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Research Article

Pharmacognostical Studies and Evaluation of Quality Parameters of *Spinacia oleracea*

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ABSTRACT
Spinach (*Spinacia oleracea*) is an edible flowering plant in the family Amaranthaceae native to central and western Asia. Its leaves are eaten as a vegetable. Spinach has a high nutritional value because it is a rich source of vitamin A, vitamin C, vitamin K, magnesium, manganese, iron , riboflavin vitamin B_6, vitamin E, calcium, potassium, and dietary fiber. Pharmacognostic standardization, physicochemical evaluation of the Leaves of *Spinacia oleracea* was carried out to determine its macro-and microscopical characters and also total ash, insoluble ash, alcohol- and water-soluble extractive values were determined for phytochemical evaluations. Preliminary phytochemical screening was also done to detect different phytoconstituents. The Proximate analysis of powder was also carried out in which extractive value, ash value, foreign matter, loss on drying were determined and also successive solvent extraction was carried out using soxhlet extractor in which ethanol was used. Aqueous extract was also carried out by maceration method. Preliminary phytochemical screening of various successive extracts of leaves was done qualitatively which revealed the presence of phytosterol, saponins, tannins, flavonoids, carbohydrates, tannins and amino acids.

Keywords: macroscopy, microscopy, phytoconstituents, R_f (retention factors).

INTRODUCTION

Spinacia oleracea which is popularly known as palak and King of iron is distributed more or less throughout in central and western Asia. *Spinacia oleracea* is included in the group of vegetable drugs (vargas) by Charaka[1]. The leaves are used in anaemia, night blindness, respiratory disorders, urinary disorders, female disorders, cancer prevention, bone disease and diabetes. Spinach is a rich source of minerals, vitamins, sugar, proteins and amino acids[2,3]. Literature revealed that pharmacognostic studies have not been reported for the leaves of this plant. Therefore, the main aim of the present work is to study the macro, microscopic and some other pharmacognostic characters and physic-chemical standards of leaves of *Spinacia oleracea* which could be used to explore this plant.

MATERIALS AND METHODS

Collection of Plant Material

Plant of *Spinacia oleracea* collected from the botanical garden of G. J. Patel Institute of Ayurvedic Studies and Research (New V.V. Nagar). The collection was done in the month of February and is authenticated by the Taxonomist, Bioscience Dept., Sardar Patel University, Vallabh Vidyanagar, Gujarat, India.

Pharmacognostic evaluation

Macroscopy

Macroscopical studies of leaves were done by naked eye and shape, color, taste and odor of Leaves were determined and reported.

Microscopy

Pharmacognostical evaluation including histochemical study was carried out by taking free-hand sections according to Wallis and powder studies according to Evans. The section was stained with phloroglucinol and concentrated HCl solution and mounted in glycerin[5,6]. A separate section was prepared and stained with iodine solution for the identification of starch grains. Powder (Sieve mesh 60 of the dried leaves was used for the observation of powder microscopical character[7]. The powdered drug was separately treated with pholoroglucinol- hydrochloric (1:1) solution.

Photomicrographs were obtained by observing free-hand sections of drug under compound binocular microscope.

Physico-chemical evaluations

Physicochemical parameters of *Spinacia oleracea* leaf powder were determined and reported as total ash, water-soluble ash, acid-insoluble ash, determination foreign matter, loss on drying, Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components[8].

Phytochemical Analysis

Preliminary phytochemical screening

The dried powdered plant material was successively extracted 70% v/v alcohol in a Soxhlet apparatus. Aqueous extracts were also prepared by using chloroform water I.P.

