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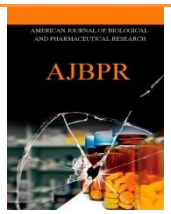

IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *EMBELIA RIBES* BURM.F. - A THREATENED MEDICINAL PLANT FROM KERALA

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ABSTRACT

Embelia ribes Burm.f. is the threatened medicinal plant belongs to the family Myrsinaceae. It is commonly known as false black pepper, is being used since the ancient times, in the form of the drug 'Vidanga'. The plant contains numerous medicinal properties and high commercial value. The root, berries and leaves of *E.ribes* is used in herbal formulas in various medicinal preparations. In the present investigation antimicrobial potential of leaves of *E.ribes* was studied against two bacterial strains. The dried powder was successively extracted with petroleum ether and acetone using soxhlet apparatus. The obtained concentrated extract was used for the antimicrobial activity study. Agar disc diffusion method was followed for the antimicrobial activity assay. The results revealed that the maximum zone of inhibition was observed against *Staphylococcus aureus* in acetone extract and minimum zone was observed against *Escherichia coli* in petroleum ether extract.

INTRODUCTION

Herbal medicine is still the mainstay of about 75-80% of the world population, particularly in the developing countries, for primary health care because of better cultural acceptability, compatibility with the human body and lesser side effects [1].

India is an emporium of medicinal and aromatic plants. It has been estimated that out of 15,000 higher plants occurring in India, 9000 are commonly used, of which 7500 are used in various systems of medicine [2]. Plant based drugs have been used worldwide in traditional medicines for treatment of various diseases. India is the largest producer of medicinal herbs and appropriately called the 'Botanical garden of the world' [3].

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The use of plant whether herbs, shrubs or tree, in parts or whole in the treatment and management of diseases dated back to pre-historic times. Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity [4]. During the last century, the practice of herbalism became main stream throughout the world. In spite of the great advances achieved in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medicinal systems. *E.ribes* is widely used as traditional herbal medicine in India. The plant is a climber with slender branches and long internodes. The leaves are elliptic, broad and covered with minute glands. The flowers are small, white racemes arranged in panicle inflorescence at the end of the branches. The fruits are berries, round, red to black colour and tipped with style [5]. In Indian system of medicine 'Ayurveda', the plant is popularly known as Vidanga or Bashmak or

Krimigna (Sanskrit); Baberangor Wawrung (Hindi); Vayuvilanga (Kannada) and it is used as one of the adjuvant in most of the drug preparations. The whole plant is used in the treatment of anti-inflammatory to relive rheumatism and fever [6]. The fruit is bitter in taste, good appetizer, cures tumors, ascites, bronchitis, jaundice and mental disorders [7]. Seeds are used as antibiotic, anthelmintic, anti-tuberculosis, alterative and stimulative [8]. Leaves are astringent, demulcent, depurative and useful in pruritus, sore throat, ulcers of mouth, indolecent, skin diseases and leprosy [9]. All the parts of this plant have the enormous medicinal properties. There was no previous work on leaves of this plant. So the present study focused on the antimicrobial properties of leaves of *E.ribes.*

MATERIALS AND METHODS

The leaves of the *Embelia ribes* Burm.f. was collected from Kakkayam, Kozhikode, Kerala and the plant material was identified by the experts at M. S. Swaminathan Research Foundation and also by literature survey.

Method of preparation of plant extract

The collected plant leaves were cleaned and shade dried for a week. 30g of powdered leaf was extracted successively with the following solvents petroleum ether and acetone using soxhlet apparatus. Finally the extracts were concentrated and kept in brown bottle at 4°C for antimicrobial activity studies.

Preparation of test organisms

The organisms selected for the present study is *Escherichia coli* and *Staphylococcus aureus.* Stock cultures of two bacteria were grown in nutrient broth at 30°C and were sub-cultured and maintained in nutrient broth at 4°C. Before swabbing, each culture was diluted (1:10) with fresh sterile nutrient broth.

Antibacterial assay

The antibacterial activity was determined by the agar disc diffusion method [10]. A suspension of the organism was added to sterile nutrient agar medium at 45°C. The mixture was transferred to sterile petriplates and allowed to solidify. Sterile disc of diameter 5 mm (made from Whatmann No. 1 filter paper previously sterilized in autoclave) was dipped in different extracts of the plant. Then the sterile disc containing test solution of the plant extracts were placed over the seeded agar plates in such a way that there is no overlapping of zone of inhibition. Standards and a blank were placed on the surface of agar plate. The plates were kept at room temperature for 2 h to allow diffusion of the test solution into the agar; they were incubated for 24 h at 37°C. After the incubation period was over, the plates were observed and zone of inhibition was measured in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the crude acetone extracts of *Embelia ribes* against *E.coli* and *S. aureus* was determined by using serial dilution technique [11]. 1 mg/ml of the sample solutions of all the extracts were prepared using Dimethyl Sulfoxide (DMSO). In this technique a large number of test tubes were used and each of the test tubes was filled with 1 ml of sterile nutrient broth media and graded doses of sample solution were added. Then these test tubes were inoculated with the selected organisms (inoculum contains 1×10^6 cells/ml) followed by incubation at 37°C for 24 hours to allow the growth of the bacteria. The test tubes which showed minimum concentration as well as clear content were selected. This lowest or minimum concentration was considered as Minimum Inhibitory Concentration (MIC). Another three test tubes containing medium, medium and sample, medium and inoculum were used as control. Bacterial growth observed was only in test tubes (solution content was cloudy) containing medium and inoculum and the other two were clear showing no growth [11].

