Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (2), 457-462

Phyto-pharmacognostical investigation w.s.r. Micrometric investigation of *sheethavar* seed (C*elosia argentea* linn.)

Research Article

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Abstract

Celosia argentea Linn. is a leading leafy vegetable of Nigerian country and India. It is herb, ornamental plant which belongs to family Amaranthaceae also having medicinal use in antidiabetic, anti-inflammatory, antioxidant, anti-bacterial, antiapoptosis etc. For proper methodical evaluation, authentication, macroscopy, microscopy, physicochemical parameters, phytochemical screening and qualitative of seed of *C. Argentea* has been observed under standard approach. The morphological study showed small black shining spherical, lenticular 3mm in diameter, greyish black in color, slightly aromatic in odour and gritty in touch. Microscopical transverse section of cock's comb seed showed outer layer of epidermis consisting radially elongated rectangular dark brown color cell, narrow collapsed celled band, outer epidermis of tegmen, followed by parenchyma zone with angular cells of perisperm loaded by starch grains and crystals. Cock's comb inner cell consists of prismatic crystals and aleurone grains. The unplumbed physicochemical parameters and qualitative screening of seed of cock's comb are as per standard procedure and useful for future analysis.

Key Words: *Celosia, C. Argentea,* Celosin, Cock's comb.

Introduction

Celosia argentea Linn. is pan tropical weed found on riverside and defoliated area. They are ornamental cultivated plant either attractive inflorescence or many grotesque configuration of fascination. Cock's comb is the oldest race of fascinated plant on historical records was probably native to India (1).

Celosia argentea Linn. is an herbaceous plant which belongs to the family Amaranthacaceae and one of the leading leaf vegetables in south-western Nigeria and India (2). The *celosia* species is member of edible and ornamental plant. The generic name has been derived from Greek word "kelos" means burned and refers to the flame-like flower heads. Wool-flowers, brain celosia or Cock's combs are the common name used for the flowers of the species if the flower heads are crested by fascination or Velvet flower [in Mexico]. *Celosia argentea* is commonly known as Silver cock's comb or Lagos spinach, is a species of the genus Celosia. It is cultivated for its edible leaves and tender shoots. Cock's comb [*Celosia argentea var. Cristata*] is known by *Mora-shikha*.

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Phytochemical compounds present in *C. argentea* are betalains, nicotinic acid, celogenamide A, celogentin A–D, Celogentin-H, celogentin-J and celogentin -K, moroidin. *C. argentea* subsists of over eighteen minerals in which the contents of Al, Fe, Ni, Mn, Cu, K, Ti, and Se were far higher than those in *C. cristata* (3). The seeds contain 11.6-17% of protein and 6.4-10.9% of fatty oil. The seeds and roots yield triterpenoid saponins (4).

The plant *C. argentea* is use as medicine for curing many diseases such as dysentery, coughs, spitting up blood, excessive menstruation, amenorrhea, intestinal bleeding, bleeding from the lungs, female disorders, hemorrhoids, UTI, blood diseases, mouth sores, retinal hemorrhage, conjunctivitis, eye diseases and to lower blood pressure. *Celosia argentea* var. *cristata* contains betalains which are water-soluble nitrogen-containing pigments that are subdivided in red-violet betacyanins and yellow-orange betaxanthins. Large number of studies suggested that, the *Celosia* species possess antidiabetic, anti-inflammatory, antioxidant, anti-bacterial, antiapoptosis, antidiarrhoeal, anthelmintic, antiaging, antimalarial, antispasmodic, hepatoprotective and immunostimulating activities (5).These research works consist of the macroscopic, microscopic transverse section and powder microscopic study, physicochemical parameters, phytochemical screening, qualitative and quantitative of *C. Argentea* Linn.

Materials and Methods

Collection & Authentication of raw drug:

Celosia Argentea Linn. Collected naturally from Bangalore, Karnataka [3200ft., 12.9°N, 77.5°E] during the month of December as per standard procedure (6) and authenticated Specimen No. BSI/AZRC/1.12012/ Tech./20-21 [PI.ID]/426 from Botanical Survey of India, Jodhpur.

Morphology

Morphological characters of *Celosia argentea* Linn. was identified and authenticated by studying their characters systematically as per the methods described in the textbooks of Pharmacognosy. The specimen was observed as such with necked eyes along with microscope (7).

