PRELIMINARY PHYTOCHEMICAL SCREENING OF SPINACIA OLERACEA L


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ABSTRACT
Spinacia oleracea in an annual herb belongs to the family Chenopodiaceae and it is widely distributed, cultivated in India. The whole plant is medicinally important. Thus the present study is conducted to identify the presence of phytochemical constituents. The fresh and dried leaves of Spinacia oleracea were subjected to cold maceration. In this method 2 polar solvents are used i.e. water and ethanol. All the four extracts were found to be rich in bioactive compounds like flavonoids, carbohydrates - reducing sugars, monosaccharides, glycosides of cardiac, coumarins, anthraquinones, and steroids. In this study effect of drying and effect of solvent on extractability is determined. The extractability is more in fresh leaves than the dried leaves. And in the solvents the extractability is more in water compared to ethanol. There is no effect of drying on phytochemical constituents of fresh and dried leaves of Spinacia oleracea.

KEYWORDS: Spinacia oleracea leaves, effect of drying, effect of solvent on extractability, preliminary phytochemical screening.

INTRODUCTION
The natural drugs are always a better substitute of synthetic drugs. Thus numerous drugs have entered the I.P through ethno botany and traditional medicine. The medicinal value of a plant lies on bioactive phytochemical constituents that produce a definite physiological action on the human body. These phytoconstituents work with nutrients and fibers to form an integrated part of defence system against various diseases and stress conditions. The most important of
these bioactive constituents of plants are tannins, flavonoids, carbohydrates, glycosides, steroids, terpenoids, lignin’s, and fats.[1]

Spinacia oleracea Linn (Family-Chenopodiaceae) is commonly known as “spinach” (English) paalak (Hindi; Gujarati & Marathi) Pasalai (Tamil) Mathubucchali (Telugu) Chhurika (Sanskrit) Palakh (Kashmiri), Palang (Bangla). In different traditional medicinal system it is known by different names. It’s Ayurvedic name is ‘paalankikaa’, in ‘Unani’ it is called as ‘palak’, where as in ‘siddha’ it is known by ‘vasaiyila-keerai’. It is an erect herb with about 30-60cm ht. It is native to South- West Asia and cultivated through the world as vegetables. Several parts of this plant are used in traditional Indian medicine for numerous therapeutic effects like laxative, diuretic, carminative, cooling, flatulence. It is a rich source of vit-A, vit-C, vit-E, vit-K, vit-B6, vit-B2, magnesium, manganese, folic acid, iron, calcium, potassium, folic acid, copper, protein, phosphorous, zinc, niacin, selenium and omega-3 fatty acids.

Spinach also packed with a number of antioxidants like polyphenols, flavonoids and carotinoids which are shown to possess anti-inflammatory effects, anti-mutagenic potential, anti neoplastic effects as well as chemo-preventive activites.[1-2]

It is an important green leafy vegetable. The leaf of this annual plant is used as major ingredient in Indian cuisine mainly due to its nutritional and therapeutic values. It is low in calories. A cool climate is best for producing spinach. During periods of warm temperatures and long days, plants are likely to produce seed stalks before making desirable foliage growth. spinach is fast growing and short lived and matures its leafy foliage in 7 weeks. Spinach then quickly goes to seed, although it produces for a long period in the cool, coastal areas before seed stalk development occurs.[3]

**RESEARCH ENVISAGED**

*Spinacia oleracea* L is a annual herb belongs to the family Chenopodacea. The plant has not been explored either pharmacognostically or pharmacologically. Hence the present study aimed to establish

1. Effect of solvents on extractability
2. Effect of drying on extractability
3. Preliminary phytochemical screening of *Spinacia oleracea*.
<table>
<thead>
<tr>
<th>Nutritional Value</th>
<th>1/2 c. Boiled</th>
<th>Primary Nutrients</th>
<th>% RDA(m/f)</th>
<th>% RDA(f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>21</td>
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<td>737 RE</td>
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</tr>
<tr>
<td>Fat</td>
<td>0.2 g</td>
<td>Folic acid</td>
<td>131 mcg</td>
<td>66</td>
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<td>9%</td>
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<tr>
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<td>Vitamin C</td>
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<tr>
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<td>3.4 g</td>
<td>Riboflavin</td>
<td>0.21 mg</td>
<td>12</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>2.0 g</td>
<td>Vitamin B6</td>
<td>0.22 mg</td>
<td>11</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td>419 mg</td>
<td>% Min. Requirement 21</td>
</tr>
</tbody>
</table>

**PLANT PROFILE**[3-8]

Regional Name: Mathubucchali (Telugu)

Synonym: Spinacia domestic a Borkh

Common name: Regional name in other language.

