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SHORT COMMUNICATION

Pharmacognostic and Phytochemical Evaluation of Staminate Flower of *Cocos nucifera* L

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Abstract

Background: Despite the medicinal value of the staminate flower of coconut (*Cocos nucifera* L) there is no data available in the archives for botanical as well as chemical standardisation of flowers of *C. nucifera*. **Aim:** Pharmacognostic characters and a phytochemical parameter can be used as a quality standard for authentication of the flower of *C. nucifera* and detection of adulteration. **Methods:** Pharmacognostic and phytochemical standardisation of the staminate flower of *Cocos nucifera* L. was performed as per Ayurvedic Pharmacopoeia of India. It includes studies like morpho-anatomical, powder analysis, preliminary phytochemical evaluation and HPTLC fingerprinting of the flower of *C. nucifera*. The solvent system in HPTLC studies was Toluene: Chloroform: Ethanol (4:4:1 v/v/v) powder analysis, histochemical test, and preliminary and phytochemical screening were carried out as per standard protocol. **Results:** Microscopic studies of petal sepals and anther show distinct characteristics that help in the evaluation of the genuine quality of flowers. Physicochemical parameters like loss on drying, total ash, and acid insoluble ash should not be more than 5.58%, 6.8061% and 0.223% respectively. However, water-soluble and alcohol-soluble extractives should not be more than 12.544% and 2.498% respectively. Six prominent bonds of R_f value (0.12, 0.37, 0.48, 0.52, 0.67 and 0.91) were visualised. This R_f value imparts significant quality control data in chromatographic (HPTLC) fingerprinting. **Conclusion:** Current findings will be helpful in plant authentication, assurance of quality and adulteration detection for staminate flower of *C. nucifera*.

Keywords: Coconut, *Cocos nucifera*, *Narikela* Flower, Standardisation

1. Introduction

Cocos nucifera L., belonging to the family Arecaceae, is commonly known as the coconut palm. The basionym of plant is *narikela*. It is an inhabitant of the coastal areas of Southeast Asia and Melanesia, most likely the Philippines, Papua New Guinea, Malaysia and Indonesia[1,2]. It is a common part of the island and the coastal area around the globe, occurring in more than 80 countries across Africa, America, Asia and Oceania[1-3]. The plant is distributed throughout India but is naturally and abundantly found in Kerala, Karnataka, Tamil Nadu and Goa. The details of the distribution of *C. nucifera* are available on India Biodiversity Portal[4]. All the parts of the plant are therapeutically active. However, the least explored one is the flower. The staminate flower has been explored in recent eras for its biological activities. The infusion prepared from the staminate flower is used to overcome the problem associated with the menstrual cycle[5]. Reported activities on the inflorescence of *C. nucifera* are antioxidant, antihyperglycemic and cytoprotective[5,6].

The inflorescence is used to cure lifestyle disorders like diabetes, hypercholesterolemia and high blood pressure. The staminate flower along with honey is used to manage diabetes. Tonic prepared from coconut flowers is commonly used by women in Kerala after childbirth. Flower-containing Pollen (PO) mixed with honey helps in lowering cholesterol. Flowers are rich in zinc and iron. Inflorescences can facilitate

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the functioning of the liver[7,8]. Despite being full of medicinal value, there is no data available in the archives for botanical as well as chemical standardisation of flowers of *C. nucifera*. In this study, the quality standard for the staminate flower of *C. nucifera* as per WHO/API was developed for the first time.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals

All the chemicals used in this study were of AR grade. The chemicals and make are as follows.

Phloroglucinol (LOBA Chemie), Safranin (LOBA Chemie), Fast green (LOBA Chemie), Dragendroff's solution (LOBA Chemie), Hydrochloric acid (S.D.Fine Chem Ltd.), Sulphuric acid (S.D.Fine Chem Ltd.), Sodium hydroxide (S.D.Fine Chem Ltd.), Sudan red III (LOBA Chemie), Ruthenium red (S.D.Fine Chem Ltd.), Ferric chloride (S.D.Fine Chem Ltd.), Alcohol (CS), Acetic acid (S.D.Fine Chem Ltd.) and Iodine solution (Thomas Bakers).

