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PHARMACOGNOSTICAL STUDIES OF LEAVES OF *SPINACIA OLERACEA* LINN.

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ABSTRACT

The paper presents the pharmacognostical studies of leaves of *Spinacia oleracea* Linn. (Amaranthaceae) commonly known as Palak, a traditional medicinal plant with high nutritional value, used as anti diabetic, anti bacterial and hepatoprotective agent. Extensive literature survey revealed that no reports were available on organoleptic, microscopic and physicochemical properties of *Spinacia oleracea* Linn. The present study was undertaken to perform pharmacognostical research which can support the identification and characterization of the plant.

INTRODUCTION: A high intake of fruit and vegetables is well known to have positive effects on human health and has been correlated to a decreased risk of most chronic diseases such as cardiovascular disease, diabetes and several forms of cancer[1, 2]. Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A (lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, iron. *Spinacia oleracea* is an edible flowering plant in the family of Amaranthaceae. It is an annual plant (rarely biennial), which grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions [3]. Apart from having nutritional value, it has been also credited with various biological activities like virus inhibitor [4], anthelmentic [5], antioxidant, [6] hepatoprotective [7] and reducing risk of breast cancer [8]. Our objective was to identify and characterize *Spinacia oleracea.*

MATERIALS AND METHODS: The Fresh leaves of *Spinacia oleracea* were collected in the month of February, 2010 from Shirpur, Dist Dhule (M.H.) India. These were identified, and authenticated by the scientists of Botanical Survey of India, Pune, India. A voucher specimen of the leaf itself is deposited in department for future reference. Collected fresh leaves were washed and used for study of organoleptic and microscopic characteristics. The powder of shade dried leaves was used for the determination of ash values, extractive values and phytochemical investigations. All chemicals and reagents used for testing were of analytical grade obtained from SD Fine Chemicals and Loba Chemicals, Mumbai (India).

Microscopy: Fresh leaves of *Spinacia oleracea* were selected for the microscopical studies. Microscopic sections were cut on a microtome and by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the leaf specimen were made and examined [9]. Histochemical reactions were applied with various reagents to reveal lignified elements; Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provided with MOTIC IMAGES PLUS 2.0 software. A powder characteristic, preliminary examination, behavior of powder with different chemical reagents and microscopical examination was carried out [10].

Micrometry: The measurements of different cells and cell contents were done with the help of calibrated ocular micrometer and MOTIC IMAGES PLUS 2.0 software.

Physicochemical parameters: Percentage of total ash, acid-insoluble ash, water soluble ash and sulphated ash were calculated as per the Indian Pharmacopoeia [11]. The total ash of the powdered leaf was tested for different inorganic constituents. Different extracts of the leaves were prepared for the study of extractive values. Fluorescence analysis of powdered leaf was carried out by standard methods [12-13].

Preliminary phytochemical analysis - For the preliminary phytochemical analysis, 5g of powdered drug was extracted with petroleum ether, chloroform, methanol, ethyl acetate, ethanol, acetone and water successively. The extracts were dried and weighed. The presence or absence of different phytoconstituents viz. proteins, alkaloids, sugars, tannins, glycosides etc. were detected by usual prescribed methods [14-15].

RESULTS:

Macroscopy: The leaves are alternate, simple, and ovate to triangular-based, too variable in size from about 2-30 cm long and 1-15 cm broad, with larger leaves at the base of the plant and small

leaves higher on the flowering stem. Apex was oval in shape, base are symmetrical in shape vein lets are AlterNet. Upper surface of leaf are smooth while lower surface was ruff.

Microscopic Features: The transverse section of the leaf showed following characters:

- The leaf is generally ovate to triangular based. The lamina portion consisted of upper and lower epidermis. The midrib consisted of spiral vascular bundles namely lignified xylem and non lignified phloem. Below the vascular region spongy parenchyma cells were observed.

a) Transverse section of leaf:

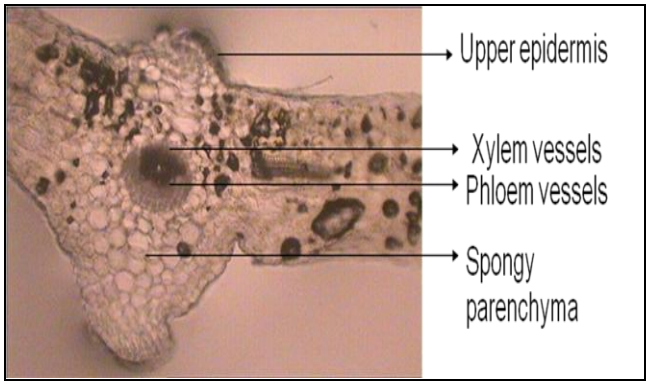

FIG. 1: TRANSVERSE SECTION OF LEAF

b) Powder characteristics: The powder was characterized on its morphological features as;
Color: Green,

Odor: Odorless,

Taste: Palatable

Microscopical examination of drug powder revealed the presence of anomocytic stomata, with lignified xylem, non lignified phloem and spongy parenchyma cells.

