International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **2(4),** 28-32, April **(2013)** *Int. Res. J. Biological Sci.*

Antibacterial Property of Two Different Varieties of Indian Mango *(Mangifera indica)* Kernel Extracts at Various Concentrations against some Human Pathogenic Bacterial strains

Prakash Alok[1], V Keerthana[1], Jha Chandan Kumar[1], Kumar Ratan[1] and Agrawal Dinesh Chand[2]*
[1]School of Biosciences and Technology, VIT University, Vellore-632014, Tamilnadu, INDIA
[2]School of Biotechnology, Banaras Hindu University, Varanasi-221005, UP, INDIA

Available online at: www.isca.in
Received 7th February 2013, revised 12th February 2013, accepted 10th March 2013

Abstract

The study was carried out to find a natural source of antibacterial agent because of the drug resistant of bacterial pathogens to the commercially available antibacterial agents in the market and at the same time to enhance the utilisation of the waste products from the mango processing industries. Mango seeds and kernels are the major by product of any mango processing industry. These kernels, as a waste product, cause tremendous investment of capital to decompose it safely and to prevent any environmental pollution. If these waste products can be converted as a raw material for the production of any bioactive compounds, then it will keep the food processing industries free from investing its capital in decomposing these wastes. Instead, it can be used efficiently for any useful products, giving them an extra profit. The mango samples studied in our work were Bagnapalli and Senthura varieties of Mangifera indica. The fruit samples were collected from Vellore district of Tamilnadu. The spread plate technique was used to determine the antibacterial property. The count of viable cells after the application of kernel extracts to the bacterial pathogens was used for the determination of antibacterial property of the kernel extracts. Bagnapalli variety was found to have more antibacterial activity than the Senthura variety of Mangifera indica. At the concentration of 10% of kernel extracts, no viable colony was found in the Petri dish of Bagnapalli kernel extract and hence it proves to be a potent antibacterial agent.

Keywords: Kernel extracts, antibacterial activity, bacterial pathogens.

Introduction

Since the beginning of human civilisation, we have been exploiting our natural resources for eradication of common human pathogen borne diseases. Various developing countries over the globe have been ravaged by bacterial pathogen borne diseases. Mostly, three diseases, namely, tuberculosis, malaria, and AIDS attributes to the majority of infectious diseases. These diseases on a whole, proves fatal for the human population of 5 million and causes sickness to the human population of 300 million each year, all over the globe[1]. The diseases caused by bacterial pathogens are mostly spread by the street vendors, where the food materials are unprotected from flies, carrying food borne pathogens[2]. These infectious diseases can be prevented by controlling the growth of food borne pathogenic microorganisms and food spoilage[3].

Mango (*Mangifera indica* L.), a fruit consumed worldwide belongs to the family Anacardiaceae, which is also well known as "Maamphazham" or "maangani" in Tamil Nadu. It is mostly found in tropical countries like India. Various products are found in India as mango processed food products. These processed food leads to enormous generation of mango seeds as a waste product. These waste products can be utilised for their antibacterial activity.

Mango is mostly used in food processing industries such as Juice industries, jam industries, jelly industries, and pickle industries. Mango kernel is the major waste product of these industries. It needs a huge capital to decompose these kernels to make sure that it does not pollute the environment. To save this investment in the disposal of mango kernels, it can be converted as a raw material for pharmaceutical industries based on the result of the current research work.

Antibacterial compounds are the agents which suppress the growth of bacteria and can also be fatal for the bacterial population. Potent antibacterial activity has been exhibited by mango kernel extracts[4]. There is rarely any antibiotic which has an inhibiting effect on all microorganisms[5].

The bacterial pathogens are becoming resistant to the commercially available antibiotics in the market[6,7]. This is because of the random use of these drugs. It creates a need to find a new bioactive source of antibacterial compounds. Thus, the research work was carried on to get an idea of antibacterial property in mango kernel extracts and its comparison at various concentrations. Since the beginning of human civilisation, various different parts of plants, such as leaves, bark, seeds, peels of fruits, and other plant organelles are being used in the form of extracts either in hot water or alcohol. These extracts are being used as ethnic drugs[8,9].

