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Pharmacognostical characterisation of *Kakamachi* (*Solanum nigrum* Linn) whole plant

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Abstract
Pharmacognostical studies play an important role in the identification of the genuine plant of various *Ayurvedic* raw drugs. Adulteration/substitution exists for many ayurvedic raw drugs due to the non-availability of genuine herbs in required quantities and controversy in the selection of source plants. *Solanum nigrum* is an important herb. Present paper deals with the standardization of the whole plant by studying the micro-morphological, anatomical, physicochemical characters and chemical comparison by HPTLC studies.

Keywords: *Kakamachi*, *Solanum nigrum* Linn, macroscopic, microscopic, powder microscopic, preliminary phytochemical and high performance thin layer chromatographic.

Introduction
Over centuries humans have relied on the plants for the basic needs such as food, clothing and shelter. In addition, plants have also formed the basis of sophisticated traditional medicine that has been used for thousands of years. Some of the earliest records of their usage of plants as drugs can be traceable from Ayurveda, which is the basis of *Ayurvedic* medicines in India [1].Safety, efficacy and inexpensiveness have led to rapid expansion of *Ayurvedic* herbal pharmaceutical industry [2,3]. Therefore, The World Health Assembly has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards [4]. Modern physico‐chemical parameters and chromatographic studies provide the unique direction and a scientific basis towards this approach. *Kakamachi (Solanum nigrum* Linn.) is herb of Solanaceae family used mostly in Traditional Medicinal prescriptions for liver and skin disorders. [5] The leaves are used as poultice for rheumatic and gouty joints skin diseases. A decoction of the berries and flowers is useful in cough, rat bite, bronchitis and diarrhea. The seeds are useful in inflammation and skin diseases. The root bark is useful in otopathy, ophthalmopathy and hepatitis. [6]
No systematic pharmacognostic and phytochemical studies of its whole plants have been reported and therefore, a detailed investigation of the powdered whole plant of *S. nigrum* L. has been carried out using various pharmacognostical and physico‐phytochemical parameters.

Materials and methods
Collection of the Sample
The whole plant was collected, by uprooting the plant without damaging any system, from the campus of Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital,
Hassan, (Karnataka, India) in the month of April 2018 [Figure 1a]. The plant specimen was authenticated by the Sri Dharmasthala Manjunatheshwara *Ayurveda* College, *Dravyaguna* Department Hassana (Karnataka). Whole parts were separated and washed under running tap water; shade dried; pulverized; sieved through 80 meshes and preserved in an airtight glass bottle.

Macroscopic Features
Macroscopic features of the aerial part, stem and root of S. nigrum L were observed under Stereo microscope (Zeiss Stemi) and the characters recorded with reference to leaf, stem, fruits And root characters reported in literature.

Microscopy
Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the

sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars [7].

Powder microscopy

Pinch of whole plant powder of Kakamachi previously sieved is put on the slide and mounted in glycerine and powder charcters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. [8]

Physicochemical evaluation

The physicochemical evaluation which includes Loss on drying. Total ash, Acid insoluble ash. Water soluble, water extractive value and alcohol soluble extractive value was carried out as per standard procedure. [9]

HPTLC

1g of Kakamachi (whole plant) powder was extracted with 10 ml of alcohol. 3, 6 and 9µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in n-hexane: Ethyl acetate (7.0: 1.0). The developed plates were visualized in under short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm. Rf, colour of the spots and densitometric scan were recorded. [10-11]

Results and Discussion

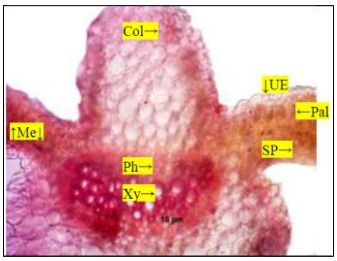
1. Macroscopy

The leaves were simple, alternate, subacute, tapering into the petiole, stem erect, berry and root Tap root consists of a few branches and numerous small lateral roots.

