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Micrometric study and physicochemical evaluation of *Solanum nigrum* Linn. leaves

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ABSTRACT

Background: *Solanum nigrum* Linn. (Solanaceae), commonly known as black night shade (Kakamchi), is an herb, traditionally used in the treatment of skin and cardiac disorders. Till date there is no scientific evaluation has been reported on its leaves. **Aims and objectives:** Present study assumes singular significance and it is supposed to contribute a great deal to the existing literature. The present investigation was, therefore, undertaken to determine the requisite pharmacognostical standards for standardization of leaves of *Solanum nigrum* Linn. **Methods:** Macroscopic and microscopic evaluation of fresh leaves and dried powder of leaves was carried out along with their micrometric study. Physicochemical and the preliminary phytochemical investigations of dried powder of *Solanum nigrum* Linn. leaves were also carried out along with fluorescence analysis. HPTLC finger profile of alcohol extract of leaves was also performed. **Results:** The pharmacognostical studies of leaves revealed the presence of single layered palisade parenchyma, anisocytic stomata and multicellular warty trichome, glandular trichomes which are the striking characters of identification of this plant material. The preliminary phytochemical screening of three extracts of the leaf powder showed the presence of alkaloids, glycosides, tannins and saponins along with other phytoconstituents. In HPTLC studies, the alcoholic extract showed presence of eleven and nine phytoconstituents at 254 nm and 366 nm wavelengths respectively. **Conclusion:** The information generated in this study will be helpful for the proper identification and authentication of leaves of this herb.

Keywords: *Solanum nigrum* Linn., Kakamchi, Phytoconstituents, Phytochemistry, Micrometry.

INTRODUCTION

The black nightshades (*Solanum nigrum* L. and related species) are worldwide weeds of arable land, gardens, rubbish tips, soils rich in nitrogen, in moderately light and warm situations which occur from sea to mountain levels.^[1] They are widely used as leafy herbs and vegetables, as a source of fruit and for various medicinal purposes. *S. nigrum* L., commonly known as black nightshade, is important amongst them belonging to *Solanaceae* family. Human consumption of its leaves as food is widespread, particularly in Africa and South East Asia.^[1] In India, this plant is noted for its antiseptic and anti-dysenteric properties and is given internally for cardiac pain and gripe.^[2] An infusion of the plant is used as an enema for infants with abdominal upsets. The plant is also a household treatment for anthrax pustules when it is applied locally.^[2] It is further reported to have emollient, diuretic and laxative properties and its decoction is regarded as both antispasmodic and narcotic.^[3] Freshly prepared extracts of the plant are apparently effective in the treatment of cirrhosis of the liver and also serve as an antidote to opium poisoning.^[4] An alcoholic extract of leaves is active against *Staphylococcus aureus* and *Escherichia coli*.^[5]

Unfortunately, there is a widespread confusion over the precise identification of the taxa involved in black nightshades, especially in those areas in which the species are most commonly used as food sources. Therefore the present investigation of *S. nigrum* L. leaves is taken up to establish pharmacognostical profile of the leaves which will help in crude drug identification, standardization of the quality and purity and also to distinguish the drug from its adulteration.

MATERIALS AND METHODS

Collection of sample

Fresh leaves of *S. nigrum* L. were collected from the campus of Gujarat Ayurved University, Jamnagar, Gujarat in the month of January 2012. The leaves were washed under running tap water; blotted dry; shade dried; pulverized; sieved through 80 meshes and preserved in an airtight glass bottle.

Macroscopic and microscopic evaluation

Macroscopic characters like shape, size, and margins were recorded as per visual observation. Thin free hand sections of the petiole and the leaf and midrib portion were taken by maceration method [6] and were treated with fluoreglucinol, hydrochloric acid and iodine for identification of various contents.[7] Photomicrographs were taken by using canon digital camera attached to Carl Zeiss binocular microscope. Powder microscopy was also done with stain and without stain and photomicrographs were taken.

Micrometric evaluation

For the micrometric evaluation of leaf, surface preparation of both surfaces was done by free hand peeling method. Stomata number, length, width, radius etc were studied and values were taken by successive three readings. Mean value was taken into consideration as per standard procedure.[8] [Table 1]

Phytochemical evaluation

The dried sample was used for physicochemical and the preliminary phytochemical investigations by standard procedure adopted by Ayurvedic pharmacopoeia of India.[9] Fluorescence analysis was done as per the method of Chase and Pratt (1949).[10]

HPTLC

A CAMAG (Switzerland) HPTLC system equipped with a sample applicator Linomat V was used for application of samples. CAMAG TLC Scanner 3, Reprostar and Wincats 4.02 were used for scanning the plates. CAMAG twin through glass chamber was used for developing the plates. Pre-coated silica gel plate was used as stationary phase and Toulene: Ethyl acetate: Formic acid (7:2:1 v/v/v) was used as mobile phase. After development, the plate was visualized under short UV (254 nm) and long UV (366 nm) and thereafter sprayed with Vaniline sulphuric acid for color reaction.