Microscopical evaluation

Sample seed material observed under distilled water under the compound microscope (40x) for the presence of primary and secondary metabolites, like starch grains which were confirmed by staining them with iodine. The sections cleared with chloral hydrate to observe the presence of various ergastic cell contents like, crystals of calcium oxalate, calcium carbonate, and silica etc (8).

Histochemical evaluation:

Sample thin sections subjected to Histochemical tests to find starch grains, tannin, calcium etc. by treating with various reagents (9).

Micrometry evaluation:

Countless approaches have been employed for measuring linear, area, and volume specimen dimensions with the microscope and a wide variety of useful techniques have emerged over the past few hundred years. The diameter of a cell or length/diameter of sub-cellular components can be easily measured using an ocular micrometer which has graduation in arbitrary units. This arbitrary graduation of the ocular micrometer is calibrated using a stage micrometer by superimposing the two scales.(7)(9)

Physico-chemical parameters:

Physico-chemical parameters like moisture content [loss on drying at 105°C], methanol soluble extractive value, water soluble extractive value, pH [5% aqueous extract], bulk & tap density, total Ash value, and acid insoluble ash value were done.(10)(11)

Phytochemical screening and quantitative estimation of phyto-chemical parameters:

Preliminary phytochemical analysis was performed in methanol extract and water extract for the confirmation of present/ absent of phytochemical such as carbohydrates, protein, amino acid, steroids, volatile oil, glycosides, alkaloids, saponin, flavonoids, terpenoids, tannin and phenolic compounds. In quantitive phyto-chemical analysis 1 mg/ml stock solution is being taken. Total Carbohydrate (12), Protein (13), Total Phenol Content (14), Total flavonoid content (15), Glycoside (16), Tannin (17) and Terpenoids (18) are being quantified using specified standards for the studies are being performed.

High Performance Thin Layer Chromatography [HPTLC]

HPTLC profile of methanolic extract was obtained were Rf values were recorded. The HPTLC analysis was performed on E. Merck aluminum plate pre-coated with silica gel 60 F254 of 0.2 mm thickness and the plate was developed in Toluene: Ethyl acetate [9:1]. Extract was applied on the plate with the help of Hamilton [Reno, Nevada, USA] Syringe and plate developed in CAMAG twin trough chamber, previously calibrated with mobile phase. After development densitometric scan was performed with a Camag TLC scanner III in reflectance absorbance mode at 254 nm, 366 nm under control of Win CATS Software [V 1.2.1. Camag](19). The chromatogram was recorded.

Observations and Results

Morphological study

Seeds are small black shining Spherical, Lenticular. They are about 3mm in diameter. Shallow depression lies near the small elevated region at the margin- micropyle.

Taste slightly bitters and unpleasant and odor is characteristic. **[Figure 1 a-d]**

Microscopical study

Diagrammatic transverse section is lenticular in outline shows outer thin dark brown color testa enclosing with wide central horizontally placed starchy perisperm with shoe shaped embryo. [Figure 2 a-b]

Detailed transverse section showed that outer layer of epidermis consisting radially elongated rectangular dark brown color cell with thick outer cuticle followed by narrow collapsed celled band and then a layer of tangentially running yellow-colored rectangular cells of outer epidermis of tegmen, cells of its inner epidermis being with fine lignified streaks are thickening on the radial and inner walls, followed by parenchyma zone with angular cells of perisperm loaded by starch grains and crystals. [Figure 2 c-f]

Through radical is circular in outline outer dermatogen and inner pleurome. Inner cells consists prismatic crystals, oil globules and aleurone grains. Endospem cells filled with starch grains and oil globules. [Figure 2 g-h]

Organoleptic character

The organoleptic character of *C. argentea* was performed and the results are depicted. [Table 1]

Table No.1 Organoleptic character of *C. Argentea* Linn. Seed powder

Parameters	Description
Color	Grayish black
Odour	Slightly Aromatic
Taste	Sweetish astringent
Touch	Gritty

Powder Microscopical study

The powder microscopy of seed powder shows following characteristics such as Brown content, epidermal cells of tegma, black debris, fibers(simple), oil globules, endosperm cells, spool cells, oil content, fragments of spiral vessel and prismatic crystals were observed in seed powder of *C.argentea* Linn.[Figure. 3 a-i]