Unlike the antimicrobial agents synthesised by chemical sources, those from natural sources are readily accepted by the consumers. Thus, the development of new antimicrobial agents is a major research opportunity for the researchers at present. Previously, the antimicrobial agents have been reported from the essential oils of spices[10], oil from fishes[11], and various plants[12].

Material and Methods

Chemicals: Nutrient media agar, ethanol, distilled water, and Ampicillin, used as the standard antibacterial agent were obtained from the Instrumental and Food analysis laboratory of School of Biosciences and Technology, VIT University, Vellore.

Sample Preparation: Mango fruits were obtained from the local market in Vellore, near VIT University, between the month of March and April 2012. Two different varieties of mango for the research include "Bagnapalli" (figure 1) and "Senthura" (figure 2). The senthura seeds (figure 3) and Bagnapalli seeds (figure 4), for investigation was manually taken out from the flesh of mango and washed to remove the adhered pulps. The seeds were then dried in the open sun for few days. It was boiled in hot water at about 100 degree Celsius for few minutes and then cut manually by knife to take out the kernel from inside the mango seed. The separated mango kernels were cut into small pieces manually (figure 5, 6), minced in mixer along with 200ml of distilled water to make it a paste. The paste was centrifuged at 12000 rpm for 10 minutes and 20ml of the clear supernatant was separated in another falcon tube. These were stored for further usage at 4^{o} C.

Bacterial cultures: The human pathogenic bacterial strains used for the present study were one strain each of gram+ve i.e. *Staphylococcus aureus* (figure 7) and gram-ve bacteria i.e. *Pseudomonas aerugenosa*. These bacterial cultures were obtained from Instrumental and Food analysis laboratory, VIT University, Vellore. The obtained bacterial cultures were sub cultured in a 100ml conical flask. The culture was streaked to obtain a pure strain of bacterial species and it was stored for further usage. Whenever required, the cultures from the Petri Plates were inoculated in a 100ml conical flask (figure 8) for overnight and were used for the research.

Figure-1
Bagnapalli Mangoes

Figure-2
Senthura Mangoes

Figure-3
Senthura seeds

Figure-4
Bagnapalli Seeds

Figure-5
Bagnapalli kernel pieces

Figure-6
Senthura kernel Pieces

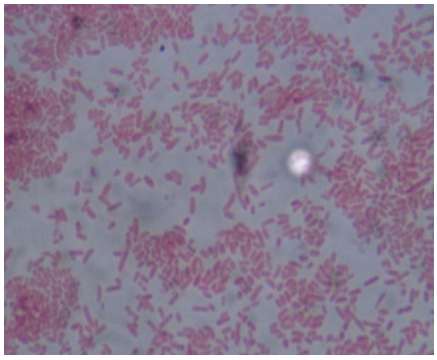

Figure-7
Microscopic study of Bacterial culture taken for study

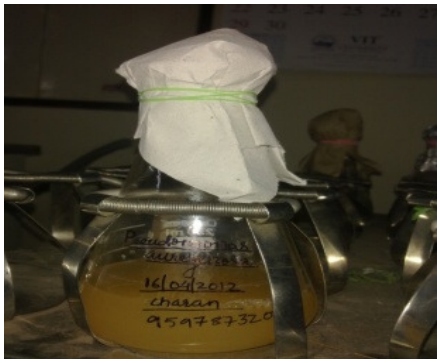

Figure-8
Subculturing of Bacterial strains

Gram staining: Gram staining was done to confirm the bacterial strains to be gram positive or gram negative. Gram stain is a differential stain. Gram-positive cell has a thick peptidoglycan cell wall that is able to retain the crystal violet iodine complex that occurs during the staining, while gram negative cells have only a thin layer of peptidoglycan. Thus gram-positive cells do not decolourise with ethanol, and gram-negative cells do decolorize. This allows the gram-negative cells to accept the counter stain safrarin. Gram-positive cells will appear blue to purple, while gram negative cells will appear pink to red.

