

EVALUATION OF ANTIMICROBIAL PROPERTIES OF *BASELLA RUBRA* METHANOLIC EXTRACTS ON SELECTED MICROORGANISMS.

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ABSTRACT

In vitro antibacterial activity of *Basella rubra* commonly used by the Indian community was analyzed in this study. Traditional uses of *Basella rubra* include general tonics and over-the-counter medications used to treat specific conditions or diseases. The present study examined the antibacterial activity of *Basella rubra* extract using the disk diffusion method as part of the process of understanding the chemistry, toxicity and efficacy of *Basella rubra* extract. Methanolic extracts of the herb were examined using a standard antimicrobial disk diffusion method. Extracts were tested against *E. coli* (MTCC No. 1652), *Pseudomonas aeruginosa* (MTCC No. 424), *Bacillus subtilis* (MTCC No. 2393) and *Aspergillus flavus* (MTCC No. 277) bacteria. The inhibition zones were significantly different in each plant extract. The methanolic extract of leaves showed activity with zone at (MIC of 3.125 mg/ml) against *E.coli* and *Pseudomonas aeruginosa* followed by *Bacillus subtilis* (MIC of 6.25 mg/ml), *Aspergillus flavus* (MIC of 12.5 mg/ml) while methanolic extract of stem showed activity with zone at (MIC of 3.125 mg/ml) against *E.coli*, followed by *Pseudomonas aeruginosa* (MIC of 6.25 mg/ml), *Bacillus subtilis* (MIC of 12.5 mg/ml), *Aspergillus flavus* (MIC of 25 mg/ml). The phytochemical components of the methanolic extracts of the leaf and stem of *Basella rubra* showed the presence of different compounds such as leaf showed presence of steroids and carbohydrates, while stem extract showed presence of Tannin flavonoids and steroids. This study serves as basis for further research on *Basella rubra* extract.

Key word: Antimicrobial activity, Disc Diffusion method, *Basella rubra*, MIC

INTRODUCTION

Medicinal plant is defined as any substance with one or more of its organ containing properties that can be used for therapeutic purposes or which can be used as precursors for the synthesis of various drugs. [Sofowora A., 1993]. In recent years, there has been an increasing interest by researchers in the use of naturally occurring biologically active compounds of medicinal value [Anandarajagopal K *et.al.*, 2011]. There has therefore been an upsurge in the interest in herbal remedies in several parts of the world with many of the herbal remedial being incorporated into orthodox medical practice [Daniyan SY & Muhammad HB., 2008]. There are many plant species available all over the world which has been used for the multi beneficial activities. India and China are the two major countries that are richer in many of the medicinal plant species. In spite of millions of chemically synthesized drug for a number of diseases; natural products of plant origin has got its own importance and has remained the most important source of new drugs. One such medicinal herb is *Basella rubra*.

Basella rubra is a wildy 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 [Kumar P., 2010].

Basella rubra has been used for many of its useful product from ancient times. Nowadays its properties have been utilized for the extraction of some useful material so that it can be used for the beneficial human activities. Some of the uses of this plant parts in the cure of certain problems occurred to humans has been explained here: The leaf juice is a demulcent, used in cases of dysentery. [Kumar P., 2010]. Stem and leaves are used as mild laxative, diuretic and antipyretic [Chou CT., 1997]. In India, it has been used for antipruritis and burn [Saikia AP *et.al.*, 2006]. The Ayurvedic treatment in India has been used *Basella rubra* leaves and stem for anticancer such as melanoma, leukemia and oral cancer [Premalatha B & Rajgopal G., 2005] However, relevant experimental work on the antimicrobial activity of the plant has not yet been explored. Therefore, the present

study is designed to evaluate the antimicrobial activity of methanolic extracts of the leaves and stem of *Basella rubra*.

MATERIAL AND METHODS

Plant material

The leaves and stem of *Basella rubra* were collected during August, 2013 to March, 2014 from University Guest House near Department of Botany, School of Life Sciences, Khandari Campus, Dr. B.R.Ambedkar University, Agra.

Phytochemical (Screening) Analysis of the extract

Then the screening of the plant extract was carried out by (Debela A., 2002) for the purpose of detecting active components like tannins, glycosides, alkaloid, terpene, steroids, phenolics, saponins, carbohydrates, proteins and flavonoids.

Preparation of Plant Extracts

Leaves and stem 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 filterates were collected and evaporated to dryness under reduce pressure. The dried extracts were stored in dry sterilized small containers at 4°C until further use (Azu N.C et.al., 2007 & Jain A., et. al., 2011). Then different concentrations of methanolic extracts of *Basella rubra* were prepared for antimicrobial sensitivity testing.

Microbial strains and culture media

The organisms were obtained from IMTECH Chandigarh were *Bacillus subtilis*, (MTCC No. 2393), *Escherichia coli* (MTCC No. 1652), *Pseudomonas aeruginosa* (MTCC No. 424) and fungal culture of *Aspergillus flavus* (MTCC No. 277).