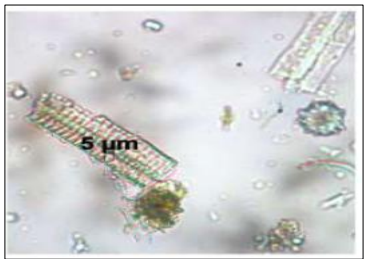
Fig 1a: Kakamachi aerial part

Fig 1b: Kakamachi stem and root

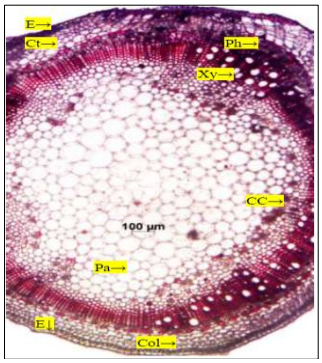
Microscopic Study



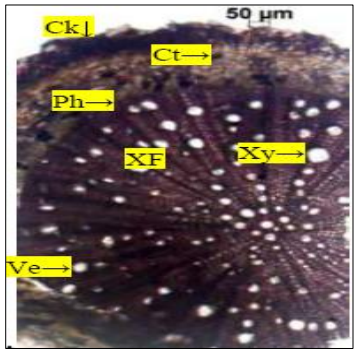
Midrib with Vascular bundle



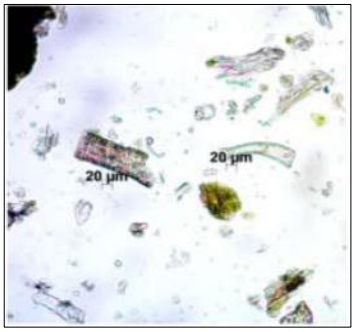
Vessels with scalariform and reticulate



T.S of petiole



T.S. of Root



Trichome and reticulate stomata

Sclereids

Col – collenchymas; LE – lower epidermis; Me – mesophyll; Pal – palisade; Ph – phloem; SP – spongy parenchyma; T – trichome; UE – upper epidermis; Xy – xylem., CC – cell content; Col – collenchymas; E – epidermis; Pa – parenchyma; SG – starch grain, AR – acicular raphide; F – fibres; Ck – cork; Ct – cortex; XF – xylem fibres; XR – xylem ray; Ve – vessel GT– ground tissue.

Fig 2: Microscopy of Leaf of Kakamachi

In 2-4 rows of tangentially elongated cells forming cork. Cortex is composed of thin walled cells with patches of lignified fibres; most of the cortical cells contain oval to round starch grains, single or compound with 2-3 components; a few cortical cells contain microsphenoidal crystals of calcium oxalate. Phloem rays are uniseriate, filled with starch grains. Xylem is composed of vessels and parenchyma; vessels arranged in groups of 2-4 in radial rows; parenchyma thick walled containing micro sphenoidal crystals of calcium oxalate.

Transverse section of petiole shows single layered epidermis covered with striated cuticle; trichomes uniseriate, 3-5 celled with pointed tips and warty walls, glandular hairs with 1-2 celled stalk and 2-7 celled head. Epidermis is followed by 2-3 layerd compactly arranged chlorenchyma; 5-8 layered parenchyma consisting or round, thin walled cells, a few containing microsphenoidal crystal of calcium oxalate. Central vascular bundle is arc shaped, bicollateral; 2 smaller bundles present laterally on either side of main vascular bundle.Midrib shows upper and lower epidermis of round to oval cells covered with striated cuticle and trichomes similar to those found on petiole; collenchyma, thin walled; vascular bundle bicollateral, arc shaped. Lamina shows dorsiventral structure, both upper and lower epidermis being covered with thick cuticle. Palisade is single layered; spongy parenchyma 4-6 layered. Trichomes are simple, uniseriate, multicellular with pointed tips and warty walls and glandular with 1-2 celled stalk and 2-7 celled head. Stomata are anisocytic and present on both of surfaces, more abundant on lower surface. Palisade ratio is 2-4, vein islet number 7-10 stomatal index, 15-17 on upper epidermis and 22-23 on lower epidermis.

Organoleptic characters of powder

Whole plant powder is creamish green in colour; bitter in taste and with characteristic odour.

