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Research Article

Pharmacognostic and phytochemical evaluation of *Spinacia oleracea* leaves

Namrata Singh*[1], Mukul Tailang[1] and S.C. Mehta[2]

[1]*SOS in Pharmaceuitical Sciences, Jiwaji University, Gwalior M.P. India*
[2]*Department of Pharmacology, G R Medical College, Gwalior M.P. India*

*Correspondence Info:

Namrata Singh
School of Studies in Pharmaceutical Sciences,
Jiwaji University, Gwalior (M.P.) India
E-mail: namrata.singhms@gmail.com

Abstract

Spincia oleracea is a leafy green vegetable that came originally from south-western Asia and is now grown in most parts of the world. Commonly it is known as Spinach/Palak (Family-Chenopodiaceae). Though Spinach is most often used as a food, and has great medicinal value. The aim of this study to evaluate pharmacognostic evaluation including examination of morphological characters, ash value, powder analysis, and extractive values were carried out. Quantitative Phytochemical screening of different extracts were carried out. This would help to scientifically justify its pharmacological activities of particular chemical constituents in different extracts.

Keywords: Flavonoids, Hepatoprotective, laxative, Carotinods, Glycolipids Alkaloids

1. Introduction

1.1 Botanical Description:

The leaves of *Spinacia oleracea* (Family: Chenopodiaceae) commonly known as Palak/Spinach. *Spinacia oleracea* useful in diseases of blood and brain, asthma, leprosy, biliousness; causes "kapha" (Ayurveda). It has been used in the treatment of urinary calculi and has poglycemic properties. Leaves are cooling, emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestiblle, anthelmentic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, soalding urine, arrest vomiting, biliousness, flatulence. And have been used in the treatment of febrile conditions. Seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, and diseases of the brain and of the heart. They have been used in the treatment of difficulty in breathing, inflammation of the liver and jaundice [1,2] .

1.2 Chemical Consituents:

1.2.1 Flavonoids: *Spinacia oleracea* is very rich in the flavonoids. Various flvonoids reported to be present are querecetin; myricetin; kampeferol[3]; apigenin; luteolin; patuletin; spinacetin; jaceidin; 5,4'-dihydroxy-3.3'-dimethoxy-6:7-methylene dioxyflavone-4'-glu-curonide[4], 5,4'-dihydroxi-3,3'-dimithoxi-6,7-methylene-dioxi-flavone ($C18H14O8$.); 3,5,7,3',4'pentahydroxi-6-methoxiflavone[5].

1.2.2 Phenolic Compounds: The polyphenols isolated from the plant are *para*-coumaric acid, ferulic acid, *ortho*-coumaric acid [6].

1.2.3 Carotinods: Spinach shows presence of different carotinoids like lutein, β-carotene, violaxanthin and 9'-(Z)-neoxanhin[7].

1.2.4 Vitamins: *Spinacia oleracea* contains high concentration of vitamin A, E, C, and K. and also folic acid, oxalic acid [7].

1.2.5 Minerals: Magnesium, manganese, calcium, phosphorus, iron, zink, copper and potash [5].

1.2.6 Glycolipids: It also contains mainly three glycolipids: monogalactosyl diacylglycerol, digalactosyl diacylglycerol, and sulfoquinovosyl diacylglycerol[8].

1.2.7 Two antifungal peptides: designated alpha- and beta-basrubrins[9].

1.3 Ethanobotanical Uses:

1.3.1 Plant: It is sweet, cooling, carminative, laxative, alexipharmic; useful in diseases of blood and brain, asthma, leprosy, biliousness; causes "kapha" (Ayurveda). It has been used in the treatment of urinary calculi. In experiments it has been shown to have hypoglycemic properties.

1.3.2 Leaves: These are cooling, emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestiblle, anthelmentic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, soalding urine, arrest vomiting , biliousness, flatulence.

1.3.3 Seeds: Seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, and diseases of the brain and of the heart (Yunani). Seeds are laxative and cooling. They have been used in the treatment of difficulty in breathing, inflammation of the liver and jaundice. The green plant is given for the urinary calculi [10].