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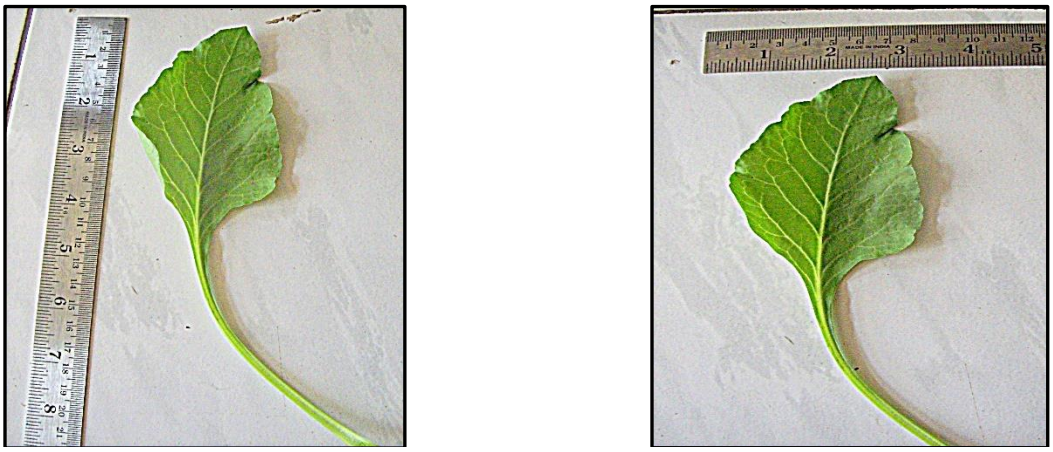


Figure 1: External morphology of plant and leaves.

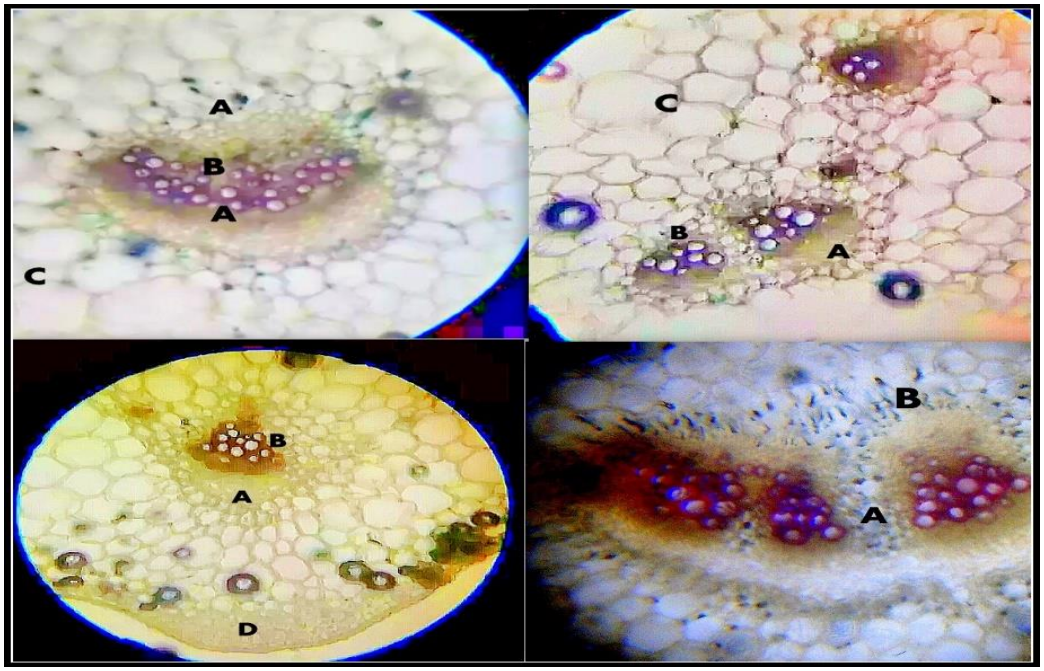
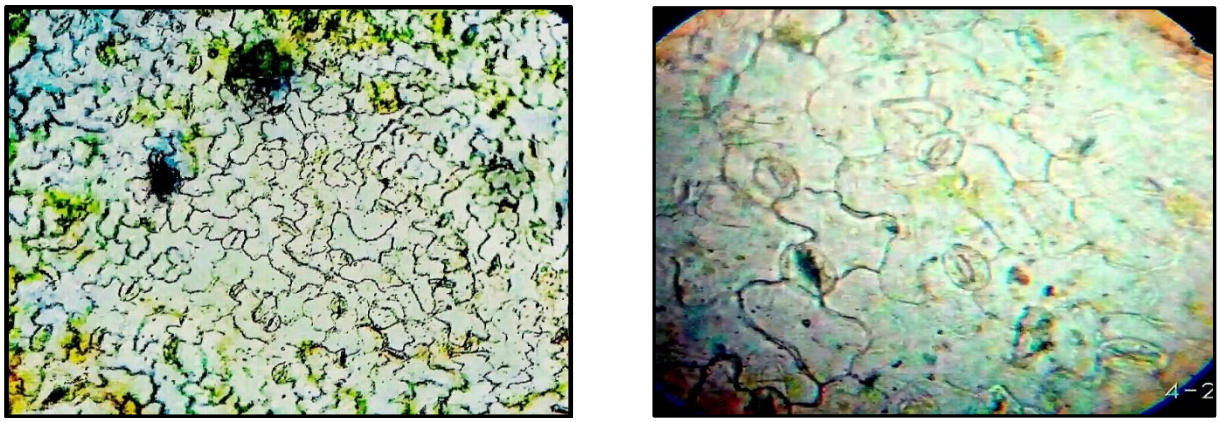


