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Research Article

PHARMACOGNOSTIC STUDY OF KAKAMACHI (*SOLANUM NIGRUM LINN)*

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ABSTRACT
Kakamachi (Solanum nigrum Linn) belongs to family Solanaceae and is being used in Ayurveda in skin diseases and diabetes. It is being sold in the market under the common name *Makoy*. In order to ensure correct botanical standardization to remove the controversy, a detailed pharmacognostic study on whole plant of *Solanum nigrum* Linn has been carried out. The review on this drug shows that the drug is known since Vedic period. This is the plant amongst the few plants, which are used widely for purposes like *Shaka Dravya* (Vegetable drug). This study confirms that *Solanum nigrum* Linn fulfill the standard parameters, decided by the Ayurvedic Pharmacopoeial committee of India. The Physiochemical standards like ash value, alcohol soluble extracts, water soluble extracts etc, were also with the limits of the values mentioned in Pharmacopoeia. The heavy metals analysis of *Kakamachi* was carried out for Cadmium, Lead, Arsenic, etc., shows that the drug, is free from the abnormal levels of heavy metals. The qualitative study shows the presence of Saponins, Tannins and Alkaloids in *Kakamachi*. The standard monogram prepared concludes that these parameters could be useful for future standard.
KEYWORDS: *Solanum nigrum* Linn, Plant Microscopy, Ayurveda, Dravyaguna, pharmacognostic Standardization

INTRODUCTION

In recent years, Herbal products are increasingly in Pharmaceutical market[1]. Hence the importance of standardization and identification of herbs is getting more and more valued[2]. The drugs mentioned in Ayurveda texts and its identity with existing pattern of presentation must be matched and implemented. Solanum nigrum is one of such plant having great importance in Ayurvedic medication.[3] It is a species in the Solanum genus, native to Eurasia and introduced in the Americas, Australasia and South Africa. The plant *Solanum nigrum* Linn (Solanaceae) commonly called as black night shade in English, *Makoi* in Hindi, *Kachchipandu* in Telugu, *Munatakali* in Tamil, *Piludi* in Gujarati & *Kamuni* in Marathi[4].

Ayurvedic Historical Review

Description of *Kakamachi* is found in all the Ayurvedic literature. The drug is explained first time in Vedic *Granthas and* was also widely used in *Samhita* period especially in the form of *Shakdravya* (vegetable). *Gadanigraha* (12th century) is the one who mentioned a special chapter in *Rasayanadikara* on Kakamachi[5]. The description of *Kakamachi* is explained first time in Vedic *Granthas* of *Koushikasutra*. It is described along with *Bhrungaraj* for the prevention of *Keshadosha*. In *Keshava Paddhati* the fruits were used for *Keshavruddhi* as *Phalamani* Bandhan[6]. All *Samhita* have described *Kakakmachi* for *Shaka Dravya* as well as *Oushadha Dravya*. It is observed that the drug is very popular in those days, which was studied well and used widely in the therapeutics and in dietary supplement. They have explained the drug in the form of therapeutic application as well as contraindicated with some combination and application[7].

Taxonomic Study

Aims and Objectives

The main aim of the study is carryout the pharmacognostic study including microscopic and macroscopic characters of the whole plant of *Kakamachi* which will be utilized for future standard monograph.

MATERIAL AND METHODS

Collection and Authentication

For experimental study the sample drug was collected from the peripheral part of Hyderabad in specified and non-contaminated region. Its external morphological feathers were detected primarily and once it was confirmed the drug was collected in the month of November, in the morning session before 11 AM. The plant was collected in its full maturity condition. The leaf, stem, fruit, petiole, and root were sent it for microscopic study.

