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An Ethnobotanical,Phytochemical and Antioxidant activity of *Spinacia oleracea, L.*

Sumit kumar,[1] Aaditya Singh (Dy.Director)[2]
[2]*Faculty of Pharmaceutical Sciences Aryakul Group of Colleges Lucknow.*

Abstract

Objective: The present study is design to evaluate an ethnobotanical, phytochemical constituents, pharmacological and physiochemical properties evaluation of Spinacia oleracia leaves.
Methods: Total phenol content, Total flavonoid content, Total tannin content for antioxidant activity.
Result: The result demonstrated the Photochemical screening shows the presence of constituents like alkaloids, tannic acid, pentosis, proteins, amino acids and flavonoids. Ethanollic extract of spinach showed the antioxidant activity of total phenol content was 4.842±10mg gallic acid equivalent/g, total flavonoid content was 27.019±10mg rutin equivalent/g and total tannin content was 0.232±10mg tannic acid equivalent/g ethanollic extract have maximum antioxidant activity .Physicochemical studies shows that the physical parameters of ash value 2%, acid value 9.53 mg KOH/g & iodine value of 116.51 mgI_2/100g respectively.
Conclusion: The results in the paper show that the Spinacia oleracia leave is a natural antioxidant and can be used for treatment of various diseases.

Keywords: Antioxidant activity, Spinach, photochemical studies.

1. INTRODUCTION-

Spinach is a topical dioecious species and edible flowering plant, which contains nutrients as iron, phosphorus, calcium, potassium, sulfur etc. [1]. *Spinacia Oleracea* is the source of spinach having family of Amaranthecеae and subfamily of Chenopodioideae , it is commonly known by the name of spinach[2]. Spinach oleracea is commonly known as Chhurika (Sanskrit), Spinach (English), Palak (Hindi), Palakh (Kashmiri), Palang (Bangla) and Pasalai (Tamil).[3]

Spinacia oleracia (palak (Hindi),spinach(Eng.) is a herb, leaves are eaten as vegitable and reported to a good source of minerals, vitamins B-complex, vitamin K, ascorbic acid and flavonoids[4] .

The leaves are simple, ovate to triangular; alternative in size with about 30-60 cm height and small leaves higher on the flowering stem, It is a rich source of iron, magnesium, vitamin A (lutein), vitamin C, vitamin E, manganese, folate ,vitamin K, magnesium, [5].

Botanical description –spinacia oleracia is the treatment of urinary calculi, It useful in diseases of blood and brain, leprosy, asthma causes kapha (ayurveda). It leaves are cold and sneezing, soalding urine, arrest vomiting, sore eye, ring worm scabies, leucoderma, biliousness, flatulence, wholesome, diuretic, laxative[6]

1.1 Ethanobotanical Uses:

Leaves: Leaves are biliousness, flatulence emollient, maturant, laxative, digestiblle ,wholesome, antipyretic, diuretic, anthelmentic, useful in urinary concretion, , sore throat, pain in joints, ring worm scabies, thirst, lumbago, inflammation of the lungs and the bowels, cold and sneezing, sore eye, leucoderma, soalding urine, arrest vomiting[7].

Stem: succulent, round, smooth, piped, sometime reddish.

Plant: It is cooling, sweet, carminative, laxative, alexipharmic, useful in diseases of blood and brain, asthma, leprosy, biliousness .It has been used in the treatment of urinary calculi. In experiments it has been shown to have hypoglycemic properties[8].

Seeds: It is useful in fevers,and diseases of the brain and of the heart , Seeds are laxative and cooling.It is treatment of difficulty in breathing, leucorrhoea, urinary discharges, lumbago ,inflammation of the liver and jaundice.

Flowers: Flowers axillary, sessile, crowded,very numerous, stamen 4, anthers twin, sessile, calyx 4- parted, very large.

Fig. 1.1 Spinach leaf

Fig. 1.2 Spinach plant

1.3 Scientific Classification-

Main classification	Plantae
Superdivision	Spermatophyta
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass Order	Caryophyllidae Caryophyllales
Genus	Spinacia l.
Species	Spinaciaoleracea l

1.4 Chemicals:

All the chemical used like KOH, Phenolphthalein indicator, HCl, carbon tetrachloride, Hager reagent, potassium Iodide, sodium thiosulphate, starch, Folin-Ciocalteus reagent, gallic acid, tannic acid, rutin were of analytical grade and purchased commercially from scientific laboratory lucknow.