Figure 2: Microscopical view of *Spinacia oleracea* leaf [A: phloem, B: xylem, C: spongy parenchyma, D: lower collenchyma].



[10x] [45x]

Figure 3: surface preparation of lower surface of leaf having anomocytic stomata.

by maceration process. The liquid extracts obtained with different solvents were collected and the consistency, color, appearance of the dried extracts and their percentage yield were noted. The extracts obtained from powder by successive solvent extraction were subjected to qualitative examination for the phytoconstituents like alkaloids, glycosides, carbohydrates, phytosterols, fixed oils, saponins, phenolic compounds, tannins and flavonoids, proteins and amino acids by the reported methods[8]. Results are shown in table no.

Thin layer chromatography of extracts

Table 1: Physico-chemical evaluations of leaves of *S. oleracea*.

Sr. no.	Parameter	Result
1.	Total ash	34 % w/w
2.	Water-soluble ash	18.5 % w/w
3.	Acid-insoluble ash	14 % w/w
4.	Water-soluble extractive value	17.25 % w/w
5.	Alcohol-soluble extractive value	8 % w/w
6.	Loss on drying	0.16 % w/w
7.	Foreign matter	Nil

W/W - weight/weight; water soluble extractive is approximately two times higher than alcohol soluble extractive.

Table 2: Preliminary profiles of Successive solvent extracts.

Sr. No.	Extract	Color in day light	Consistency	% w/w
1	Ethanol	Dark green	Semi solid	60 % w/w
2	Aqueous	Greenish brown	semi solid	40% w/w