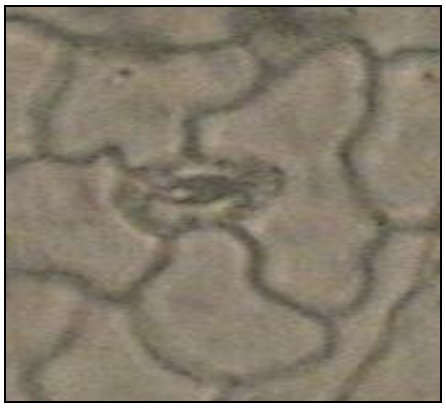

FIG. 2: STOMATA

FIG. 3: VASCULAR BUNDLES

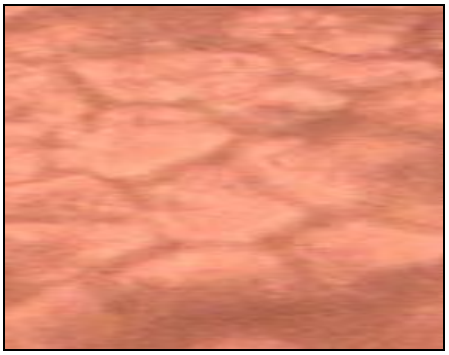

FIG. 4: SPONGY PARANCHYMA

TABLE 1: HISTOCHEMICAL COLOR REACTIONS

Reagents	Observations	Characterization
Phloroglucinol+ Conc. HCl	Pink	Vascular bundles
Dilute HCl	Soluble	Calcium oxalate
Sulphuric acid (60%w/w)	Soluble	Calcium oxalate
Dragendorff's reagent	Light orange	Lamina

Physicochemical Parameters: The percentage presence of acid insoluble ash, water soluble ash, water insoluble ash, and loss on drying are tabulated in **table 2**.

TABLE 2: ASH VALUES OF SPINACIA OLERACEA LEAF

Types of Ash Values	Results
Total ash	23.66 %w/w
Acid insoluble ash	9% w/v
Acid soluble ash	13% w/v
Water insoluble ash	6% w/v
Water soluble ash	4.6% w/v
Loss on Drying	0.15% w/w

Extractive value: The results revealed the highest extractability of powdered leaf drug was found in methanol and water. Extractive values of different solvent for the extractability are shown in **table 3**.

TABLE 3: EXTRACTIVE VALUES WITH DIFFERENT SOLVENTS

Types of Solvent	% Extractability v/v
Petroleum ether	8.0%
Chloroform	5.60%
Ethyl acetate	9.60%
Ethanol	13.6%
Methanol	61.60%
Water	32.80%
Acetone	10.40%

TABLE 4: LEAF CONSTANTS FOR *SPINACIA OLERACEA*

Leaf constants	Value
Stomatal number	50-55-58
Stomatal index	22-23-24.5
Vein –islet number	6-10-12

Florescence Analysis: The extracts were prepared by the maceration, further they were treated with various reagent and the color changes were observed under ultra-violet light. All the results are shown in **table 5**.

TABLE 5: RESULT OF FLUORESCENCE ANALYSIS OF ALCOHOLIC EXTRACT

Chemicals	Day light	Short ultraviolet	Long ultraviolet
Water	Light Yellow	Dark green	Black
Concentrated HCL	Dark Green	Light green	Black
Concentrated H_2SO_4	Dark Green	Dark Green	Black
Concentrated HNO_3	Light green	Light Green	Black
Acetic anhydride	Green	Green	Black
NaOH	Green	Green	Black
Methanol	Green	Light Green	Black
Acetone	Green	Light Green	Black
0.1N NaOH	Green	Light Green	Black
0.1m HCl	Greenish Brown	Dark Green	Black