Taxonomical Classification

Division - Embryophyta
Sub-division - Angiospermae
Class - Dicotyledoneae
Order - Tubeflorae
Sub-order - Solanales
Family - Solanaceae
Genera - Solanum

Characteristics Of Solanaceae Family

Leaves, Stem & Roots - Members of this Family are often climbers or at least scrambling plants, often with hairy stems and leaves. The leaves are variable, and may be entire or dissected, without stipules, and are usually alternate. The calyx has five parts, which may be joined, and it often remains and enlarges around the fruit, as in Cape gooseberry (*Physalis*) or the Shoo-Fly Plant (*Nicandra*).

Flowers - The flowers have five petals and are generally regular in shape. They may be round and flat or star-shaped, but are often bell shaped or tubular. They usually occur in groups in the leaf axils, although they may be solitary. There are five stamens attached to the corolla tube.

Seeds - The ovary is superior (inside the flower), and the fruit is either a berry or a capsule, often containing many light brown disc-shaped seeds.

Members of this Family usually have regular flowers with five petals, alternate leaves, five stamens attached to the corolla tube and superior ovary containing many yellowish disc-shaped seeds[8]

Distribution

Throughout India, Ceylon- all temperate and tropical regions of the world[9]

Macroscopic and microscopic evaluation

Macroscopy

The Morphological study shows the root with few branches and numerous small lateral roots, externally it is smooth pale brown. The bark is thin and easily peeled off exposing pale yellow wood. The stem is erect, glabrous or pubescent green, rounded at the basal region and angular at the apical region. It is slightly woody and branched. The leaf is simple, 2.5-8.5 cm long and 2.5 cm wide, ovate or oblong, sinuate, toothed or lobed, narrowed at both ends and the petiole is thin.

The flowers are small, extra axillary, sub-umbellate, 3-8 flowered cyme, peduncle 6-20 mm long, pedicles 6-10 mm long, very slender; calyx 2-3 mm long, glabrous, five lobed, oblong, obtuse, 1.25 mm long; corolla 4-8 mm long, divided more than half way down into 5 oblong sub-acute lobes, white or pale violet; filaments short, flattened, hairy at base; anther 1.2-2.5 mm long, yellowish, oblong, obtuse notched at apex; ovary globose, glabrous; style cylindric, hairy in lower part. The fruits are berry, 6mm diameter, obtuse, usually purplish-black; smooth shining. The seeds are discoid, 1.5 mm in diameter, smooth, minutely pitted and yellow.

Microscopic Study

Schleiden (1947) used microscope for the identification of the drug. For the microscopic study following methods were adopted.

Materials and Methods

Slides from the microtome sections used for the study were prepared as follows-

Soaking of the Samples

All the samples were cut into thin slices in order to expose inner regions and to permit light to penetrate through the object. The samples were cut into 0.5 to 0.75 cm. thick slices and are carefully labeled. In the pharmacognostic study leaf and other vegetative parts of *Solanum nigrum* were fixed in FFA (Formaldehyde - Acetic acid - Alcohol) and processed for microtomming by usual paraffin Infiltration method (Johanson 1940). Sections at 4-8 µm stained with Crystal Violet and Basic Fuschin and permanent slides were prepared using Canadabalsam and mountant.

Observations- (Figure- 1-9)

Root- Shows cork consisting of 2-4 rows of tangentially elongated cells; cortex of large, slightly elongated, thin-walled cells having patches of lignified sclerenchymatous fibers, most of the cortical cells contain oval to round, starch grains, single or with two or rare 3 components; a few parenchyma cells contain microphenoidal crystals of calcium oxalate; phloem consists of thin-walled, polygonal cells, phloem rays uniseriate, filled with starch grains; xylem composed of vessels and thick-walled parenchyma containing microphenoidal crystals of calcium oxalate; rays composed of thin-walled, radially elongated cells.

