# SCREENING OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *SPINACIA OLERACEA*

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**ABSTRACT**

Spinacia Oleracea is green leafy vegetable belonging to family Amaranthaceae, subfamily Chenopodioideae. It is commonly known as Spinach or Palak. It is often used as food. It can be used as therapeutic and curative medicine against several diseases because of presence of important active phytochemicals and its antibacterial activity. The different types of leaf extracts of S. Oleracea were prepared (quath, aqueous, ethanolic extract). Antibacterial activity through agar disc diffusion was carried out. Phytochemical analysis was done for different extracts.

The present study investigated the antibacterial activity of aqueous, ethanol extract at different concentrations (25%,50%,100%) along with pure extract(extract **without** solvent).The spinach leaf pure extract without any solvent inhibited the growth of selected bacteria Staphylococcus aureus and Klebsiella pneumoniae. The aqueous and ethanolic extract had no effect on the growth of the tested bacteria. A Phytochemical analysis of different plant extract (quath, aqueous,ethanol extracts) shows the presence of carbohydrates, protein, alkaloids, flavonoid, glycosides, cardiac glycosides, phenolic compounds, tannin, saponin, terpenoids, steroids.

This study revealed that Spinach shows presence of antibacterial agents. Spinach consist of important Phytochemical constituents like alkaloids, flavonoid, phenolic compounds, tannin, saponin, cardiac glycosides etc. which suggests that Spinach is a good nutrient rich vegetable with great medicinal value.

**KEYWORDS:** Antibacterial Activity, Spinacia Oleracea, Phytochemical Analysis

## INTRODUCTION

Spinach (*Spinacia Oleracea*) is an annual leafy green plant from family Amaranthaceae, subfamily Chenopodioideae. Spinach is packed with vitamins, mineral and number of antioxidants components like polyphenols, frauoroids and carotinoids which are shown to possess antiinflammatory effects antimutagenic potential, antineoplastic effectsas well as chemo-preventive activities [1], [2].

Spinach is a rich source of vitamins A, C, E, B6, B2 and minerals such as magnesium, manganese, iron,calcium, potassium and low levels of proteins and carbohydrates [3].

Scientific experiments on the antimicrobial properties of the plant compounds were first documented in the late 19th century [4].

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, alkaloids and fiavonoid which have been found in-vitro to have antimicrobial properties [5]. Spinach as Basella rubra contained phytochemicals as tannins and alkaloids which have been found to possess antimicrobial activity against some organisms [6], [7].

## MATERIALS AND METHODS

### Collection of Plant

Fresh samples of spinach (*Spinacia oleracea*) were purchased from local market of Osmanabad. By shelf the vegetables were randomly sampled. The plants were collected with plastic zip lock bags and brought to the laboratory which thereafter washed thrice with tap water to remove any debris and then rinsed with distilled water as well shed dried and used for extraction [8].

### Preparation of Plant Extracts

Extraction was done by three different methods Aqueous extract, Quath extract [9] and Ethanoic extract [8].

**Aqueous extract**- 5 gm of dry powder was taken in conical flask having 100 ml of deionized water flask was heated at $90^0$c in water bath for 1 hour. After 1 hour flask was taken out from the water bath and kept at room temperature for cooling purpose. Then the extract was filtered with the help of Filter paper and stored at $4^0$c [10].

**Quath Extraction**- For quath extraction fresh leaves were used. Firstly leaves washed carefully and then crushed in automatic grinder to make paste. 100 ml of paste was mixed with 300 ml distilled water in the beaker and heated at 100 degree Celsius until the final volume remain 100 ml Cool down the quath extract filter with muslin cloth and Store at $4^0$c[10].

**Ethanolic Extract**- 5 gm of powdered plant sample was soaked with 50 ml of ethanol. The mixture was incubated at 4 $^0$c for 48 hrs. After incubation period was over, the mixture was filtered and centrifuged at 10000 rpm at 40 $^0$c. The extracts were concentrated to dryness in rotary evaporator and stored at 40 degree Celsius [8].

### Phytochemicals Analysis

Phytochemical analysis of the test sample was carried out according to standard methods [11].