- Sanskrit : Chhurika
- English : Spinach
- Hindi : Paalak
- Tamil : Pasali
- Kashmiri : Palakh
- Bangla : Palang

**Biological source**: Dried or fresh leaves, stem and roots of *Spinacia oleracea Linn* belonging to the family Chenopodiaceae.

**Distribution**: Cultivated worldwide in temperate areas and in the cooler parts of the tropic. Spinach is an important leafy vegetable in temperate climates. Leaves are eaten raw or cooked. Tender young leaves can be added to salads, older leaves are cooled and is used in soups.

**Habitat**: The spinach is an annual plant, long cultivated for the sake of its succulent leaves, a native of Asia, probably of Persian origin being introduced into Europe about the first century.

**Plant parts used**: Leaves, stem, root, and seeds.
Plant morphology

**Stem:** Erect from 30-60 cm high, round, smooth, piped, succulent, sometime reddish.

**Leaves:** Alternative, the lower ones very long petioled, variously lobed with lobes of an acute triangular shape smooth on both sides.

**Flowers**

**Male:** Flowers on long terminal glomerate spikes and on short ones from the axil, very numerous, sessile, calyx-4 parted, stamen-4, anther twin, very large.

**Female:** Flowers axillary, sessile, crowded. Calyx-2 tipped with a projecting horn in each side, growing into spines when the seed is ripe styles generally-4, while tapering. Capsule 1-celled, 1-valved, armed with 2 opposite short horns and crowned with the small remaining calyx.

**CHEMICAL CONSTITUENTS:** the following class of chemical constituents like Flavonoids, Phenolic compounds, Carotionoids, Steroids, Glycosides, Vitamins, Minerals reported.

**USES**
- It is used in the treatment of Urinary calculi.
- It is used in the treatment of difficulty in breathing, inflammation of liver and lungs.
- It is also used in the treatment of leucorrhoe

**TAXONOMICAL CLASSIFICATION**

- **Kingdom** : Plantae
- **Sub Kingdom** : Trachiobionta
- **Superdivision** : Spermatophyta
- **Division** : Magnoliophyta
- **Class** : Magnoliopsida
- **Subclass** : Caryophyllidae
- **Order** : Caryophyllales
- **Family** : Chenopodiaceae
- **Genus** : Spinacia L
- **Species** : Spinacia oleracea L
LITERATURE REVIEW\textsuperscript{[9-19]}

Shattrohan Lal, Ratan Nilesh Kumar, Abhay K. Singhai reported Ameliorative effects of Spinacia oleracea L. seeds on carbon tetrachloride - induced hepatotoxicity. In this article in vitro and in vivo hepatoprotective effects of Spinacia oleracea seeds were examined and suggests that these seeds acts as therapeutic agent in liver diseases.

Merry Evelyn A. Toledo, Yoshinori UEDA, Takashi SHIROSAKI investigated the changes in Ascorbic acid contents in various market forms of spinach (Spinacia oleracea L.) during postharvest storage in light and dark conditions. In the leaves of whole spinach plants, the decline in ascorbic acid contents, specifically in mature leaves which comprise the bulk of the plants, was effectively minimized with the provision of supplemental lightening. Light seems to be an effective environmental factor in the maintainance of spinach.

Singh and Rajesh kumar investigated the levels of some heavy metals in edible portions of spinach (Spinacia oleracea) The metals were analysed using Atomic Absorption Spectrophotometer. Bacterial loads were also determined. Biochemical characterization shown the possibility of Bacillus and Streptococcus strains. Consumption of these vegetable may not pose possible health hazards to humans at the of study.

