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Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *EUGENIA JAMBOLANA* SEED EXTRACT AGAINST HUMAN PATHOGENS

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ABSTRACT

Much work had been carried out on *Eugenia jambolana* that determine its pharmacological importance. The present study was devised to investigate the antimicrobial activity of *Eugenia jambolana* seeds, that was not utilizing by population as food and thrown as waste. The aqueous, ethanolic, methanolic, acetic acid and petroleum ether extracts of the *Eugenia jambolana* seeds were evaluated for its antibacterial activity against four gram positive, (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Bacillus megaterium*) and six gram negative (*Escherichia coli, Salmonella typhi*, *Salmonella paratyphi A, Salmonella paratyphi B, Pseudomonas aeruginosa* and *Proteus vulgaris)* pathogenic strains. The preliminary investigation of different solvent extracts shows aqueous and ethanolic extract had significant antimicrobial activity against tested microorganism as compared to acetic acid and petroleum ether extract. Methanolic extract show no antimicrobial activity. The *Eugenia jambolana* seeds show antimicrobial activity against *Salmonella paratyphi A, Proteus vulgaris, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Salmonella typhi and Pseudomonas aeruginosa.* However, effectiveness will varies depending upon the nature of solvent used for extraction, type of microorganisms against which it was tested and the dose of extract being administrated. Effective antimicrobial activity was demonstrated at 10mg/ml against tested microorganisms. The phytochemical analysis shows presence of flavanoids, cardiac glycosides, steroids and tannin that might be contributing for antimicrobial activity of the plant material. Hence, the seeds of *Eugenia jambolana* can be used as potential sources of antimicrobial agents.

Keywords: Antimicrobial, *Eugenia jambolana,* Jamun, Phytochemical.

INTRODUCTION

Eugenia jambolana, belongs to the member of family Myrtaceae, commonly known as jambul, black plum[1]. The *Eugenia jambolana* tree is large sized found in various countries like India, Bangladesh, Nepal, Pakistan, Srilanka, Indonesia, South-East Asia and Eastern Africa[2, 3]. *Eugenia jambolana* had been reported to contain phytochemicals like coumarin, flavanoids, glycosides, phenols, tannins and steroids[4]. The various part of *Eugenia jambolana* had got therapeutic applications. The bark of plant was used for anaemia and diabetes, the fruit was used for dysentery and leaves show antibacterial properties as well as used for increasing the strength of teeth and gums[5]. Seeds of *Eugenia jambolana* was reported to have hypoglycemic[6], anti-inflammatory[7], antibacterial[4,8], antiviral[9] and antidiarrheal effects[10]. This study was carried out with the aim to evaluate the antibacterial activity of *Eugenia jambolana* seed using different solvent extractions. It was reported earlier that the leaves of *Eugenia jambolana* had antimicrobial activity against *Salmonella typhi, Shigella dysentriae, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus*[11]. This study focus on extraction from seeds material as fruit was utilized as food and the seed obtained from it was contributed to waste. Hence, the study device to demonstrate the waste source (seeds) of *Eugenia jambolana* as potential source of antimicrobials.

MATERIALS AND METHODS

Collection of plant material

The seeds of *Eugenia jambolana* plant were collected from local market of Surat city during the study period. The authentication of plant material was carried out by expert at Bhagwan Mahavir College of M. Sc. Biotechnology, Surat. The collected seeds were washed with tap water to remove the unwanted material that may hinder investigation process. The washed seed materials were dried in the incubator at 40°C and ground into powder with the help of mortar and pestle. The ground materials were stored in air tight container for further use.

Extraction of Plant Material

The aqueous extracted was obtained by maceration techniques[12]. 30 grams of ground seed material were soaked in 140 ml of distilled water and the mixture was allowed to extract for 3 days at room temperature on a rotary shaker (Remi Instrument.) at 150 rpm. The aqueous layer was separated and air dried to make it concentrated. The ethanolic, methanolic, acetic acid and petroleum ether extracts were obtained by the Soxhlet extraction method[12] and the extract were then subjected to drying in open air. The extracts were stored at 4°C until investigation was completed.

Preparation of extract for antimicrobial activity

The extract obtained using different solvent system was dissolved in DMSO for antimicrobial activity assay to avoid false positive results.

Test bacterial strains

Pathogenic microbial strain like *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus*, *Bacillus megaterium, Escherichia coli, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Proteus vulgaris* and *Pseudomonas aeruginosa* were used to evaluate the antimicrobial activity of *Eugenia jambolana* seed extracts. All the pathogenic strains were obtained from Microbiology Diagnostic Centre, Surat, Gujarat, India. All bacterial cultures were maintained on Nutrient agar slant and stored at 4°C. Periodic subculturing of these strains was carried out.