2.1.2 Instruments

Stereo microscope LEICA S9D and EC4 camera, Olympus microscope BX 43 and LC camera 30, Nikon D7200 camera and rotary evaporator REMI were used in the study.

2.1.3 Plant Materials

The flower of *C. nucifera* was collected from the campus of Regional Ayurveda Research Institute, Kothrud, Pune, dated 19/08/2019. The *C. nucifera* flower was collected and authenticated with the help of Eflora of India. The collected drug is kept in the pharmacognosy section of R.A.R.I., Pune (Ref. No. PCG/2021/ 24/ a).

2.2 Macroscopic and Organoleptic Analysis

Macroscopic characters and organoleptic **characters of** *C. nucifera* flower **were observed and noted**[9,10].

2.3 Microscopic Characterisation

The Transverse Section (TS) of the flower was sliced manually. The section was stained using a 0.1% w/v solution of safranin dye. The Image of TS was taken using an Olympus BX43 and LC30 camera[11,12].

2.4 Powder Microscopy

The powder samples were stained with suitable staining reagents (Iodine solution, Phloroglucinol and HCl) and seen under a microscope for evaluation of characters. The microscopical characteristics of the prepared slides were observed using Olympus BX43 and LC30 cameras[13].

2.5 Histochemistry

TS and powder of sample were taken on slides and stained with Dragendorff's reagent, Phloroglucinol + dilute Hcl, Sudan red -III, Iodine solution, Ruthenium red to distinguish alkaloids, Lignified Cells (LGC), cuticular cells, aleurone grains, fats, volatile oils, mucilage, starch grains, calcium oxalate and calcium carbonate crystals[14].

2.6 Fluorescence Analysis

Powdered flower material was observed under visible light and ultraviolet light after treatment with various organic/inorganic reagents like double distilled water, dilute HCl, nitric acid, sulphuric acid, glacial acetic acid, sodium hydroxide, potassium hydroxide, iodine solution, ferric chloride and ammonia[12].

2.7 Physiochemical Analysis

It includes moisture content, extractive value and ash value as per Ayurvedic Pharmacopoeia of India (API)[14].

2.8 Preliminary Phytochemical Screening

Alcoholic and aqueous extract (5%) of *C. nucifera* flower was prepared by cold maceration techniques. 5g powders of plant sample were added in 100ml of solvents (either absolute alcohol or double distilled water), shaken vigorously at regular intervals of 30 minutes, the process was repeated after 6 hours and then allowed to stand for the next 18 hours. The mixture was filtered through the Whatman filter grade 1. The filtrate was evaporated using a rotary evaporator to get the extract. The extracts were tested for the presence and absence of secondary metabolites[15,16].

2.9 High-performance Thin Layer Chromatography

The analysis was carried out using the CAMAG HPTLC apparatus. The stationary phase used in the study was E

marck silica gel 60 F_{254} glass TLC plates, 10 × 10cm and 200µm layer thickness. The mobile phase comprised Toluene: Chloroform: Ethanol (4:4:1 v/v/v). The volume of the mobile phase was 10mL. Application type: band; Front: 60mm; Time for development: 14 minutes; Drying: 5 minutes; Detection at 254 and 366nm and after derivatisation at visible light with anisaldehyde and sulphuric acids[17].

3. Results and Discussions

3.1 Macroscopic or Organoleptic Evaluation

The male flower is evaluated morphologically by visuals in the natural habitat as well as in the laboratory Figure 1(A-D). The entire male flower as well as the calyx, corolla and androecium were evaluated using a stereo-zoom microscope (Leica SD9). The images captured are depicted in Figure 1 (a–h). Detailed macroscopic and organoleptic characters are as follows:

Male flower having bracts and bracteoles-bracteate and ebracteolate; attachment of flower-sessile; presence of floral whorls-incomplete; symmetry-actinomorphic; presence of reproductive organs-unisexual (staminate); number of floral parts-trimerous; special character-pistilodes saw; arrangement of floral organs-cyclic and colour-pale yellow. Perianth having, tepals- 6; arranged in two series, inner odd tepal posterior in position; cohesion-polyphyllous; aestivation-valvate for both the series; colour-pale yellow. Androecium having stamens-6; fertility-all fertile; cohesion of stamens-polyandrous; adhesion of stamens- free; sequence-arranged in two series of three, antiphyllous; position of stamens-exerted; number of locules-dithecous; attachment of filament to anther-basifixed; opening of anther-introrse.

