PHYTOCHEMICAL STUDIES ON VARIOUS EXTRACTS AND MOISTURIZING USE OF SPINACH LEAF

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ABSTRACT - Spinach is green leafy vegetable which is native to India. Spinach are natural antibacterial agent with numerous nutritional and medicinal benefits to human being. Spinach has the possible health benefits are the Diabetes Management, Cancer prevention, Asthma prevention, Lowering the blood pressure, Bone health, promote digestive regularity, make healthy skin and hair. Therefore, spinach is versatile vegetable and can be eaten raw or cooked. The presence of phytochemicals including phytosterols, saponins, alkaloids, phenolic compounds and tannins, proteins, glycosides, flavonoids, carbohydrates, acidic compounds, oxalic acid were determined. Vitamin A and Vitamin C are also present in the extract such as water, ethanol, ethyl acetate, chloroform: acetone(1:1). The inorganic constituents such as magnesium, calcium, sodium, potassium, iron were found. Spinach having large quantity of Vitamin A which moderate the oil in skin pores and hair follicles to produce moisturizing activity in the skin and hair.

Key Words: Spinach, Health Benefits, Phytochemicals, Vitamins, Inorganic Constituents, Moisturizing activity.

INTRODUCTION-

Spinach (spinalis oleracea) is an edible flowering plant in the family Amaranthaceae. A small genus of annual herbs, distributed from the eastern Mediterranean to central Asia & Afghanistan. One species, S. oleracea, native to south-west Asia, is extensively cultivated in India for its nutritional leaves [1]. This plant grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, ovate to triangular - based, varying in size from about 2-30cm long and 1-15cm broad. The larger leaves are at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3-4mm in diameter, maturing into a small, hard, dry, lumpy fruit cluster about 5-10 mm across containing several seeds [2-3].

CULTIVATION-

Spinach is popular because of its high yield, wide adaptability to varying soil & climatic conditions & high nutritional value. Spinach is a cold-season crop. It can be grown pure or as a mixed crop with peas, cabbage & other comparatively longer-duration vegetables. It is sown during September-November in the plains & during Feb-April in the hills [4].

SPINACH NAMEING-

HINDI-Isfanaj, palak
BENG-palang, pinnis
MARATHI & GUJ-Palak
TAM-vasayleykiray
ORIYA-palaksag, mithapalanga
PUNJAB-palak, isfanaj, valayati sag
ASSAM-malangas[5].
SCIENTIFIC CLASSIFICATION-
Spinach
Kingdom-Plantae
Class-Angiosperm
Subclass-Eudicots
Order-Caryophyllenes
Family-Amaranthaceous
Genus-Spinalis
Species-S. oleracea
Binomial Name- Spinacia.oleracea\[^5\].

MICRONUTRIENTS-
Analysis of the edible portion(87%) of spinach gave; moisture,92.1%; protein,2.0%; Fat,0.7%; Fibre,0.6%; mineral matter,1.7%; carbohydrates2.9%; calcium,73; oxalic acid,6.58; magnesium,84; potassium,206; iron,10.9; phosphorus,21; sodium,58.5; copper,0.01; sulphur,30; chloride,54mg./100g. Other minerals present in the leaves (dry basis) are: nickel,0.42; manganese,9.61; molybdenum,0.08; zinc,13.53; and strontium,0.077mg./100g. Cobalt (0.007-0.12mg./100g), selenium, and iodine(20.1µg./100g) are also present\[^6\]-\[^11\].

Spinach is a good source of minerals, vitamin-B complex, ascorbic acid and carotene; it is also an important natural source of vitamin K. The composition of vitamins in the leaves is as follows: vitamin A,9300 I.U.; thiamine,0.03; riboflavin,0.07; nicotinic acid,0.5; and ascorbic acid,28 mg./100g; ascorbic acid values of 60 mg./100g have also been reported. The total content of folic acid in spinach is 0.12mg./100g. (free folic acid,0.05mg./100g) \[^5\].