Steven R. Boese and Norman P. A. Huner reported effect of growth temperature and temperature shifts on spinach leaf morphology and photosynthesis. The growth kinetics of Spinacia oleracea grown at $5^\circ$ or $16^\circ$ were determined. The second leaf pairs reached full expansion at a plant age of 32 and 92 days for the $16^\circ$ and $5^\circ$ plants respectively. Growth at $5^\circ$ resulted in an increased leaf area dry weight and leaf thickness. $5^\circ$ expanded leaves were
found to be more resistant photoinhibition at $5^\circ$ than were $16^\circ$ expanded leaves. Thus it is concluded that spinach grown at low temperature is not stressed. It appears that leaf aging during the temperature shift period can account for the reduction in photosynthesis.

Luka, C.D., Abdulkarim et al. evaluated the potential of aqueous extract of spinacia oleracea leaf in the treatment of anaemia in phenylhydrazine treated albino rats. The extract was administered to anaemic and normal rats at $100\text{mg/kg bwt.}$ Once daily for 28 days. Extract was found to be rich in phytochemicals and improve in the body weight of anaemic and normal treated rats.

Amarnath kanchana, Isha agarval et al. reported the synthesis of biogenic silver nanoparticles from Spinacia oleracea and Lactuca sativa and their potential antimicrobial activity. This article determines the efficacy of synthesized nanoparticles as antimicrobial agents, against B.subtilis, S.aureus, K. pneumonia and E. faecalis strains. And the results specify that the bactericidal properties of the nano particles present a direct interaction with bacteria.

Nilesh Kumar, Abhay K. Singhai reported Ameliorative effects of Spinacia oleracea L. seeds on carbon tetrachloride - induced hepatotoxicity. In this article in vitro and in vivo hepatoprotective effects of Spinacia oleracea seeds were examined and suggests that these seeds acts as therapeutic agent in liver diseases.

Merina paul Das, Souvik Chatterjee evaluated the antibacterial potential of Spinacea oleracia L against urinary tract pathogens. It was revealed the presence of a wide range bioactive compounds in polar solvent extract of this plant was responsible for the inhibition of the urinary tract infections.

Rajesh kumar verma, R Sisodia and A.L. Bhatia investigated the radioprotective efficacy of spinach against radiation induced oxidative stress, since its leaves are rich in antioxidants.

This article indicates the possible role spinacia as radioprotector due to synergistic effect of antioxidant constituents present in the spinach.

Sara Bergquist reported the effects of some factors during production and postharvest handing on the concentration and composition of bio active compounds in baby spinach.

Gaikwad Priyanka Subhash, Shete Rajkumar Virbhadrappa, Otari Kishore Vasant reported pharmacological activities of Spinacia oleracea such as antioxidant, protection against
gamma radiation, anticancer activity. Various secondary metabolites like flavonoids, carotenoids, phenolic compounds have been reported from this plant.

PHARMACOLOGICAL SCREENING REPORTS OF SPINACIA OLERACEA
Antioxidant property
The chemical fraction of natural antioxidant (NAO) components in *Spinacia oleracea* was reported by Grossman. The study demonstrated the presence of both flavonoids and p-coumaric acid derivatives as antioxidant components of the aqueous extract of spinach leaves.

Protection Against Gamma Radiation
The protective effect of 1100 mg/kg/day of 50% methanolic extract of *Spinacia oleracea* L. (MESO) against radiation-induced oxidative stress were evaluated in terms of lipid peroxidation (LPO) product and tissue levels of glutathione. LPO values were significantly lower in the MESO pre-treated irradiated mice as compared to respective untreated-irradiated mice at all intervals, which reached normal values from day 7 onward.

**Inhibition of Mammalian DNA Polymerases**
The purification of the major glycolipids in the class of monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG), from green vegetable spinach (*Spinacia oleracea* L.) was reported. MGDG was the potent inhibitor of replicative DNA polymerases such as α, δ and ε. monogalactosyl monoacylglycerol (MGMG), a monoglycerol form of MGDG. MGGMG inhibited the activities of the all mammalian DNA polymerases including repair-related DNA polymerases β with IC_{50} value of 8.5-36 µg/ml and the inhibition by the MGGMG was stronger than that by the MGDG.

**Anticancer activity:** The spinach glycoglycerolipid fraction can inhibit mammalian pol activity, human cultured cancer cell growth, and in vivo solid tumour proliferation with oral administration. This fraction could help to prevent cancer and be a functional food with anti-cancer activity.