Table No.2: Histochemical study

Reagents	Observation	Characteristics	Results
Phloroglucinol + Hcl	Pink color	Lignin content	+ve
Iodine	Blue color	Starch grains	-ve
Sudan III	Pink color	Oil globules	+ve
$FeCl_3$	Blue color	Cellular constituents	+ve

Micrometric Evaluation

The micrometric evaluation scientifically observed under the microscope measuring cellular constituents randomly taken mean value under consideration. [Table No.3]

Table No.3 Micrometric analysis of *C. argentea* Seed powder

Sr.No.	Components	Measurements [µm= micrometer]
1	Seed	2 μm^2
2	Oil globules	0.2 μm^2
3	Endosperm cells	0.5x0.9µm
4	Epidermal cells of tegma	1x1.7 µm
5	Prismatic crystals	0.8x0.7 µm

Mean [n=3]

Note: Observations are carried out under 40x measurement. [Figure 4 a-e]

Physico-chemical analysis

The Physico-chemical parameters of *C. argentea* seed powder were performed and the results are depicted. [Table No.4]

Table No. 4: Physico-chemical analysis of *C. argentea* seed powder

Powder parameters	Results
PH [Aqueous 5 %]	6.3
Loss on drying [%w/w]	1.52±0.08
Ash value [%w/w]	4.79±0.02
Acid insoluble ash [%w/w]	0.24±0.16
Alcohol soluble extractive [%w/w]	5.19±0.07
Water soluble extractive [%w/w]	3.19±0.24
Bulk density	0.70±0.18

Mean±SD [n=3]

Preliminary phyto-chemical qualitative and quantitative analysis of *C. Argentea* powder are depicted. [Table 5] and [Table no.6]

Table 5: Qualitative Phyto-chemical analysis of *C. Argentea.* Seed powder

Phyto-constituents	Test	Water extract	Methanol extract
Protein	Biuret test	++	++
Amino acids	Ninhydrin test	++	++
Volatile oil	Direct manual test	++	++
Glycosides	Keller-killiani test	++	++
	Bontrager test	++	++
Alkaloids	Dragondraff test	--	--
Saponins	Foam test	++	++
Flavonoids	Direct manual test	++	++
Tannin and Phenolic Compounds	Lead acetate test	++	++
Steroids & Terpenoids	Salkowski test	++	++

Present ++ Absent –

Table 6: Quantitative phyto-chemical analysis of *C. Argentea.* Seed powder

Name of the test	Quantitative extract [in µg/
Total Carbohydrates content	145.56±54.62
Total Protein content	11.39±5.57
Total Phenolic content	78.21±15.65
Total Flavonoid content	9.25±4.05
Total Glycoside content	67.29±11.82
Total Tannin content	55.43±17.24
Total Terpenoid content	130.48±36.87

Mean±SD [n=3]

Table 7: HPTLC chromatogram of *Celosia argentea* Linn. Methanolic extract

Condition	Wavelength 254nm		Wavelength 366 nm	
	No. of spots	R_f values	No. of spots	R_f values
CA [1mg/ml]	7	0.03, 0.17, 0.21, 0.25, 0.7, 0.9, 0.96	3	0.03, 0.17, 0.97

The methanolic extract of *C. argentea* sample showed 7 spots under short UV [254nm], 3 spots under long UV [366 nm] [Figure 5 a-b]

Discussion

Plants have strongly influenced the development of biology and have contributed to many important scientific advances. Former screened data of *Celosia Argentea* showed amplitude of bioactive components (20). The *C. Argentea* pantropical weed with configuration of fascination, its dark brown color testa

enclosing with wide central horizontally placed starchy perisperm with shoe shaped embryo. This structure contains thick outer cuticle followed by narrow collapsed celled band and angular cells of perisperm loaded by starch grains and crystals. Outer dermatogen and inner pleurome have circular outline. Inner cells consist prismatic crystals, oil globules and aleurone grains. However, the qualitative and quantitative data revealed the presence of Carbohydrates followed by Protein, amino acid, steroids, volatile oil, glycosides, alkaloids, saponin, flavonoids, terpenoids, Tannin and phenolic compounds in plant. HPTLC fingerprint developed under above mentioned conditions can be used for authentication and standardization of Seed of *C. argentea* collected from Bangalore. The separated bands can be used for drug discovery of new lead molecules and structural elucidation which may concern as a new medicine in future health care system. All sample used are genuine and adulteration free.