Phytochemicals are naturally occurring components in fruits, green vegetables, legumes and grains. Plants are getting specific odor, flavor, smell and are part of plant’s natural defense system i.e., disease resistance. Phytochemicals are bioactive, non-nutrients plant compounds in fruits, vegetables, grains and other plant foods have been linked with reducing the risk of major degenerative diseases\[^12\]-\[^13\].

Therefore, there is need to evaluate the potential of vegetables in relation to the provision of basic micronutrients and phytochemicals, which will help to provide crucial data for the selection of proper green leafy vegetables.

MATERIALS AND METHODS-
Collection of plant materials-

The plant material, Spinacia oleracea was collected from the rural agricultural field of Junner Tehsil, Pune District, Maharashtra, India. The plant material was collected in plastic zip lock bags and brought to the laboratory, washed five times with tap water to remove all debris and then washed with doubled distilled water. The cleaned material was shed dried and used for the extraction.

Preparations of plant leaf extracts-

The collected plant samples were dried and crushed to powder form. Five gram of powdered plant sample was soaked separately in 50ml of extraction solutions, such as water; ethanol; ethyl acetate; chloroform: acetone (1:1). The entire mixture was incubated at 4°C for 48 hrs. After the incubation period is over, the mixture was filtered and centrifuged at 10000 rpm at
4°C. The extracts were concentrated to dryness in rotary evaporator (IKA-RV 10 Control) and were stored at 4°C until for further used ended.

**PHYTOCHEMICAL SCREENING**-

Phytochemical screening of test sample was carried out according to standard methods [14-16].

**CHEMICAL TESTS FOR DETECTION OF ORGANIC CONSTITUENTS**-

1) Test for carbohydrates-

Molisch's test (General test): To 2-3 ml extract, add few drops of alpha-naphthol solution in alcohol, shake and add conc. sulphuric acid from side of test tube. Violet ring is formed at the junction of two liquids.

2) Test for proteins-

Biuret test (General test) - To 3ml of extract solution. add 4% NaOH and few drops of 1% CuSO₄ solution. Violet pink color appears.

3) Test for steroid-

Liebermann’s reaction - mix 3ml extract with 3ml acetic anhydride. Heat and cool. Add few drops of conc. sulphuric acid. blue color appears.

4) Test for Glycosides-

Legal’s test (Test for cardenoloids) - To extract, add 1ml pyridine and 1ml sodium nitroprusside. pink to red color appears.

5) Test for Tannins and Phenolic compounds-

To 2-3 ml of extract add few drops of lead acetate solution, white ppt observed.

**CHEMICAL TESTS FOR DETECTION OF ACIDIC COMPOUNDS**-

Test solution with red litmus paper. Paper turns blue.

**CHEMICAL TESTS FOR DETECTION OF VITAMINS**-

1) Test for Vitamin A-

Dissolve a quantity equivalent to 10-15 units in 1ml of chloroform and add 5ml of antimony trichloride solution, a transient blue color is produced immediately.

2) Test for Vitamin C (Ascorbic Acid)-

To 2ml of 2% w/v solution add 2ml of water, 0.1 g of sodium bicarbonate and about 20 mg of ferrous sulphate, shake and allow to stand; a deep violet color is produced. Add 5ml of 1M Sulphur acid, the color disappears.
CHEMICAL TESTS FOR DETECTION OF INORGANIC CONSTITUENTS:

Prepare ash of drug material. Add 50%v/v HCL or 50%v/v HNO₃ to ash. Keep for 1 hour or longer time. Filter. With filtrate perform this test.

1) Test for Calcium-
To 10 ml filtrate, add 1 drop dil. NH₄OH and saturated ammonium oxalate solution. White ppt. Of calcium oxalate forms. Ppt is soluble in HCL bit insoluble in acetic acid.

2) Test for Magnesium-
Filtrate and add ammonium carbonate solution. Give white ppt.

3) Test for Sodium-
10ml ash extract+2ml of potassium pyroanthllcollate gives white ppt.

4) Test for Potassium-
To 2-3ml extract add few drops of sodium cobalt nitrite solution. Yellow ppt. of potassium cobalt nitrite is observed.