**COLLECTION AND AUTHENTICATION**[3]
Stem 30-60 cm high, round, smooth, piped, succulent, sometime reddish, leaves alternative, the lower ones very long petioled, variously lobed with lobes of an acute triangular shape.
smooth on both sides. Male Flowers on long terminal glomerate spikes and on short ones from the axil, very numerous, sessile, calyx-4 parted, stamen-4, anther twin, very large, and Female Flowers axillary, sessile, crowded. Calyx-2 tipped with a projecting horn in each side, growing into spines when the seed is ripe styles generally-4, while tapering. Capsule 1-celled, 1-valved, armed with 2 opposite short horns and crowned with the small remaining calyx. Distributed worldwide in temperate areas and in the cooler parts of the tropic. 

It was authenticated by Mr. A. Lakshma reddy, (retired professor), Dept. of Botany, Nagarjuna government College (Autonomous) Nalgonda. The plant herbarium was prepared and deposited in the Department of Pharmacognosy in Nalanda college of pharmacy. The plant was identified as Spinacia oleracea L and was certified under Voucher NO. NCOP-NLG/ph’ cog/2014-2015/18.

MATERIALS AND METHODS

Chemicals required
Benzene, chloroform, ethanol, distilled water, acetic acid acetic anhydride, hydrochloric acid, sulphuric acid, sodium hydroxide, phloroglucinol, iodine, sodium nitropruside, pyridine, ferric chloride, ammonia, lead acetate, copper sulphate, potassium hydroxide, Benedict’s reagent, barfoed’s reagent, million’s reagent, dragendorff’s reagent, Mayer’s reagent, Hager’s reagent, ninhydrin solution, Fehling’s solution.

Equipment required
Electronic balance, heating mantle, micro pipettes, test tubes, stirrer, spatula, china dish, beakers.

PHARMACOC Gnostic STUDY

Macroscopic Observation
The fresh plant of Spinacia Oleracea Linn was used for macroscopical study. The size, shape, color, taste, and odour were observed. The plant was investigated in different organoleptic features by repeated observations. Morphological studies, such as shape, size, base, taste and odour of leaves were carried out.
PROXIMATE ANALYSIS\[^{[11]}\]

**Determination of moisture (loss on drying)**
Weighed about the 10 gms of the powdered drug and was added in to a flat and thin porcelain dish. Dried in the oven for 1 to 3 hours. Cooled in a dessicators and watch. The loss in weight was recorded and reported in results showed in table no. 5.1. The water present in the leaves was determined and it was found to be 95.8%.

\[
\text{Loss on drying} = \frac{w_2 - w_3}{w_2 - w_1} \times 100
\]

\(w_1\) = weight of empty sample.
\(w_2\) = weight of sample.
\(w_3\) = weight of bottle with sample after 1 to 3 hrs Drying in vaccum.

**DETERMINATION OF EXTRACTIVE VALUES**
- Useful for the evaluation of the crude drug.
- Extractive values were performed to understand the nature of chemical constituents present in a crude drug.
- Useful for the estimation of specific constituents, soluble in that particular solvent.

**Determination of alcohol (ethanol)-soluble extractives**
Weighed about 5g of the powdered drug in a weighing bottle and transferred to dry 250 ml conical flask. A 100 ml graduating flask was filled with 90% alcohol. The flask was corked and set aside for 24 hrs, shaking frequently. (Maceration). Filtered in to a 50 ml cylinder. When sufficient filtrate was collected, complete drying in an oven at 100\(^0\) C Cooled in desiccators and weighed. Transferred 25 ml of the filtrate to weighed, thin porcelain dish, as used for ash values determinations. Evaporated to dryness on water bath. The percentage w/w of extractive with reference to air dried drug was calculated and reported in the table 5.1 and 5.2

**CALCULATION**
25ml of alcoholic extract gives = x g of residue
100 ml of alcoholic extract gives = 4x g of residue.
- 5 g of air dried gives 4x gm of alcohol soluble residue.
- 100 gm of air dried drug gives 80x g of the alcohol with reference to soluble residue.
- Alcohol soluble extractive value of the sample = 80x\%. 
Determinaton of water soluble Extractive value

Macerated about 5 gm accurately weighed powdered drug with 100 ml of chloroform water in stoppered flask for 24 hrs. Shaked frequently during first 6 hrs. Filtered into 50 ml cylinder. When sufficient filtrate was collected, transfer 25 ml of the filtrate to a weighed thin porcelain dish, as used for the ash values determinations. Evaporated to dryness on a water bath and completed the drying in an oven at 100°C. Cooled in a desiccators’ and weighed. The percentage w/w of extractive with reference to the air dried drug was calculated and reported in the table 5.1,5.2.