3.2 Transverse Section of Staminate Flower of *C. nucifera*

Image of TS of flower is depicted in Figure 2. TS of the sepal is crescent-shaped. The OE is single-layered and made up of sub-rectangular cells. Beneath that BSC is present, cells are irregularly polygonal, lignified and with narrow lumen. COL are thick-walled, polygonal cells embedded with BSC, LGC and patches of SCL cells. SCL cells are thick-walled, lignified and narrow lumen. The IE is also single-layered. It is small compared to the OE.

The TS of the petal shows the OE, which is made up of single-layered, sub-rectangular cells. These cells are smaller than the LE. Below the E, PA cells are present. Here the cells are deposited, thin-walled and polygonal. SCL cells are present beneath the PA cells and the cells are 11-12 layered, thick-walled, lignified, sharply polygonal and with a lumen. 3 to

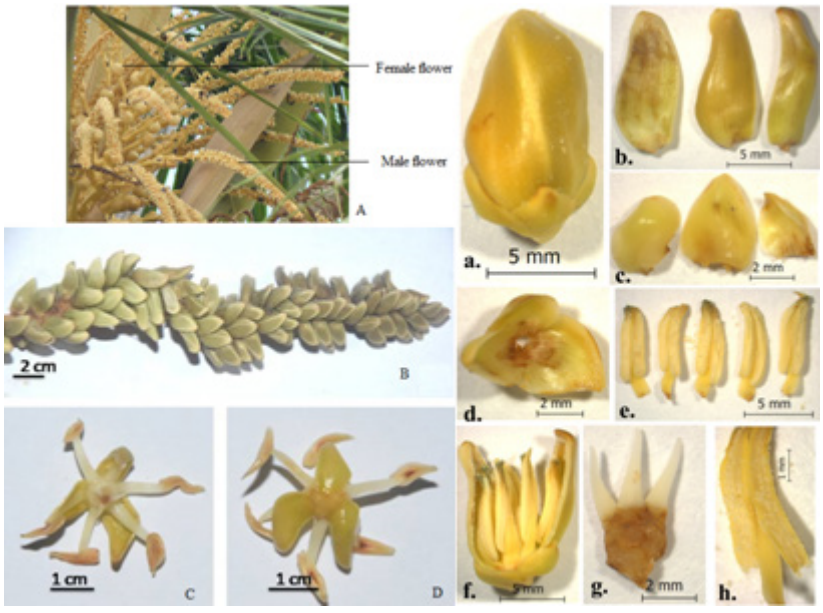

Figure 1. Morphology of flower. (**A.** Inflorescence in natural habitat; **B.** Male flower; **C.** Upper surface of male flower; **D.** Lower surface of flower) (Stereo microscopy image showing *Cocos nucifera* L. Male flower; **a.** Entire flower, **b.** petals, **c.** Sepals, **d.** Calyx cup, e. Stamens, **f.** Stamens inside calyx cup, **g. h.** Stamen).

