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Research Article

Unveiling the Antimicrobial Efficacy of Fruit Extracts of Indian Spinach (*Basella rubra* L.) for *Bacillus subtilis, Escherichia coli* and *Saccharomyces species*

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Abstract

Indian spinach, *Basella rubra* L. has been traditionally used in Indian medicine to treat specific diseases. The present investigation was carried out to examine the antimicrobial activity of the fruit extracts of Basella rubra fruits by measuring the zones of inhibition using Disc Diffusion method and Agar well Diffusion method against three species of microorganisms viz., Bacillus subtilus, Escherichia coli and Saccharomyces species. The fruit extract of Basella was dissolved in methanol to obtain different concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml). 0.5ml of methanol was used as negative control (solvent control). The extract showed significant antimicrobial activity against *Bacillus subtilus, Escherichia coli* and *Saccharomyces species* and the inhibition zones were significantly different in each dilution of the fruit extract. The minimum inhibitory concentration of the extract against the different strains was found to be 25mg/ml.

Keywords: Basella Rubra; Antimicrobial Activity; Disc Diffusion Method; Agar Well Diffusion Method; Inhibitory Concentration

Introduction

India is one of the main countries having a rich source of medicinal plant species. The natural products of plant origin find a place in synthesis of drugs to cure several diseases. Indian spinach *Basella rubra* L. (Basellaceae) is a herbaceous annual or biennial climbing herb found in tropical and sub-tropical areas of India (Wealth of India, 2000). The leaves have glossy green upper surface and red-violet lower surfaces and are 3–7 inches in length, and are rich in betalain pigment (Cyunel, 1989). Likewise, the stalk, petioles and fruits are red-violet which are also rich in betalain pigment (Palada and Crossman, 1999). In ancient Indian and Chinese traditional medicine the plant has been utilised for its medicinal properties to treat constipation and also as a diuretic and an anti-inflammatory material. *Basella rubra* L. is a wildly cultivated, cool season vegetable with climbing growth habit. It is a succulent, branched, smooth, twining herbaceous vine, several meters in length. Stems are purplish or green. Leaves are fleshy, ovate or heart-shaped, 5 to 12 cms long, stalked, tapering to a pointed tip with a cordate base. Spikes are axillary, solitary, 5-29 cm long. Fruit is fleshy, stalkless, ovoid or spherical, 5-6 mm long, and purple when mature. Mainly leaves and stems are used for the medicinal purpose [1].

Basella rubra L. has been used since ancient times for its medicinal importance. The leaf juice is a demulcent, used to cure dysentery [1]. Stem and leaves are used as mild laxative, diuretic and antipyretic [2]. In India, it has been used for antipruritis and burn [3]. The Ayurvedic treatment in India has been using the leaves and stem for anticancer such as melanoma, leukaemia and oral cancer [4]. However, relevant experimental work on the antimicrobial activity of the fruits has not yet been explored thoroughly. Therefore, the present study was undertaken to evaluate the antimicrobial activity of fruit extracts of *Basella rubra* L.

Materials and Methods

Plant material

The fruit extracts of *Basella rubra* L. were collected during 2023 - 2024 from the plants maintained at the Department of Vegetable Science, Horticultural College and Research Institute, TamilNadu Agricultural University, Coimbatore, TamilNadu, India. The experiment was conducted in the Centre for Post Harvest Technology, Agricultural Engineering College and Research Institute, TamilNadu Agricultural University, Coimbatore, TamilNadu, India.

Physical screening of the fruits

The physical characteristics of the fruits harvested during were recorded as given in table 1.

Preparation of fruit extracts

Fruits were shade dried, powdered and then extracted with methanol for 48 hours or till the solvent in the siphon tube of an extraction become colourless using Soxhlet apparatus. The filtrates were collected and evaporated to dryness under reduce pressure. The dried extracts were stored in dry sterilized small containers at 4ºC until further use [5,6]. Then different concentrations of methanolic extracts of *Basella rubra* L. were prepared for antimicrobial sensitivity testing.