Antibacterial activity test: The antibacterial activity tests of various kernel extracts were obtained by Spread plate method and counting the viable bacterial cells. The supernatant liquid obtained from centrifugation of the kernel extract was added in three different volumes of 2ml, 5ml, and 10ml to create 2%, 5%, and 10% concentrations of kernel extracts in the three different conical flasks containing 100ml of Nutrient Hi-Veg agar, after it is autoclaved. The respective plates were made for different concentrations of mango kernel extracts and 0.2 ml of the bacterial culture from 10^{-2} dilution factor was swabbed over the hardened agar. The plates inoculated with bacterial cultures were incubated at 37^{o}C for 24 hours. The colony count was done by the help of electronic colony counter. The positive control (figure 9) and negative control (figure 10) was performed to check any contamination. Positive control has only the bacterial strain without any kernel extract solution and the negative control do not have the kernel or the bacterial strain under study. Ampicillin was used as the standard antibacterial agent, in the concentration of 2%, 5%, and 10% for the comparison of efficacy of the antibacterial activity of the mango kernel extracts with the available drug in the market.

Figure-9
Positive control with *Pseudomonas aerugenosa* and without Kernel extracts

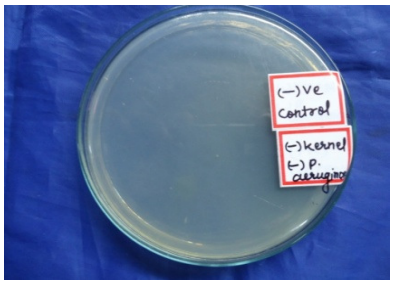

Figure-10
Negative control without Kernel extracts as well as *Pseudomonas aerugenosa*

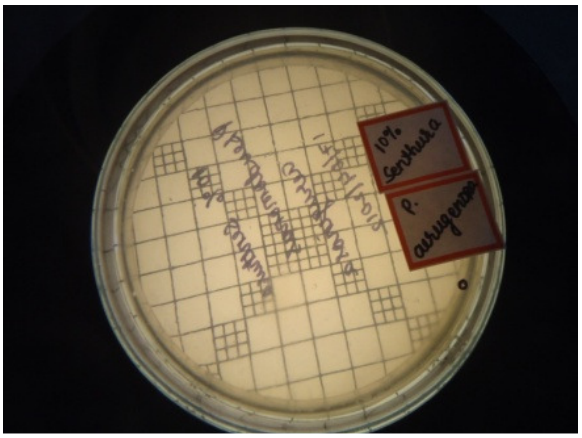

Figure-11
Plate with 10% Kernel extract

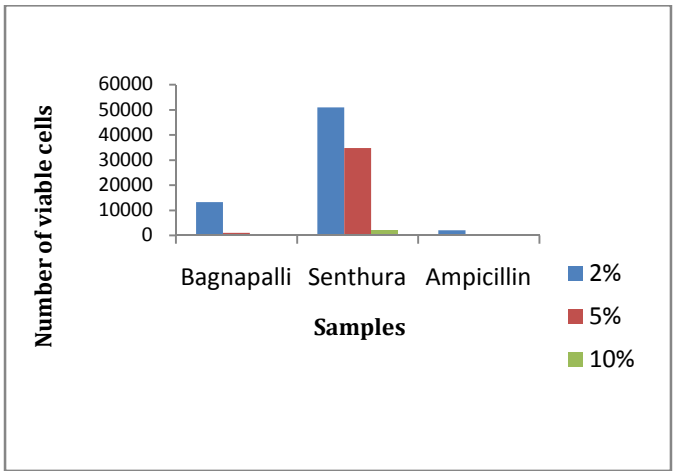

Figure-12
Antibacterial activity of samples against *Staphylococcus*

Statistical Analysis: All the experiments were performed thrice, and the average value was considered for the final calculation. The dilution factor in our study was 10^{-2} and the test volume used for the study of antibacterial activity was 0.2ml. The number of viable cells per ml of the culture was calculated by the following equation:

$$\text{Number of cells/ml} = \frac{\text{Number of colonies}}{\text{Test volume} \times \text{Dilution factor}}$$

Results and Discussion

The biologically active compounds used for the treatment of various infectious diseases have always been found to be obtained from many medicinal plants[13].