Table.1. Chemical costituents-

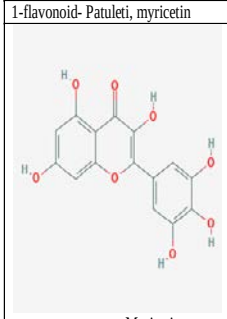
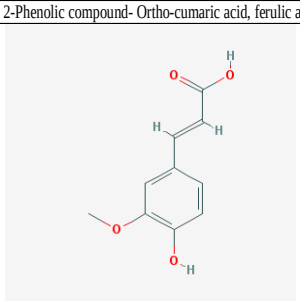
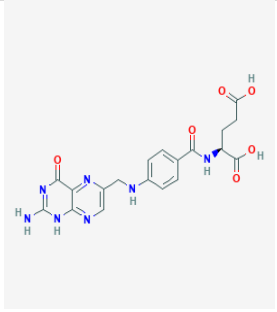
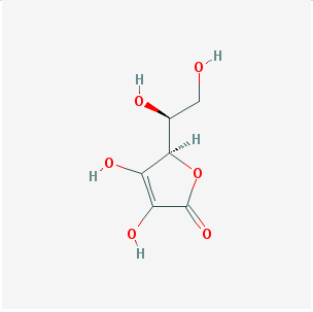
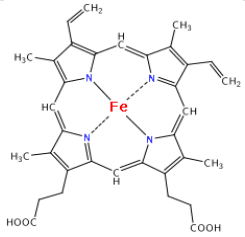
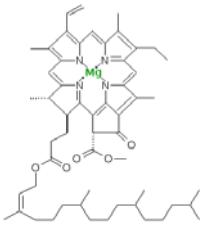
1-flavonoid- Patuleti, myricetin	2-Phenolic compound- Ortho-cumaric acid, ferulic acid
 Myricetin	 Ferulic acid
3-Vitamins-A,E,C and K & folic acid.	
 Folic Acid	 Ascorbic acid (Vitamin C)
4-Minerals- Magnesium(Chlorophyll A), phosphorus, zink, copper, iron(Heme, a constituent of hemoglobin).	
 Heme, a constituent of hemoglobin	 Chlorophyll A

Figure-1-flavonoid- myriceti, 2-Phenolic compound- ferulic acid, 3-Vitamins- folic acid, ascorbic acid 4-Mineral Magnesium(Chlorophyll A), Iron(Heme, a constituent of hemoglobin).

2. Plant Material:

The Fresh leaves of *Spinacia oleracea* were collected in the month of April, 2018 from Sarojini nagar, Dist lucknow (U.P.) India. These were identified, and authenticated by the scientists of Mr. Dileep Singh, Technician Central Instrumentation Facility (CIF) CSIR- National Botanical Research Institute Lucknow-226001, India. A voucher specimen no (NBRI/CIF/595/2018) has been submitted.

2.1 Preparation of Extract:

Collected fresh leaves were washed and dried in an oven (30-40^{0}C) temp, dried seed were grind to course powder by using electric grinder and sheaved, stored in air tight plastic container until use. The powder of shade dried leaves was used for study of morphological and microscopic characteristics. The dried powder leave were extracted by Soxhlet apparatus using need some solvents Petroleum ether, Chloroform, Methanol, Ethanol and Acetone successively and the dried residue was collected and stored in refrigerator for further experimentation .The extract used for the determination of ash values, extractive values and phytochemical investigations.

3. Phytochemical screening:

Phytochemical screening is done according to method of C. K. Kokate, Practical Pharmacognosy; for Petrolium ether, chloroform, ethanol, methanol and Acetone extract[7] and results were tabulated in table 2.

4. Physiochemical studies:

The physiochemical parameters in ethanol extract were determined using methods reported in spinach leave extract with state, colours, ach value acid value, iodine value and results were tabulated in table 3.

5. In-vitro antioxidant activity:

Estimation of Total Phenolic Content, estimation of total flavonoid content[9] and estimation of tannin content, expressed in terms of mg gallic acid equivalent/g, mg rutin equivalent/g, mg tannic acid equivalent/g based on the calibration curves of standards i.e gallic acid, rutin and tannic acid respectively and result were tabulated in table 4.