Conclusion

Botanically Cock's comb or Lagos spinach is identified as *Celosia argentea* Linn. This investigation is very useful in identification, authentification and assessing the quality of *C. argentea* by means of pharmacognostical, phytochemical and HPTLC profiling. It will be useful as a reference tool to properly verify the correct plant material and to track quality for finished formulation with the use of *C. argentea* part for the therapeutic purpose.

Acknowledgement

Authors express their thanks to the Director, Head of departments and staff members of institute for their cooperation in bringing out this work successfully.

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Figure 1

[a]Fresh plant **[b] Dried Flowers bunch of plant with seeds** **[c] Natural seeds with measurement** **[d] Powder of seeds**

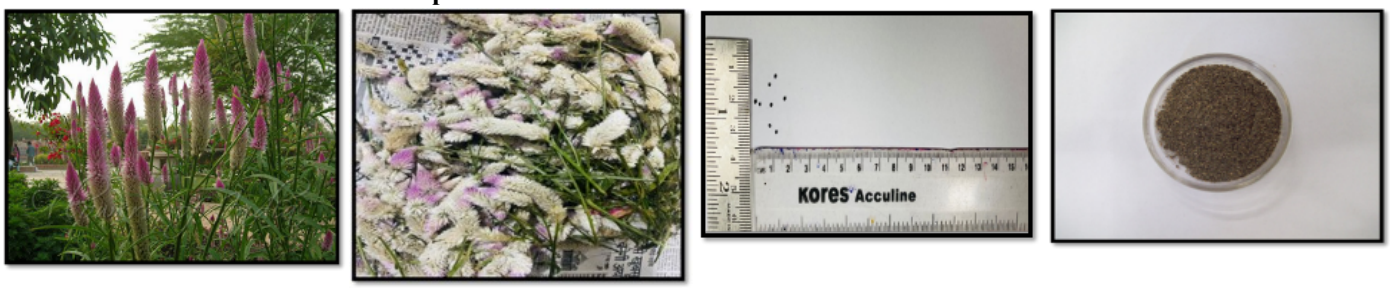
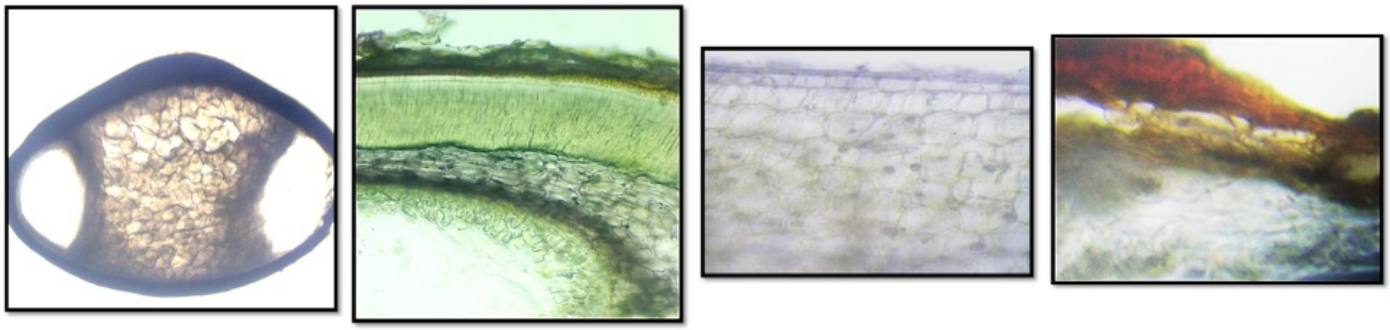


Figure 2

[a]Diagrammatic section of seed **[b] Outer epidermis consisting radially elongated rectangular dark brown color cell** **[c] Narrow Collapsed Cells** **[d] Yellow-colored rectangular cells of outer epidermis of tegmen**



[E] Fine lignified streaks on inner epidermis **[f] Parenchyma zone with angular cells of perisperm loaded by starch grains and crystals.** **[g] Inner cells consist prismatic crystals, oil globules and aleurone grains** **[h] Inner epidermal cells filled with starch grains and oil globules.**