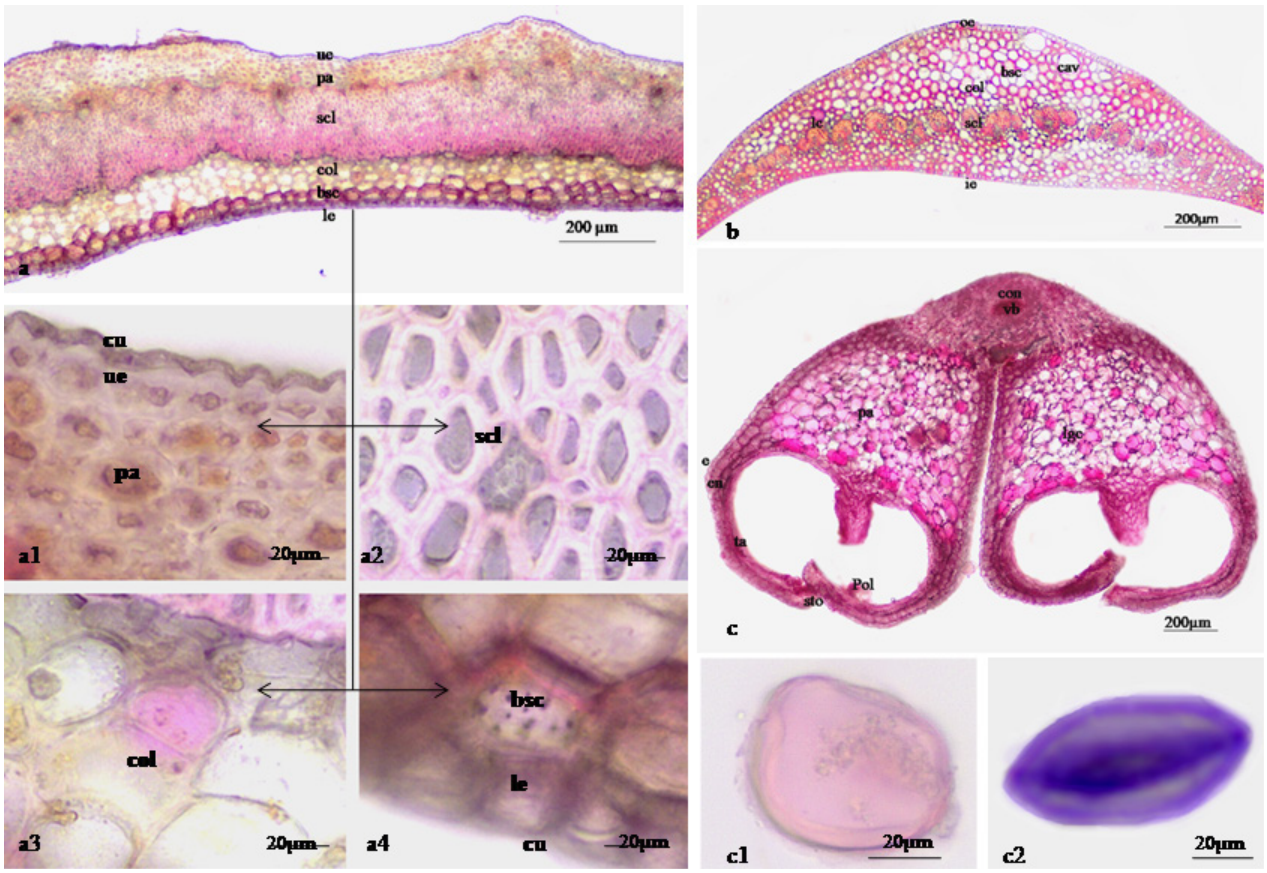

Figure 2. TS of the male flower of *Cocos nucifera* L. showing a. TS of petal; a1 to a4 showing zoom images of petal; b. TS of sepal; c. Ts of tetrasporangiate anther; c1 and c2. Pollens image.(Brachysclereid (BSC), Cavity (CAV), Collenchyma Cells (COL), Connectives (CON), Cuticle (CU), Endothecium (EN), Epidermis (E), Inner Epidermis (IE), Lignified Cells (LGC), Lower Epidermis (LE), Outer Epidermis (EO), Parenchyma Cells (PA), Pollen (PO), Sclerenchyma Fibre (SCL), Stomium (STO), Tapetum (TA), Upper Epidermis (UE), Vascular Bundle (VB).

4 layers of COL are present below the SCL layer. These cells are rounded to polygonal and some cells are lignified. Below this, a single layer of sclereid and BSC type of scleried/ stone cells are present. The cells are lignified, pitted, broad lumen and thick-walled. The LE is again single-layered and made up of sub-rectangular cells.

