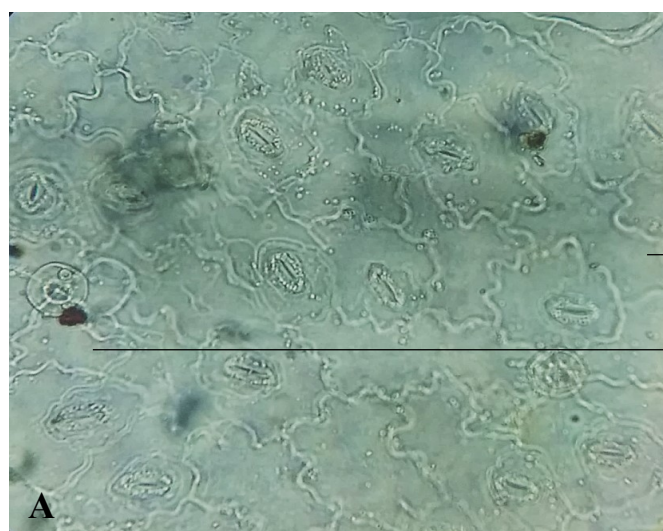
Microscopic examination of the upper epidermal layer of the leaf of *H. cancellata* revealed epidermal cells with sinuous wall, diacytic stomata, sessile glandular trichomes and cystoliths.

Microscopic examination of the lower epidermis of the leaf of *H. cancellata* revealed epidermal cells with sinuous wall, diacytic stomata, multicellular covering

trichomes as well as sessile glandular trichomes.

Microscopic examination of the transverse section of the leaf and stem of *H. cancellata* revealed collateral arrangement of vascular bundles with a large central pith in the stem.

Microscopic examination of the powder of the leaf of *H. cancellata* revealed prismatic calcium oxalate crystals, sclereids and starch granules.