6. Thin Layer Chromatography

Prepare a solution of acetone and methanol. Place just enough of this solution in a TLC jar to cover the bottom of the jar. Tightly cap the jar while it is not in use. Obtain two TLC plates, and in pencil, mark a straight line across the plate 1 cm from the edge. TLC plates were prepared by using silica gel G, and were left for air drying. These Plates were activated by hot air drying in hot air oven at 100^0 C for 1 hr. Extracts from different solvents was spotted on TLC plates. The plates were dried and developed in suitable solvents for rapid screening. The plates were run in the following solvent system and dried at room temperature. Detection of TLC plate was done by Iodine chamber and UV chamber. R_f value of different spots available is calculated by using formula:

R_f value = Distance travelled by the solute / Distance travelled by the solvent

6.1 TLC Analysis of Extract

Different solvent systems were tried for developing TLC system for identification of constituents in the extract keeping in mind the chemical nature of constituents.

1. Methanol :Hexane: Ethyl acetate (1:3:1)
2. n-butanol: acetic acid: water (4:4:2)
   This solvent giving maximum resolution and separation of constituents.

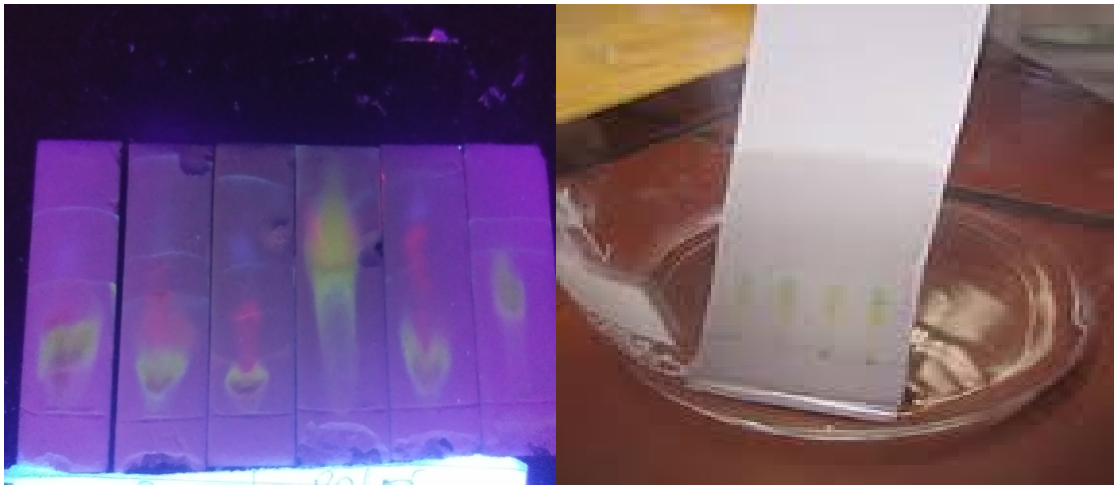


Fig. 6.3 TLC performance in Laboratory

7. RESULT:

Table.2. Preliminary phytochemical screening of leave extracts of spinach.

Plant constituents test/reagent used	Petroleum ether	Acetone	Chloroform	Ethanol	Methanol
1-Alkaloids					
a-Meyer's reagent	+ve	-ve	+ve	+ve	-ve
b-Dragendroff's reagent	+ve	+ve	+ve	+ve	+ve
c-Hager's reagent	+ve	+ve	+ve	+ve	+ve
d-Wagner 's reagent	+ve	+ve	+ve	+ve	+ve
e-Tannic acid	+ve	+ve	+ve	+ve	+ve
2-Carbohydrate & glycosides					
a-Fehling's solution	-ve	-ve	-ve	-ve	-ve
b-Molish's reagent	-ve	-ve	-ve	-ve	-ve
c-Benidict's reagent	-ve	-ve	-ve	-ve	-ve
d-Barfoed's solution	-ve	-ve	-ve	-ve	-ve
e-Pentosis test	+ve	+ve	+ve	+ve	+ve
3-Anthraquinone glycoside test					
a-Borntrager's test	-ve	-ve	-ve	-ve	-ve
b-hydroxi-antraquinone glycoside	-ve	-ve	-ve	-ve	-ve
4-Cardic glycoside test					
a-Raymond's test	-ve	-ve	-ve	-ve	-ve
b-Legal's test	-ve	-ve	-ve	-ve	-ve
c-Bal jet's test	-ve	-ve	-ve	-ve	-ve
5-Saponin -					
a-Saponin test	-ve	-ve	-ve	+ve	+ve
b-Fourth formation test	-ve	-ve	-ve	-ve	-ve
6-Phenolic compound & tannin					
a-Ferric chloride test	-ve	-ve	-ve	-ve	+ve
b-Lead acetate test	-ve	-ve	-ve	-ve	-ve
c-Dilute iodine test	-ve	-ve	-ve	-ve	+ve
7- Amino acid & protien					
a-Millon's reagent	+ve	+ve	-ve	-ve	-ve
b-Ninhydrin reagent	- ve	-ve	+ve	-ve	-ve
8-Flavonids					
a-Alkaline reagent	-ve	+ve	+ve	+ve	+ve
b –zinc hydroxide test	-ve	-ve	-ve	+ve	-ve