Anther male flower of *C. nucifera* is tetrasporangiate. In the TS, the anther is made up of two distinct sporogenous cells. It is connected with CON provided by the VB Sporogenous cells are made up of PA cells which are polygonal, thin-walled and embedded with lignified cells. The single sporogenous cell is covered with single-layered, sub-rectangular, elongated and LGC of the E. In surface view, these are polygonal, striated cells. Followed by 1-2 layers of endothecium cells which are rectangular, striated and LGC. TA is also single-layered, rectangular and elongated. STO is the point of dehiscence of PO. PO is monocolpate and tenuimarginate, both elongated as well as spheroidal in shape.

3.3 Powder Microscopy

Image of powder microscopic character of flower is depicted in Figure 3. Powder microscopy shows fragments of the E of petals revealing the sectional view with compact and tabular cells covered with thick CU while the surface view with polygonal and straight-walled cells (mostly 6-7 angled). A sectional view of a BSC cell is present with rectangular, lignified, broad lumen and pitted cells while surface view with polygonal, lignified, broad lumen and pitted cells. A sectional view of outer and inner COL shows rounded-oval cells deposited with starch grains, LGC and oil globules. The only difference was seen in size. The outer COL are smaller than the inner COL cells.

Sepal shows fragments of COL in sectional and surface view rounded and deposited with BSC while surface view shows polygonal cells deposited with BSC cells. The sectional view of the sclerenchymatous fibre is seen with polygonal cells, lignified with the lumen.

Anther shows epidermal fragments. The sectional view shows single-layered, compact and tabular cells while the surface view shows sub-rounded cells. The sectional view of endothecium cells is single-layered, sub-rectangular, lignified and striated while the surface view is elongated, polygonal and lignified with striations. The sectional view of tapetal cells is 2-3 layered. The cells are non-lignified and elongated. The sectional view of PA cells reveals polygonal-shaped cells. The