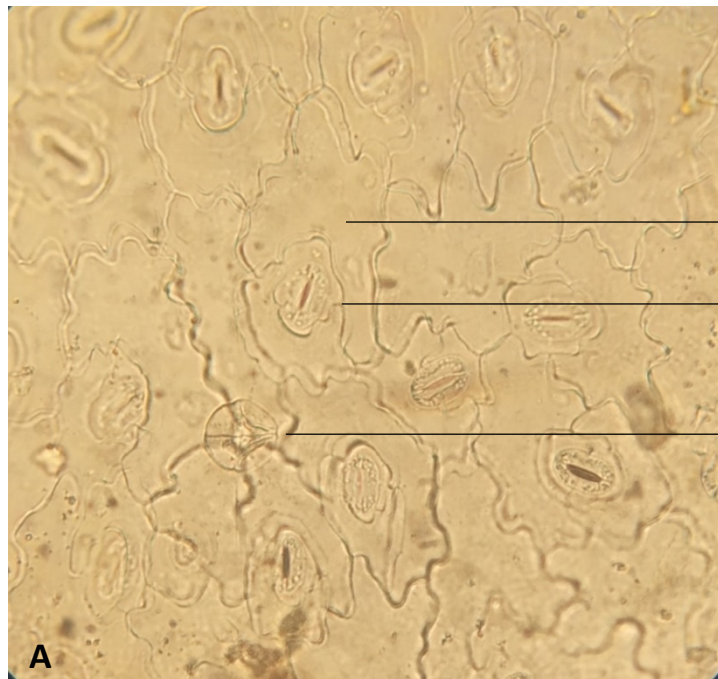


Epidermal cell  
Sessile glandular trichome



Cystolith  
Diacytic stoma

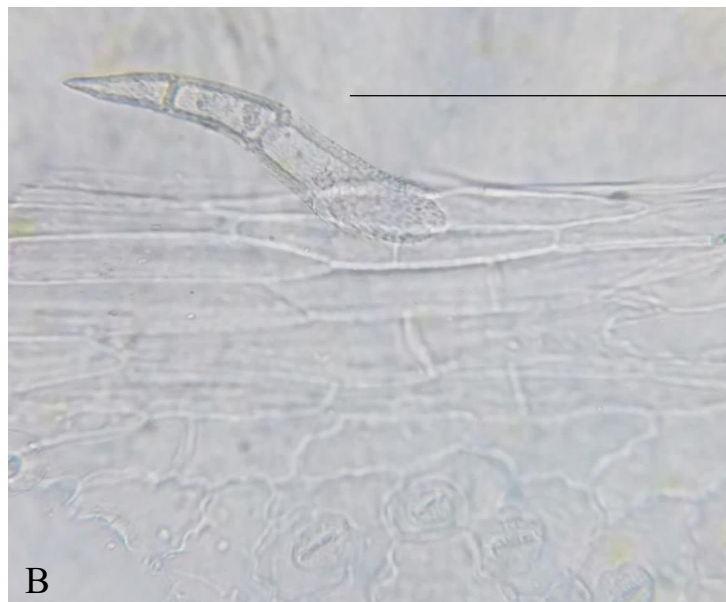
**Plate II: Photomicrograph of upper epidermal layer of *H. cancellata* leaf showing sinuous walled epidermal cells, sessile glandular trichomes, cystolith and diacytic stomata (Mag. ×400)**