CALCULATION

25 ml of water extract gives = x g of residue.
100 ml of water extract gives =4x g of residue.
- Therefore 5 g of air dried drug gives – 4x g of alcohol(90%) soluble residue.
- Therefore 100 g of air dried drug gives – 80x g of the alcohol with reference (90%) soluble residue.
- Alcohol (90%)soluble extractive value of the sample = 80x %

U. V FLUORESCENCE ANALYSIS

Powder leaf of Spinacia oleracea subjected to analysis under Ultra violet light after treatment with various chemical and organic reagents. Three parameters were taken into account i.e observation under long U. V(365 nm), short U.V (256 nm) and normal day light.

Procedure

2 gm of powdered drug sample was taken in a beaker and dissolved in 5 ml methanol. The sample is transfered to a watch glass and observed under U.V chamber for color and fluorescence reported.

EXTRACTION OF LEAVES BY COLD MACERATION

The plant leaves (fresh and dried) was subjected to cold maceration by using ethanol and water.

Initially 150 gm of fresh plant material was taken in a beaker. To this added 100 ml of water and kept it aside for 7 days. Filtered it .The extract was evaporated,observed for physical properties, weighed and the chemical tests were performed to the obtained residue. And for
the ethanol extract, ethanol was added and repeated the same as of the water extract. The same procedure was followed for the dried leaves.

**PRELIMINARY PHYTO CHEMICAL SCREENING OF EXTRACTS**[^12]

The extracts were obtained by the maceration technique (solvents: water, ethanol). These extracts were subjected to qualitative tests for the identification of various plant constituents. The tests which were performed have given us a broad idea of the organic chemical constituents.

**TESTS FOR CARBOHYDRATES**

**Molish’s test (general test)**

To 2-3 ml aqueous extract, add few drops of alpha naphthol solution in alcohol shake and add con. sulphuric acid from sides of the test tube. Voilet ring is formed at the junction of the two liquids.

**Tests for reducing sugars**

a) **Fehling’s test**: Mix 1 ml of Fehling’s A and Fehling’s B solution boil for 1 min. Add equal volume of test solution in test tube. Heat in boiling water bath for 5-10 min. First yellow, then brick red ppt is observed.

b) **Benidict’s test**: Mix equal volume of Benedict’s reagent and tests solution in test tube. Heat in boiling water bath for 5 min. solution appears green yellow or red depending on amount of reducing sugars present in test solution.

**Tests for monosaccharide’s**

**Barfoed’s test**: Mix equal volume of barfoed’s reagent and test solution. Heat for 1-2 min boiling water bath and cool. Red ppt is observed.

**Tests for pentose sugars**

Mix equal volume of test solution and Hcl. Heat and add a crystal of phloroglucinol Red color appears.

**Tests for hexose sugars**

a) **Selwinoff’s test**

Heat 3 ml selwinoff’s reagent and 1 ml test solution in bearing water bath for 1-2 min. Red color is formed.
B) Tollen’s phloro glucinol test for galactose
Mix 2-5 ml con HCl and 4 ml 0.5% phloro glucinol. Add 1-2 ml test solution. Heat. Yellow to red color appears.

Test for Non reducing poly saccharide (starch)
Iodine test: Mix 3 ml test solution and 3 drops of dilute iodine solution. Blue color appears, it dis appears on cooling.

TESTS FOR PROTEINS
Biuret test (General test): To 3 ml test solution add 4% NoaH few drops of CusO₄ solution, violet or pink color appears.

Millions test: Mix 3 ml tests solution with 5 ml reagent white ppt warm ppt turns brick red or the ppt


TESTS FOR AMINO ACIDS
Ninhydrin test: Heat 3 ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath for 10 min. purple or bluish color appears.

Test for tyrosine: Heat 3 ml of test solution and add 3 drops of million’s reagent. Solution shows dark red color.

