

**COMPARATIVE STUDIES ON PHYTOCHEMICAL AND ANTIMICROBIAL  
ACTIVITIES OF *SPINACIA OLERACEA* L. AND *BASELLA RUBRA* L.**

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**ABSTRACT**

**Objective:** The present study aimed to compare the phytochemical and antimicrobial potential of two Indian spinaches *Spinacia oleracea* L. and *Basella rubra* L. **Methods:** Methanolic extract of *Spinacia oleracea* and *Basella rubra* leaves were obtained using Soxhlet extraction techniques. The present study was investigated for *invitro* antimicrobial activity against selective pathogens using agar well diffusion method. In parallel study was performed to identify the distribution and the concentration of the phytochemicals in the leaves of both plant extracts. **Results:** The methanolic extract yields were obtained both the spinach as *S. oleracea* (2.26 g (4.52%)) and *B. rubra* (2.14 g (4.28%)). The total phenol was estimated in *S. oleracea* and *B. rubra* in methanolic extract as 684.5 mg/g of dry extract and 574.2 mg/g dry extract respectively. *S. oleracea* is found to be better antibacterial activity than the *B. rubra*. Anticandidal activity was observed in both extract but no activity was observed in *A. niger*. Both the extracts have all the tested phytoconstituents except coumarins and glycosides. **Conclusion:** This comparison study clearly indicated that, the antimicrobial and phytochemical importance of both Indian spinaches. However, the spinach *S. oleracea* shown higher antimicrobial activity than *B. rubra* and there is little variation in the phytochemical constituents.

**KEYWORDS:** Indian spinach, *Spinacia oleracea*, *Basella rubra*, Phytochemistry, Antimicrobial activity.

**INTRODUCTION**

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. The medicinal plants are being used since the human civilization for their ailments to cure various diseases and symptoms<sup>[1]</sup>. Medicinal plants are known to produce various secondary metabolites which employ to develop new eco-friendly bio-pesticides and other pharmaceutical and nutraceuticals relevance<sup>[2]</sup>. Traditional medication would be the first hand medical practices all around the world by the healers about 80% world's population<sup>[3]</sup>. Recent decades, the biological and pharmacological potentials of plant secondary metabolites have been well established. However, various biological activities were addressed on the isolation and screening of bioactive potential secondary metabolites such as antimicrobial, antioxidant, anticancer, antiviral, anti-inflammatory and anti-diabetic from plants sources<sup>[4]</sup>. The various phyto-constituents like, alkaloids, flavonoids, terpenoids, saponins, coumarins, glycosides, tannins, etc. were responsible for the various bioactivities<sup>[5]</sup>, hence it indicating the

pharmacological background of the plant can be identified. On the other hand, ethno botanical information's are also standing right clue to choose right plant to targeted activity in addition to scientific validation of that particular activity determined<sup>[6]</sup>.

*Basella rubra* and *Spinacia oleracea* possess various nutritional factors such as vitamins, minerals and other phytochemicals which is very essential for day to day life<sup>[7]</sup>. Common spinach, *Spinacia oleracea* is well known that has been used as leafy vegetables. Apart from the various nutritious benefits of the plant; there are lot of pharmacological potential have been reported worldwide<sup>[8]</sup>. These both leafy vegetables commonly called Indian spinach. To best of our knowledge, no systematic work on comparison on phytochemical and antimicrobial potential has been do so far. With this background, the present study has been aimed to investigate comparison of phytochemical and antimicrobial activity of the Indian spinach such as *Spinacia oleracea* L. and *Basella rubra* L. leaf extracts.

**MATERIALS AND METHODS**

**Collection of plant samples**

The leaf of the medicinal plants were used for the present study, the plants of *Spinacia oleracea* L. and *Basella*

*rubra* L. were obtained from Coimbatore localities, Tamil Nadu, India. The plant parts were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

### Phytochemical Analysis

The collected leaf samples were washed thoroughly two times with running tap water and once with sterile water, air-dried, powdered using a pulverizer and used for extraction. About 50 grams of air-dried and coarsely powdered plant material was extracted successively with 250 ml of methanol using a Soxhlet extractor for at least 15 reflux. After complete extraction, the extract in the round bottom flask were removed and condensed using rotary evaporator. After solvent evaporation, extracts were weighed for the percentage yield calculation. The thick syrup plant extract were labeled and stored at 5°C in sterile screw-capped vials for further use.

Preliminary phytochemical screening of methanol extract of *S. oleracea* and *B. rubra* leaf was carried out to detect the phyto-constituents using standard conventional protocols<sup>[9-11]</sup>. Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

### Antimicrobial Activity

#### Test-pathogenic microorganisms

The Gram negative *Pseudomonas aeruginosa* (MTCC 424), Gram positive *Staphylococcus aureus* (MTCC 96) bacterial pathogens and a yeast pathogen of *Candida albicans* (MTCC 227), mycelial pathogen of *Aspergillus niger* (MTCC 872) were used for *in vitro* antimicrobial activity. These selected pathogenic strains were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, Punjab, India.