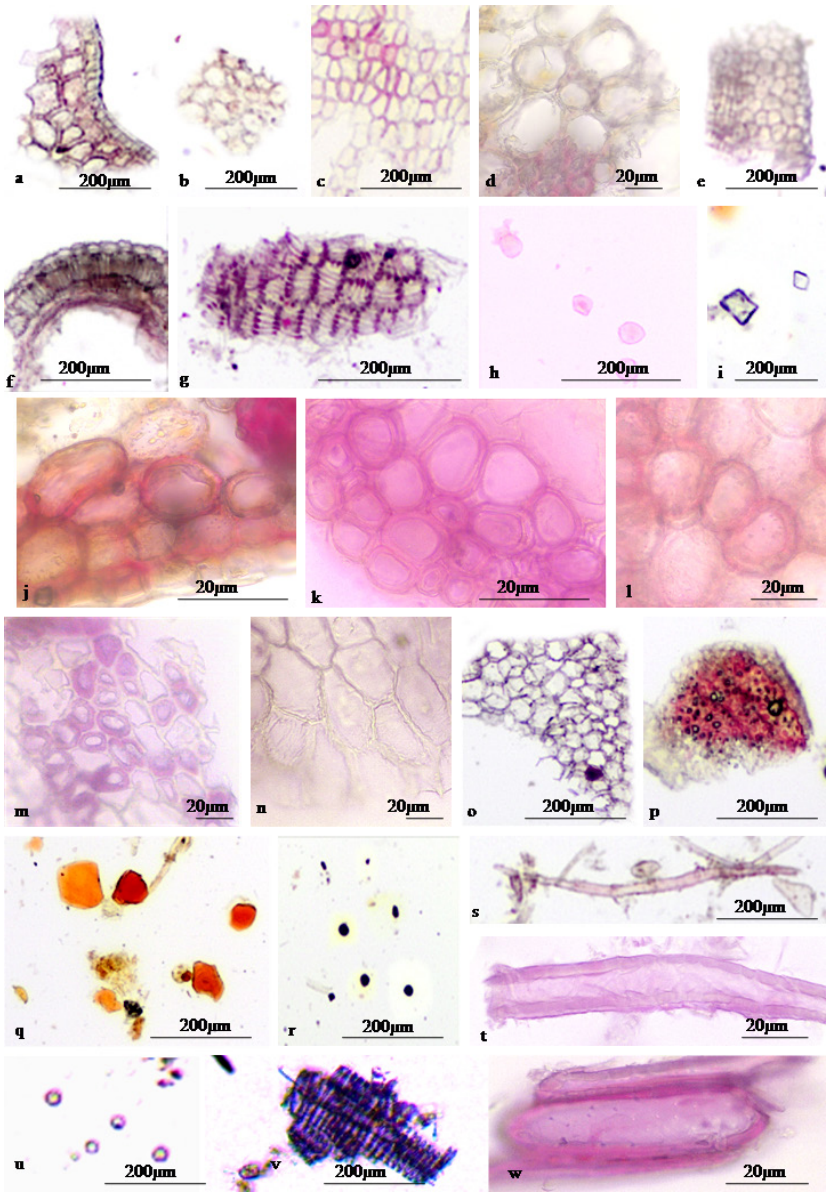

Figure 3. Powder microscopy of the male flower of *Cocos nucifera* L. (a. Sectional view of the IE and BSC cells of petals; b. Sectional view of outer COL of the petal; c. Surface view of the IE of petals; d. Sectional view of inner COL of petal; e. Surface view of the E of the anther; f. Sectional view of the E, endothecium and tapetal layer cells of the anther; g. Surface view of endothecium cells; h. PO from anther; i. Prism crystals of calcium oxalate; j. Sectional view of the E and BSC of petals; k. LGC of petals; l. Sectional view of PA and BSC cells of sepal; m. Surface view of PA and BSC cells of sepal n. Surface view of PA cells of CON of the anther; o. Sectional view of PA cells of the anther; p. Sectional view of SCL cells of sepal; q. Brown matter; r. Simple and compound starch grains; s. Non lignified fibre; t. Lignified sclereid; u. Oil globules; v. Surface view of uncoiled endothecium cells; w. BSC.

surface view of the filament shows elongated, polygonal and parenchymatous cells. PO is monocolpate, and tenuimarginate, both elongated as well as spheroidal in shape, medium-sized ranging from 36-54µm.

Powder showing elongated non-lignified fibre as well as lignified sclerenchymatous fibres are seen from sepal and petal. Spheroidal oil globules, prism crystals of calcium oxalate, simple and compound starch grains and brown content are present in the powder.

3.4 Histochemical Test

The analysis result (three separate batches) is depicted in Table 1.

3.5 Fluorescence Analysis

The observation of **Fluorescence analysis** is depicted in Table 2.

3.6 Preliminary Phytochemical Screening

Tested phytoconstituents are depicted in Table 3.

3.7 Determination of Physicochemical Parameters

The value of physicochemical parameters are given in Table 4.

Table 1. Histo-chemical analysis

S. No.	Test	Chemical	Observation	Result
1	Alkaloid	Dragendorff's Reagent	Brick Red	-
2	Lignified cell	Phloroglucinol + HCl	Pink to Cherry Red	+
3	Cuticular cell	Sudan Red -III	-	+
4	Aleurone grains	Iodine	-	-
5	Fats and volatile oils	Sudan Red- III	-	+
6	Mucilage	Ruthenium Red	-	-
7	Starch	Iodine	Blue or Reddish-Blue	+
8	Calcium oxalate crystals	Hydrochloric Acid	Dissolved	+
9	Calcium carbonate crystals	Hydrochloric Acid	-	-

+ Present; - Absent

Table 2. Fluorescence analysis

Test	Day Light	254nm	36nm
Powder as such	Fulvous	Umber	Brown Vinaceous
Powder + H_20	Cinnamon	Light Pale Purplish Grey	Light Pale Purplish Grey
Powder + HCl	Buff	Light pale Purplish Grey	Light Pale Purplish Grey
Powder + HNO_3	Luteous	Dark Herbage Green	Greenish Glaucous
Powder + H_2SO_4	Fulvous	Greenish Glaucous	Olivaceous
Powder + GAA	Ochreous	Dark Brick	Pale Mouse Grey
Powder +18N HCl	Ochreous	Pale Mouse Grey	Purplish Grey
Powder + 50% HNO_3	Light sienna	Dull Green	Dark Citrine
Powder + 50% H_2SO_4	Ochreous	Mouse Grey	Pale Purplish Grey
Powder + 50% GAA	Ochreous	Purplish Grey	Dark Brick
Powder + 1N NaOH	Chestnut	Dark Brick	Chestnut
Powder + 1N KOH	Sepia	Luteous	Luteous
Powder + 0.1N Iodine	Bay	Herbage Green	Herbage Green
Powder + 5% $FeCl_3$	Light Citrine	Chestnut	Sepia
Powder + Liquid NH_3	Fulvous	Umber	Dark Brick