### Test for Carbohydrates

**Molisch Test-** 1ml of aqueous extract was treated with 2drops of alcoholic alpha-naphthol solution in a test tube and then 500 ul of concentrated sulphuric acid was added carefully along sides of the test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

**Test for Reducing Sugars**

**Fehling's Test -** To 500µl of extract, 500µl of Fehling's A and 500µl of Fehling's B solution were added in a test tube and heated on a water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

**Benedict's Test -** 500µl of Benedict's reagent and extract were mixed in a test tube and heated on a water bath for 5-10 minutes. The solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicates the presence of reducing sugar.

**Tests for Protein and Amino acids**

**Ninhydrin Test-** One ml of the test solution was heated with 1 drop of 5% Ninhydrin solution on a water bath for 10 minutes. Formation of the blue color indicates the presence of amino acids.

**Biuret Test-** The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of the violet or pink color indicates the presence of proteins.

**Tests For Alkaloids -** To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed.

**Mayer's Reagent Test -** To 1 ml of filtrate, few drops of Mayer's reagent was added along sides of the tube. Formation of creamy precipitate indicates the presence of alkaloids.

**Hager's Test -** To 1 ml of filtrate, few drops of Hager's reagent was added in a test tube.

Formation of yellow color precipitate indicates the presence of alkaloids.

**Wanger's Test -** To 1 ml of filtrate, few drops of Wanger's reagent was added in a test tube.

Formation of reddish brown precipitate indicates the presence of alkaloids.

**Test for Flavonoids**

**Alkaline reagent test -** One ml of the extract was treated with few drop of sodium hydroxidesolution separately in a test tube. Formation of intense yellow color, which becomes colorless onthe addition of drops of dilute acid, indicates the presence of flavonoids.

**Tests for Glycosides**

**Borntrager's Test -** To 1 ml of test solution, dilute sulphuric acid was added, boiled for 5minutes and filtered. To the cold filtrate, 1 ml of benzene or chloroform was added and it was shaken well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates the presence of anthraquinones glycosides.

**Test for Cardiac Glycosides**

**Keller-Killani Test -** To 1 ml of a test solution, 1.5 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Carefully few drops of concentrated sulphuric acid were added to the sides of the test tube. Formation of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

**Tests for Tannin and Phenolic Compounds**

**Ferric chloride test 5% -** A small amount of extract was dissolved in distilled water. To this solution 500µl of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates the presence of phenolic compounds.

**Lead Acetate Test -** One ml of extract was dissolved in distilled water. To this solution, few drops of lead acetate solution were added. Formation of white precipitate indicates the presence of phenolic compounds.

**Test for Saponins**

**Froth test-** One ml of the extract was diluted with 2ml of distilled water and shaken in agraduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

**Tests for Terpenoids**

**Salkowski's Test-** One ml of the extract was treated with 1ml of chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, a steroid is present. Presence of golden yellow layer at the bottom indicates the presence of triterpenoids.

**Test for Steroids**

2ml of acetic anhydride was added to 2ml extract of each sample followed by careful addition of 2ml $H_2SO_4$. The colour changed from violet to blue or green indicate the presence of steroids.

**Preparation of Bacteria Inoculums**:

A 24 hrs old culture of bacterial isolate was emulsified in sterile normal saline and adjusted to 0.5 Mcfarland standard (by compared the desired inoculums with 0.5 Mcfarland standard) [8].

**Bacterial Sensitivity Testing:**

**Disc Diffusion Method**: For each aqueous extract and pure extract (without any solvent), a solidified Muller Hinton agar plate was assigned to it and aseptically each plate was seeded by flooding with specific bacteria solutions (*Staphylococcus aureus* and *Klebsiella pneumoniae*) and then allowed to settle for 10 min. Then sterile disc containing 20 ul each extract were applied on bacterial lawn. Incubated at $37^0$c for 24 hrs.

## RESULTS AND DISCUSSION

Successful predication of botanical compounds is largely dependent on the type of solvent used in the extraction procedure traditional healers use primarily water as the solvent [12] but in our studies we found spinach leaf pure extract without any solvent demonstrated antimicrobial activity compared to those aqueous extract and ethanolic extract. In present study, spinach leaf pure extract without any solvent exert the antibacterial activity against Staphylococcus aureus and Klebsiella pneumoniae. Aqueous extract and ethanolic extract at different concentrations (25%, 50%, and 100%) were not effective against these two test organisms. (Table 1).