Subsidiary cell

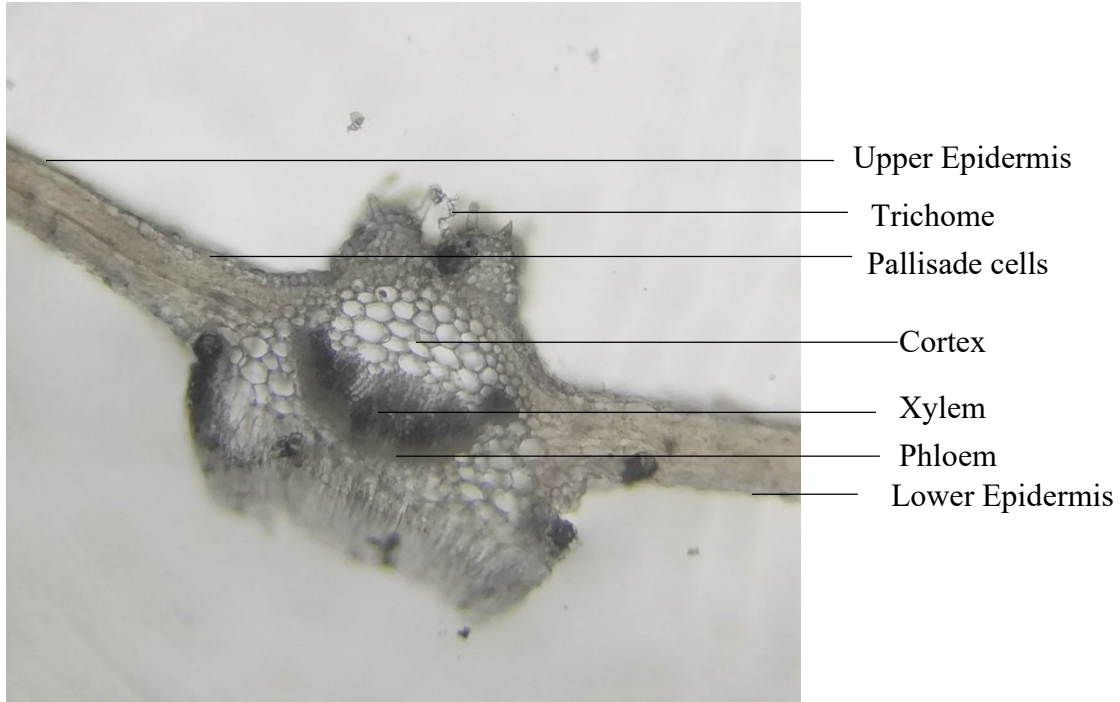
Stoma

Sessile glandular  
trichome

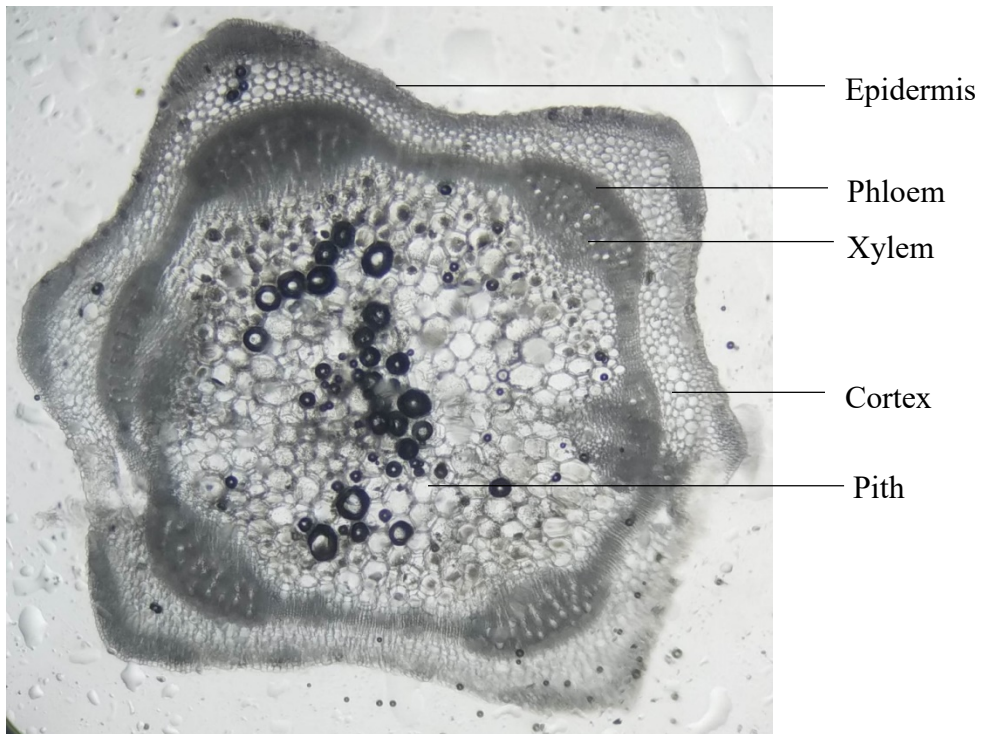


Multicellular  
Trichome

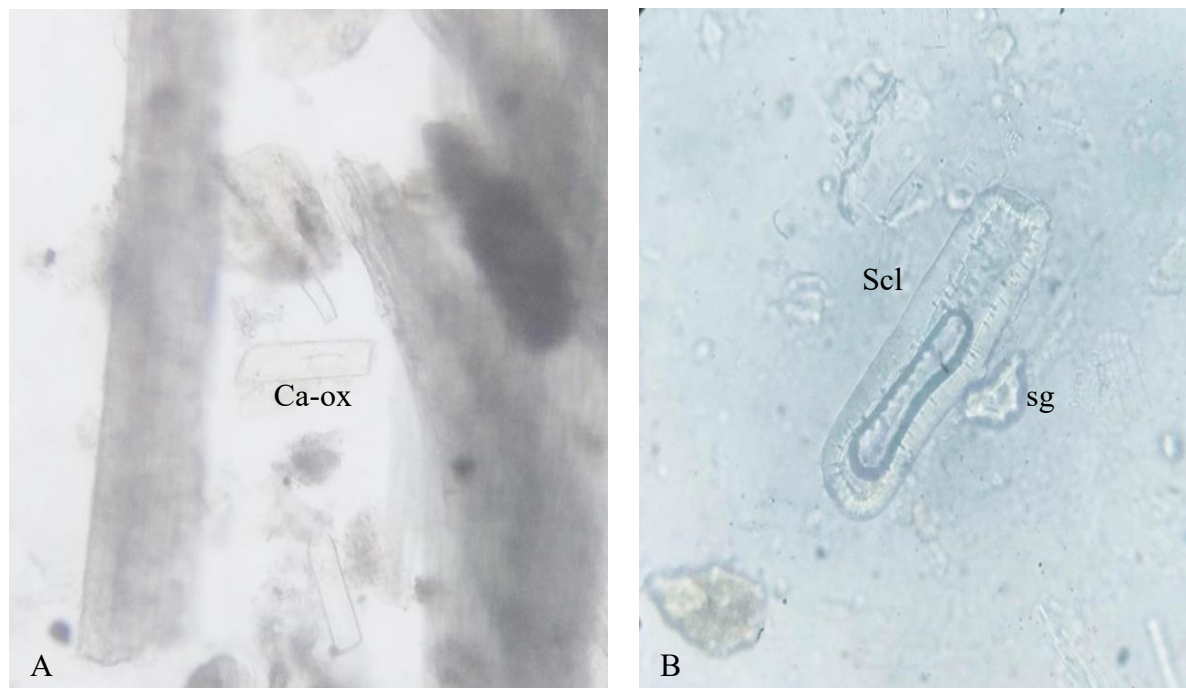
**Plate III: Photomicrograph of the lower epidermal layer of *H. cancellata* leaf showing sinuous epidermal cells, diacytic stomata, sessile glandular trichome and a multicellular covering trichome (Mag.  $\times 400$ )**



**Plate III: Photmicrograph of the Transverse section of the leaf of *H. cancellata* (Mag. ×400)**



**Plate IV: Photmicrograph of the Transverse section of the stem of *H. cancellata* (Mag. ×400)**



**Plate V (A-C):** Photomicrograph of the powdered sample of *H. cancellata* showing (A) prismatic calcium oxalate crystals (Ca-ox) (Mag.×400) and (B) sclereid (Scl) and starch granules (sg) (Mag. ×400)

**Quantitative Microscopic Characteristics of the Leaf of *H. cancellata***

In the quantitative microscopic examination of the leaf of *H. cancellata*, parameters such

as stomatal numbers and indexes for the upper and lower epidermises of the leaf were evaluated. Other evaluated features are palisade ratio, vein islet number and veinlet termination number.

**Table 2: Quantitative microscopical Features of *H. cancellata* leaf**

Evaluative feature	Values *	
	Upper	Lower
Stomatal number	4.80±0.06	8.00±1.22