Stem- Shows single layered, epidermis of barrel-shaped cells, covered with thick, slightly striated cuticle; trichomes multicellular, uniseriate; secondary cortex composed of 2-4 layered collenchyma, tangentially elongated, oval parenchymatous cells, some containing numerous microsphenoidal crystals of calcium oxalate, oval to round starch grains; endodermis single layered; pericycle consist of intermittent ring of patches of fibers; vascular bundle-collateral, conjoint and open; cambium 2-4 layered; xylem vessels arranged radially, smaller (protoxylem) being towards center, showing endarch condition with spiral thickening and simple perforations; tracheids pointed at the apex and with pitted walls; xylem rays homogenous, uniseriate; internal phloem is in small or large patches, accompanied by fibers, embedded in peri-medullary zones; large pith, composed of thin walled parenchymatous cells with intracellular spaces, a few cells containing micro-sphenoidal crystals of calcium oxalate are also found in the pith.

Leaf- Petiole- Shows single layered epidermis of oval or tangentially elongated cells, covered with striated cuticle; covering trichomes, uniseriate, glandular hairs with 1-2 celled stalk and 2-7 celled head; Mesophyll consists of 2-3 layered chlorenchyma, compactly arranged; 5-8 layered parenchyma consisting of round, thin-walled cells with smaller intercellular spaces, a few containing microsphinoidal crystals of calcium oxalate; central vascular bundle shallow, arc-shaped, bicollateral; two smaller bundles present laterally or either side of main vascular bundles one in each lateral wing of the petiole.

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

Lamina- Dorsiventral, both upper lower epidermis single layered, composed of oval to tangentially elongated cells covered with thick cuticle; spongy parenchyma 4-6 layered; warty hairs with pointed tips and glandular hairs are present, epidermis with irregular outline, stomata anisocytic, scattered on both surfaces but more abundant in lower surface, palisade ratio 2-4; vein islet number 7-10, stomatal index 15-17 on upper epidermis and 22-23 on lower epidermis.

Fruit- Shows thin, papery epicarp, pulpy mesocarp and axile placentation, seeds lie free in pulp of fruit.

Powder- Creamish-green, shows fragments of vessels with spiral thickening, a few broken pieces of pointed, unicellular hairs, single, oval to round and compound with three components of starch grains.

Standardization according to WHO / API Guidelines[10]

Determination Of Foreign Matter

The sample was free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below. The sample was spread in a thin layer in a suitable dish and examined in daylight with unaided eye. Later, the suspected particles were transfered to a petri dish, and examine with 10x lens in daylight.

Results- Percentage of Foreign matter-not more than 1

Determination Of Ash Value

Incinerated about 3 g accurately weighed, of the drug in a silica dish at a temperature not exceeding 4500 until free from carbon, cool and weigh. Collected the residue on an ashless filter paper and incinerated the residue and filter paper. Added the filtrate and evaporated up to dryness, and ignited at a temperature not exceeding 4500. Later,

percentage of ash with reference to the air-dried drug was calculated.

Results- Percentage of Total ash- 11.29%

Determination Of Acid Insoluble Ash

A crucible containing total ash was taken and added 25 ml of *dilute hydrochloric acid*. Later, collected the insoluble matter on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Then the filter paper containing the insoluble matter was transferred to the original crucible. It was dried on a hot-plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and weighed without delay. The content of acidinsoluble ash was calculate with reference to the air-dried drug.

Results- Percentage of acid insoluble ash- 6.2%

Determination Of Alcohol Soluble Extractive

A 5 g of the air dried drug, coarsely powdered was macerated, with 100 ml of Ethanol in a closed flask for twenty-four hours, shook frequently during six hours and allowed to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvent and evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish. It was dried at 1050, to constant weight and weigh. The percentage of Ethanol soluble extractive was calculated with reference to the air-dried drug.

Results- Percentage of ethanol- soluble extractive – 5.2%

Determination Of Water Soluble Extractive

Instead ethanol, chloroform-water was used and the whole process of alcohol soluble extractive was followed to determine water soluble extractive.

Results- Percentage of water- soluble extractive - 17%

Heavy Metal Analysis By Inductively Coupled Plasma (ICP)

Sample Preparation: This procedure is intended for the determination trace metals (excluding Hg) in the sample. The following preparation procedure was used for sample preparation for analysis by for the acid digestion of the sample.