1.4 Reported Activities:

Antimicrobial activity [11], Protection against gamma radiation [12], Antioxidant activity [13-14]. Inhibition of mammalian DNA polymerases [15], Sulphite oxidase activity [16], Hepatoprotective activity [17], Inhibition of clastogenisity[18], Anticancer activity[19], CNS depressant effect[7], Inhibition of proliferation of human gastric adenocarcinoma cells[20], Anthelmintic activity[21].

1.5 Classification of *Spincia oleracea* plant

Kingdom	**:**	*Plantae*
Subkingdom	**:**	*Tracheobionta*
Superdivision	**:**	*Spermatophyta*
Division	**:**	*Mognoliophyta*
Class	**:**	*mangoliopsida*
Order	**:**	*Caryophyllales*
Family	**:**	*Chenopodiaceae*
Genus	**:**	*Spinacea*
Species	**:**	*oleracea* L.

Figure 1: *Spincia oleracea* Plant

2. Material & Methods

The present section deals with the detailed description of various methods and techniques employed for carrying out different studies categorized in to following heading.

2.1 Collection and authentication of the plant leaves:

The leaves of *Spincia oleracea* were collected from outfield medicinal garden near to Gwalior (M.P.) that show the green color with rough surface. The plant leaves was washed thoroughly in tap water, dried in shade, finely powdered and used for extraction. Plant was identified and herbarium specimen was submitted in Department of Pharmacognosy for future references.

2.2 Extraction of Plant Material

Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. The extraction was done by following general procedure. Powdered material (leaves) was packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was subjected to further extraction process by dichloromethane, methanol and water. All the extracts were concentrated under vacuum. After completion of total, the extracted powder was discarded and the extracts so obtained were further processed . The excess solvent in the extracts were removed by distillation and the concentrated extracts so obtained were further dried at a temperature not exceeding 40°C in water bath. The extracts were then collected kept in Petri dish and stored in desiccators at room temperature. The yield values and other physical properties were observed [22].

The % Yield of the Petroleum ether, dichlomethane, Methanol, & Aqueous extract of was calculated by using the following formula.

$$\%\ \text{Yield} = \frac{\text{Net weight of powder in gram after extraction}}{\text{Total weight of leaf powder in gram taken for extraction}} \times 100$$

2.3 Determination of Physicochemical Parameters

Various physicochemical parameters were analyzed for the confirmation of identity & purity. The extractive values with alcohol and water were also determined.

2.3.1 Moisture content:

Moisture is an inevitable component of crude drugs, which must be eliminated. The moisture content should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination.

The powdered sample was weighed accurately and kept in *IR moisture balance*. The loss in weight was recorded as percentage moisture with respect to air-dried sample of crude drug [22-23].

$$\%\ \text{Moisture} = \frac{(Fw-Pw)}{W} \times 100$$

Where: Fw = Final constant weight of drug along with container

Pw = Weight of empty container

W = Total weight of drug taken

2.3.2 Ash values

I. Total ash value:

For the determination of total ash, 2 gm of the air dried crude drug was weighed in the tarred silica dish and incinerated at a temperature 450°C until free from carbon in Muffule furnance and then was cooled and weighed. The residue was collected on an ash less filter paper and then incinerated until the residue is white or nearly so. The percentage of ash was calculated with reference to the air-dried drug.

II. Acid insoluble ash value:

The ash obtained from the previous process was boiled with 25ml of 2M HCI for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a dessicator and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug.

III. Water soluble ash:

The ash was boiled with 25ml of water for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited for 15min. at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water-soluble ash. Percentage of water-soluble ash was calculated with reference to the air-dried drug [22-24].

2.4 Phytochemical screening

Preliminary phytochemical screening was performed for all the extracts [25-26].

2.4.1 Detection of Carbohydrate:

500 mg of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Molisch's Test:

To 1 ml of filtrate, 2 drops of Molisch's reagent was added in a test tube and 2 ml of concentrated sulphuric acid added carefully along the side of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrate.