**Table 1: Antibacterial Activity of *Spinacia oleracea* Leaf**

| Extract | *Staphylococcus aureus* | *Klebsiella Pneumoniae* |
|---|---|---|
| Extrct+$H_2o$ (25%) | Resistant | Resistant |
| Extract+$H_2o$ (50%) | Resistant | Resistant |
| Extract+$H_2o$ (100%) | Resistant | Resistant |
| Extract+Etheno(25%) | Resistant | Resistant |
| Extract+Etheno(50%) | Resistant | Resistant |
| Extract+Ethenol(1%) | Resistant | Resistant |
| Pure Extract (Withoutany solvent) | Sensitive | Sensitive |

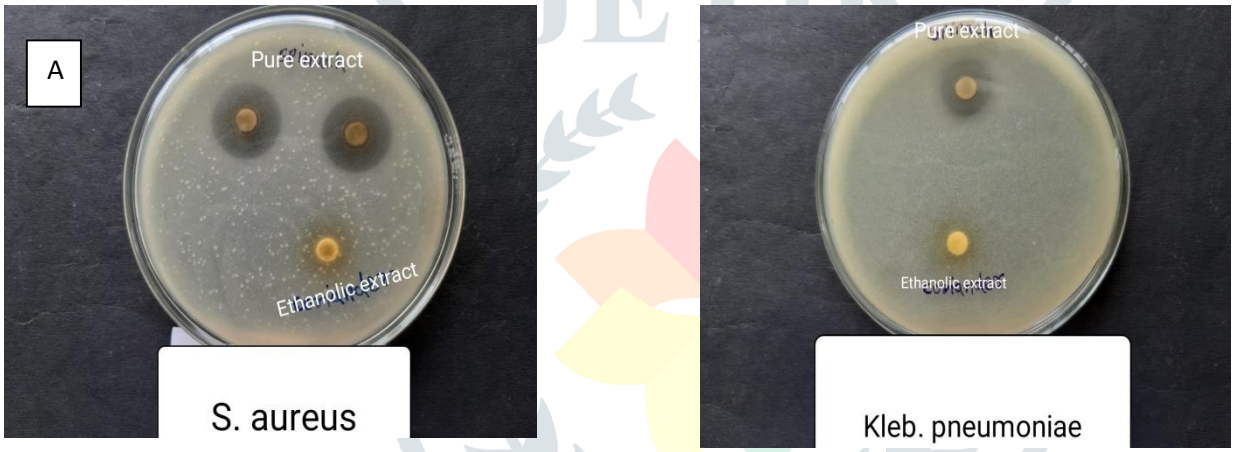


**Fig:1 Antimicrobial activity of Spinach extract against *Staphylococcus aureus* (A) and Klebsiella pneumoniae (B).**

This study confirms the potential antibacterial activity of Spinach oleracea leaf pure extract without any solvent. The result of the phytochemical test carried out on the extracts was recorded as shown in Table-2. This screening showed the presence of most of the important phytoconstituents like carbohydrates, reducing sugar, alkaloids, flavonoids, glycosides, cardiac glycosides, tannins, saponin, amino acid, protein, terpenoids and steroids.