800 mg of sample accurately weighed and heated with 10 ml of nitric acid (70%), followed by gentle heating with the addition of 8 ml, perchloric acid (70%), until the solution become colorless. After cooling, 30 ml water was added and heat was resumed for 10 min. Finally the solution was cooled, and made to 100 ml volume with water. The instrument was optimized with the operating condition as given below. Calibrations were performed using external standards prepared from 1000 ppm single element stock, made-up appropriate with 2% Nitric acid.

Operating Conditions-

- Equipment- - ICP-OES and Micro Wave Digestion
- Plasma RF Power - 1500W
- Sample depth - 9.5 mm from load coil
- Carrier gas flow - 1.1 L/min
- Spray Chamber temperature - 2^{o}C
- Sample flow rate - 240 µL/min
- Nebulizer - Agilent microflow (PFA)
- Interface - Nickel sample and skimmer cones.

Observations and Results

Heavy metal analysis of Kakamachi- The results are expressed in percentage (Table No-1)

Florescence Study

This study gives some clues about the presence of some active principles. Hence analysis was done by this method. Some of the chemical constituents in the samples of the drug found to give a fluorescent color when exposed to normal light and UV light.

Materials and Method-

2 grams of the dried powder of the whole plant of *Solanum nigrum* Linn is soaked under the solvents and the colors of the extracts were observed under ordinary light and UV light for the fluorescent color.

Observations and results: (Figure 10**)**

The fluorescence study reveals that the *Kakamachi* whole plant powder shows different colors in ordinary light as well as in UV light. In Ordinary light, the extract of Acetone, Chloroform and ethyl acetate shows dark green color; whereas green color observed in Ethanol and distilled water. The faint green color and gray color were observed in methanol and powder-as-such respectively of ordinary light. In UV light, the extract of Acetone, Chloroform, Ethanol and ethyl acetate shows Red color; whereas the brown color was observed in Methanol. Yellowish green color was observed in distilled water and faint green color of powder-as-such under the UV light.

Preliminary Phytochemical Study

The preliminary phytochemical analysis is a qualitative chemical evaluation, which indicates spectrum of chemical constitution present in the plant drug. The chemical tests also help in proper identification of verities of the crude drugs.

In the present study, the phytochemical tests were carried out on *Kakamachi* (*Solanum nigrum*) whole plant powder to find out the presence of Saponins, Tannins, Flavonoids, Triterpenoids and Alkaloids as mentioned below:

Materials and Methods:

- **Test for Saponin**- Shook 2 gms of powdered drug with 5 ml of water. The formation of foam, which remained for more than 10 mins which indicated the presence of Saponin.
- **Test for Tannins**- Took 2 gms of powdered drug in a test tube, add 5 ml of ferric chloride solution which imparted dark greenish black color, which indicated the presence of tannins.
- **Test for Flavonoids**- Took 2 gms of powdered drug in 5 ml of methanol in a test tube, added few magnesium foils and conc. HCl slowly from the sides of the test tube. Turning into pink color indicated the presence of Flavonoids.
- **Test for Triterpenoids**- Took 2 gms of powdered drug in equal quantity of Chloroform and Acetic anhydride in a test tube, added conc. H_2SO_4 slowly along the side of the test tube. The green coloration at upper layer indicated the presence of Triterpenoids.
- **Test for Alkaloids**- Took 2 gms of powdered drug in 5 ml of Chloroform in a test tube, added Dragendorff's reagent (Potassium Bismuth Iodide Solution) from the sides of the test tube. The reddish brown color indicated the presence of Alkaloids.

Observation and Results- Preliminary Phyto-chemicals Analysis (Table No-2)

RESULTS AND DISCUSSIONS

Exclusive review right from the Vedic period to till date was taken. The review shows that the description of *Kakamachi* was related with its utility in various therapeutic applications. It has also been observed through review that *Kakamachi* was also screened for its various activities such as Hepato-protective, Anti-pyretic, Anti Inflammatory, Antibacterial, Anti-hypertensive, Anti-fungal, etc. These reviews indicate that the drug possesses broad spectrum of actions which attracted researchers to work upon it.