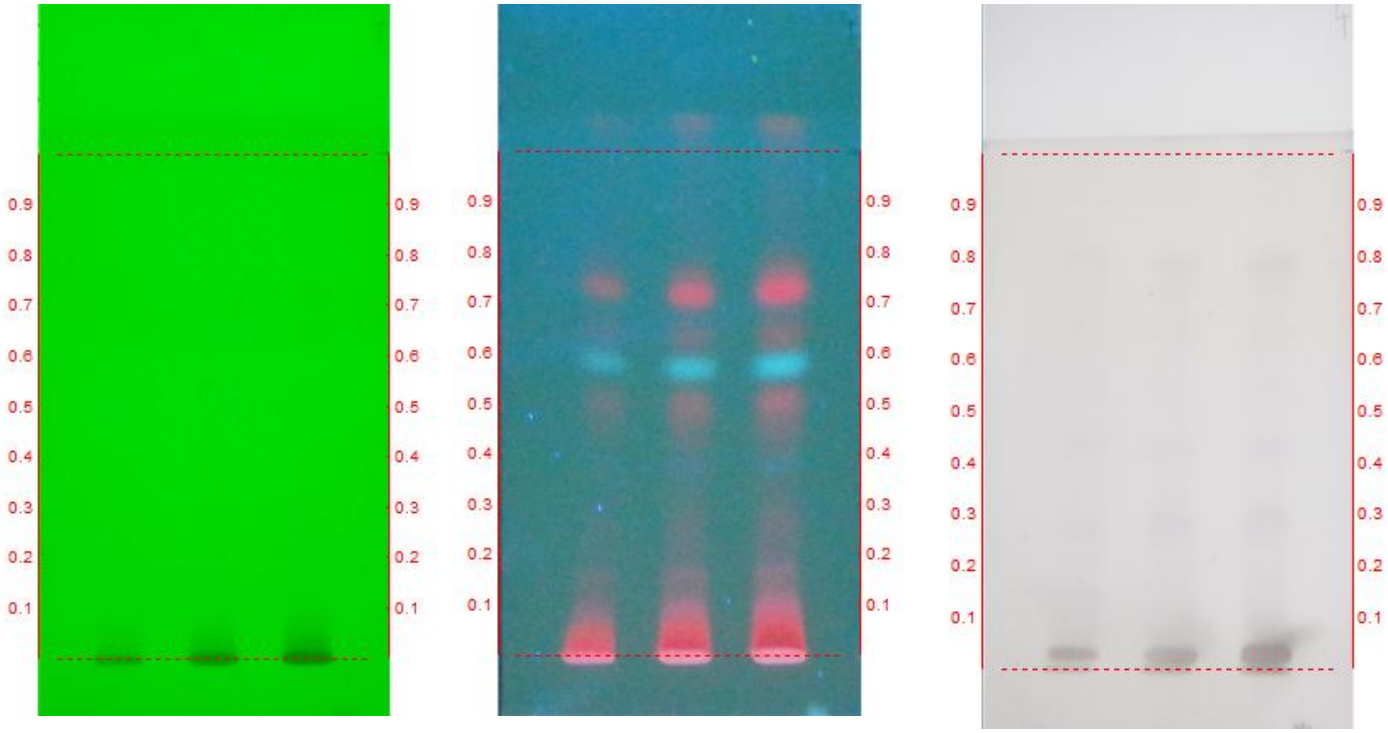
Table 1: Results of standardization parameters of whole plant of *Kakamachi*

Parameter	Results n = 3 %w/w
	Average ±SEM
Loss on drying	17.62±0.01
Total Ash	14.38±0.10
Acid Insoluble Ash	0.00±0.00
Water soluble Ash	7.47±0.23
Alcohol soluble extractive value	4.41±0.00
Water soluble extractive value	20.90±0.005

Table 2: Results of preliminary phytochemical screening of aqueous extract of *Kakamachi*

Test	Inference
Alkaloid	+
Steroid	+
Carbohydrate	+
Tannin	+
Flavanoids	-
Saponins	+
Terpenoid	-
Coumarins	+
Phenols	-
Carboxylic acid	-
Amino acids	+
Resin	+
Quinone	-

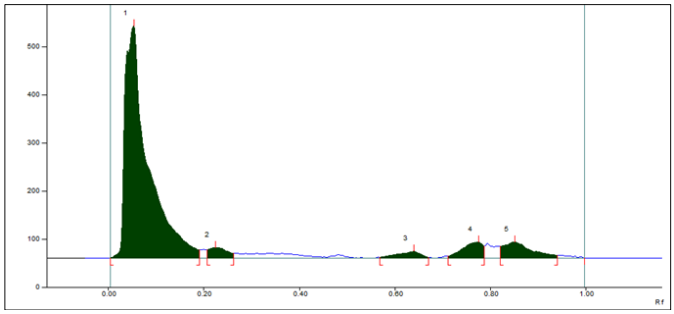
(+) present, (-)- absent



Short UV **Long UV** **Post derivatisation**

Track 1: *Kakamachi* - 3µl
Track 2: *Kakamachi*- 6µl
Track 3: *Kakamachi*- 9µl
Solvent system: Toluene: Ethyl aetate (7:1)