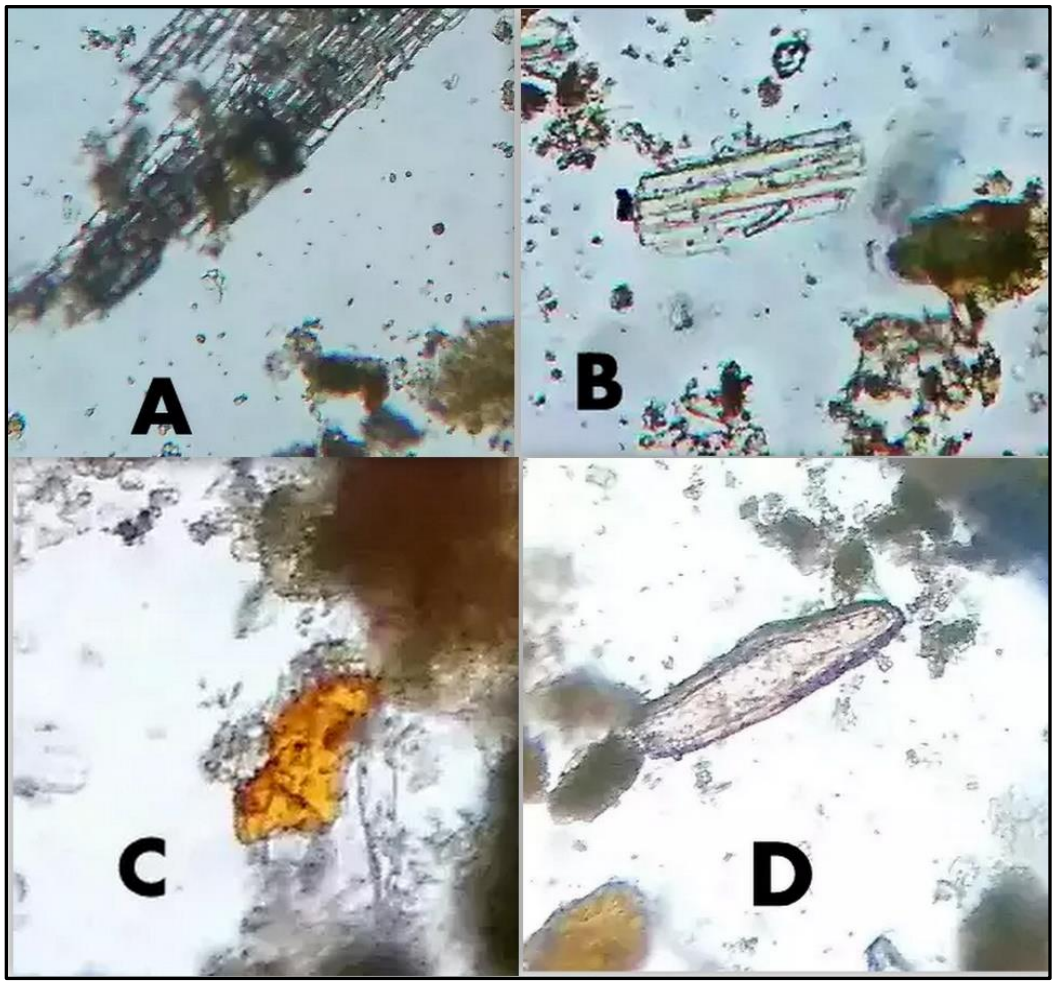


Figure 4: powder study of leaves of *S. oleracea*.
A – Xylem parenchyma, B- epidermal cells C- orange mass, D- fiber

FThe alcohol and water extracts were also subjected to thin layer chromatographic studies on silica gel – G as adsorbent and different detecting reagents using reported methods[9]. Results are shown in figure – and table no.4