The results obtained for determination of foreign matter, ash value, acid insoluble ash, alcohol soluble extractive and water soluble extractive are matching with the standard parameters, decided by the Ayurvedic Pharmacopoeial committee of India. This indicates the uniformity in the experiments and strengthening the standards. By determining the preliminary phytochemical analysis, a qualitative study shows the presence of Saponins, Tannins and Alkaloids in *Solanum nigrum* Linn. The heavy metals analysis of the study drug was also carried out for Cadmium, Lead, Arsenic, etc. It showed that the drug is free from the abnormal levels of heavy metals and considered as safe to administer for therapeutic application.

In conclusion *Kakamachi* is non-controversial easy available and cheaper drug. The standard monogram prepared concludes that these could be useful for future standard.

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Table No-1: Heavy Metal Analysis Of *Kakamachi* Results in percentage

S. No.	Elements-	*Kakamachi* whole plant	*Kakamachi* Ash
1.	Lead	0.00083	0.004
2.	Arsenic	0.00004	Not Detected
3.	Nickel	0.00023	0.00061
4.	Mercury	Not Detected	Not Detected
5.	Cadmium	0.000004	0.0002

Table No-2: Preliminary Phyto-Chemicals Analysis

No.	Observations	Interpretation
1.	Formation of foam which remain for more than 10 minutes	Saponins are present
2.	Greenish black coloration	Tannins are present
3.	No Pink color	Absence of flavonoids
4.	No Green coloration at upper layer	Absence of Triterpenoids
5.	Presence of Reddish brown color	Alkaloids are present

ABBREVIATIONS: C- Cortex, **S-**Schlerenchyma Fibers, **F-** Phloem, **M-** Medullary Rays, **X-**Xylem, **T-**Trachieds, **O-**Ca Oxalate Cells, **P-**Pith, **R-** Pericycle, **E-**Endodermis, **T-**Secondary Cortex, **M-**Care Cambium, **I-**Epicarp, **L-** Cuticle, **H-** Parenchyma, **V-** Vasuclar Bundle, **N-**Cholenchyma, **A-** Mesocarp, **D-** Endocarp, **LM-** Leaf Margin

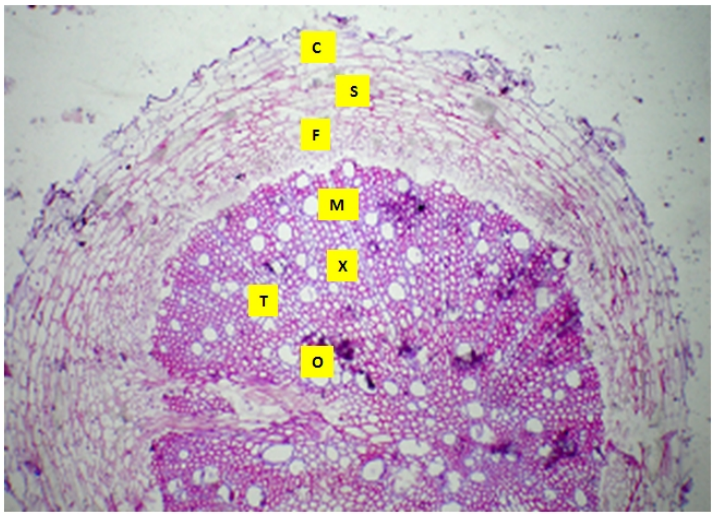

Figure 1- T.S. Root of mature *Solanum nigrum* with Sclerenchyma fibers, Medullary rays, Phloem and Cells of Calcium oxalate.