Fehling's Test:

To 1 ml of filtrate, 4 ml of Fehling's solution was added in a test tube and heated for 10 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

2.4.2 Detection of Glycosides:

0.5 gm of extract was hydrolyzed with 20 ml of dilute hydrochloric acid (0.1 N) and filtered. The filtrate was used to test the presence glycosides.

Modified Borntrager's Test:

To 01 ml of filtrate, 02 ml of 1% ferric chloride solution was added in a test tube and heated for 10 minutes in boiling water bath. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. Formation of rose pink or cherry colour in the ammonia layer indicates the presence of glycoside.

Killer Killiani Test:

Small portion from the respective extracts was shaken with 1 ml glacial acetic acid containing a trace of ferric chloride. 1 ml of conc. sulphuric acid (H_2SO_4) was added carefully by the sides of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquids indicate the presence of glycosides.

2.4.3 Detection of Alkaloids:

0.5 gm of extract was dissolved in 10 ml of dilute hydrochloric acid (0.1 N) and filtered. The filtrate was used to test the presence of alkaloids.

Mayer's Test:

Filtrates were treated with Mayer's reagent; formation of yellow cream coloured precipitate indicates the presence of alkaloids.

Dragendorff's Test:

Filtrates were treated with Dragendroff's reagent; formation of red colored precipitate indicates the presence of alkaloids.

Hager's test:

Filtrates were treated with Hager's reagent; formation of yellow coloured precipitate indicates the presence of alkaloids.

2.4.4 Detection of phytosterols and triterpenoids:

0.5 gm of extract was treated with 10 ml chloroform and filtered. The filtrate was used to test the presence of phytosterols and triterpinoids.

Salkowaski Test:

To the test extract solution added few drops of conc. H_2SO_4 shaken and allowed to stand, lower layer turns reddish brown or golden yellow indicating the presence of triterpenes.

2.4.5 Detection of Protein and Amino acid:

`100 mg of each extract was taken in 10 ml of water and filtered. The filtrate was used to test the presence of protein and amino acids.

Millon's Test:

2 ml of filtrate was treated with 2 ml of Million's reagent in a test tube and heated in a water bath for 5 minutes, cooled and added few drops of Sodium Nitrate solution. Formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acid.

Ninhydrin Test:

To 2 ml of filtrate, 0.25% Ninhydrin reagent was added in a test tube and boiled for 2 minutes. Formation of blue color indicates the presence of amino acids.

Biuret test:

2 ml of filtrate was treated with 2 ml of 10% sodium hydroxide solution in a test tube and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violet colour indicates the presence of proteins.

2.4.6 Detection of Fixed oils and Fats:

Oily spot test:

One drop of each extract was placed on filter paper and solvent was allowed to evaporate. An oily stain on filter paper indicates the presence of foxed oil.

2.4.7 Detection of Phenolics and Tannins:

100 mg of each extract was boiled with 1 ml of distilled water and filtered. The filtrate was used for following tests.

Ferric chloride test:

To 2 ml of filtrate, 2 ml of 1% ferric chloride solution was added in a test tube. Formation of bluish black colour indicates the presence of phenolic nucleus.

Lead Acetate Test:

To 2 ml of filtrate, few drops lead acetate solution was added in a test tube. Formation of yellow precipitate indicates the presence of tannins.

2.4.8 Detection of Flavonoids:

Alkaline Reagent test

To 100 mg of extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow colour that becomes colorless on addition of few drops of dilute acid (HCl) indicates the presence of Flavonoids.

2.4.9 Detection of Saponin:

Foam Test

Extracts were diluted with distilled water to 20 ml and Shaken in a graduated cylinder for 15 minutes. Formation of one cm layer of foam indicates the presence of Saponin.

2.4.10 Detection of Mucilage:

10 ml of the aqueous extract was tested for mucilage; the extract was added with 25 ml of 95% alcohol with constant stirring. The so formed precipitate was centrifuged and washed with alcohol, the dissolved in water (10 ml) and reprecipitated. After washing the precipitate was collected & dried in desiccators. On addition of a drop of water and allowed to stand for some time, it swelled to give a viscous mass which gave indication for presence of mucilage.

