Journal of Scientific and Innovative Research 2015; 4(6): 232-236
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2015; 4(6): 232-236

Received: 20-12-2015
Accepted: 02-01-2016

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Antimicrobial activity of *Eugenia jambolana* seeds against foodborne isolates

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Abstract

Food borne diseases encompass a wide spectrum of illnesses associated with the ingestion of food contaminated by microbes. Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compounds with anti-microbial activity, with possibly new modes of action. *Eugenia jambolana* has been reported to contain phytochemicals like coumarin, flavanoids, glycosides, phenols, tannins and steroids. Diluted meat samples in PBS were inoculated onto culture plates of S S Agar, and Chromogenic agar and incubated at 370C overnight. After 48 hours incubation, cultures were examined for significant growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 hours. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation. Antibacterial activities of the plant extracts were tested on Mueller-Hinton agar by well diffusion method. Acetone, methanol and ethanolic *Eugenia jambolana* extracts against *S. aureus*, *Pseudomonas* species and *Salmonella* species showed a significant zone of inhibition. The objectives of the present study were as follows: Isolation of bacteria from meat, Study of biofilm production by the isolated bacterias and Screening of *Eugenia jambolana* extract for antibacterial activity against the isolated bacteria.

Keywords: Foodborne, *Eugenia jambolana*, Antimicrobial, Well diffusion.

INTRODUCTION

Doughari reports that long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Still these traditional medicines are included as part of the habitual treatment of various common infectious diseases [1]. On the same line Venkataswamy, states that since time immemorial plant based natural constituents have been derived from any part of the plant like bark, leaves, flowers, roots, fruits, and seeds [2]. Food borne diseases encompass a wide spectrum of illnesses associated with the ingestion of food contaminated by bacteria, viruses, parasites and chemicals as well as bio-toxins and they are a significant cause of morbidity and mortality worldwide [3].

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by the physicians; several are already being tested on humans. Scientists realize that the effective life span of any antibiotic is limited, so new sources; especially plant sources are also being investigated. Second, the public is becoming increasingly aware of the problems with the over prescription and misuse of traditional antibiotics. In addition many people are interested in having more autonomy over their medical care. A multitude of plant compounds is readily available over the counter from herbal suppliers, national food stores and the self medication with these substances is a common practice to certain extent [4].

Eugenia jambolana belongs to the member of family Myrtaceae, commonly known as jambul, black plum [5]. The Eugenia jambolana tree is large sized found in various countries like India, Bangladesh, Nepal, Pakistan, Srilanka, Indonesia, South-East Asia and Eastern Africa [6, 7]. *Eugenia jambolana* had been reported to contain phytochemicals like coumarin, flavanoids, glycosides, phenols, tannins and steroids. The various part of *Eugenia jambolana* had got therapeutic applications. *Eugenia jambolana* plant parts have been used in traditional system of medicine where its bark is acrid, sweet, digestive, and astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst, biliousness,

dysentery, blood impurities and to cure ulcers [8]. The seeds of *Eugenia jambolana* have been reported to have hypoglycemic, anti-inflammatory, antibacterial, antiviral and ant diarrheal effects. This study was carried out with the aim to evaluate the antibacterial activity of *Eugenia jambolana* seed using different solvent extractions. The objectives of the present study were as follows: Isolation of bacteria from meat, Study of biofilm production by the isolated bacterias and Screening of *Eugenia jambolana* extract for antibacterial activity against the isolated bacteria.

MATERIALS AND METHODS

Collection of meat samples

Raw meat sample was collected within 8 hours post-slaughter in order to minimize the microbial changes due to environmental temperatures and post-slaughter timings from Tiruchengode market, Namakkal district, India.

Meat sample preparation

Five grams of collected meat sample was weighed and transferred to a sterile flask containing 100 ml of phosphate buffer saline (PBS). The sample was grinded using a mortar and pestle under aseptic conditions and was stored for further analysis.

Bacterial isolation and identification

Diluted meat samples in PBS were inoculated onto culture plates of S S Agar, and Chromogenic agar and incubated at 37^0C overnight. After 48 hours incubation, cultures were examined for significant growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 hours. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation. Characterization and identification of the isolates was done using the methods of Cheesbrough [8]. The test organism was isolated from the meat sample.

Plant material

The seeds of *Eugenia jambolana* were collected from in and around Namakkal district. I was able to identify the plant by the help of Dr. Jegadeeshkumar, director, Chromopark Research Center Nammakal, India. The seeds of the plant were washed with de-ionized water and shade dried for 3 days. The shade dried seeds were powdered using a mechanical grinder and passed through 40 mesh sieve.