Preparation of Media

The nutrient agar medium was prepared as per instruction manual from manufacturer (Hi-media, Mumbai). The media were boiled, sterilized at 121°C for 15 minutes in laboratory autoclave. 20 ml of sterilized medium was poured into sterile petridish and allowed to cool and solidify. The nutrient agar plates were dried at 37°C for 30 mins and used for the well diffusion method.

Preparation of the Inoculums

The inoculums of each test organisms were prepared prior to begin antimicrobial assay. Sub-culturing of each test organisms were carried out by taking loopful of microorganism from their slant and inoculating into test tubes containing nutrient broth. The inoculated test tubes were kept in incubator at 37°C for 24 hours. The inoculum having approximately 10^5CFU/ml was prepared to be used for the assay.

Screening for Antimicrobial Activity

Screening for antimicrobial activity of *Eugenia jambolana* seeds extracted using different solvent system was carried out by agar well diffusion assay[13] (Table 1). The loopful of inoculums of test microorganisms was spread onto the nutrient agar plate with the help of sterile glass spreader. The bore of 6 mm size was prepared into the agar plate using sterile cup borer. 1 ml of plant extract was poured into well. Antibiotics such as tetracycline (1mg/ml) (Table 2) were used as positive controls, while DMSO was used as negative controls. All the plates for antimicrobial activity were incubated at 37°C for 24hrs. The plates was examined for zone of inhibition and measured in mm. All the bioassays were conducted in triplicate to minimize the error.

Phytochemical screening

The presence of phytochemical in aqueous, ethanolic, methanolic, acetic acid and petroleum ether were investigated using the method adopted by various researchers. Phytochemical screening were carried out to detect the presence of Alkaloids[14], Tannin[15], Saponins[16], Flavonoids[16], free Amino acid[16], Cardiac glycoside[16], steroids and Terpenoids[16-17] (Table 3).

Dose dependent Antimicrobial susceptibility Test

The dose dependent antimicrobial susceptibility of *Eugenia jambolana* seeds was carried out for each extract. 1 mg/ml, 2 mg/ml, 5 mg/ml and 10 mg/ml doses of extract was prepared and was tested against test microorganisms for their antimicrobial activity. The plates for antimicrobial activity were incubated at 37°C for 24hrs. The plates was examined for zone of inhibition and compared with the tetracycline control. The zone was measured in mm (Table 4).

RESULTS AND DISCUSSIONS

The seeds of *Eugenia jambolana* show various therapeutic applications as hypoglycemic[6], anti-inflammatory[7], antibacterial[4, 8], antiviral[9] and antidiarrheal effects[10] as reported by different authors. Much work has been reported that demonstrate pharmacological significance of various plants material as natural products are widely used to cure diseases. The present study on *Eugenia jambolana* seed extract using various solvent systems was carried out to explore the potential antimicrobial activity of each seed extract. Antimicrobial activities of *Eugenia jambolana* seed tested against pathogenic microorganism's shows significant antimicrobial activity as reported by different researchers[4,8,11]. Preliminary screening for antimicrobial compounds, *Eugenia jambolana* aqueous extract was shown to be effective against *Bacilus subtilis, Bacillus megaterium, Bacillus cereus, Salmonella paratyphi A and Proteus vulgaris*. The dose dependent antimicrobial susceptibility study using aqueous extract show antimicrobial activity against *Bacillus cereus and Proteus vulgaris* only at concentration of 10mg/ml. *Eugenia jambolana* methanolic extract shows no zone of inhibition against tested microorganisms. The ethanolic extract shown to be effective against *Salmonella paratyphi A, Proteus vulgaris, Bacillus cereus, Bacillus megaterium* while dose dependent studies show effectiveness against *Proteus vulgaris* only at extract concentration of 10mg/ml. *Eugenia jambolana* seed acetic acid and petroleum ether extract shows zone of inhibition but was not significant to be considered positive however dose dependent study show effective antimicrobial activity against only *Salmonella typhi and Pseudomonas aeruginosa* respectively at 10 mg/ml extract concentration respectively. Hence, *Eugenia jambolana* seeds extract can be utilize as potential natural source of antimicrobial agents. The effectiveness of seeds as antimicrobial agent varies depending on the nature of extraction and microorganisms being tested. All the results of antimicrobial susceptibility testing were compared with tetracycline used as positive control. DMSO was used as negative control for each tested extract. The dose dependent studies reveal the effectiveness of extract as antimicrobials will increase with increase in dosage of extract.