5) Test for Iron-
To 5ml extract add few drops 2% potassium ferrocynide. Dark blue coloration observed.

RESULT AND DISCUSSION-

Table 1: -Phytochemical screening of various Spinach leaf extract.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Phytochemicals</th>
<th>Water</th>
<th>Alcohol</th>
<th>Ethyl Acetate</th>
<th>Chloroform: Acetone (1:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>+++</td>
<td>+</td>
<td>---</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Proteins</td>
<td>++</td>
<td>+</td>
<td>---</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>Steroids</td>
<td>---</td>
<td>+</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td>++</td>
<td>++</td>
<td>---</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Tannins and Phenolic compounds</td>
<td>---</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Acidic compound- Oxalic acid</td>
<td>++</td>
<td>---</td>
<td>+</td>
<td>---</td>
</tr>
<tr>
<td>7.</td>
<td>Vitamin A</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>8.</td>
<td>Vitamin C</td>
<td>+++</td>
<td>---</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

*Illustrative: +++ (high amount); ++ (moderate amount); + (less amount); --- (absent)*

Table 2: -Inorganic constituents in Spinach leaf.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Phytochemicals (Inorganic)</th>
<th>S. oleracea</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Calcium</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Magnesium</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Sodium</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Potassium</td>
<td>+++</td>
</tr>
<tr>
<td>5.</td>
<td>Iron</td>
<td>+</td>
</tr>
</tbody>
</table>

*Illustrative: +++ (high amount); ++ (moderate amount); + (less amount); --- (absent). *
The result of phytochemical screening on various extracts of spinach leaf were recorded as mention in Table 1 and Table 2. The biomolecule such as carbohydrates, proteins, glycosides were as absent in ethyl acetate extract and present in other extracts such as water, ethanol and chloroform: acetone (1:1). The steroid gives presence in only ethanol extract. Tannis and other phenolic compound was present in ethanol, ethyl acetate and chloroform: acetone (1:1). Acidic compound present in water and ethyl acetate, and shows absence in ethanol and chloroform: acetone (1:1) extracts.

Vitamin A was present in water, ethanol and chloroform: acetone (1:1) extracts but not in ethyl acetate extract. Vitamin C present in water, ethyl acetate and chloroform: acetone (1:1) extracts absent in ethanol.

Inorganic constituents give presence in Spinach leaf such as calcium, magnesium, sodium, potassium, Iron.

The spinach leaf extract with their phytochemical constituents are reported for anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities [17].

Spinach is high in fiber and water, both of which help to prevent constipation and promote a healthy digestive track and gives healthy skin.

Spinach is an excellent source of antioxidant. Vitamin A, one of the fat-soluble vitamins. Excess amount is store in the liver and fatty tissues. It plays a significant role in fending off infection and illness. The body needs Vitamin A for growth and repair, Vitamin A is also an antioxidant that protect cell from oxidation damage. Vitamin A is essential for normal vision and is necessary for normal cell growth and division, the development of bone and teeth and for the health of skin, mucus membrane and the tissue that lines the intestines, airways and other organs [18-19].

CONCLUSION-

The commonly consumed green leaf vegetable in India selected for the present study contain maximum number of phytochemicals, which are helpful in prevention of some deadly disease [20-26]. Based on phytochemical study spinach has large quantities of vitamin A which moderates the production of oil in the skin pore and hair follicles to moisturize the skin and hair. It is this oil that can buildout to cause acne. Vitamin A is also necessary for growth of all bodily tissues, includes skin and hair. The phytochemicals were not affected by cooking except for flavonoids and alkaloids. This indicates that the fear of losing these plant chemicals as a result of cooking need not arise [27-32]. Spinach and other leafy green high in vitamin C are crucial for the the building and maintenance of collagen, which provides structure to skin and hair. Iron deficiency is a common cause of hair loss, which may be prevented by an adequate intake of iron rich food, such as spinach. Hence, spinach having vitamins other phytochemicals produce the moisturizing activity to skin and hair.

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