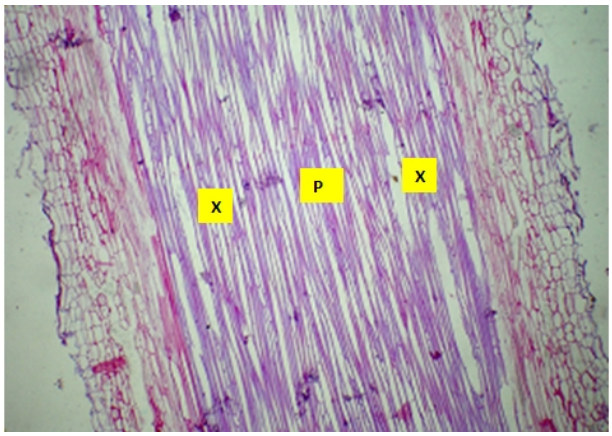

Figure 2- L.S. Root of mature *Solanum nigrum* with Pith and Xylem

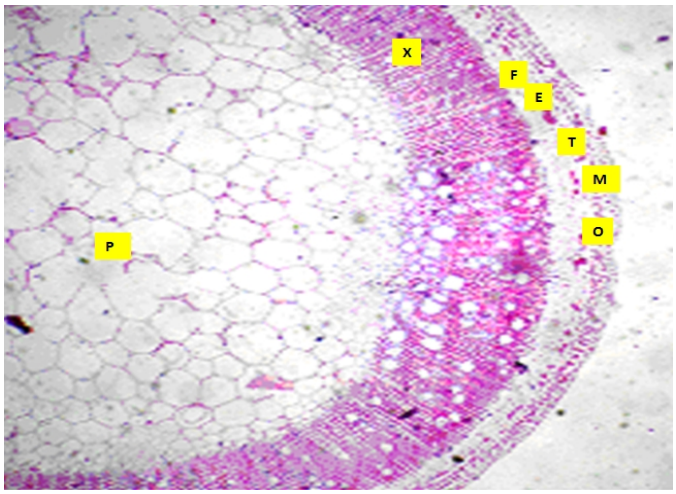

Figure 3- T.S. Stem of mature *Solanum nigrum* with Pith, Phloem, Pericycle, Secondary cortex and Xylem.

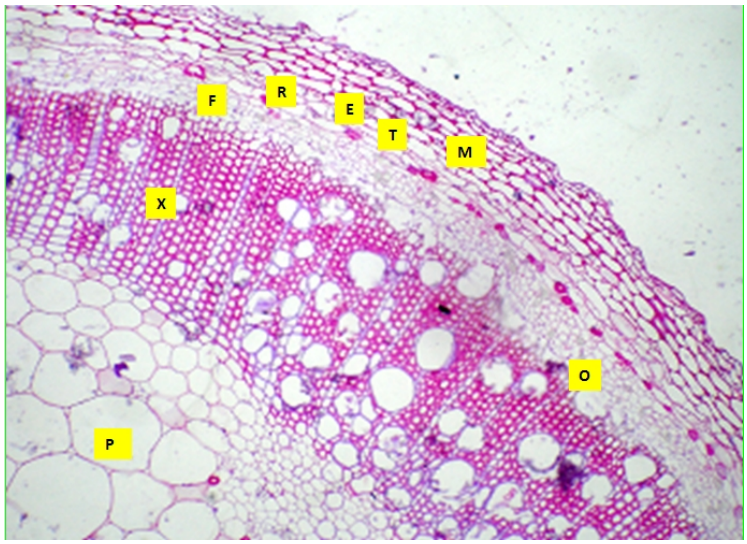

Figure 4- T.S. Stem of mature *Solanum nigrum* with Cells of Calcium Oxalate, Care cambium, Secondary cortex, Endodermis, Xylem and Phloem.