Table 3. Preliminary phytochemical screening

S. No.	Phytoconstituents	Aqueous Extract	Alcoholic Extract
1	Carbohydrate	+	+
2	Protein	+	+
3	Lipid	+	+
4	Alkaloids	-	-
5	Glycoside	-	-
6	Phenolics	+	+
7	Flavonoids	-	-
8	Tannins	-	-
9	Saponin	-	-
10	Steroids		-
11	Volatile oils	-	-
12	Triterpenoids	-	-

Table 4. Physico-chemical parameter

S. No.	Parameter	Result
1	Loss on drying	It should not be more than 5.58 %
2	Total Ash	It should not be more than 6.8061%
3	Acid Insoluble Ash	It should not be more than 0.223%
4	Water-Soluble Extractive	It should not be less than 12.544%
5	Alcohol Soluble Extractive	It should not be less than 2.498%
The data depicted in the table is the mean of three samples.		

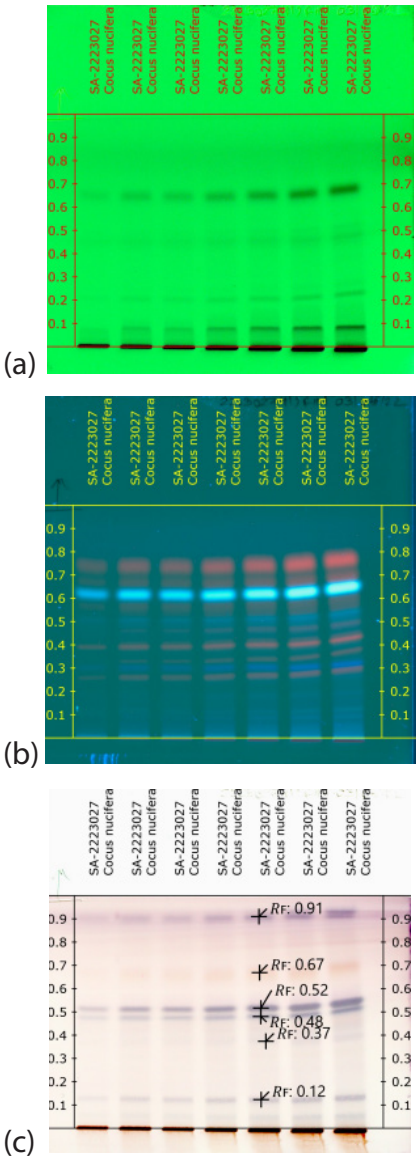

Figure 4. HPTLC chromatogram, **a.** at 254 nm, **b.** at 366 nm and **c.** at visible light after derivatisation.

3.7.1 HPTLC Fingerprinting

Six prominent bonds of R_f value (0.12, 0.37, 0.48, 0.52, 0.67 and 0.91) were visualised as seen in Figure 4. This R_f value imparts significant quality control data in chromatographic (HPTLC) fingerprinting.

The outcome of the current research would provide standard data for quality control of the staminate flower of *C. nucifera*. Morphological and organoleptic assessment plays a significant role in the identification of the flower of *C. nucifera* and the detection of adulteration. The TS allows the identification of the male flower of *C. nucifera* by developing histological character. Powder microscopic evaluation and developed data play similar role as TS of flower if the drug is available in powder form. The Pharmacognostic and physicochemical parameters will be helpful in the assurance of quality and detection of adulteration of genuine drugs.

4. Conclusion

Pharmacognostic characters, physicochemical parameter data and HPTLC fingerprinting collectively or individually can be used as the standard for plant

authentication, assurance of quality and adulteration detection.

5. Acknowledgements

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