ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2010; 1(3):267 - 270

ORIGINAL RESEARCH ARTICLE

In-Vitro Antimicrobial Activity of Fruits Extract of *Embelia ribes* Burm.

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Received 18 April 2010; Accepted 5 June 2010

ABSTRACT

Embelia ribes is a medicinal plant used intraditional Indian medicine for the treatment of various ailments. This plant was selected to evaluate their potential antibacterialactivity. To determine antibacterial activity and phytochemicals in the crude extracts of this medicinal plant used in traditionalIndian medicine for the treatment of various ailments like rheumatism, piles fever, skin diseases and snake bite. The antibacterial activity of aqueous and ethanolic extracts of this plant was determined by disc diffusion and broth dilution techniques against gram-positive bacterial strains (*Bacilus subtilis, Staphylococcus aureus*) and gram-negative bacterial strains (*Escherichia coli, Pseudomonas aeruginosa*). Results revealed that the aqueous and ethanol extracts of *Embelia ribes* exhibited significant antibacterial activity against gram-positive and gram-negative strains with minimum inhibitiory concentration (MIC) ranging from 1.5 to 100 mg/ml. The most susceptible organism to the ethanolic extract was B. subtilis and P. aeruginosa. The presence of phytochemicals such as alkaloids,tannins, triterpenoids, steroids and glycosides in the extracts of this plant supports their traditional uses as medicinal plants forthe treatment of various ailments. The present study reveals potential use of these plants for developing new antibacterial compoundsagainst pathogenic microorganisms.

Key words: Antibacterial, *Embeliaribes* fruits, Bacilussubtilis, Staphylococcus aureus, Escherichia coli, Pseudomonasaeruginosa.

INTRODUCTION

Embelia ribes Burm. is a threatened woody shrub belongs to the family Myrsinaceae, which is sparsely distributed in the moist deciduous forests of the Western Ghats, India, SriLanka,Malaysia and South China [1]. 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 [2]. The fruit is bitter in taste, good appetizer, cures tumors, ascites, bronchitis, jaundice and mental disorders [3]. Seeds are used as antibiotic, anthelmintic, antituber-culosis, alterative and stimulative[1].

Leaves are astringent, demulcent, depurative and useful in pruritus, sore throat, ulcers of mouth, indolecent, skin diseases and leprosy [4]. The traditional medical practitioners residing in the vicinity of the Lakkinakoppa forest range of Bhadra Wild life Sanctuary, are being used the tender leaf paste of this species to cure cut wounds and leprosy.

Fruits contain a quinone derivative embelin (3-undecyl 2,5- dihydroxy, 1,4-benzoquinone), an alkaloid christembine [5] and a volatile oil vilangin; its chemical constituentis 2,5-dihydroxy-4-undecyl-3, 6-benzoquinone [6]. The biological activities of this species have been evaluated for anti spermatogenic effect [7], urinary tract infections [8]. Literature review indicated that only the fruits of this species have been subjected to rigorous

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phytochemical and pharmacological studies. This paper reports the isolation of embelin from the leaves and comparative screening of wound healing property of embelin and the ethanol extract of the leaves on albino rats.

MATERIAL AND METHODS

Plant material

Embelia ribes fruit, Burm (Myrsinaceae), procured from locally Mandsaur District, (M.P), India, in August 2008, were authenticated by Dr. GyanenderTiwari (Head, Department of Aromatic and Medicinal Plant), K.N.K. College of Horticulture Mandsaur (M.P), India. The voucher specimen (BRNCP/Z/003/2008) was submitted in the Department of Pharmacognosy; B. R. Nahata College of Pharmacy, Mandsaur (M.P), India.

Preparation of ethanol extract

The *Embelia ribes* fruits were air dried in shade and were made to coarse size. The 1.5 kg coarse sized fruits were weighed and used for the extraction by using the soxhlet apparatus. These coarse sized fruits were defatted with petroleum ether for 72 hr. on 40 ^{0}C temperature. Then alcoholic extraction with ethyl alcohol was done 44 to 48 hr. at 40 ^{0}C temperature. After extraction, solvent was recovered by distillation. The concentrated extract was dried on water bath at 50 ^{0}C, made in powder form and the yield was 7.05 % w/w.

Preliminary Phytochemical Analysis

Preliminary phytochemical screening was performed to identify phytochemicals in the ethanolic extract of *Embelia ribes* fruits used in this study. This extract was subjected to preliminary phytochemical tests as described earlier[9]. Briefly, following tests has been performed for identifying the class of compounds.

Test for alkaloids

Of each extract 2 ml was acidified with a few drops of dilute hydrochloric acid and then 1 ml of Dragendorff reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

Test for tannins

To 2 ml of each extract, a few drops of 10% lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

Test for saponins

To 1 ml of each extract taken in a measuring jar, 9 ml of distilled water was added and shaken vigorously for 15 s and extracts were allowed to stand for 10 min. Formation of stable foam (1 cm) indicates the presence of saponins.

Test for steroids

Chloroform 10 ml was added to 2 ml of all the three plant extracts. To these extracts, 1 ml of acetic anhydride was added: then, 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Colour formation at the junction is noted. The appearance of blue-green colour indicates the presence of steroids.

Test for triterpenoids

The test for triterpenoids is same as that for steroids. The appearance of red, pink or violet colour at the junction indicates the presence of triterpenoids.

Test for cardiac glycosides

To 1 ml of each extract, a few drops of glacial acetic acid and ferric chloride, and 3-4 drops of concentrated sulphuric acid were added. The appearance of blue-green colour indicates the presence of glycosides.