Experimental Design

Plant active components were extracted using the decoction extraction method [9]. Acetone, methanol and ethanol were used for the extraction. 100ml of pure ethanol, acetone and methanol were added to 5g portions of the plant powder in sterile conical flasks individually and allowed to boil in a boiling water bath for 48 hours with a water bath set at 40°C. The filtrate was obtained by means of filtering through a Whattman no.1 filter paper. The filtrate was evaporated in a weighed flask in a hot air oven set at 50°C. Extracts were reconstituted by re-dissolving in respective solvents. Sterile extracts obtained were stored separately in labeled, sterile capped bottles, in a refrigerator at 4°C.

Preparation of plates

Mueller Hinton Agar (MHA) medium (7.98 g) was mixed with100 ml of sterile distilled water and sterilized by autoclaving at 120°C for 20 minutes. Under aseptic conditions, in the laminar flow hood 15 ml of MHA of medium was dispensed into pre-sterilized petri dishes to yield a uniform depth of 4 mm. After solidification of the medium, the bacterial cultures were inoculated by spread plating technique. In this study, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas* and *Shigella* species were used as the test organisms.

Grouping of plates

A total of 48 plates were prepared and they were divided into four groups of 12 plates each.

Group I: 12 MHA plates were inoculated with Salmonella species and they were further sub-divided into groups of 3s and each group was inoculated with different concentrations of ethanolic, methanolic and acetone plant extracts.

Group II: 12 MHA plates were inoculated with Staphylococcus aureus and then sub-divided into 3 groups and then inoculated with varying concentrations of ethanolic, methanolic and acetone plant extracts.

Group III: 12 MHA plates were inoculated with Pseudomonas species and then sub-divide into 3 sub-groups and each group was inoculated with varying concentrations of plant extracts of ethanol, methanol and acetone.

Group IV: 12 MHA plates were inoculated with Shigella species and then sub-divided into 3 sub-groups and each was inoculated with different concentrations of ethanolic, methanolic and acetone plant extracts.

Test microorganisms used:

The following microorganisms were used in this study; *Shigella species, Salmonella species, S. aureus species* and *Pseudomonas species.*

Culture media and Solvents used:

The following culture media and solvents were used in this study; Chromogenic media, S S agar, Nutrient broth, Biofilm media and Muller Hinton agar, Acetone, Ethanol and Methanol.

Determination of antibacterial activity

Agar well diffusion method

Antibacterial activities of the extracts were tested on Mueller-Hinton agar by well diffusion method. The inoculums were spread evenly over the entire surface by swabbing in three directions using sterile cotton swab (Anusha *et al.,* 2009). After the medium was solidified a well was made in petriplates with the help of a sterile metal borer (6mm). The different concentrations of each extracts were filled in each well by using adjustable volume digital Finn pipette. Different concentration of plant extract used for the determination of antibacterial activity was: 25, 50, 75 and 100 µl. After that the plates were incubated at 37^0C for 24hrs. After proper incubation, antibacterial activity was determined by measuring the diameter of the zone of the inhibition around the well. The above mentioned procedure was fallowed for all plant extracts [7].

Determination of biofilm producing isolates

Freeman *et al*. (1995) have described a simple qualitative method to detect biofilm production by using Congo Red Agar (CRA) medium. CRA medium was prepared with brain heart infusion broth 37 g/L, sucrose 50 g/L, agar 10 g/L and Congo red indicator 8 g/L. First Congo red stain was prepared as a concentrated aqueous solution and autoclaved (121ºC for 15 minutes) separately from the other medium constituents. Then it was added to the autoclaved brain heart infusion agar with sucrose at 55ºC. CRA plates were inoculated with test organisms and incubated at 37ºC for 24 h aerobically. Black colonies with a dry crystalline consistency indicated biofilm production.

RESULTS

Isolation of food borne pathogens

The beef meat sample that was used in this study showed high bacterial count as shown in Table 1, Fig 1 and Fig 2. Gram-negative bacteria such as *Salmonella species, Shigella species, and Pseudomonas species* predominated in the meat sample, whereas frequently observed Gram-positive bacteria included *Staphylococcus aureus* species. In general, a total of 7 potential pathogenic bacterial isolates were obtained from the collected meat sample yielding a number of isolates. Among the 4 bacterial isolates *Salmonella spp* was highly predominant in my present study.

Table 1: Bacterial isolates from the meat sample on SS agar and Chromogenic mediums

S. No.	Bacteria	Colour of colony
1.	*Shigella species*	Pink
2.	*Salmonella species*	Black
3.	*E. Coli*	Red
4.	*K. pneumonia*	Blue to Purple
5.	*Staphylococcus aureus*	Golden yellow
6.	*Enterococcus faecallis*	Blue to green
7.	*Pseudomonas species*	Colorless

Determination of biofilm producing bacterial pathogens

In the current study, two isolates were strong positive for the biofilm production in vitro (Table 2 & Fig 3). The results of the biofilm production by the Congo red agar method showed three types of results (Fig 3). The results were confirmed with various colour formations on the Congo red medium (Fig. 3). The two, isolates produced black with dry crystalline consistency which is indicative of strong biofilm formation. One isolate was black in colour but was not dry and crystalline and hence was moderate for biofilm formation. One isolate displayed pink colonies occasionally darkening at the centers on typical biofilm, which result indicate as weak positive (Table 2 & Fig 3).