RESULTS

Macroscopic features

Leaves are green simple alternate, petiolate 2.5-8.5cm long and 2.5cm wide, ovate or oblong, toothed or lobed, base slightly unequal, narrowed at both ends, and thin. [Fig 1 a and b]

Microscopic study

T.S. of petiole

It shows single layered epidermis of oval or tangentially cells, covered with striated cuticle; covering trichomes, uniseriate, 3-5 celled having pointed tips and warty walls, glandular trichomes with 1-2 celled head. Epidermis single layered; collenchyma 2-3 layered, compactly arranged; 5-8 layered parenchyma consisting of round, thin-walled cells with smaller intercellular spaces, a few containing starch grains and brownish content material; central vascular bundle shallow, arc-shaped, bicollateral; two smaller bundles present laterally on either side of main vascular-bundles, one in each lateral wing of the petiole. [Fig 1 c]

T.S. of the leaf through midrib

It shows upper and lower epidermis of round oval cells, covered with cuticle, similar to those found on petiole; epidermis having warty and glandular trichomes, collenchyma 2-3 layered on both surfaces; parenchyma 4-6 layered, thin-walled with small intercellular spaces; arc shaped bicollateral vascular bundle placed centrally. [Fig 1 d]

Lamina is dorsiventral, both upper and lower epidermis single layered, composed of oval to tangentially elongated cells covered with thick cuticle; palisade parenchyma single layered [Fig 1 f]; spongy parenchyma 4-6 layered containing chloroplasts with intercellular spaces [Fig 2 b]; a few vessels with thickenings, beneath the palisade parenchyma. Central vascular bundle shallow, arc-shaped, bicollateral; Phloem towards lower epidermis and xylem towards upper epidermis, Metaxylem towards lower epidermis and protoxylem towards upper epidermis with some xylem fibres. [Fig 1 e]

Plate 1

Figure 1: a & b: Macroscopic features of leaves; c: Transverse section of leaf; d,e,f: Transverse section of leaf through midrib

Organoleptic characters of powder

Color of powder is dull green; taste astringent-mucilaginous and characteristic odor.

Powder microscopy

The diagnostic characters of powder microscopy shows fragments of palisade parenchyma [Fig 2 c], Fragments of spongy parenchyma [Fig 2 b], warty multicellular trichomes [Fig 2 h], glandular trichomes [Fig 2 g], spiral vessels [Fig 2 a], and Stomata (Type Anisocytic) [Fig 2 e].

Physicochemical tests, preliminary phytochemical screening, test of fluorescence of S. nigrum L. leaf extract

The results are shown in Tables 2, 3, and 4 respectively.

Chromatographic study

HPTLC finger profile of alcohol extract revealed the presence of number of phytoconstituents present at 254 nm and 366 wavelengths (Table 5) [Fig 3 a to e].

Plate 2

Figure 2: Characters seen in powder microscopy of leaf
a: Spiral vessels, **b:** Fragments of spongy parenchyma, **c:** fragments of palisade parenchyma, **d:** fragments of spiral vessels, **e:** Anisocytic stomata, **f:** lignified fibre, **g:** Glandular trichome, **h:** warty multicellular trichome

Plate 3

Figure 3: HPTLC profile of alcohol extract of leaf
a: After spray in normal light; **b:** spots at 254 nm wavelength; **c:** spots at 366 nm wavelength; **d:** peak display at 254 nm wavelength; **e:** peak display at 366 nm wavelength.

Table 1: Micrometric values of *S. nigrum* L. leaf

Parameters	Results
Stomata length Upper epidermis	12.1 μm
Stomata length Lower epidermis	11.40 μm
Stomata width Upper epidermis	9.50 μm
Stomata width Lower epidermis	9.75 μm
Stomata radius Upper epidermis	92.50 μm^2
Stomata surface area Lower epidermis	93.35 μm^2
Upper epidermal cell	212.94 μm^2
Lower epidermal cell	203.19 μm^2
Palisade ratio	4-6
Stomata number Upper surface	44-70
Stomata number Lower surface	70-100
Stomata index Upper surface	10-18
Stomata index Lower surface	20-23
Vein islet number	25-30
Veinlet termination number	38-42