Circumference of fruit (cm)	Fresh weight of 100 fruits (g)	Fresh weight of 100 seeds after removing pulp (g)	Fresh weight of pulp(g)	TSS (°Brix)
3.00	21.62	7.07	11.00	5.0

Table 1: Physical characteristics of ripe fruits.

Preparation of test sample

The methanolic extract was dissolved in methanol to obtain the different concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml). 0.5ml of methanol was used as negative control (solvent control).

Microbial strains

The organisms *viz*., *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces species* were used for testing the antimicrobial activity of the fruit extract.

Preparation of test microorganisms

Test microorgamisms of bacteria and yeast *viz*., *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces species* were subcultured to respective media such as Nutrient agar medium (yeast culture), or Luria Bertani broth medium (*Bacillus* and *E. coli*) and Yeast Glucose Chloramphenicol Agar (*Saccharomyces*) and incubated for 24-48 hr. These pure cultures were then used for disc diffusion and agar well assay.

Disc Diffusion method

Agar plates were inoculated with standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, were placed on the agar surface. The Petri dishes were incubated under 37 ^{0}C for 24 hours. Generally, antimicrobial agent diffuses into the agar and inhibits the growth of the test microorganism and then the diameters of zone of inhibition were measured after 24 hours of incubation.

Agar-well Diffusion Method

Using 25ml of sterile Nutrient agar medium (yeast culture), or Luria Bertani broth medium (*Bacillus* and *E. coli*) and Yeast Glucose Chloramphenicol Agar (*Saccharomyces*) was poured into sterile culture plates and allowed to solidify. 0.5ml of 24 hours old culture of test organism was layered onto the medium. The seed medium was then allowed to dry at room temperature for about 30 minutes.

With the aid of a sterile cork borer, wells of about 8mm in diameter were punched on the plates. About 0.5ml of each dilution of the extracts, 0.5ml of streptomycin and niacin (absolute control control) and sterile distilled water (control) was dispensed into the wells and the plates were incubated at 37ºC for 24 hours for bacterial cultures and for fungal culture it was incubated at room temperature for 48 hours. After incubation, inhibition zones formed on the medium were evaluated in mm.

Minimum Inhibitory Concentration (MIC)

The experiment was carried out according to two fold serial dilution method. The stock solution of test solution (extracts) was prepared at concentration of 100µg/ml in nutrient broth and serially diluted up to five times. Six assay tubes were taken for screening of minimum inhibitory concentration of each strain. In the first tube 1ml of the sterilized nutrient broth was inoculated and then 1ml of the test solution was added and thoroughly mixed. Further dilutions of this solution were made by inoculating 1ml from first tube into second assay tube serially and 0.1ml of each test inoculums were added in each tube and were done in duplicate. The procedures were conducted under aseptic conditions. The inoculated tubes were kept at 37° C at 24 hours for bacterial assay and kept for 48 hours for fungal assay during the incubation period. After the incubation period, tubes were removed and observed for any deposits or turbidity in the solution and shaken to suspend bacteria that might have been settled down. These concentrations were observed and recorded as minimum inhibitory concentration (MIC).

Micro organism	Concentration(mg/ml)	Zone of inhibition (mm)	Streptomycin (µg/ml)	Niacin(µg/ml)
Bacillus subtilus	25	0.75 ± 0.070	76	89
	50	1.15 ± 0.070	62	71
	100	1.25 ± 0.070	45	56
Escherichia coli	25	0.65 ± 0.070	83	101
	50	0.85 ± 0.070	66	95
	100	1.1 ± 0.141	42	84
Saccharomyces species	25	0.22±0.120	126	212
	50	0.37±0.050	110	196
	100	0.39±0.061	99	187

Table 2: Antimicrobial activity of *Basella rubra* L.Fruit against test microorganisms.