Table.3. Physicochemical properties of leaves of Spinacia oleracea

S.No	Property	Unit	Value
1.	State of leave	-	Powder
2.	Colour of leave	-	Green
3.	Total Ash	%	2
4.	Acid value	mgKOH/g	9.53
5.	Iodine value	mgI2/100g	116.51
6.	Moisture content	%	3

Table.4. Total phenolics, flavonoids & tannins content of extracts of Spinacia oleracea

Plant extracts	Total phenolics (mg gallic acid equivalent/g)*	Total flavonoid (mg rutin equivalent/g)*	Total tannin (mg tannic acid equivalent/g)*
Ethanolic extract	4.842±10	27.019±10	0.232±10

Table.5. TLC of Acetone & Ethanol extract of spinach leave-

S. No	Fractions	Solvent system	Detecting agent	color	Number of spot	Rf value of spots
1.	Acetone	Methanol :Hexane: Ethyl acetate (1:3:1)	Vanillin sulfuric acid	Red, Black, Violet, Green	4	0.84 0.68 0.35 0.22
2.	Acetone	n-butanol: acetic acid: water (4:4:2)	Vanillin sulfuric acid	White, Red, Green	3	0.92 0.83 0.22
3.	Ethanol	Methanol :Hexane: Ethyl acetate (1:3:1)	Vanillin sulfuric acid	Red, Brown, Green, Black	4	0.94 0.43 0.35 0.15
4.	Ethanol	n-butanol: acetic acid: water (4:4:2)	Vanillin sulfuric acid	White, Green, Brown	3	0.90 0.61 0.21

DISCUSSION

As per table 1 chemical constituent, 2 Various important phytochemicals like alkaloids, glycosides, carbohydrates, phytosterols, saponin, phenolic compounds, tannins, proteins, amino acids are present in petroleum ether, chloroform, acetone, methanolic and ethanolic extracts of Spinacia oleracea which are very valuable for various therapeutic activities.

As per table 3 The acid value was 9.53 mgKOH/g this indicates that oil of Spinacia oleracea is safer for human consumption and also does not undergoes deterioration. Iodine value was 116.51 mgI2/100g indicates that it is more suitable for biodiesel production and is a drying leaves.

Antioxidant potential of many plants is due to total phenolic components which includes phenolic acid, phenolic diterpines etc. As per table 4 total phenol content was 4.842±10mg gallic acid equivalent/g, total flavonoid content was 27.019±10mg rutin equivalent/g and total tannin content was 0.232±10mg tannic acid equivalent/g ethanollic extract which shows the plant have antioxidant properties.

Table 5 shows the TLC were fractions of acetone, ethanol in solvent system Methanol: Hexane: Ethyl acetate (1:3:1), n-butanol: acetic acid: water (4:4:2) which shows the colours and Rf value of spots.

CONCLUSION

Spinacia oleracea belonging to family Amarantheceae is a plant which is also used as food; it is very easily available and can be helpful in the prevention of some deadly disease. This work also showed that the *Spinacia oleracea* is one of the most cherished vegetables in India which is very rich in most of these phytochemicals because the results in the paper shows that the Spinacia oleracea leaves is a very good natural antioxidant.

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