Test for cysteine: To 5 ml test solution and few drops of 40%NaoH and 10% lead acetate solution. Boil. Black Ppt of lead sulphate is formed.

TESTS FOR GLYCOSIDES
Tests for cardiac glycosides
Baljet’s test: A thick section shows yellow to orange color with sodium picrate.
Legal’s test: To aqueous or alcoholic extract, add1 ml pyridine and 1 ml sodium nitroprusside. Pink to red color appears.

Keller Killiani test: To 2 ml extract add glacial acetic acid and one drop of 5% ferric chloride and con. Sulphuric acid. Reddish brown color appears at the junction of the two liquid layers and upper layer appears bluish green.
**Liebermann’s test**: mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops of con.sulphuric acid from the side of the test tube. First red, then blue and finally green color appears.

**Tests for anthraquinone glycosides**

**Borntrager’s test**: To 3 ml extract add dil. Sulphuric acid. Boil and filtrate. To cold filtrate add equal vol benzene or chloroform. Shake well. Separate the organic solvent and add ammonia. Ammonical layer turns pink or red.

**Modified Borntrager’s test**: To 5 ml extract add 5ml 5% ferric chloride and 5 ml dilute HCl. Heat for 5 min in boiling water bath. Cool and add benzene or any other organic solvent. Shake well. Separate organic layer. Add equal vol dil. Ammonia. Ammonical layer turns pinkish red color.

**Tests for saponin glycosides**

**Foam test**: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

**Hemolytic test**: Add drug extract or dry powder to one drop of blood on glass slide. Hemolytic zone appears.

**Tests for cyanogenitic glycosides**

To dry drug powder or extract add 3% aqueous mercury nitrate solution. Metallic mercury forms.

**Tests for coumarin glycosides**

Alcoholic extract when made alkaline, shows blue or green fluorescence.

**TESTS FOR FLAVONOIDS**

**Shinoda test**: To dry powder or extract, add 5 ml 95% ethanol, few drops con.HCl and 0.5 g magnesium turnings. Pink colored observed. To small qty of residue, add lead acetate solution. Yellow Ppt is observed.

**TESTS FOR STEROIDS AND TRITERPINOIDS**

**Salkowski reaction**: To 2 ml extract, add 2 ml chloroform and 2 ml con. Sulphuric acid. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
Liebermann Buchard reaction: Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and add 2 drops of con. Sulphuric acid from the side of test tube. First red, then blue and finally green color appears.

Sulphur powder test: Add small amount of sulphur powder to the test solution, it sinks at the bottom.

TESTS FOR ALKALOIDS

Dragen dorrfl’s reagent: Alkaloids gives reddish brown Ppt with this reagent. (mercuric iodide solution).

Mayer’s reagent: Alkaloids give cream color Ppt with Mayer’s reagent. (Potassium mercuric iodide).

Wagner’s reagent: Alkaloids give reddish brown Ppt. (Iodine potassium iodide solution).

Hager’s reagent: Alkaloids give yellow Ppt (saturated solution of picric acid).

Picrolonlic acid test: Alkaloids give yellow Ppt.

TESTS FOR PHENOLIC COMPOUNDS

Ferric chloride test: Treat the extract with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Test for chlorogenic acid: Treat the test solution with aq. Ammonia and exposure to air gradually green color is developed.

Potassium dichromate: red Ppt.

Bromine water: discoloration of bromine water.

FATS AND FIXED OILS

Saponification test: Add few drops of 0.5N alcoholic potassium hydroxide to a small qty of various extracts along with a drop of phenolphthalein separately and heat on a water bath for 1-2 hrs. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils.
RESULTS AND DISCUSSION

MACROSCOPY OF PLANT
Habit : Annual herb
Habitat : Endemic
Ecological status : Commo
Flowers : Axillary racemes
Stem Type : Erect stems, round and sometimes reddish.
Leaf Arrangement : Alternative
Leaf Shape : Ovate
Leaf Base : Rounded

Color : green
Odour : characteristic
Taste : agreeable

PROXIMATE ANALYSIS
Proximate Analysis of fresh leaves
Proximate analysis of Spinacia oleracea L. leaves were determined by standard procedures and the results were tabulated in Table 1.