RESULT AND DISCUSSION

Zone of inhibition was evaluated for *E. ribes* using *E. coli* and *S. aureus* against the leaf extracts obtained from acetone and petroleum ether (Table 1). The extract constituted with acetone has shown maximum zone of inhibition than that of petroleum ether extract. The zone of inhibition was found to be more in *S. aureus* than that of *E. coli* (Figure 1).

The minimum inhibitor concentration (MIC) of *E. ribes* leaves also studied and the result was displayed in Table 2. MIC value observed against *S. aureus* was 20µg/ml and *E. coli* was 45µg/ml. The result confirmed that low concentration of leaf extract show inhibition against *S. aureus*. The zone of inhibition also observed more in S. *aureus*

The demonstration of antibacterial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds [12]. The previous report on antimicrobial activity studies of this plant also supported our results. Chitra *et al.* [13] reported that embelin (100 µg) exhibited significant antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Shigella flexneri, Shigella sonnei* and *Pseudomonas aeruginosa*. Feresin *et al.* [14] reported that embelin inhibited both methicillin sensitive and methicillin-resistant strains of *Staphylococcus aureus* with MICs of 250 and 62 µg/ml, respectively. While the MIC for the methicillin-sensitive strain of *Staphylococcus aureus* was 250 µg/ml. Moreover, the MIC for *Escherichia coli* was 50 µg/ml. The plant has the potent activity; it may due to the presence of potential phytoconstituents embelin present in this plant.

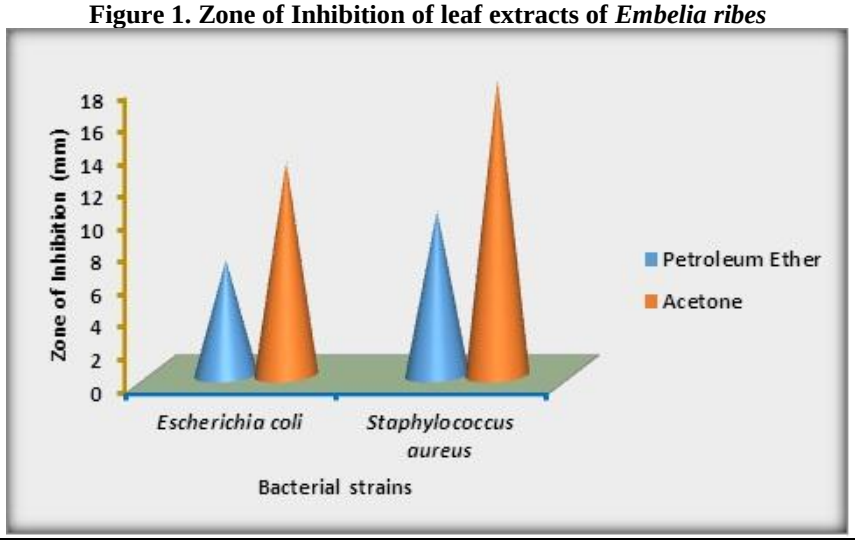
Table 1. Antibacterial Activity of leaf extracts of *Embelia ribes*

S. No	Solvents	Zones of Inhibition (mm)	
		Escherichia coli	*Staphylococcus aureus*
1.	Petroleum Ether	7.0	10.0
2.	Acetone	13.0	18.0

Table 2. MIC Values of leaf extracts (µg/ml) of *Embelia ribes* against tested bacteria

Solvent	Organisms	
	Escherichia coli	*Staphylococcus aureus*
Acetone	45.00±0.00µg/ml	20.00±0.00µg/ml

Figure 1. Zone of Inhibition of leaf extracts of *Embelia ribes*



CONCLUSION

In the present study, significant antibacterial activity was observed in leaf extracts of *Embelia ribes* against both gram positive and gram negative bacteria. Based on previous reports, the plant showed maximum activity against bacteria due to the presence of active principle embelin. Most of the studies represent its presence in all other parts of the plant specifically fruits of the plant. In the present study, we confirmed that it was also present in leaves based on the zone of inhibition. In future, active principle of the plant will be isolated for the preparation of drugs in pharmaceutical companies.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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