All data shown in **Table. 1**

Table 1: Preliminary phytochemical analysis of the plant extracts *Embelia ribes*

Extract	Tannins	Saponins	Triterpenoids/ Steroids	Cardiac Glycosides	Alkaloids
Aqueous	-	+	-	-	+
Ethanolic	+	+	+	-	+

Antibacterial Activity

Microorganisms:

The antibacterial activity of the extracts was tested individually on gram-positive and gram-negative bacterial strains.

The gram-positive bacterial strains used were *Bacillus subtilis* and *Staphylococcus aureus* and gram-negative bacterial strains used were *Escherichia coli*,and *Pseudomonas aeruginosa*. Bacterial strains were maintained on nutrient agar at 4°C and sub-cultured every month in our laboratory.

Agar disc diffusion assay

The antibacterial activity of the extracts was determined by the disc diffusion method [10].

Briefly, overnight bacterial cultures were diluted in the Mueller-Hinton broth (O.D. 600=0.08) to obtain a bacterial suspension of 108 CFU/ ml. Petri plates containing 20 ml of Mueller-Hinton agar media were inoculated with 200 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Five filter paper discs (Whatman No. 1, 6 mm diameter) were placed on the inoculated agar surface. A 20 µl of the extracts (100 mg/ml) were loaded on to the filter paper discs and were allowed to dry completely. Standard antibiotics ciprofloxacin (10 µg) and 20 µl of DMSO were placed as controls. Plates were incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicate. All data shown in **Table. 2**

Table2. Results of antimicrobial activities of extracts of Embelia ribes

S/No	Organism	Zone of Inhibition (mm)		
		Aq. Extract	Ethanolic Extract	Std.
1	*S. faecalis*	6	12	28
2	*S. aureus*	7	23	27
3	*E. coli*	5	10	32
4	*B. subtilis*	8	24	35
5	*P. aerugenosa*	10	25	29

Std. = Standard.

Determination of minimum inhibitory concentration

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 h. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. Briefly, the stock solutions of the extracts were subjected to two-fold serial dilution in the Muller- Hinton broth to obtain concentrations from 100 mg/ml to 0.19 mg/ml. Standard antibiotics ciprofloxacin and DMSO were placed as controls. A 10 µl of 107 (CFU) bacterial cultures were added to the tubes and were incubated at 37°C for 18 h. MIC was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration [10]. All data shown in **Table. 3**

RESULTS

Phytochemical constituents present in the plant extract included tannins, saponins,sesquiterpenes, alkaloids, and phlobatamins. Results of the antimicrobial activity of the plant extracts are shown in **Table 2.**The result showsorganisms. The highest activity (diameter ofzone of inhibition 27mm) was demonstrated bythe ethanolic extract of *Embelia ribes* fruits against*Pseudomonas aerugenosa*while the lowest activity(diameter of zone of inhibition 2mm) wasdemonstrated by the water extract against*Escherichia coli*. The aqueous extractgenerally showed lower activity against the testorganisms compared to the ethanolic extract.Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are shown in **Table 3.** The result showed that *Streptococcus faecalis* had the highest MIC (16 mg/ml) and MBC (18.5 mg/ml), while thelowest MIC of 6 mg/ml was shown by*Pseudomonas aerugenosa*.

Table3. Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanolic extracts of Embelia ribes

S/No	Organism	MIC mg/ml	MBC
1	*S. faecalis*	16	18.5
2	*S. aureus*	8	17
3	*E. coli*	15.5	18
4	*B. subtilis*	8.5	17
5	*P. aerugenosa*	6	16

DISCUSSION

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromaticcompounds are secondary metabolites of plantsthat serve as defense mechanisms against predation by many microorganisms, insects andherbivores [11,12]. This may therefore explain the demonstration of antimicrobial activity by thefruits extracts of *Embelia ribes*. The demonstration of antibacterialactivity against both gram positive and gramnegative bacteria may be indicative of thepresence of broad spectrum antibiotic compounds [13]. This will be of immense advantagein fighting the menace of antibiotic refractivepathogens that are so prevalent in recent times.The result also showed that the ethanolic fruit extracts are more effective than the aqueousextract. This may be due to the fact that theethanolic extract contain was more phytochemicals thanthe aqueous

extractas it reported by phytochemical screening. Out of the two solvents used forextraction, the ethanolic extract showed thehighest activity against the test organisms, followed by the aqueous extract. Different solvents have been reportedto have the capacity to extract differentphytoconstituents depending on their solubilityor polarity in the solvent [12]. Ethanol extract inthis study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity. The demonstrationof antimicrobial activity by aqueous extractprovides the scientific basis for the use of theseplants in the traditional treatment of diseases, since most traditional medicine men use wateras their solvent in which the decoctions areprepared. Although the plant is used as adecoction with other plants as skin cleanser, allthe plant extracts tested did not show any antimycotic activity against any of the fungi atthe tested concentrations. Their cleansingactivity may be as a result of their synergy withcomponents from other plants and some othermetabolites.

CONCLUSION

The demonstration ofantibacterial activity by *Embelia ribes*mayhelp to discover new chemical classes ofantibiotic substances that could serve asselective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of thisplant in drug development for human consumption possibly for the treatment ofgastrointestinal, urinary tract and wound infections. The effect of thisplant on more pathogenic organisms andtoxicological investigations and furtherpurification however, needs to be carried out.

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