

In vitro* Evaluation of Antifungal Activity of the Seed Extract of *Embelia Ribes

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Antifungal activity of *Embelia ribes* was evaluated on eight different fungal species by employing various concentrations of seed extract (0.5-2.0 mg). All the concentrations of seed extract inhibited the fungal growth, whereas maximum activity was observed at 2.0 mg concentration of seed extract. Among different doses, the diameter of inhibition zones ranged from 9 to 18 mm in various fungal species and increased with the increase in the concentration of test solution. Among all the fungi, high inhibition zones were observed in *Colletotricum crassipes* (18 mm). This was followed by *Cladosporium* (17.5 mm), *Armillaria mellea* (17 mm), *Colletotricum capsici* (17 mm), *Aspergillus niger* (16.5 mm), *Rhizopus oryzae* (16.5 mm), respectively. *Aspergillus terreus* and *Candida albicans* showed less inhibition zones (15.5 and 16.0 mm) compared to other organisms. The present study clearly demonstrated the antifungal properties of *Embelia ribes*.

Key words: Antifungal activity, bioassay, *Embelia ribes*, seed extract, zone of inhibition

Higher plants are the source of numerous chemicals of commercial significance. They have impressive biological properties like antifungal, antibiotic, insecticide, pharmacological activities and are used as drugs, fragrance, food flavours, colours. In recent years, there is a great demand for plant-based products because of broad biological activities, low impact on environment and safety to non-target organisms. During last few decades, many plant species were screened and plants with high bioactive compounds were identified. *E. ribes* is an important medicinal plant with rich source of insecticidal and antimicrobial compounds.

Embelia ribes Burm. (Family-Myrsinaceae) commercially known as *Vidang* or *Baibirang* is one of the important medicinal plants of 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^[1]. The seed resembles so much to pepper and often referred as false pepper and pepper is also used to adulterate *Embelia*.

The fruits are astringent, carminative and stimulants. Traditionally the seeds are employed as a remedy for toothache, headache and snakebite. The seeds are mainly used for maintaining healthy skin and to support the digestive function^[2]. It is also effective in the treatment of fevers and for the diseases of chest. The plant is used in more than 75 Ayurvedic commercial formulations. Embelin is the principle chemical compound reported from the seeds^[3]. It is also used as antifungal agent in ringworm infection and other chronic dermatitis. It has laxative activity and used as anthelmintic agent^[4]. Seed extract possess antioestrogenic properties and specially used as contraceptive^[5]. The present study has been taken up

to evaluate the antifungal activity of *Embelia ribes* seed extract against various plant pathogenic fungi.

E. ribes seeds were collected from a private firm near Hyderabad, which were originally brought from the state of Kerala and authenticated in the Department of Botany, Osmania University, Hyderabad. Healthy, fresh seeds were washed with tap water for several times and dried under shade. Air-dried, finally powered seeds (500 g) were extracted with petroleum ether (60-80°) in a Soxhlet apparatus. The plant extract was further concentrated in rotavapour and residues were weighed. Different concentrations of stock solutions (0.5 mg to 2.0 mg) were prepared by dissolving the plant extract in dimethyl sulphoxide (DMSO).

Eight test organisms, *Aspergillus niger* (MTCC 281), *Rhizopus oryzae* (MTCC 262) *Aspergillus terreus* (MTCC 1281), *Cladosporium species* (MTCC 1003), *Colletotricum crassipes* (MTCC 2223), *Collectotricum capsici* (MTCC 2071), *Armillaria mellea* (MTCC 409) and *Candida albicans* (MTCC 183) were obtained from the Institute of Microbial Technology, Chandigarh and maintained on potato dextrose agar (PDA).

Agar cup bioassay was employed for testing of antifungal activity of plant extract^[6]. The ready-made PDA medium (Himedia, 39 g) was suspended in distilled water and autoclaved at pressure of 15 lb/sq in for 20 min. Seven day old cultures of test organisms (0.5 ml) were inoculated onto the medium. After inoculation, cups were scooped out from Petri plates with 8 mm sterile cork borer. To each cup, different concentrations of test solutions (0.5 to 2.0 mg) were added. Controls were maintained with DMSO and Bavistin (5 µg) was used as standard. The treated and the controls were kept in an incubator at 26° for 24 h to 78 h and inhibition zones were

TABLE 1: ZONE OF INHIBITION OF SEED EXTRACT OF *EMBELIA RIBES* FOR ANTIFUNGAL ACTIVITY

Test fungi	Concentration of seed extract (mg)										
	0.5	0.7	0.9	1.1	1.3	1.5	1.6	1.7	1.8	1.9	2.0
<i>Aspergillus niger</i>	9	10	10	11	12	12	13	13	13.5	14.5	16.5
<i>Aspergillus terreus</i>	9	10	11	12	13	14	14	14.5	14.5	15	15.5
<i>Rhizopus oryzae</i>	10	11	12	13	14	15	15.5	15.5	16	16.5	16.5
<i>Cladosporium species</i>	11	12	12	13	14	15	16	16.5	16.5	17	17.5
<i>Armillaria mellea</i>	10	11	12.5	13	13.5	14	15.5	16	16	17	17
<i>Colletotricum capsici</i>	10	11	12	12.5	13	14	15	15.5	16	16.5	17
<i>Colletotrichium crassipes</i>	10	11.5	12.5	13	14	14.5	15	16	17	17	18
<i>Candida albicans</i>	9	10	11	12	13	14.5	15	15.5	15.5	16	16

*Average values of four replicates.

measured. Three to four replicates were maintained for each treatment.

Effect of different concentrations (0.5-2.0 mg) of *E. ribes* seed extract against eight different fungi is given in the Table 1. The antifungal activity was observed in all the concentrations tested, but less effective below the 0.5 mg concentration of seed extract, hence the data was not included. The minimum activity of extract at lower concentrations may be due to the crude nature of the test solution. Among different doses, the diameter of inhibition zones ranged from 9 to 18 mm among different fungal species and increased with the increase in concentration of test solution. Maximum antifungal activity was observed at 2.0 mg concentration of seed extract. A similar study of screening the natural plant extracts against different fungal and bacterial pathogens was well recorded^[7-10]. Since plants have co-evolved with pathogens, it is reasonable to expect a variety of such compounds with specific as well as general antifungal activity^[11].

Different concentrations of the test solution inhibited all the fungal species with varying degree of sensitivity. Among all, high inhibition zones were observed in *Colletotricum crassipes* (18 mm). This was followed by *Cladosporium* (17.5 mm), *Armillaria mellea* (17 mm), *Colletotricum capsici* (17 mm), *Aspergillus niger* (16.5 mm), *Rhizopus oryzae* (16.5 mm) respectively. *Aspergillus terreus* and *Candida albicans* showed less inhibition zones (15.5 mm, 16.0 mm) compared to others organisms.

The present study clearly showed that the seed extract of *E. ribes* exhibited antifungal activity against many fungal strains, which included plant pathogens also. Thus there is a possibility of

developing *Embelia ribes* as an important source of biopesticide and antifungal agent. However, further studies are needed for isolation and purification of bioactive constituents.

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