Table 1. Measurements of different proximate values of fresh leaves of spinach:

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameters</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Loss on drying</td>
<td>0.96 w/w</td>
</tr>
<tr>
<td>2.</td>
<td>Water soluble extractive value</td>
<td>11% w/w</td>
</tr>
<tr>
<td>3.</td>
<td>Ethanol soluble extractive value</td>
<td>4% w/w</td>
</tr>
</tbody>
</table>
Proximate Analysis of dried leaves

Proximate analysis of *Spinacia oleracea* L. leaves were determined by standard and the results were tabulated in table 2

Table 2: Measurements of different proximate values of dried leaves of spinach:

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameters</th>
<th>%Yield</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water soluble extractive value</td>
<td>9.7% w/w</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol soluble extractive value</td>
<td>3.5% w/w</td>
</tr>
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</table>

COLD MACERATION EXTRACTION OF FRESH AND DRIED LEAVES

The whole plant (leaf and stem) was subjected to cold maceration technique. The solvents used in this technique were alcohol and water. Both fresh and dried leaves were subjected to maceration by using water and ethanol. The percentage yield and physical characteristics of extracts obtained was calculated and reported in the following.

Table 3. Cold maceration, colour fluorescence character and percentage yields of solvents

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fresh leaves</th>
<th>Dried leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>Ethanol</td>
<td>Water</td>
</tr>
<tr>
<td>Color day light</td>
<td>Dark Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Short UV</td>
<td>Green</td>
<td>Light green</td>
</tr>
<tr>
<td>Long UV</td>
<td>Blackish green</td>
<td>Green</td>
</tr>
<tr>
<td>Percentage yield</td>
<td>4% w/w</td>
<td>11% w/w</td>
</tr>
</tbody>
</table>

PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACTS

Fresh leaves of spinacia oleracea were subjected to cold maceration with water and ethanol. The obtained extracts was subjected to preliminary phytochemical screening according to the standard procedures mentioned. Findings were tabulated below.

Table: Preliminary Phytochemical Screening of water and ethanol extracts of fresh leaves of *Spinacia oleracea*

<table>
<thead>
<tr>
<th>PHYTO CONSTITUENTS</th>
<th>ETHANOL</th>
<th>WATER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (reducing sugars, pentoses, hexoses)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides(cardiac)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Dried leaves of *Spinacia oleracea* were subjected to cold maceration with water and ethanol. The obtained extracts was subjected to preliminary phytochemical screening according to the standard procedures mentioned. Findings were tabulated below.

**Table: Preliminary phytochemical screening of water and ethanol extracts of dried leaves of *Spinacia oleracea***

<table>
<thead>
<tr>
<th>PHYTO CONSTITUENTS</th>
<th>ETHANOL</th>
<th>WATER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (reducing sugars, pentoses and hexoses)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides (cardiac glycosides)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol’s and Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fats</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**CONCLUSION**

*Spinacia oleracea* is a neutraceutical medicinal plant. In the literature review it was claimed for its use in the treatment of inflammation of liver, lungs and jaundice. It was reported to have anti oxidant, emollient and laxative properties when used traditionally. It is evident from the literature review that *Spinacia oleracea* contains flavonoids, steroids, and saponins.

The present study confirms the presence of fats, carbohydrates (reducing sugars, pentoses and hexoses), glycosides particularly anthraquinone (-o- and –c- form) glycosides and coumarin glycosides. In this study we compared the effect of solvent extractability and effect of drying in fresh and dried leaves with reference to the phyto constituents.

In solvent extraction we found that the extractability is more in water compared to alcohol. And on drying the extractability is reduced in dried leaves and it was more in fresh leaves.
was also found that there was no change in the phytochemical constituents present in fresh and dried leaves of spinach. From the above study we concluded that the anti inflammatory, laxative and antioxidant property may due to be the presence of glycosides like: coumarins, anthroquinones, steroids and flavonoids respectively. Loss of water content on drying has no effect on the extractive values of leaves and phyto constituents. So the dried leaves can be used for its medicinal values and can be stored till its use. A further work on the effect of drying on the nutritive values of leaves need to be carried out on dried leaf powder.

Further scientific work is required carry out to prove the pharmacological activities of Spinacia oleracea in relation to the newly identified class of chemical constituents like steroidal glycosides and anthraquinoes.

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