3. Results and Discussion

Successive solvent extraction values in various organic solvent were observed as n-hexane, dichloromethne, methnol, water as shown in (Table 1).

The proximate analysis revealed the moisture content, total ash, acid insoluble ash, & water soluble ash values were observed to be 11.5%, 3.1 %, 9.2 %, 5.7 %, respectively as shown in (Table 2).

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolic compounds as shown in (Table 3).

Table 1: Different extracts with their appearance and % yield (in gm)

Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
n- hexane extract of *S. oleracea*	Dark Brown	Sticky	08 %
Dichloromethane extract of *S.oleracea*	Dark Green	Dried Powder	15 %
Methanolic extract of *S. oleracea*	Dark Orange	Sticky	13%
Water extract of *Spincia oleracea*	Dark Brown	Resinous	10 %

Table 2: Evaluation of physicochemical parameter

S.No.	Parameters	Values obtained (%w/w) dry weight basis
1.	Moisture content	3.1
2.	Total ash	8.3
3.	Acid insoluble ash	2.8
4.	Water soluble extractive	4.9

Table 3: Qualitative chemical analysis of *Spincia oleracea* by chemical tests

S. No.	Natural Product	Test performed	n-hexane	Dichloromethane	Methanolic	Aqueous
1.	Carbohydrate	➢ Molish test ➢ Felling test	- -	+ +	+ +	+ +
2.	Glycosides	➢ Borntrager test ➢ Killer killani test	- -	+ +	+ +	+ +
3.	Alkaloid	➢ Mayer test ➢ Dragendorff's test	- -	+ +	+ +	- -
4.	Phytosterol and Triterpinoids	➢ Salkowaski test	-	+	+	-
5.	Protein and Amino acid	➢ Millon's test ➢ Biuret test ➢ Ninhydrin test	- - -	+ + +	+ + +	- - -
6.	Fixed oil and Fats	➢ Oily spot test	-	-	-	-
7.	Phenolic and Tannins test	➢ Ferric chloride test ➢ Lead acetate test	- -	- -	+ +	+ +
8.	Flavonoids	➢ Alkaline Reagent test	-	+	+	+
9.	Saponin	➢ Foam test	-	-	-	-
10.	Gum and Mucilage	➢ Mucilage test	-	-	-	-

Note: + *ve* indicates positive result, whereas – *ve* indicates negative result

4. Conclusion

The preliminary pharmacognostic and phytochemical analysis revealed the successive solvent extraction value in different solvents, moisture content, total ash, acid insoluble ash, sulphatd ash, & water soluble ash , different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolic compounds, has shown in different extracts.

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References

[1] Kirtikar KR, Basu BD. Indian Medicinal plants. Vol. 8. Deharadun: International Book Distributors; 2005, 2078-2079.

[2] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants (Including the Supplement). New Delhi: Council of Scientific and Industrial Research; 1956.

[3] Gupta RS and Singh D. Amelioration of CCl4- induced hepatosuppression by *Spinacia oleracea* L.leaves in wistar albino rats. *Pharmacologyonline*, 2006: 3: 267-278.

[4] Longnecker MP, Newcomb PA, Mittendorf R, Greenberg R and Willet W. Intake of Carrots, Spinach, and Supplements Containing Vitamin A in Relation to Risk of Breast Cancer. Cancer Epidemiology, Biomarkers & Prevention, 1997. 6: 887-892.

[5] Annonymus. The wealth of India. New Delhi: National Institute of Science, Communication & Information Resources (CSIR); 2004, Vol 5 (R-Z). pp 146-7.

[6] Andjelkovic M, Bunea A, Socaciu C, Bobis O, Neacsu M, Verhe R, *et al*. Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.). *Food Chem*; 2008, 108: 649–56.

[7] Guha D, Das S. CNS depressive role of aqueous extract of *Spinacia oleracea* L. leaves in adult male mice albino rats. *Indian J Exp Biol;* 2008, 46: 185-190.