Antibacterial and antifungal activity of above mentioned extracts were tested using the agar well diffusion method described<sup>[13]</sup>. All the above-mentioned bacteria were inoculated into nutrient agar medium and fungi inoculated to potato dextrose agar medium. The well of 8 mm diameter was punctured in the culture medium using sterile cork borer. Different extracts were administered to fullness in each well. Culture plates were incubated at 37°C for 24 h in bacteria and incubated at 37°C for 4 days in fungi. Bioactivity was determined by measuring diameter of inhibition zones in mm. Solvents used for extraction served as control.

## RESULTS AND DISCUSSION

### Plant Collection and extraction

Qualitative phytochemical analyses for alkaloids, carbohydrates, tannins, phenols, gums and mucilage, fixed oils and fats, saponins, proteins, volatile oils, flavonoids and steroids were screened in methanolic extracts of the selected Both spinach *S. oleracea* and *B. rubra*. The screening of the extract indicated the presence of alkaloids, tannins and saponin in the

methanolic extracts of leaves (Table 1). Thick syrup were obtained both the spinach *S. oleracea* and *B. rubra* were yielded as 2.26 g (4.52%) and 2.14 g (4.28%), respectively. The plant extract yield percentage on the usage of methanol agreed with the earlier reported by Jamuna *et al.*<sup>[14]</sup> obtained in *Hypochaeris radicata* L. The plant extract obtained using soxhlet is varied among the herbal plants to plant. In a plant, different parts having differently yielded<sup>[15]</sup>.

Total phenol content was estimated using folin phenol reagent under standard assay conditions. It was estimated that, *S. oleracea* methanolic extract possessed 684.5 mg GAE/g of dry extract (GAE: gallic acid equivalent) whereas, *B. rubra* methanolic extract possessed 574.2 mg GAE/ g dry extract. The *S. oleracea* methanolic extract possess alkaloids, flavonoids, terpenoids, phenolic compounds, saponins and cardiac glycosides in their phyto-constituents. On the other hand, *B. rubra* methanolic extract possess all tested components such as., alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, tannins, cardiac glycosides, except coumarins. Although, these secondary metabolites possess various bioactive potentials have been demonstrated in various plants<sup>[16]</sup>. Both the spinach possesses most of the valuable and bioactive secondary metabolites with their phytoconstituents.

Medicinal plants are well known for its antibacterial activity is well established. The comparisons on antibacterial activities of the both plant methanolic extracts were tested against two pathogenic bacterial strains using agar well diffusion method. In comparison of both plants extracts on the antibacterial activity, the *S. oleracea* showing highest activity as against both Gram negative and positive bacterial pathogens at all the tested concentrations (Figure 1; Table 2). Similarly, several types of plant ethanol and acetone extracts were tested against gram-positive and gram-negative pathogenic bacteria. Ethanolic extract showed considerably more activity than the acetone and aqueous extract<sup>[17]</sup>. The yeast pathogen *C. albicans* was effectively controlled by *S. oleracea* methanolic extract than *B. rubra* methanolic extract.

*B. rubra* methanolic extract did not show any activity against 50 µg/well amended well whereas, *S. oleracea* showed zone of inhibition of 16 mm anticandidal activity. No activity was observed in both extracts towards *A. niger*. The methanolic plant extracts from 24 different plant showed various spectrum of antimicrobial activity, among them few of the methanolic possess anti-candidal effect but did not showed any mycelial fungal inhibition. This was agreed with our results finding both the spinach methanol extract<sup>[18]</sup>. It was evident that, both spinach plants possess very good phyto-constituents in their plant parts but, in terms of biological properties, Indian spinach (*S. oleracea*) found to be best than the Malabar spinach (*B. rubra*).

This study clearly demonstrated that the phytochemical and antimicrobial potential comparison of both Indian spinach (*S. oleracea*) and Malabar spinach (*B. rubra*). The phytochemical richness of both spinaches was given newer pavement to isolate various bioactive pharmaceutical leads. Though, both spinaches were eaten as leafy vegetables as nutritious but also possess many bioactive ingredients. The excellent antibacterial spectrum was indicated that, the continuous usage of

these both spinaches will definitely remove the harmful bacteria populations. This comparative study leads the further bioactive potential can be evaluated with the aid of modern techniques and instrumentation may definitely come with newer chemical entity. On the basis of the this study, Indian spinach (*S. oleracea*) found to be better in terms of high antimicrobial potential and other phyto-constituents presences than Malabar spinach (*B. rubra*).

