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# ANTIMICROBIAL POTENTIAL OF MEDICINAL PLANT – *EMBELIA BASAL*

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## ABSTRACT

*Embelia basal* (R. & S.) A. DC is a shrub from family Myrsinaceae. Various species of Embelia are used in Ayurvedic system of medicines and mainly promoted as antibacterial as well as anthelmintic mediator. In order to search for antimicrobial activity of secondary metabolites, screening of different extracts of fruits of *E. basal* was carried out. Powdered fruit material was extracted using solvents of increasing polarity from non polar (n-hexane), semi-polar (Acetone) to polar (methanol). Extracts were analysed for their antimicrobial activity against six bacterial strains with two antifungal strains. The antimicrobial activity was determined by using disc diffusion method and inhibitions of microorganisms were correlated by using Streptomycin as a standard. *Bacillus cereus, Staphylococcus aureus, pseudomonas aeruginosa* was found to be more susceptible strains, in which methanol extract showed promising activities. Therefore this was selected for further investigation to determine its therapeutic potential and MIC calculation.

## KEY WORDS

*Embelia* basal, Antimicrobial activity, *Pseudomonas aeruginosa*, Methanol extract, MIC.

## INTRODUCTION

The increasing failure and side effects of popularity used chemotherapeutic and appearance of multiple drug resistance phenotypes in pathogenic bacteria led to the search of new compounds with antimicrobial activity. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. The demand on plant based therapeutics is increasing in both developing and developed counties due to growing recognition that they are natural products, non narcotic, easily biodegradable producing minimum environmental hazards, having no adverse side effects and easily available at affordable prices. Therefore researchers are progressively more turning their attention to natural products, looking for new leads to develop better drugs against microbial infections and screening of several medicinal plants for their potential antimicrobial activities [1].

The genus Embelia has been investigated for a variety of purposes in Ayurveda [2]. One of the species, *E. ribes* is used in dental caries [3]. It is also used in the process of formulating Anti-AIDS Ayurvedic pharmaceutical compositions [4]. This species shows an antispermatogenic [5] effect and also acts as a contraceptive [6]. The antibacterial activity of embelin, isolated from berries of *E. ribes* and *E. robusta* has been reported [7] but apparently no work has been reported on isolation or identification of phytoconstituents from the fruits of *E. basal*. It is a shrub from family Myrsinaceae, an Indian variety, is widely distributed throughout India and commonly known as Vidanga. The larger elliptical leaves of the plants are used in combination with ginger, are used as a gargle for sore throats. The dried bark of the root is used as a remedy for toothache and the finely powered berries are formulated as an ointment for treating pleurities [8]. *E. basal* is highly esteemed in Ayurvedic medicine as a powerful anthelmintic [9] and also an important constituent of number of formulations [10-11]. In addition decoction is widely used in the treatment of insanity and heart diseases [11]. The present work is carried out in order to evaluate the efficacy of the plant for its antimicrobial activity.

## MATERIALS AND METHODS

***Plant Pharmaceutical Sciences with branch Novel drug delivery system***) by keeping for 24 hours at room temperature. Solvent was recovered under reduced pressure to obtain crude extracts. Extracts were studied against antimicrobial strains, where methanol was the potent extract. Thus bioguided broad fractionation of methanol extract was carried out to get five major fractions as: hexane (A), chloroform: hexane (B, 1:4), chloroform: hexane (C, 1:2), chloroform (D) and methanol (E)

***Bacterial strains:***

On the basis of pathogenic importance, six pathogenic bacterial strains (*Escherichia coli* ATCC-11246; *Staphylococcus aureus* ATCC-6538; *Salmonella typhimurium* ATCC-23564; *Pseudomonas aeruginosa* ATCC-27853; *Proteus vulgaris* ATCC-13315; *Bacillus cereus* ATCC-11778) and two antifungal strains (*Saccharomyces cerevisiae* ATCC-9763; *Aspergillus niger* ATCC-16404) were selected. All bacterial strains were maintained at 4$^{o}$C on nutrient agar (Hi media) salts and cultured at 37$^{o}$C using same agar medium.

*Antimicrobial bioassay:*

The disc diffusion method was used to determine antimicrobial activity. Samples of each extract (200 mg) were dissolved in

respective solvents (1 ml). Sterile 5mm diameter disc were impregnated with 40 µl of these solvent extracts (8mg/disc). The bacterial stains were inoculated on nutrient broth and incubated for 24 hours at 37 ± 0.1°C, while antifungal strains were inoculated on nutrient broth and incubated for 48 hours at 25 ± 0.1°C. Adequate amount of Muller Hinton Agar and Chloramphenicol Yeast Glucose Agar were dispensed into sterile plates and allowed to solidify under aseptic conditions. The count of the bacterial and antifungal stains was adjusted to yield $1x10^7$ to $1x10^8$ $ml^{-1}$ and $1x10^5$ to $1x10^6$ $ml^{-1}$ respectively. The test organisms (0.1 ml) were inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates inoculated with test organism were incubated for 1 hr. before placing the extract impregnated paper disc on the plates. Following this, the sterile discs impregnated with different samples were placed on Agar plates. The bacterial plates were incubated at 37± 0.1°C for 24 hrs while antifungal plates were incubated at 25± 0.1°C for 48 hours. After incubation all the plates were observed for zones of growth of inhibition and the diameter of the zones was measured in millimeters. All the testes were performed under sterile conditions. The results were compared with Streptomycin (10 µg/disc) and fluconazol discs (50 µg/disc) which is used as a positive controls.