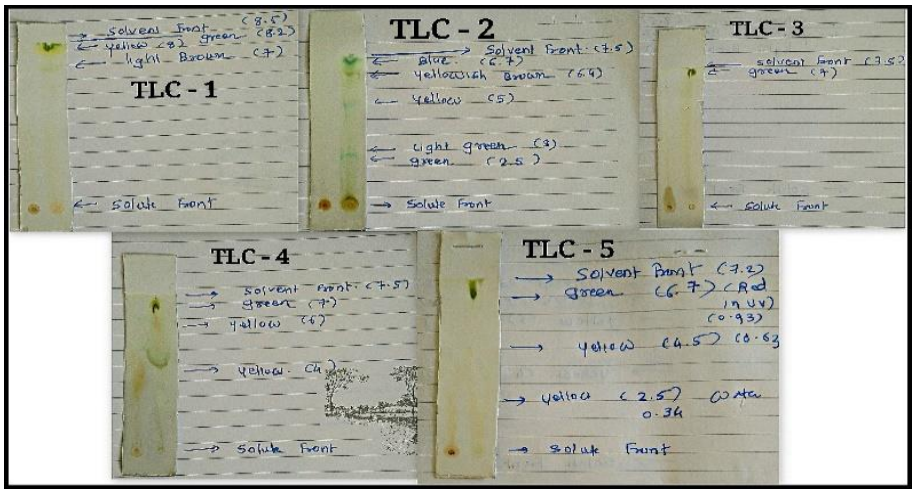


Figure 5: TLC of alcohol extract under visible light.

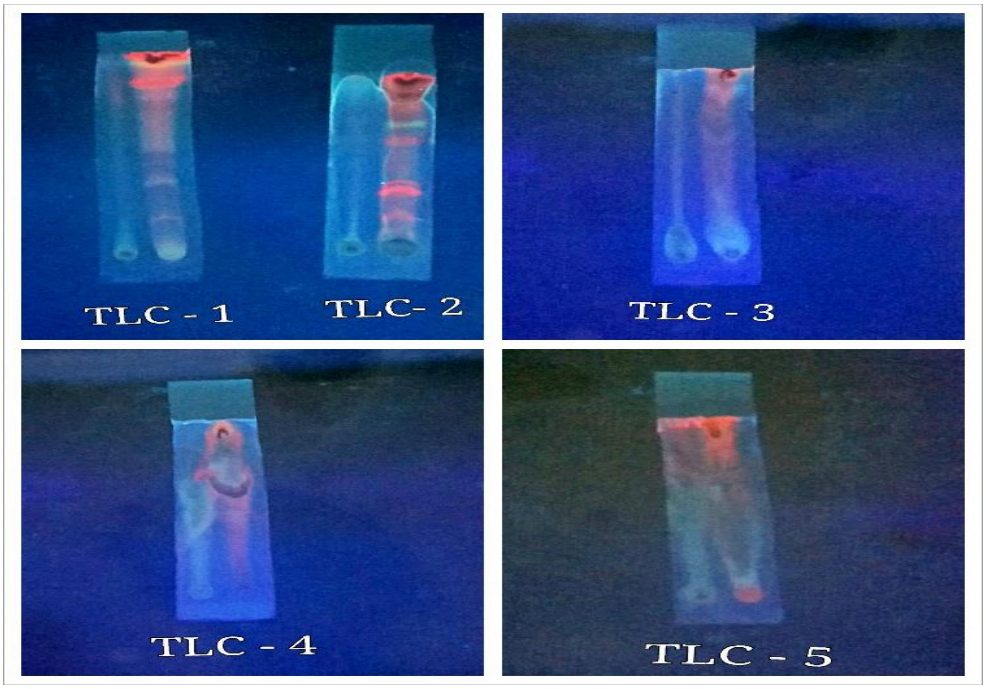


Figure 6: TLC of alcohol extract under UV light.

Table 3: Phytochemical Screening.

Sr. No.	Constituents	Ethanol	water
1.	Phytosterol	+	+
2.	Triterpenoids	-	_
3.	Saponins	+	+
4.	Tannins	+	+
5.	Carbohydrates	+	+
6.	proteins	+	+
7.	Vitamins	-	-
8.	Flavonoids	+	+
9.	saponin	+	+
10.	Alkaloids	-	-

RESULT AND DISCUSSION

Macroscopy

The leaves are alternate, fleshy, smooth, succulent and dark green in color. They are generally 5-8 cm in length and one to one and half cm in width. The bottom of leaf is shiny with thick veins running across.

Microscopy

Transverse section of leaf

Surface preparation of leaf

Powder microscopy of dried powder of leaves

The powder of *S. oleracea* was greenish yellow, without characteristic odor and with slightly bitter taste. When powder was mounted with chloral hydrate, phloroglucinol and HCl the following elements were observed:

Table 4: Data of TLC study.