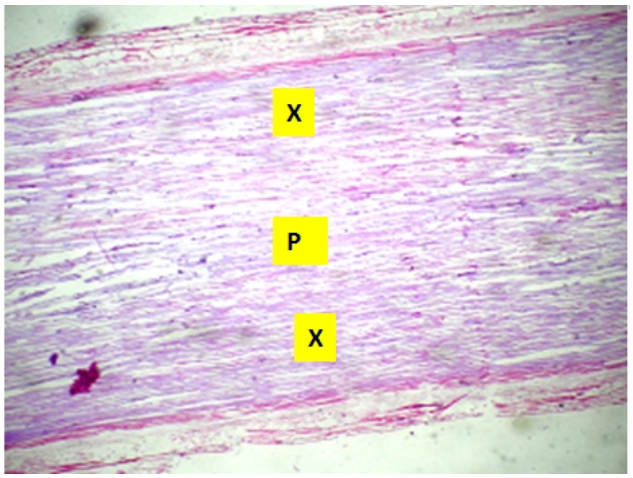

Figure 5- L.S. Stem of mature *Solanum nigrum* with Pith and Xylem

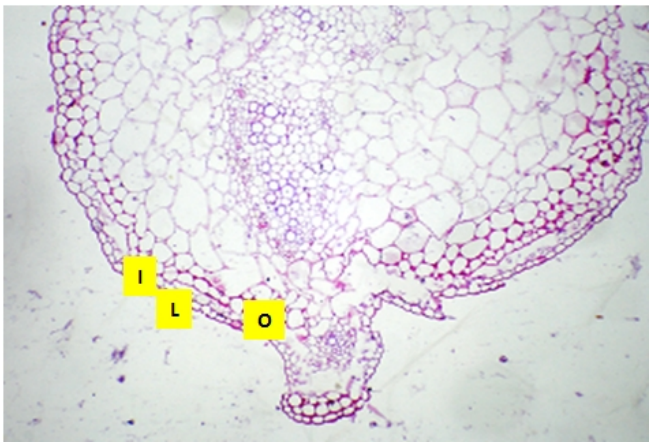

Figure 6- L.S. Stem of mature *Solanum nigrum* with Pith and Xylem.

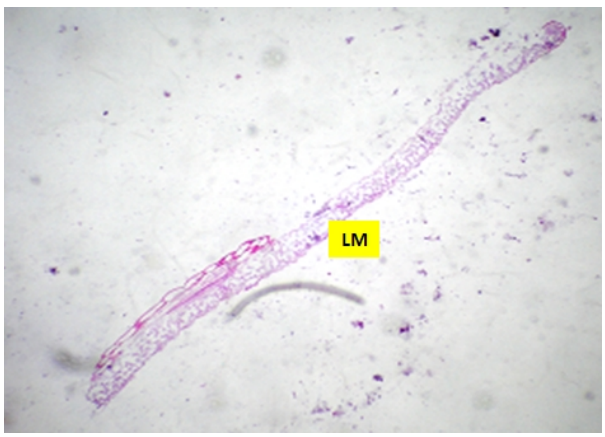

Figure 7- Leaf Margin of *Solanum nigrum*

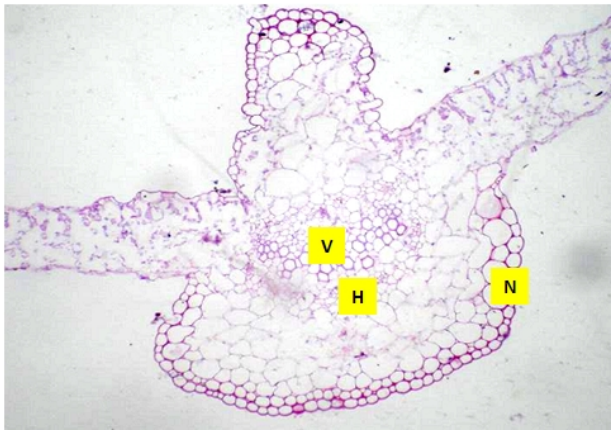

Figure 8- T.S. Leaf of *Solanum nigrum* with Collenchyma, Parenchyma and Vascular bundle