Stomatal index (%)	21.62±2.93	38.55±2.60
Palisade ratio	5.58±0.51	
Vein islet number	7.20±1.02	
Veinlet termination number	4.6±1.03	

\* Values are expressed as mean ± Standard Error of mean (SEM)

### Micrometric Characteristics of *H. cancellata*

The sizes of some diagnostic microscopic features such as stomata, sessile glandular trichome and cystolith were determined and the average values were calculated as shown in the table below.

**Table 3: Micrometric Measurements of the Leaf of *H. cancellata***

S/N	Parameter	Average (µm)*
1	Stomata (length)	25.5±1.00
	Stomata (width)	13.75±0.66
2	Sessile glandular trichome (diameter)	28.75±1.67
3	Cystolith	296±28.61

\* Values are expressed as mean ± Standard Error of mean (SEM)

**Table 4: Chemo-microscopic Features of the whole Plant of *H. cancellata***

Constituent	Inference
Cellulose	+
Lignin	+
Suberin	+
Gum and mucilage	-
Calcium carbonate	+
Calcium oxalate crystals	+
Starch	+

**Key: Present (+) Absent (-)**

### Physico-chemical Properties of *H. cancellata*

The physico-chemical evaluation of the powdered *H. cancellata* showed that water has a higher extractive capacity on the plant material than ethanol. It also showed a low moisture content of 6.67%. other physico-chemical parameters evaluated are the total, acid insoluble and water- soluble ash values.