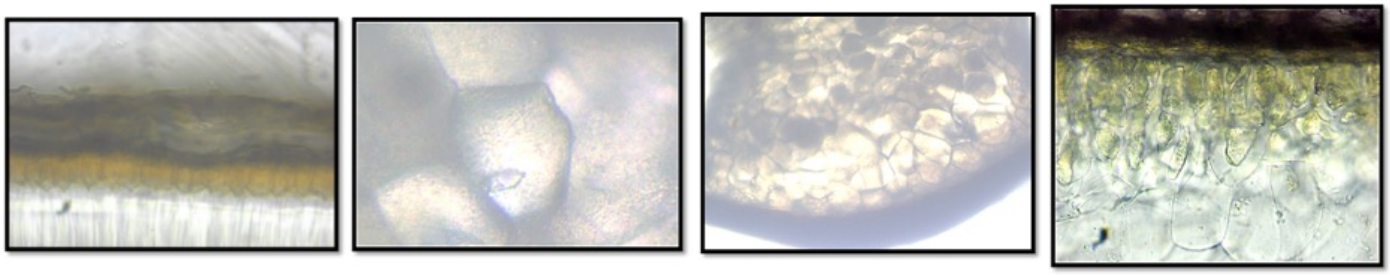
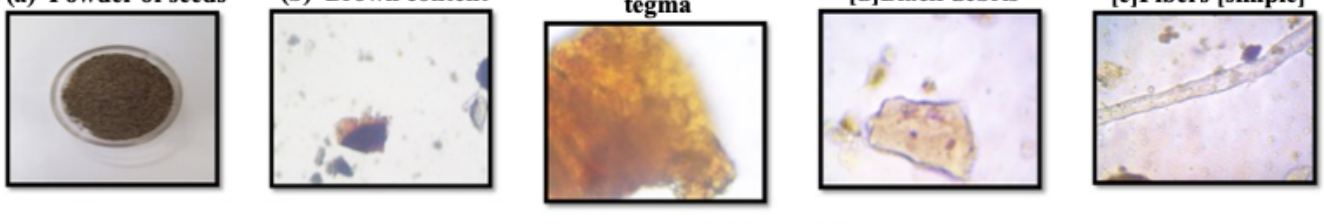


Figure 3

(a) Powder of seeds **(b) Brown content** **[c]Epidermal cells of tegma** **[d]Black debris** **[e]Fibers [simple]**



[f]Oil globules **[g]Prismatic crystal** **[h]Fragments of spiral vessel** **[i] Endosperm cells**

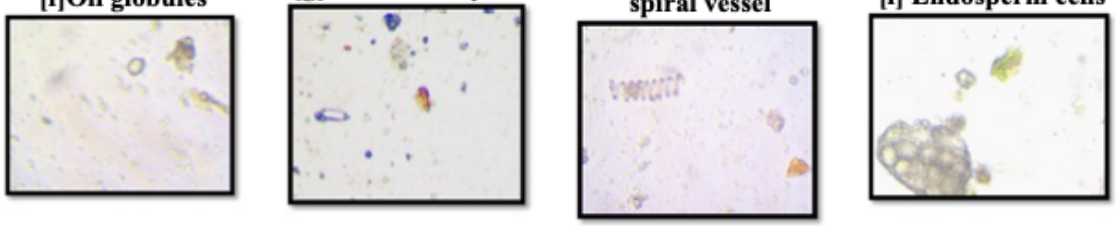
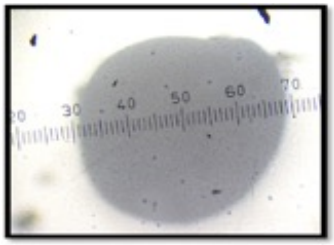
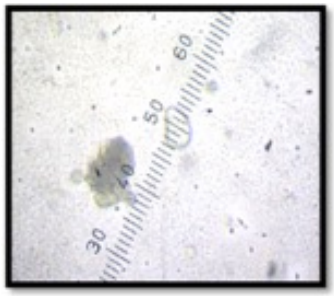
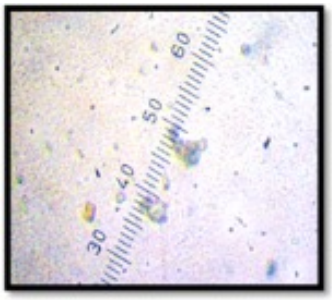
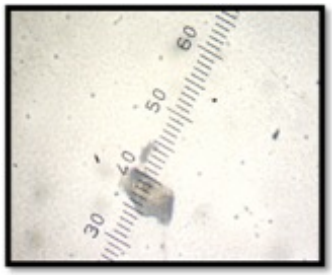