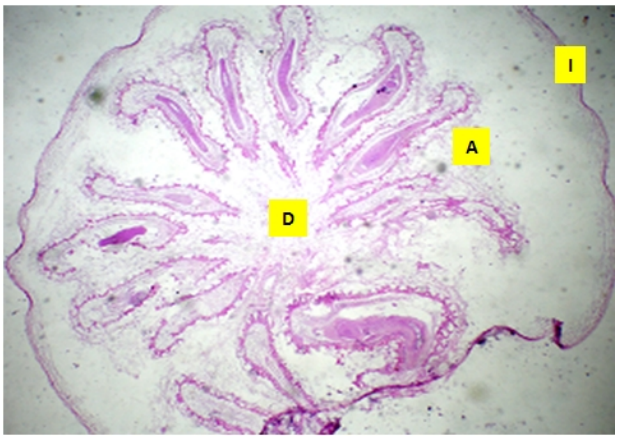

Figure 9- T.S. fruit of *Solanum nigrum* with Epicarp, Mesocarp and Endocarp

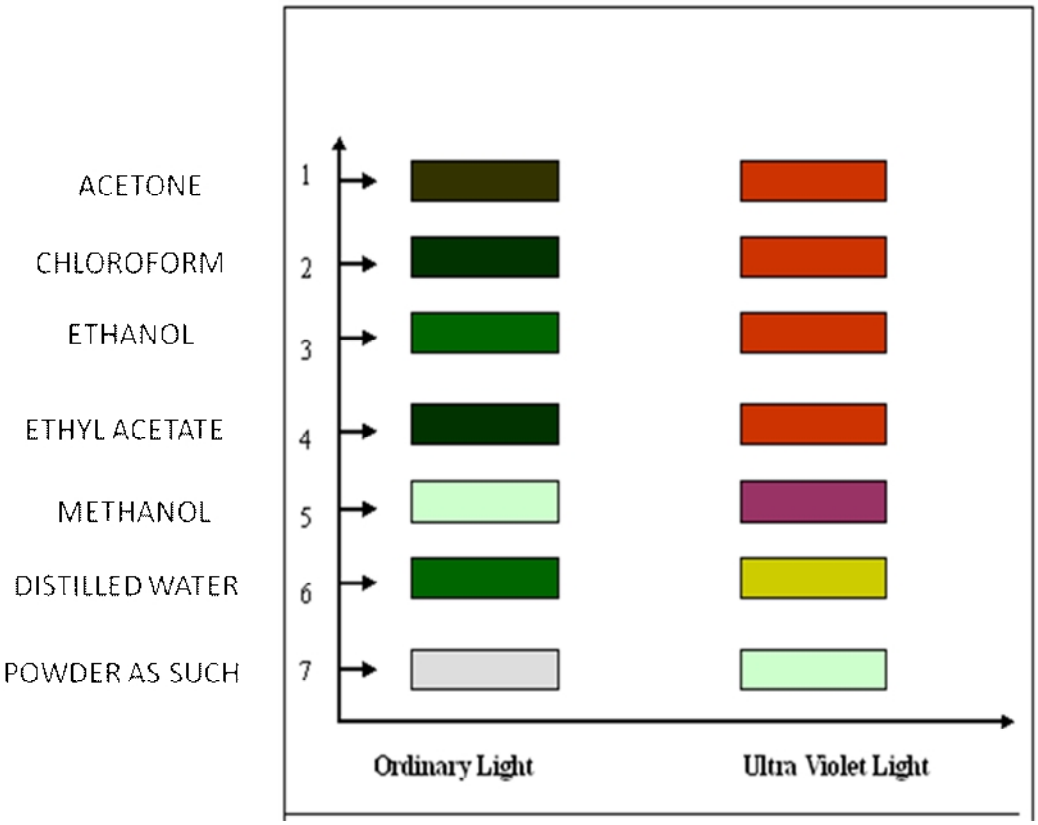

Figure 10- Florescence analysis of whole plant of Kakamachi (*Solanum nigrum* Linn.)

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