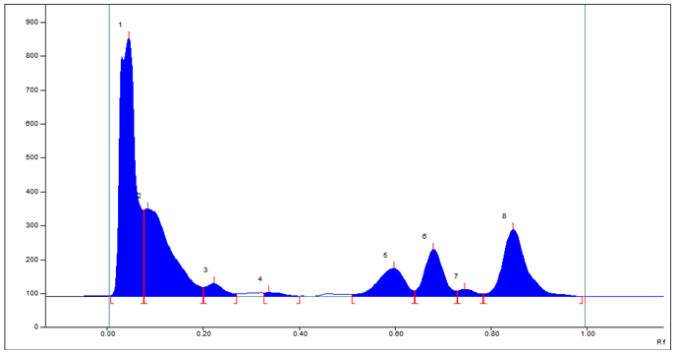
Fig 3: HPTLC Photo documentation of sample of *Kakamachi*



Track 3, ID: Kakamachi extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.7 AU	0.05 Rf	481.8 AU	82.37 %	0.19 Rf	17.0 AU	16514.5 AU	81.97 %
2	0.21 Rf	17.2 AU	0.22 Rf	22.1 AU	3.78 %	0.26 Rf	9.5 AU	631.0 AU	3.13 %
3	0.57 Rf	1.8 AU	0.64 Rf	14.6 AU	2.49 %	0.67 Rf	1.8 AU	498.3 AU	2.47 %
4	0.71 Rf	4.3 AU	0.78 Rf	32.9 AU	5.62 %	0.79 Rf	25.2 AU	1044.7 AU	5.19 %
5	0.82 Rf	24.1 AU	0.85 Rf	33.6 AU	5.74 %	0.94 Rf	5.7 AU	1458.8 AU	7.24 %

Fig 4a: At 254nm



Track 3, ID: Kakamachi extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.5 AU	0.05 Rf	762.3 AU	50.42 %	0.08 Rf	54.7 AU	17969.9 AU	40.39 %
2	0.08 Rf	255.3 AU	0.08 Rf	258.7 AU	17.11 %	0.20 Rf	27.0 AU	10524.8 AU	23.66 %
3	0.20 Rf	27.0 AU	0.22 Rf	38.4 AU	2.54 %	0.27 Rf	7.8 AU	1028.5 AU	2.31 %
4	0.33 Rf	11.1 AU	0.34 Rf	11.7 AU	0.78 %	0.40 Rf	0.7 AU	332.6 AU	0.75 %
5	0.51 Rf	6.0 AU	0.60 Rf	82.9 AU	5.49 %	0.64 Rf	16.5 AU	3333.7 AU	7.49 %
6	0.64 Rf	16.7 AU	0.68 Rf	138.5 AU	9.16 %	0.73 Rf	16.2 AU	3804.7 AU	8.55 %
7	0.73 Rf	16.5 AU	0.75 Rf	21.6 AU	1.43 %	0.78 Rf	7.7 AU	534.4 AU	1.20 %
8	0.79 Rf	7.8 AU	0.85 Rf	197.8 AU	13.08 %	0.99 Rf	0.0 AU	6960.9 AU	15.65 %

Fig 4b: At 366nm

Discussion

Macroscopic and organoleptic characters of a plant, helps for identification of the plant. In the present study, the macroscopic features of whole plant corroborates with the literature description (API)

Microscopic study of a plant reveals the characteristic microscopic features of different parts of the plant and helps in identifying different species and detects adulteration. In the present study, microscopic examination of root, petiole and leaf reveals distinct features which will aid in identification.

Physiochemical analysis of a herb evaluates the nature of adulteration and also reveals the quality, purity of the herb. Physicochemical analysis of the whole plant of *S. nigrum* has not been referred to in previous literature and the results of the present study may be used as a reference standard for future studies. Preliminary phytochemical evaluation reveals the major chemical components present in the herb. In the present study the whole plant of *S. nigrum* possess. Alkaloid, Steroid, Carbohydrate, Tannin, Saponins, Coumarins, Amino acid and Resins.

HPTLC helps to evaluate chemical constituent for the standardization of plant. In the present study the Kakamachi extract reveals five peaks at 254 nm and eight peaks at 366 nm. Identification and isolation of these components for its bio efficacy is a scope for further study which may lead for prospective compounds for cure.

Conclusion

The present pharmacognostic characterisation of whole plant of *S. nigrum* will help to supplement information on identification and standardisation. Further research on isolation of phytoconstituents will lead to marker compounds for identity.

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