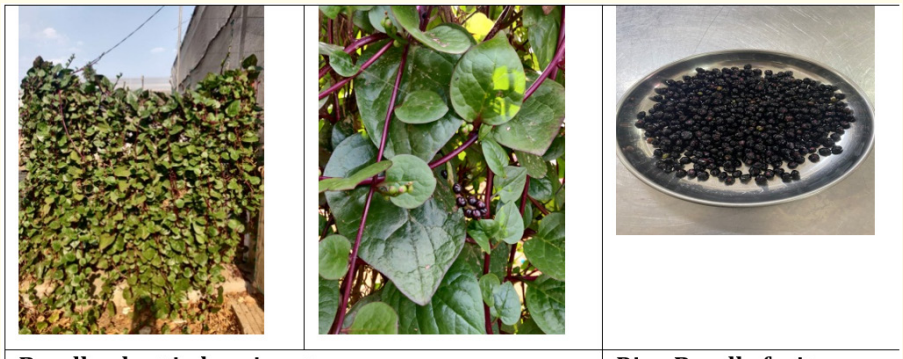
Results and Discussion

In the present study, methanolic extract of fruit of *Basella rubra* L.were found to express antimicrobial activity against all test organisms. The methanolic extract of fruits showed activity with zone at (MIC of 3.12 mg/ml) against *Bacillus subtilus*, *E.coli* and *Saccharomyces* species. Similar results have been obtained by Krishnapriya., *et al*., (2015) and Reshmi., *et al*.,(2012) [7,8].

Results revealed that as the concentration of the extract decreased, the activity also decreased; however degree of toxicity of different concentrations of different fractions of fruit extract may differ from one microorganism to another. The present study establishes the antibacterial activities of the plants extracts which may be attributed to the presence of phytochemicals.

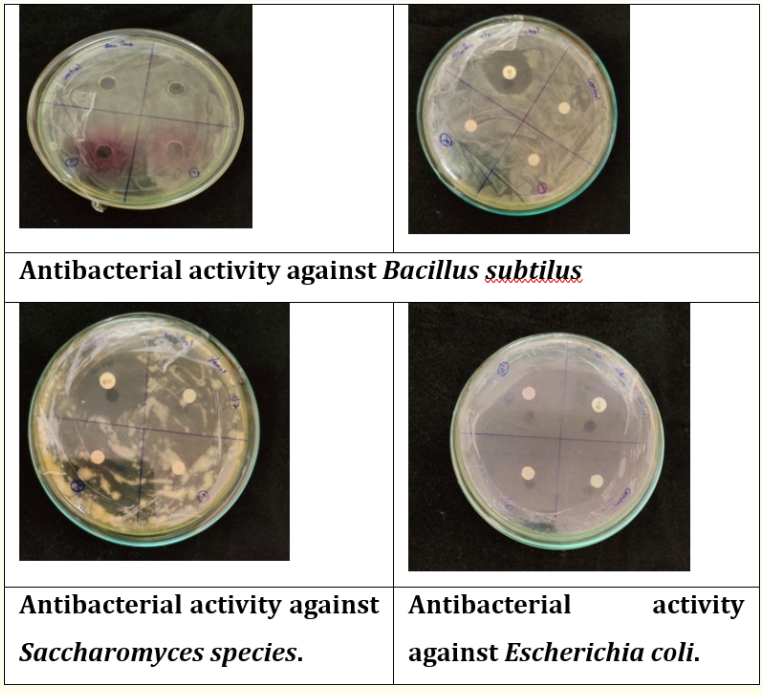
Test Microorganisms	MIC (mg/mL)
Bacillus subtilus	12.5
Escherichia coli	6.25
Saccharomyces species	25

Table 3: The Minimum Inhibitory Concentration of *Basella rubra* L. Fruit on different strains of test microorganisms.



Basella plant in bearing stage | **Ripe Basella fruits**

Figure 1



Antibacterial activity against *Bacillus subtilus*

Antibacterial activity against *Saccharomyces species*. | **Antibacterial activity against *Escherichia coli*.**

Figure 2

Conclusion

It can be concluded that the fruit extract of *Basella rubra* L. could be used for the treatment of infections caused by the microorganisms *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces* species. This study serves as basis for further research on *Basella rubra* extract.

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