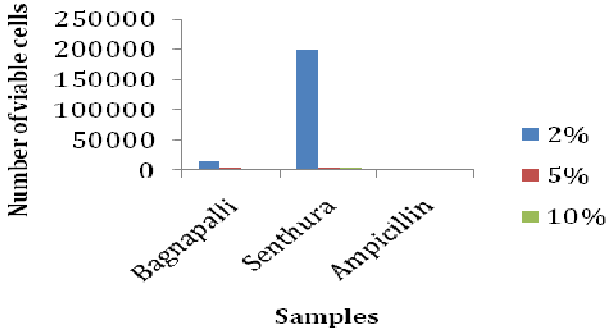

Figure-13
Antibacterial activity against Pseudomonas

Table-1
Number of viable cells for different concentrations of kernel extracts

Bacterial species	Mango variety	% of mango kernel extract	Average Colony count/plate	Average no. of cells /ml
Staphylococcus aureus	Bagnapalli	2	27	13300
Staphylococcus aureus	Bagnapalli	5	2	1000
Staphylococcus aureus	Bagnapalli	10	0	0
Staphylococcus aureus	Senthura	2	102	51000
Staphylococcus aureus	Senthura	5	69	34800
Staphylococcus aureus	Senthura	10	4	2000
Staphylococcus aureus	Ampicillin	2	4	2000
Staphylococcus aureus	Ampicillin	5	1	500
Staphylococcus aureus	Ampicillin	10	0	0
Pseudomonas aerugenosa	Bagnapalli	2	29	14500
Pseudomonas aerugenosa	Bagnapalli	5	7	3600
Pseudomonas aerugenosa	Bagnapalli	10	0	0
Pseudomonas aerugenosa	Senthura	2	401	200000
Pseudomonas aerugenosa	Senthura	5	6	3830
Pseudomonas aerugenosa	Senthura	10	5	4330
Pseudomonas aerugenosa	Ampicillin	2	3	1500
Pseudomonas aerugenosa	Ampicillin	5	1	500
Pseudomonas aerugenosa	Ampicillin	10	0	0

Antibacterial effect of mango seed kernel extract against several bacterial species has been recognised and considered as one of the most important properties linked directly to their possible biological applications. The results of antibacterial effects of mango seed kernel extracts and the colony count of bacterial strains are given in the (table 1). The concentration of the kernel ranges from 2% to 10%. In the 2% mango kernel extract there were enormous colonies whereas there were rarely any colonies in 10% mango seed kernel extract (figure 11). Bagnapalli kernel extracts showed a better antibacterial activity compared to Senthura kernel extracts and also, Bagnapalli has a better effect against *Pseudomonas* when compared to *Streptococcus* strain of human pathogenic bacteria. Figure 12 and figure 13 can be observed for a better picture of the results obtained above.

Mango kernel extracts of Bagnapalli variety can be utilised as an alternative antibacterial agent in the treatment of infectious diseases caused by the human pathogenic bacteria considered for the research study. Further studies must be done in order to elucidate the mechanism of action of the active compounds in the sample extracts which contribute to the antibacterial activity. It is very much needful that the effects of the compounds on animals and human cells should be investigated, along with the toxicity, the positive or negative reactions, and also the action mechanisms, so that it should be confirmed that the compounds are safe and have no side effects on human and animals health[14].

Conclusion

All extracts showed antibacterial property with the bacterial strains under study. Bagnapalli showed more effective result. There were no colonies in 10% kernel extract of Bagnapalli variety but there were few colonies growth in 10% of senthura kernel extracts. Bagnapalli variety of *Mangifera indica* showed the antibacterial activity nearly same as the Ampicillin, taken as the standard antibacterial agent. The bacterial colonies were enormous in 2% kernel extracts solution. This property of Bagnapalli and senthura kernel extracts can be used in various industrial applications in food additives and pharmaceutical compositions for the drugs being used against various human bacterial pathogens. The dosage of the extract has to be standardized for human being. It needs further studies and trials for its effective usage.

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