**Table 1: Phytochemical screening of spinach plant methanolic extract**

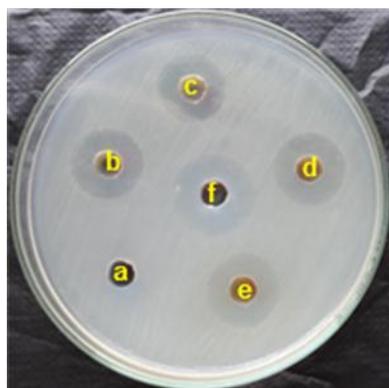
| Phyto-constituents | <i>S. oleracea</i> | <i>B. rubra</i> |
|--------------------|--------------------|-----------------|
| Alkaloids          | +                  | +               |
| Flavonoids         | +                  | +               |
| Terpenoids         | +                  | +               |
| Phenolic Compounds | +                  | +               |
| Saponins           | +                  | +               |
| Tannins            | +                  | +               |
| Glycosides         | +                  | +               |
| Cardiac Glycosides | +                  | +               |
| Coumarins          | -                  | -               |

+: presence; -: absence

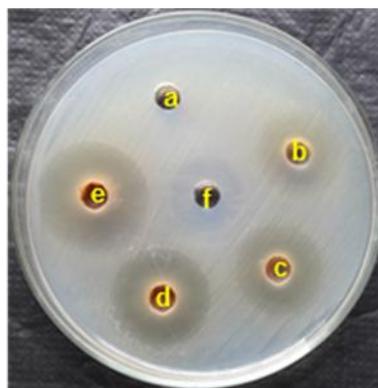
**Table 2: Antimicrobial activity of both spinach *S. oleracea* and *B. rubra* methanolic extract**

| Name of the pathogen         | Antimicrobial activity (mm)* |     |     |     |                 |     |     |     |         |
|------------------------------|------------------------------|-----|-----|-----|-----------------|-----|-----|-----|---------|
|                              | <i>S. oleracea</i>           |     |     |     | <i>B. rubra</i> |     |     |     | Control |
| Conc. §                      | 50                           | 100 | 150 | 200 | 50              | 100 | 150 | 200 | 30      |
| <i>Staphylococcus aureus</i> | 14                           | 15  | 16  | 17  | 7               | 9   | 11  | 15  | 19      |
| <i>Klebsiella pneumonia</i>  | 11                           | 14  | 17  | 21  | 8               | 10  | 13  | 16  | 18      |
| <i>Candida albicans</i>      | 16                           | 17  | 18  | 19  | -               | 6   | 8   | 9   | 20      |
| <i>Aspergillus niger</i>     | -                            | -   | -   | -   | -               | -   | -   | -   | 25      |

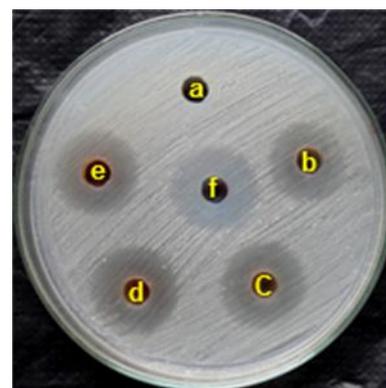
\*: zone of inhibition; §: µg/well ; -: No activity



*Staphylococcus aureus*

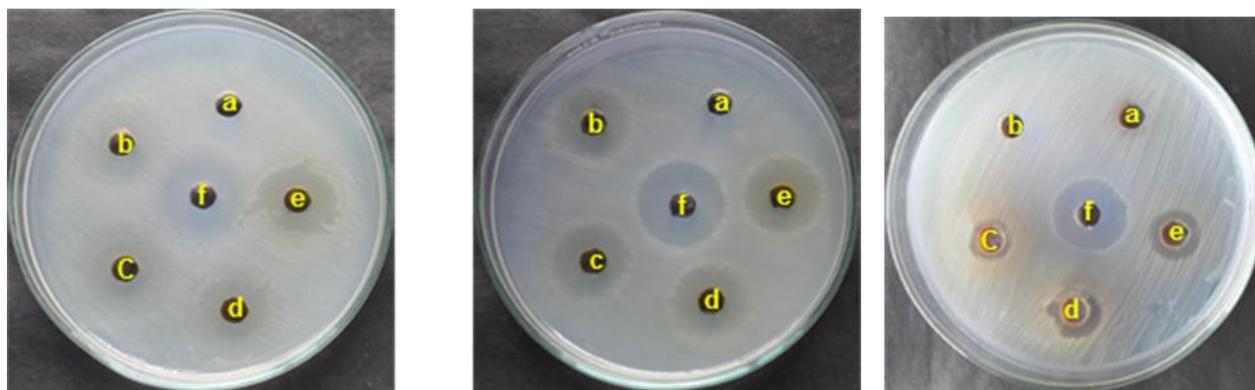


*Klebsiella pneumoniae*



*Candida albicans*

*Basella rubra L.*



*Staphylococcus aureus*                      *Klebsiella pneumoniae*                      *Candida albicans*  
 a: Solvent control; b: 50 µg/well; c: 100 µg/well; d: 150 µg/well; e: 200 µg/well; f: 30 µg/well Azithromycin (bacteria) or Clotrimazole (fungi)

**Figure 1: Antimicrobial activity of methanolic crude extract from leaves of *S. oleracea* and *B. rubra***

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