TABLE 6: RESULT OF FLUORESCENCE ANALYSIS OF ACETONE EXTRACT

Chemicals	Day light	Short ultraviolet	Long ultraviolet
Water	Dark Green	Dark green	Black
Concentrated HCl	Dark Green	Dark green	Black
Concentrated H_2SO_4	Reddish Black	Greenish Green	Black
Concentrated HNO_3	Yellow	Green	Green
Acetic anhydride	Blackish Green	Green	Green
NaOH	Green	Green	Black
Methanol	Dark Green	Green	Black
Acetone	Green	Green	Black
0.1N NaOH	Blackish Green	Green	Black
0.1m HCl	Light Green	Green	Black

TABLE 7: RESULT OF FLUORESCENCE ANALYSIS OF CHLOROFORM EXTRACT

Chemicals	Day light	Short ultraviolet	Long ultraviolet
Water	Black	Green	Black
Concentrated HCl	Dark Green	Dark Green	Black
Concentrated H_2SO_4	Black	Dark Green	Black
Concentrated HNO_3	Pink	Green	Black
Acetic anhydride	Green	Green	Black
NaOH	Black	Green	Black
Methanol	Blackish Green	Dark Green	Black
Acetone	Yellowish Green	Green	Black
0.1N NaOH	Blackish White	Blackish Green	Black
0.1m HCl	Blackish Green	Dark Green	Black

TABLE 8: RESULT OF FLUORESCENCE ANALYSIS OF PET ETHER

Chemicals	Day light	Short ultraviolet	Long ultraviolet
Water	Yellowish Black	Yellowish Black	Black
Concentrated HCl	Light Green	Dark Green	Black
Concentrated H_2SO_4	Light Green	Light Green	Dark Green
Concentrated HNO_3	Light Orange	Light Green	Black
Acetic anhydride	Dark Green	Dark Green	Black
NaOH	Yellowish Green	Light Green	Black
Methanol	Yellowish Green	Dark Green	Black
Acetone	Yellowish Green	Light Green	Black
0.1N NaOH	Yellowish Green	Light Green	Black
0.1m HCl	Dark Green	Light Green	Black

TABLE 9: RESULT OF FLUORESCENCE ANALYSIS OF ETHYL ACETATE

Chemicals	Day light	Short ultraviolet	Long ultraviolet
Water	Dark Green	Dark Green	Black
Concentrated HCl	Dark Green	Dark Green	Black
Concentrated H_2SO_4	Dark Green	Dark Green	Black
Concentrated HNO_3	Yellowish White	Light Yellow	Green
Acetic anhydride	Black	Black	Black
NaOH	Yellowish Green	Dark Green	Black
Methanol	Yellowish Green	Dark Green	Black
Acetone	Yellowish Green	Green	Black
0.1N NaOH	Yellowish Black	Light Green	Black
0.1m HCl	Yellowish Green	Dark Green	Black

TABLE 10: RESULT OF FLUORESCENCE ANALYSIS OF AQUEOUS EXTRACT

Chemicals	Day light	Short ultraviolet	Long ultraviolet
Water	Brown	Light Green	Black
Concentrated HCl	Light Green	Light Green	Black
Concentrated H_2SO_4	Yellow	Light Green	Black
Concentrated HNO_3	Yellow	Light Green	Black
Acetic anhydride	Light Yellow	Light Yellow	Green
NaOH	Light Yellow	Light Yellow	Green
Methanol	Light Yellow	Light Yellow	Green
Acetone	White Yellow	Light Green	Black
0.1N NaOH	Dark Yellow	Light Green	Black
0.1m HCl	Light Yellow	Dark Green	Black

Preliminary Phytochemical Analysis: The ethanolic, methanolic, aqueous, petroleum ether, chloroform, ethyl acetate, and acetone extract was used for the preliminary phytochemical analysis for their presence of the constituents. It

showed the presence of Alkaloids, tannins, proteins and carbohydrates in all the extract except in petroleum ether.

DISCUSSION: The pharmacognostical study is one of the major criteria for identification of plant drugs. The present study on pharmacognostical characteristics of *Spinacia oleracea* leaf will provide useful information for its correct identity. Powder test and extractive value are performed as per stander method; LOD and all the result are in the specific criteria.

CONCLUSION: The current study will serve to become a ready reference for its identification and standardization on the basis of microscopy and chemical analysis. The preliminary phytochemical investigation will further help in isolation of important compounds in future.

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