**Table 2: Phytochemical analysis of *Spinacia oleracea* leaves**

| Sr.No. | Photochemical Test | Qath | Aqueous | Ethenolic |
|---|---|---|---|---|
| 1 | Test for Carbohydrates | | | |
| | A)Molisch Test | -ve | +ve | +ve |
| 2 | Test for Reducing Sugar | | | |
| | A) Fehling's Test | +ve | +ve | +ve |
| | B) Benedict's Test | +ve | +ve | +ve |
| 3 | Tests for Protein &Amino acids | | | |
| | A) Ninhydrin Test | -ve | +ve | +ve |
| | B) Biuret Test | -ve | +ve | +ve |
| 4 | Tests for Alkaloids | | | |
| | A) Mayer's Test | +ve | +ve | +ve |
| | B) Hager's Test | +ve | +ve | +ve |
| | C)Wagner's Test | +ve | -ve | +ve |
| 5 | Test for Flavonoids | | | |
| | A) Alkaline Regen | +ve | +ve | -ve |
| 6 | Test for Glycosides | | | |
| | A) Borntrager Test | +ve | +ve | +ve |
| 7 | Test for Cardiac Glycosides | | | |
| | A) Keller Killani Test | +ve | +ve | -ve |
| 8 | Tests for Phenolic &Tannin | | | |
| | A) Ferric chloride Test | +ve | -ve | +ve |
| | B) Lead Acetate Test | +ve | +ve | +ve |
| 9 | Test for Saponin | | | |
| | A) Froth test | +ve | +ve | +ve |
| 10 | Tests for Terpenoids | | | |
| | A) Salkowski's test | -ve | +ve | +ve |
| 11 | Test for Steroids | +ve | +ve | +ve |

Carbohydrates present in aqueous and ethanolic extract but absent in quath extract while reducing sugar was detected in all extract. Amino acids and proteins are present in quath extraction. Ninhydrin test and Biuret test shows positive result for aqueous and ethanolic extract. Proteins and carbohydrates are necessary for the animal body for repair and maintenance [13] .Some plants exhibited the presence of proteins and carbohydrates thus reflecting their nutritional importance as protein and carbohydrate supplement which cannot be ignored [14].

Alkaloids are a class of nitrogenous organic compounds of plant origin which have diverse and important physiological effects on humans and other animals [9]. The entire test for alkaloids i.e. Mayer's test, Hager's test and Wagner's test shows negative with aqueous extraction and positive with quath and ethanolic extract.

Flavonoids are the most abundant polyphenols in our diet. The free radicals produced in the body are neutralized by flavanoids, which are well known for their antioxidant properties. In addition to antioxidant function, flavanoids may also modulate cell signaling pathways and could have marked effects on cellular function by altering protein and lipid phosphorylation and modulating gene expression [15] .Quath and aqueous extraction shows presence of flavonoids but ethanolic extraction shows absence of flavonoids.

Test for Glycosides is positive for all extract. Cardiac glycosides are class of organic compounds that increase the output force of the heart and decrease its rate of contraction by acting on the cellular Na-K ATPase pump [9]. Quath and aqueous extract shows positive result for cardiac glycosides but negative with ethanolic extract.

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [16]. Phenolic compounds possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function as well as inhibition

of angiogenesis and cell proliferation activities [17]. Tannins are astringent in nature and useful in treating intestinal disorders such as diarrhea and dysentery [18]. It also aids in wound healing [19]. Quath, aqueous and ethanolic extract shows positive result for phenolic and tannin test except aqueous extraction by ferric chloride test.

The presence of saponins will reduce the blood pressure and cholesterol level in the blood pressure and cholesterol levels in blood. Saponins have been found to be potentially useful in the treatment of hypercholesterolemia which suggests that saponins might interfere with the intestinal absorption of cholesterol [20], [21]. Saponin is present in all extracts.

Terpenoids reduce complications associated with diabetes and lowers the sugar level in blood [22]. Terpenoids have been found to be very useful in anti-aging and overall beauty enhancement [23]. Steroids have been reported to have antibacterial properties [24]

and they are very important compound especially due to their relationship with compounds such as sexhormones [25] Terpenoids are present in aqueous and ethanolic extraction. Steroids are detected in all the extracts

## CONCLUSION

The commonly consumed green leafy spinach vegetable in Osmanabad selected for the present study contain substantial amount of phytochemicals which are helpful in the prevention of some deadly diseases [26-29]. This work showed that *S.oleraceae* is rich in phytochemicals such as carbohydrates, amino acids, proteins, alkaloids, flavonoids, cardiac glycosides, phenolic compounds, tannins, saponin, terpenoids and steroids in different extracts. Methanol is highly toxic compound so we went for etanol which is comparatively less toxic. Further

studies are required to isolate the active compound from pure extract of *Spinacia oleracea* responsible for antibacterial effect.

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