Table 2: Biofilm production on Congo red media

S. No	Bacteria	Colour on Congo red media
1.	*Pseudomonas species*	Black with dry crystalline consistency
2.	*Shigella species*	Black with dry crystalline consistency
3.	*Staphylococcus aureus*	Black but not dry
4.	*Salmonella species*	Pink

Antibacterial activity of plant extract against food borne pathogens

Different solvent extracts of *Eugenia jambolana* seeds showed antibacterial activity against all the test organisms**.** Acetone, methanol and ethanol extracts were tested against various Gram-negative and Gram-positive bacteria (Table 3.1 to 3.4, Fig. 4 to 7). The extracts assayed, seed extracts of *Eugenia jambolana* exhibited good growth inhibitory activity against *Shigella species* and the diameter of the zone of inhibition was found to be 35mm (Fig. 7 and table 3.4). This was followed by *S. aureus* (Fig. 4 and table 3.1), *Pseudomonas species* (Fig. 5 and table 3.2) and *Salmonella species* (Fig. 6 and table 3.3) with zone of inhibition measuring 30mm, 27mm, and 21mm respectively. Ethanol and acetone extracts also showed good inhibitory activity followed by methanol extract against the above mentioned isolates. In this study, same extract exhibited same measurements i.e., 75 µl and 50 µl of methanol extract measured 23mm against *S. aureus species* (table 3.1). Among the 4 isolates, *Shigella species* was highly suppressed by the plant extract followed by *S. aureus*. The result was tabulated in table 3.1 to 3.4.

Table 3.1: Antimicrobial activity of *Eugenia jambolana* against *S. aureus*

S. No	Solvent used	Zone of inhibition in mm			
		100µl	75µl	50µl	25µl
1.	Acetone	30	25	24	22
2.	Ethanol	30	24	22	20
3.	Methanol	25	23	23	20

Table 3.2: Antimicrobial activity of *Eugenia jambolana* against *Pseudomonas* species

S. No	Solvent used	Zone of inhibition in mm			
		100µl	75µl	50µl	25µl
1.	Acetone	27	25	23	16
2.	Ethanol	24	15	13	11
3.	Methanol	23	20	18	14

Table 3.3: Antimicrobial Activity of *Eugenia jambolana* against *Salmonella* specie

S. No	Solvent used	Zone of inhibition in mm			
		100µl	75µl	50µl	25µl
1.	Acetone	12	10	12	11
2.	Ethanol	21	17	16	14
3.	Methanol	18	14	13	11

Table 3.4: Antimicrobial Activity of *Eugenia jambolana* against *Shigella* species

S. No	Solvent used	Zone of inhibition in mm			
		100µl	75µl	50µl	25µl
1.	Acetone	30	28	24	21
2.	Ethanol	35	29	27	20
3.	Methanol	26	25	21	20

DISCUSSION

Most of the bacterial biofilm formation is growth dependent. Hence, it is important to know whether the biofilm formation is growth dependent or growth independent. The regular and improper use of antibiotics may lead to drug resistance and will make the drugs ineffective against common microbial infections. The main factor contributing to microbial resistance is the biofilm formation by the microbes that allow them to withstand extreme environmental conditions and antimicrobial agents. The biofilm forming bacteria are resistant to antimicrobial agents due to the lack of penetration of antimicrobial agents [7].