The phytochemical screening of *Eugenia jambolana* seeds show presence of flavanoids, steroids and cardiac glycosides in aqueous extract. The methanolic extract shows presence of flavanoids, steroids, cardiac glycosides and tannin. The ethanolic extract show presence of only steroids and tannin whereas acetic acid extracts show presence of only steroids. The petroleum ether extraction show absent of above tested phytochemicals. Hence, the extractions of pharmacological compounds responsible for antimicrobial activity are solvent dependent.

In the present study, the antibacterial activity of *Eugenia jambolana* seeds extracts against clinically significant pathogenic microorganisms was reported and it was thus demonstrated that the active component in the plant material was extracted using different solvent system. However, significant antimicrobial activity was demonstrated by aqueous and ethanolic extract. The dose dependent studies reveal the effectiveness of extract as antimicrobials will increase with increase in dosage of extract. Majority of the antimicrobial activity will demonstrated at 10mg/ml against tested microorganisms.

Table 1: Preliminary Screening of *Eugenia jambolana* Seeds Extract for Antimicrobial Activity

Pathogens	Zone of Inhibition (mm)				
	Aqueous extract	Methanolic extract	Ethanolic extract	Acetic Acid extract	Petroleum ether extract
Escherichia coli	-	-	-	5	3
Bacillus subtilis	14	-	11	-	-
Bacillus megaterium	15	-	13	3	-
Bacillus cereus	13	-	14	6	-
Salmonella typhi	-	-	-	7	-
Salmonella paratyphi A	12	-	15	-	-
Salmonella paratyphi B	-	-	-	-	-
Proteus vulgaris	16	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	6
Staphylococcus aureus	12	-	16	5	-

Table 2: Antimicrobial Activity of Tetracycline Control

Pathogenic Organisms	Zone of Inhibition (mm)
Escherichia coli	11
Bacillus subtilis	14
Bacillus megaterium	10
Bacillus cereus	10
Salmonella typhi	6
Salmonella paratyphi A	12
Salmonella paratyphi B	10
Proteus vulgaris	5
Pseudomonas aeruginosa	7
Staphylococcus aureus	21

Table 3: Results of Phytochemical Screening of Eugenia jambolana Seeds Extracts

Phytochemical Test	Zone of Inhibition (mm)				
	Aqueous extract	Methanolic extract	Ethanolic extract	Acetic Acid extract	Petroleum ether extract
Alkaloids	-	-	-	-	-
Flavonoids	+	+	-	-	-
Steroids	+	+	+	+	-
Cardiac glycosides	+	+	-	-	-
Saponins	-	-	-	-	-
Tannins	-	+	+	-	-
Terpanoids	-	-	-	-	-
Amino acid	-	-	-	-	-

+ indicates = Positive results, - indicates = Negative results.

Table 4: Dose Dependent Antimicrobial Susceptibility Testing of *Eugenia jambolana* Seed Extracts

Pathogens	Seeds extract																			
	Aqueous mg/ml				Methanol mg/ml				Ethanol mg/ml				Acetic acid mg/ml				Petroleum ether mg/ml			
	1	2	5	10	1	2	5	10	1	2	5	10	1	2	5	10	1	2	5	10
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	2	5	2	7	-	-	-	-
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	-	-	3	4	9	10	2	3	2	6
Salmonella paratyphi A	2	6	9	11	-	-	-	-	5	5	5	10	-	-	-	-	-	-	-	-
Salmonella paratyphi B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	5	9	4	12	-	-	-	-	3	5	6	12	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4	8	10
Bacillus cereus	3	6	6	10	-	-	-	-	2	5	4	9	2	4	7	8	-	-	-	-
Bacillus subtilis	1	6	8	11	-	-	-	-	3	1	2	7	-	-	-	-	-	-	-	-
Bacillus megaterium	1	3	4	8	-	-	-	-	2	3	2	8	4	2	8	9	-	-	-	-
Staphylococcus aureus	2	3	4	9	-	-	-	-	2	1	1	6	1	2	5	7	-	-	-	-

CONCLUSION

The study of *Eugenia jambolana* seeds extract reveals that the seed material which was contributed to waste shall be utilizing to formulate the drug against pathogenic microorganisms and provide the directives to explore such pharmacological active compounds from natural source and to investigate the molecule(s) responsible for antimicrobial activities.

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