[8] Maeda N, Kokai Y, Ohtani S, Sahara H, Kumamoto-Yonezawa Y, Kuriyama I, Hada T, Sato N, Yoshida H, Mizushina Y. Anti-tumor effect of orally administered spinach glycolipid fraction on implanted cancer cells, colon-26, in mice. *Lipids*; 2008, 43(8):741-8.

[9] Wang H, Ng TB. Antifungal peptides, a heat shock protein-like peptide, and a serine-threonine kinase-like protein from Ceylon spinach seeds. *Peptides;* 2004, Jul; 25(7):1209-14.

[10] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants (Including the Supplement). New Delhi: Council of Scientific and Industrial Research; 1956.

[11] Nasim F H, Andleeb S, Iqbal M, Ghous T, Khan A N, Akhtar K *et al*. *African Journal of Microbiology Research*; 2012, 6(29): 5847-5851.

[12] Bhatia AL, Jain M. *Spinacia oleracea L.* protects against gamma radiations: a study on glutathione and lipid peroxidation in mouse liver. *Phytomedicine*; 2003; 11(7): 607-615.

[13] Verma RK, Sisodia R, Bhatia AL. Role of Spinacia oleracea as Antioxidant: A Biochemical Study on Mice Brain after Exposure of Gamma Radiation. *Asian J Exp Sci.;* 2003, 17:51.

[14] Grossman S, Bergman M, Varshavsky L, Gottlieb HE. The antioxidant activity of aqueous spinach extract: chemical identification of active fractions. *Phytochemistry*; 2001, 58:143-152.

[15] Mizushina Y, Murakani C, Kumagai T, Hada T, Kanekazu U, Nakazawa S. Effect of glycolipid from spinach on mammalian DNA polymerases. *Biochem Pharmacol*; 2003, 65:259-267.

[16] Jolivet P, Bergeron E, Meunier JC. Evidence for sulphite oxidase activity in spinach leaves. *Phytochemistry*; 1995, 40: 667-672.

[17] Gupta RS, Singh D. Amelioration of CCl4-induced hepatosuppression by *Spinacia oleracea* L. leaves in wistar albino rats. *Pharmacologyonline*; 2006; 3: 267-278.

[18] Edenharder R, Krieg H, Kottgen V, Platt KL. Inhibition of clastogenicity of benzo[*a*]pyrene and of its *trans*-7, 8-dihydrodiol in mice *in vivo* by fruits, vegetables, and flavonoids. *Mutation Res*.; 2003, 537: 169–181.

[19] Maeda N, Kokai Y , Ohtani S, Sahara H, Kumamoto-Yonezawa Y, Kuriyama I, *et al*. Anti-Tumor Effect of Orally Administered Spinach Glycolipid Fraction on Implanted Cancer Cells, Colon-26, in Mice. *Lipids*; 2008; 43 (8): 741-748.

[20] He T, Huang CY, Chen H, Hou YH. Effects of spinach powder fat-soluble extract on proliferation of human gastric adenocarcinoma cells. *Biomed Environ Sci*; 1999; 12: 247-252.

[21] Patil UK, Dave S, Bhaiji A, Baghel US, Yadav SK, Sharma VK. In-vitro Anthelmintic Activity of Leaves of *S. oleracae* Linn. *Int J Toxicol Pharmacol Res*; 1 (1): 21-23.

[22] Mukherjee PK. “Quality Control of Herbal Drugs”, Business Horizons Pharmaceutical Publishers, 2009, 1st Edn., 2002, p.186-189, 193, 256-370.

[23] Khandelwal SK. “Practical Pharmacognosy”, Vth Edition, Nirali Prakashan; 2003: p.149-155, 157-8, 159.

[24] “Indian Pharmacopoeia”, IV Edition, Vol-II, Controller of Publications, Govt. of India, New Delhi; 1996, p. A-54.

[25] Kokate CK. Practical Pharmacognosy. Delhi, Vallabh Prakashan; 1996: 107-111.

[26] Khandelwal KR. Practical Pharmacognosy. Pune, Nirali Prakashan; 2006, 149-155, 157- 159.