The antibacterial activities of hexane, acetone, ethanol, methanol and aqueous extracts were assayed in vitro by Agar disc diffusion method against six different bacterial strains and two antifungal strains **(Table I)**. Antimicrobial activity of fractions of methanol extract was presented **(Table II)** and (**Table III, Fig 1)**, indicated the Minimum Inhibitory Concentration (MIC) of fraction C.

**Table I**
***Antimicrobial activity of plant extracts.***

| Micro organism | Gram | Diameter of zone of inhibition (mm)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | Hex | Ace | EtOH | MeOH | Aq. Ext. | Std. | |
| *Escherichia coli* | -ve | - | - | - | - | - | 19 |
| *Staphylococcus aureus* | -ve | - | - | - | 11 | 8 | 20 |
| *Salmonella typhimurium* | +ve | - | - | - | - | - | 15 |
| *Pseudomonas aeruginosa* | -ve | - | - | - | 13 | 9 | 15 |
| *Proteus vulgaris* | -ve | - | - | - | - | - | 22 |
| *Bacillus cereus* | +ve | - | - | - | 10 | 9 | 29 |
| *Saccharomyces cerevisiae* | | - | - | - | - | - | 20 |
| *Aspergillus niger* | | - | - | - | - | - | 40 |

[a]*Zone of inhibition including the diameter of filter paper disc (5 mm)*
**-** *: No activity*

**Table II**
***Antimicrobial activity of fractions of methanol extract.***

| Fractions | Diameter of zone of inhibition(mm)[a] |
|---|---|
| A | - |
| B | 07 |
| C | 11 |
| D | 09 |
| E | 10 |

[a]*Zone of inhibition including the diameter of filter paper disc (5 mm)*

**Table III**
***MIC of Fraction C***

| Fraction | Concentration (µg) | Diameter of zone of inhibition(mm)[a] |
|---|---|---|
| C | 400 | 15 |
| | 350 | 15 |
| | 300 | 10 |
| | 200 | 08 |
| | 150 | 06 |
| | 100 | 06 |

[a]*Zone of inhibition including the diameter of filter paper disc (5 mm)*

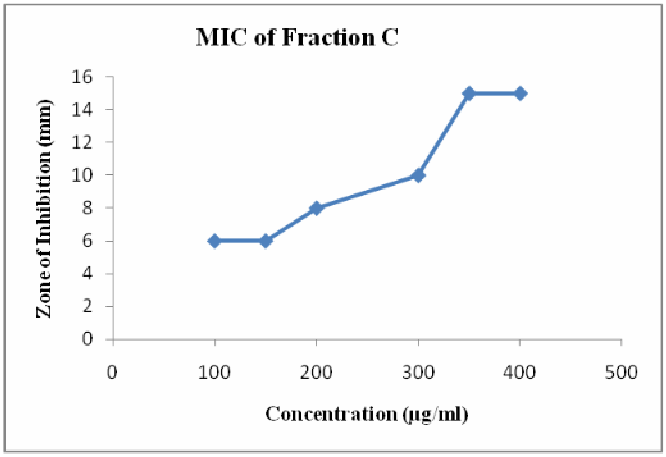


**Figure 1**

## RESULTS AND DISCUSSION

The results of the antimicrobial assay of the different extract of *E. basal* are presented **(Table I)**. Non polar and semi polar extracts do not show any activity against the test organisms while polar methanol and aqueous extracts shows the activity against *Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus*. This is the first report showing significant inhibitory potential of *Pseudomonas aeruginosa* by methanolic extract of *E. basal*. Antimicrobial activity of the fractions of this extract against *Pseudomonas aeruginosa* represented **(Table II)**. Fraction (C, 1:2) chloroform: hexane is found to be more effective. A concentration of 400µg/disc is found to inhibit most of the test organism. The results **(Table III)** indicate that 400 µg/disc is the lowest concentration required to inhibit maximum microorganism. Therefore 350µg is the concentration required to attain 50% inhibition of *Pseudomonas aeruginosa.* This study has confirmed the antimicrobial potential of the plant and thus supporting its promising possibility of finding new clinically effective natural source of bioactive compounds.

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