### Chemo-microscopical Features of the Powdered *H. cancellata*

Chemo-microscopic examination of the powdered *H. cancellata* indicates the presence of cellulose cell wall, lignin, suberin, calcium carbonate, calcium oxalate crystals and starch, but mucilage and gum were absent.

### Phytochemical Constituents of *H. cancellata*

Preliminary phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, saponin, flavonoids and steroids, however, anthraquinones, tannins and alkaloids are absent.



**Table 5: Physico-chemical characteristics of powdered whole plant of *H. cancellata***

Parameters	Values (%)*
Alcohol soluble extractive	19.00±0.67
Water soluble extractive	28.00±0.88
Moisture content	6.67±0.11
Total ash	8.00±0.20
Acid insoluble ash	1.50±0.17
Water soluble ash	3.50±0.17

\*Values are expressed as mean ± Standard Error of mean (SEM)

**Table 6: Preliminary phytochemical constituents of the methanol extract of *H. cancellata***

Name of Test	Inference
Carbohydrate	+
Reducing Sugar	+
Anthraquinone	-
Cardiac Glycosides	+
Saponin	+
Flavonoids	+
Tannins	-
Steroids/Triterpenes	+
Alkaloids	-

Key: Present (+) Absent (-)

## DISCUSSION

Macroscopically, the leaves of *Hypoestes cancellata* have shown some important features that can be used to identify the plant preliminarily. These features include colour, shape, margin, base, apex and surface.

Microscopically, the upper and lower epidermal surfaces of the leaf showed similar microscopic characters except for the fact that the lower epidermis shows more stomata than the upper. Diacytic stomata have been reported in some members of the Acanthaceae (Patil and Patil, 2011) as well

as the presence of cystolith (Gabel *et al.*, 2021). Transverse sections of the leaf and stem showed a collateral arrangement of vascular bundles, as well as wide central pit in the stem.

Microscopic studies of the powdered *H. cancellata* revealed some features that are of diagnostic importance. Microscopy is of significant importance in the evaluation of crude drugs in powdered form because most of the diagnostic morphological features are lost in broken and powdered plant materials (Gurav and Gurav, 2014). Some quantitative

microscopic characters of the leaf of *H. cancellata* such as stomatal number, stomatal index, palisade ratio, vein islet and veinlet termination numbers also revealed values that are of diagnostic importance. These features cannot be easily studied by general microscopy, thus making quantitative microscopy an important tool in the evaluation of medicinal plants (Kumar and Doble, 2011). These features can be used to easily differentiate the desired medicinal plant from other closely related species for example, the stomatal index of any given species is always constant regardless of the age of the leaf (Evans, 2009), many plant species show constant values for veinlet termination and vein islet numbers (Trease and Evans, 1996), many plants exhibit a constant palisade ratio irrespective of their geographical location; therefore, this feature may be of significance in obtaining general subjective information for the authentication of a plant (Mukherjee, 2002).

Results of physicochemical parameters of the powdered *H. cancellata* showed a higher water soluble extractive value than the ethanol soluble extractive value suggesting the presence of more polar constituents in the plant. It also showed a low moisture content which is essential to prevent the plant material from microbial action and possible hydrolysis. Moisture content above 15% is considered high and poses the risk of decay in the drug material (Heinrich *et al.*, 2012). The ash values determine quality and purity of a crude drug by indicating the presence of various contaminants such as carbonate, silicate, oxalate etc. (Majid *et al.*, 2021).

Preliminary phytochemical screening of the methanol extract revealed the presence of some phytoconstituents which can be responsible for the claimed medicinal uses of the plant.

## CONCLUSION

The pharmacognostic features of *Hypoestes cancellata* established from this study can be useful in the detection of adulteration, contamination and establishment of a suitable monograph of the plant crude drug.

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