Table 2: Physicochemical analysis of *S. nigrum* L. leaves

Parameters	Leaf
Foreign matter	Nil
Loss on drying (% w/w)	7.86
Ash value (% w/w)	12.45
Acid insoluble ash (% w/w)	0.49
Water soluble extractive value (% w/w)	34.08
Alcohol soluble extractive value (% w/w)	12.80
pH	6

Table 3: Preliminary phytochemical screening of *S. nigrum* L. leaves

Tests for phytoconstituents	WE	ME	CE
Carbohydrates	+	+	+
Proteins	+	-	-
Amino acids	+	-	-
Steroids	-	-	-
Alkaloids	+	+	+
Glycosides	+	+	+
Cardiac glycosides	+	-	-
Phenols	+	-	-
Flavonoids	+	+	+
Terpenoids	-	-	-
Tannins	+	+	+
Saponins	+	+	+
Anthraquinone	-	-	-
Volatile oil	+	+	+

WE: Water extract, ME: Methanol extract, CE: Chloroform extract, +: Present, -: Absent

Table 4: Fluorescence analysis of *S. nigrum* L. leaves

Treatment	Observations under		
	Ordinary light	UV light 254 nm	366 nm
1 gm powder + Methanol	Green	Colorless	Red
1 gm powder + Water	Yellow	Green	Parrot green
1 gm powder + Chloroform	Dark brown	Colorless	Red
1 gm powder + Hexane	Olive green	Colorless	Red
1 gm powder + 6 N HCL	Green	Green	Green
1 gm powder + 4 % NaOH	Green	Brown	Pink

Table 5: Chromatographic results of alcoholic extract of *S. nigrum* L. leaf

Conditions	Number of spots	Rf value
Short U.V. (254 nm)	11	0.01, 0.11, 0.21, 0.38, 0.44, 0.49, 0.59, 0.63, 0.67, 0.75, 0.89
Long U.V. (366 nm)	9	0.01, 0.10, 0.21, 0.28, 0.38, 0.44, 0.59, 0.68, 0.75
After spraying (Vaniline sulphuric acid)	9	0.15, 0.38, 0.48, 0.55, 0.59, 0.66, 0.72, 0.77, 0.94

DISCUSSION

Substitution of herbal drugs and adulteration is the major drawback in the promotion of the herbal products. Adulteration can be explained as mixing or substituting the original drug material with other spurious, inferior, defective, spoiled, useless other parts of same or different plant or harmful substances or drug which do not confirm with the official standards.

Some of the herbal adulteration is due to the carelessness of herbal collectors and suppliers. Collection from other plant by ignorance, due to similarity in the appearance, color, lack of knowledge may also lead to adulteration. In such case the detailed pharmacognostical study through morphological, phytochemical and physicochemical analyses are the most important tools not only for plant identification but also to lay down standardization parameters with the help of which adulteration can be identified and prevented. For example leaves of *Solanum nigrum*, *Phytolacca americana* and *Ailanthus glandulosa* are adulterated with the leaves of belladonna herb. In such case the histological characters help to distinguish them. Palisade ratio varying from 2 to 4 is seen in *S. nigrum* L. whereas *phytolacca* leaves show idioblasts of acicular calcium oxalate crystals and *ailanthus* leaves have cluster crystals of calcium oxalate near vein.

For example, in place of *S. nigrum* L. leaves the leaves from other plants of Solanaceae family may be collected due to similarity in their appearance. Thus in such case micrometric evaluation of leaf (stomata number, stomatal index etc) gives more appropriate scientific values regarding the proper plant identification.

The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy,

physiochemical and phytochemical parameters. Thus various tests in the present study provide referential information for correct identification and standardization of *S. nigrum* L. leaves. The pharmacognostical studies reveal that the presence of single layered palisade parenchyma, anisocytic stomata and multicellular warty trichome, glandular trichomes are the striking characters of identification of this plant material. The results of physicochemical analysis can be considered as standard parameters to be included in the Ayurvedic pharmacopoeia. In preliminary phytochemical screening, the qualitative study of three extracts of leaf powder shows the presence of alkaloids, glycosides, tannins and saponins along with other phytoconstituents. Among the modern analytical tools HPTLC is playing an important role since it is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. It simply helps in rapid and simple authentication and comparison of the suitable difference among samples with identical plant resource but different geographic locations.^[11] In present HPTLC study, the alcoholic extract of leaves shows presence of eleven and nine phytoconstituents at 254 nm and 366 nm wavelengths respectively.

CONCLUSION

The information generated in this study is helpful to differentiate *S. nigrum* L. from the other closely related species of Solanaceae family and moreover this scientific validation of leaves will also provide a standard reference for the further research works.

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