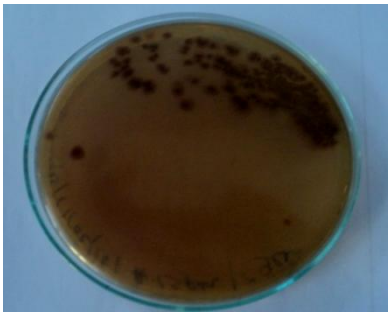

Fig 1: Isolates obtained from SS agar

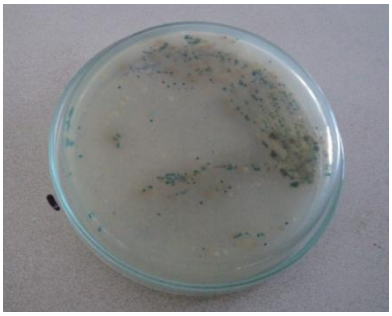

Fig 2: Isolates obtained from Chromogenic media

Fig 3: Biofilm formation by the isolates

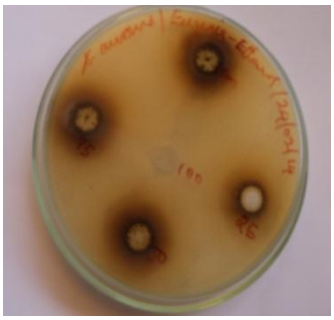

Fig 4: Antimicrobial activity of *E. jambolana* against *S. aureus*

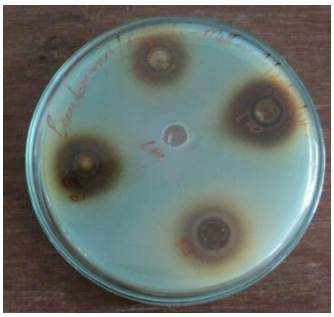

Fig 5: Antimicrobial activity *of E. jambolana* against *Pseudomonas* species

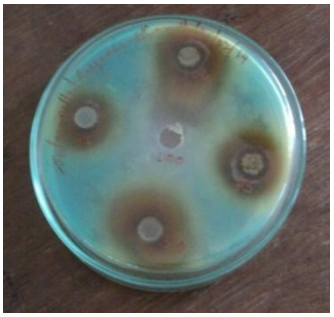

Fig 6: Antimicrobial activity of *E. jambolana* against *Salmonella* species

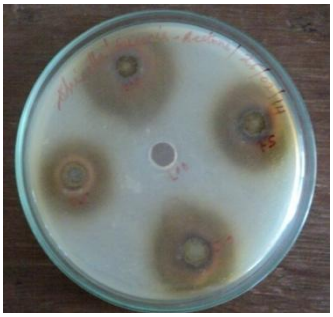

Fig 7: Antimicrobial activity of *E. jambolana* against *Shigella* species

In recent years, much of the research has been focused to identify alternative medicines to treat the infections caused by the drug resistant organisms. Various chemicals have been tested for their antibiofilm activities. Unfortunately, those chemicals cannot be used as drug molecules to treat the diseases associated with the biofilm. The alternative to the chemical antibiofilm agents is natural source. Plant derived molecules have found potential applications in pharmaceutical industry. Plant extracts and other biologically active compounds isolated from leaves, stems, and roots have gained interest in the antibiofilm activity. Against these backdrops, in the present study, food bacterial isolates were isolated and identified as biofilm producing strains. The biofilm producing ability and antibiofilm activity of plant extracts against 4 bacterial species have been reported.

In this study, 4 types of bacterial species namely *S. aureus, Pseudomonas, Salmonella* and *Shigella species*, were obtained from a meat sample. These bacterial pathogenic isolates were observed by selective media. All isolates were subjected to biofilm formation with Congo red agar plate method. Among the 7 isolates, 4 were biofilm producers. The seed extracts of *Eugenia jambolana* exhibited good activity against *Shigella* at 100µl for example; 35 mm was recorded as diameter of zone of inhibition. This was followed by 30 mm, 27 mm and 21mm, for *S. aureus, Pseudomonas,* and *Salmonella species* respectively. Ethanol and acetone showed good inhibitory activity. A parallel study by the authors employing ethanolic, aqueous and acetone extracts of fruits of *Emblica officinalis* against *Staphylococcus aureus* and *E. coli* revealed that the extracts were antibacterial against gram positive than gram negative group of bacteria, Saeed and Tariq also observed effective activity of *Emblica* against *S. aureus* [10]. Patil has observed maximal antibacterial activity for methanol extract of fruits against *S. aureus* whereas the acetone and aqueous extracts inhibited the growth of *E. coli* and *K. pneumonia* maximally [11].

The antibacterial activities of four extracts were assayed *in vitro* by agar well diffusion method against 4 different bacterial species. The results revealed that all extracts exhibited antimicrobial activity with different efficacy for different pathogens. All the extracts exhibited the zones of inhibition ranging from 11 to 35 mm against *S, aureus, Shigella, Pseudomonas and Salmonella.* In this study all plant extracts showed that Gram positive bacteria are susceptible to plant extracts more as compared to Gram-negative bacteria as reported previously by Mbwambo**,** Moshi and Bartfay [12, 13]. This difference can be attributed to membrane compositions of bacteria.

CONCLUSION

Based on the results described, we may conclude that the methanol, ethanol and acetone extracts of *Eugenia jambolana* seeds has significant antimicrobial activities *against S, aureus, Shigella, Pseudomonas and Salmonella* isolates. However further studies are needed to find out the active compounds of this plant. It is possible to find the better therapies for many microbial diseases from the plants. The *Eugenia jambolana* would be helpful in the treatment of microbial diseases.

Acknowledgement

I give my gratitude and heartfelt thanks to Kenyatta University for sponsorship of the project work.

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