Antimicrobial Screening Test

The sensitivity of the test organisms to methanol streptomycin and fluconazole was determined by standard disc diffusion method (Mukherjee et al., 1995). The medium was prepared using nutrient agar and agar plates previously inoculated with 24 hours, old cultures of test organisms respectively. Empty sterile discs having a diameter of 6 mm were impregnated with 25 ml of each concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml) respectively of extract solution and then incubated for 15 minutes for proper diffusion of extract. On the other hand, aseptically pack up some colonies from the pure culture was mixed in nutrient both. This broth was inoculated on entire surface of nutrient agar plate with the culture moistened cotton swab. Then wait for 5-6 minute after inoculation to allow the liquid culture to soak on agar surface and then with the help of sterile forceps herbal extracts containing disc were placed on inoculated surface of agar plate. These plates were incubated for 24 hours (in case of bacteria) and 48 hours (in case of fungal) at 37°C and the zone of inhibition was measured in mm.

RESULT AND DISCUSSION

PHYTOCHEMICAL SCREENING OF THE EXTRACTS –

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. A number of medicinal plants have been chemically investigated (Ambasta SP et.al., 1986, Kokate CK et.al., 1998). The methanolic extracts of *Basella rubra* shows the presence of steroids, triterpenoids and carbohydrates in the leaf while stem extracts of the plant, contain phenols, flavonoids tannin, steroids and triterpenoids (Table –1).

Table 1 – Phytochemical component's of the methanolic extras of *Basella rubra*

Phytochemical Components	Leaf Extract	Stem Extract
Alkaloids	-	-
Tannins	-	-
Phenals	-	+
Proteins and amino acids	-	-
Steroids and triterpenoids	+	+
Carbohydrates	+	-

(+) = present, (-) = absent

In the present study, methanolic extract of *Basella rubra* leaves and stem the were found to exert the antimicrobial activity against all test organisms. The methanolic extract of leaves showed activity with zone at (MIC of 3.125 mg/ml) against *E.coli* and *Pseudomonas aeruginosa* followed by *Bacillus subtilis* (MIC of 6.25 mg/ml), *Aspergillus flavus* (MIC of 12.5 mg/ml) while methanolic extract of stem showed activity with zone at (MIC of 3.125 mg/ml) against *E.coli*, followed by *Pseudomonas aeruginosa* (MIC of 6.25 mg/ml), *Bacillus*

subtilis (MIC of 12.5 mg/ml), *Aspergillus flavus* (MIC of 25 mg/ml) (table-2 & 3). 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 plant may differ from one microorganism to another. The methanolic extracts exhibited marked antimicrobial activity against gram positive and gram negative bacteria and fungi. *Basella rubra* showed good inhibitory activity against *Aspergillus niger*. [Premakumari KB et.al. 2010) This credit to antimicrobial activity of methanolic extract was supposed to methanol being an organic solvent and will dissolve organic compounds better, hence liberate the active component required for antimicrobial activity (Ekwenye UN & Elegalam NN., 2005).

CONCLUSION

This study has revealed the presence of secondary metabolites like Steroids and triterpenoids in the leaves and stem of *Basella rubra*. It has further confirmed that the leaf extract could be used for the treatment of infections caused by the microorganisms *E.coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus flavus*. The result on *Aspergillus flavus* lend credence to the folkloric use of this plant in treating microbial infections and shows that *Basella rubra* could be exploited for new potential antibiotics.

Table 2 : Antimicrobial activity of Methanolic extracts of *Basella rubra*

Test Organism	200	100	50	25	12.5	6.25	3.125	Drug
<i>E. coli</i>	13.5 ± 0.42	13.0 ± .72	12.5 ± .47	12.0 ± .17	11.0 ± 0.06	10.5 ± 0.00	7.7 ± .13	17
<i>P. aeruginosa</i>	13.5 ± .41	12.5 ± .84	12.0 ± 0.33	11.0 ± 0.33	8.0 ± 0.2	8.0 ± 0.00	-	14
<i>B. subtilis</i>	12.5 ± 0.24	11.5 ± 0.29	11.0 ± 0.24	8.0 ± 0.02	7.4 ± 0.1	-	-	8
<i>A. flavus</i>	11.5 ± 0.22	11.4 ± 0.29	8.0 ± 0.41	7.5 ± 0.25	-	-	-	7

Table – 3

Stem	200	100	50	25	12.5	6.25	3.125	Drug
<i>E. coli</i>	14.5 ± 0.24	13.5 ± 0.42	13.0 ± 0.72	12.5 ± 0.47	11.5 ± 0.76	10.5 ± 0.08	8.0 ± 0.04	17
<i>P. aeruginosa</i>	13.5 ± 0.22	12.5 ± 0.47	12.0 ± 0.42	11.5 ± 0.76	11.0 ± 0.24	10.5 ± 0.08	8.0 ± 0.02	12.5
<i>B. subtilis</i>	13.0 ± 0.24	12.0 ± 0.41	12.5 ± 0.19	11.0 ± 0.39	10.5 ± 0.00	8.0 ± 0.03	-	11
<i>A. flavus</i>	11.5 ± 0.24	11.3 ± 0.44	8.0 ± 0.41	7.5 ± 0.25	6.6 ± 0.05	-	-	9

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