Figure 4

[a]Micro-measurement of seed	[b]Micromeasurement of starch grains	[c] Micromeasurement of Oil globules	[d] Micromeasurement of Endosperm cells
			

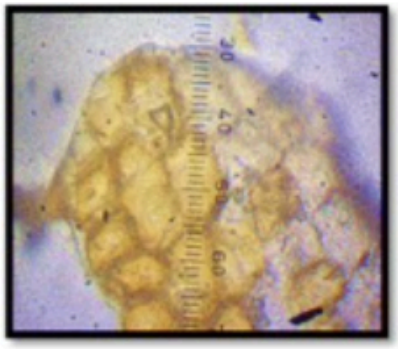
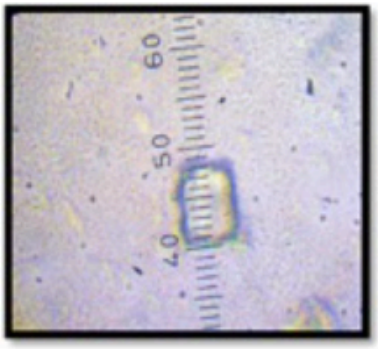
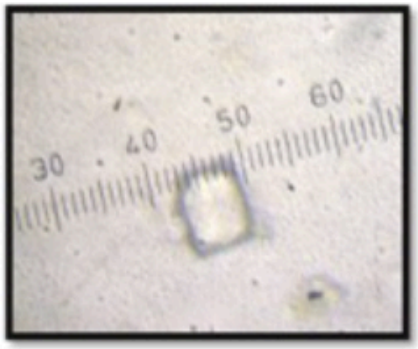
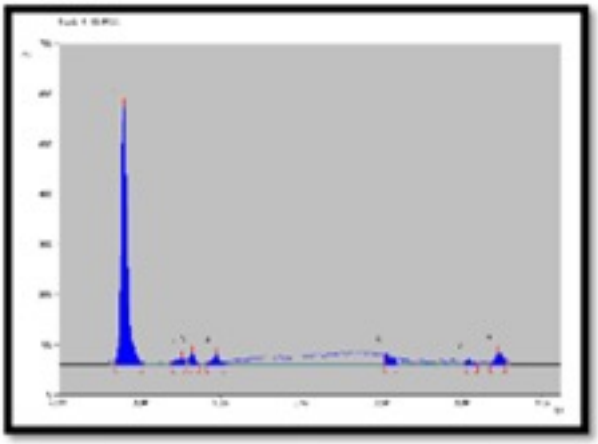
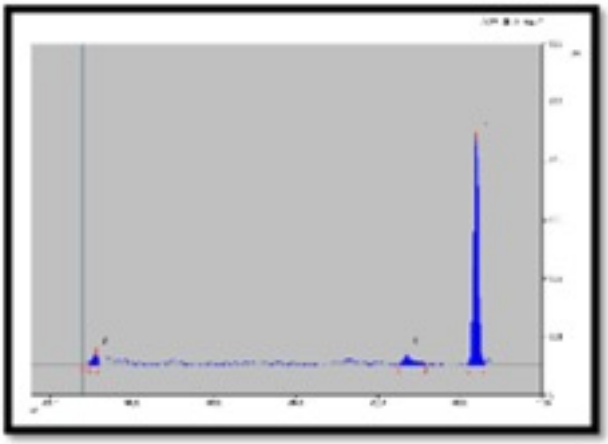
[e] Micromeasurement of Epicarp cells	[f] Micromeasurement of Prismatic crystals	[g] Micromeasurement of Prismatic crystals
		

Figure 5: HPTLC densitogram for methanolic extract of *C. argentea*

[a]Scanned at 254nm	(b) Scanned at 366nm
	

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