NO	Phytoconstituents	Mobile system	Alcohol extract Rf value and color of spot	Water extract Rf value and color of spot	Detection
TLC-1	Cardioactive sterol	Ethyl acetate: methanol: water (100:13.5:10)	Rf 0.82 (light brown) Rf 0.94 (Yellow) Rf 0.96 (Green)	-	UV 254 or 365 nm
TLC-2	carbohydrates	Ethyl acetate: water (1:1)	Rf 0.33 (pink) Rf 0.4 (light pink) Rf 0.66 (yellow) Rf 0.85 (yellowish brown) Rf 0.89 (blue)	-	10% ethanolic sulphuric acid
TLC-3	Flavonoids	Ethyl acetate: formic acid: glacial acetic acid: : water (100:11:11:26)	Rf 0.57 (blue) Rf 0.93 (green)	Rf 0.24 (yellow)	UV 254 or 365 nm
TLC-4	Saponin Glycosides	Chloroform: glacial acetic acid: methanol: water (64:32:12:8)	Rf 0.53 (yellow) Rf 0.8 (Yellow) Rf 0.93 (green)	Rf 0.53 (yellow)	Vanillin sulphuric acid
TLC-5	Tannins	Ethyl acetate: acetic acid: formic acid: water (75:2:3:20)	Rf 0.63 (Yellow) Rf 0.93 (Green) (Red under UV)	Rf 0.34 (yellow)	Vanillin sulphuric acid

Physico-chemical evaluations

Phytochemical Analysis

Preliminary profiles of Successive solvent extracts

The powder of *Spinacia oleracea* leaves were extracted with ethanol by soxhlet apparatus. And aqueous extract was prepared by maceration. The results are described in Table no. 2

Preliminary phytochemical screening

All the above extracts were tested with various reagents and the results for the same are reported in table no.3. The various extracts showed the presence of triterpenoids, saponins, flavonoids, phenolic compounds and tannins.

TLC study of alcohol and water extract

CONCLUSION

As there is no pharmacognostical anatomical work on records for this traditionally much valued herb, present work is taken up in the view to lay down the macroscopic and microscopic standards, which could be used in deciding the genuineness of the herb, irrespective of their collection from different sources. The colored photographs of the leaves of the above mentioned plant might facilitate the researcher for identification. The results of the phytochemical screening, histochemical tests can be considered as distinguishing parameters to identify and decide the authenticity of *Spinacia oleracea* and thus can be used as standards for reference purpose also. The outcome of the quantitative parameters described on the above-mentioned plant parts (leaves) might be useful in determining the authenticity of the drugs. Further work can be done to identify the markers present in plant by HPTLC, HPLC and GC.

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REFERENCES

1. http://www.wisdomlib.org/definition/p%C4%81la%E1%B9%85ky%C4%81.

2. https://en.wikipedia.org/wiki/Spinach.
3. http://www.medicalnewstoday.com/articles/270609.php.
4. http://www.whfoods.com/genpage.php?tname=foodspice&dbid=43.
5. Kokate CK, Practical Pharmacognosy, Edn 4, Vallabh Prakashan, Delhi, 2005, 7, 14, 107.
6. Anonymous, Indian Pharmacopoeia, Vol. 2, Ministry of Health and Family Welfare, Govt. of India, Controller of Publication, New Delhi, 1996, p. A-47, A-53, A-54.
7. Quality controls methods for medicinal plant materials", World Health Organization, Geneva AITBS publisher and distributors, Delhi 2002; 28-30, 38-40, 64-73.
8. Harborne JB. Phytochemical Methods. London: Chapman and Hall; 1998.
9. Stahl Egon, "Thin Layer chromatography ", A Laboratory Hand book, Published by Springer Private Limited, New delhi, India, Second edition,2005, pg no. 855-904.