



RESEARCH ARTICLE

Study of Antibacterial Activity of Different Organic Solvent Extract from Three Mango Cultivar Kernel on *Staphylococcus aureus*

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Abstract

A study of the antibacterial activity of two solvents, aqueous and methanol extract, from three varieties of mango seeds kernel, was done against gram-positive *Staphylococcus aureus* bacterial strain. Antibacterial assay was studied by “agar disc diffusion method”. Three antibiotics Rifampicin, Gentamicin and Amoxicillin were taken as references in this study. Among the three antibiotics, gentamicin showed greater inhibitory activity against *S. aureus*. The methanolic kernel extract of ‘Banganapalle’ and kernel aqueous extract of ‘Himsagar’ showed higher anti-microbial activity against test pathogen suggesting the presence of active phytochemicals among the three varieties of mango which can be used as an alternate source of medicine for the treatment of bacterial infections. This finding could be utilized as an alternative cheaper source of natural anti *staphylococcus* resources.

Introduction:

Mango fruit (*Mangifera indica*) of genus *Mangifera* and Anacardiaceae family, originated in South Asia and thereafter dispersed to the rest of the world (Omutubga *et al.*, 2012). A total of 69 species of *Mangifera* genus is known and are mostly restricted to tropical Asia (Soong & Barlow, 2004; Ashoush & Gadallah, 2011).

It has been reported that for over 4000 years, indigenous medical systems and Ayurvedic, used different parts of the mango tree due to its various medicinal properties (Litz, 2009). These medicinal properties of seed kernel are thought might be due to the phytochemicals present in it which acts against various diseases reported. Earlier reports were Abdullah *et al.*, (2011) available on the anti-inflammatory activity of alcoholic mango seed extract against acute, sub-acute and chronic cases. Antibacterial activity of ethanolic mango seeds extract was reported against microorganisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus pyogenes* etc. by Sowmiya *et al.* (2009). The compounds responsible for the antimicrobial property in mango seed are mainly due to the presence of phytochemicals like flavonoids, terpenes, tannins, coumarins and phenolic, etc., (Maisuthisakul & Gordoan, 2009; Gupta *et al.*, 2015). Mango seed kernels are also a rich source of various phenolic compounds viz. tannic acid, gallic acid, epicatechin etc. which may act as natural antioxidants

(Abdallla *et al.*, 2007; Nithitanakool *et al.*, 2009; Soong & Barlow, 2004). Antimicrobial and antifungal activity of kernel powder of some South African mango variety was reported against few microorganisms like *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli* etc., (Maisuthisakul & Gordon, 2009). These compounds found in mango kernels are known to inhibit extracellular microbial growth and avoid oxidative phosphorylation, affecting the survival of the microorganisms (Kaur *et al.*, 2010). It has been reported that oil extracted from mango kernel can also be used to cure hyperacidity, vomiting, diarrhoea etc. (Jiamboonsri *et al.*, 2011; Gulcin *et al.*, 2004). Seed kernel oil is also a rich source of PUFA like “oleic acid”, “linoleic acids” “palmitic”, and “linoleic acids” (Jeeva, 2009; Oriajogun *et al.*, 2014).

The major byproducts of the mango juice industry are the seed kernels. The mango seed constitutes 20-60% of the whole fruit (W/W), while the kernel carries 45-75% of the seed according to different varieties (Shah *et al.*, 2010; Ahmed *et al.*, 2005). Kernel of mango seed composed of ash (2.58%), fat (11.55%), protein (6.02%), crude fibre (10.6%), and carbohydrates (59.34%), with 9.89% moisture content (Ashoush & Gadallah, 2011). Extract of mango seed seems to possess antioxidant, anti-tyrosinase, hepatoprotective activities (Jeeva, 2009). The seed kernel waste is growing day by day due to increased commercialization of the fruit production and processing industry creating serious

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environmental problems (Gupta *et al.*, 2015; Sowmiya *et al.*, 2009).

S.aureus is an important pathogen, well known to cause skin infection. It also causes several diseases like osteomyelitis, pneumonia, endocarditis, meningitis; toxic shock syndrome etc. significance of these diseases has risen due to the rapid development of antibiotic resistance pathogens (Dorta, 2012).

This study has been taken to test the antimicrobial activity of seed kernel from three selected mango cultivars against staphylococcus aureus. The three varieties of mango kernels used are Banganapalle, Himsagar and Dasherri extracted in two extraction solvents i.e. methanol and distilled water to evaluate their antimicrobial activity.

Materials and methods:

Preparation of mango seed kernels: to study the antimicrobial activity of mango kernels, three varieties of mango namely, Banganapalle, Himsagar and Dasherri were taken from the market of Bolangir district. The seed kernels of different mango were collected, sliced into small pieces, and kept in an airtight container in deep freeze (-80°C for 3 days). Excessive moisture has been removed using a freeze dryer. The samples were stored at 4°C for future use. Dried pieces were ground separately using grinder. Kernel powders were used for extraction.

Culturing of the test organism: *Staphylococcus aureus* was taken as a test organism to examine antimicrobial properties which were grown in Mannitol Salt Agar (MSA) media. MSA is a selective medium having sodium chloride (7-9%). 30 g kernel powder was suspended in 250 ml sterile distilled water by stirring and boiling. Then Mannitol Salt agar was stored at 4°C after sterilization. Pure *S.aureus* cultures were obtained after repeated sub-culturing on MSA media. Pure viable cultures were stored in the refrigerator (4°C) after sub-culturing and incubation onto fresh agar slants at 37°C for 24 hours.

Extraction procedure: the dried powder was used for extraction. Methanol and distilled water were used as extraction solvents. 100 gm of each variety dry seeds kernels powder of *Mangifera indica* were used for extraction with methanol and distilled water in Soxhlet apparatus for eight hours till colourless solvent appeared. Extracts were concentrated by evaporating the extra colourless solvent rotary evaporator. Finally, the extract was dried in air at room temperature.

Antibacterial assay: *S.aureus* strains were inoculated in 50 ml of MSA and incubated at 37°C. The antimicrobial study was performed by using the "Disc Diffusion Technique". Bacterial suspension culture of bacterial strains was kept overnight Mueller-Hinton agar (MHA) media for activation. 8 mm round-shaped disc was prepared by punching filter paper, it was then autoclaved for sterilization. Sterile discs were impregnated with 10 ml

of both the extract i.e., methanolic and aqueous of three cultivar mango seed kernel extracts respectively for Banganapalle (a), Himsagar (b) and Dasherri (c). Methanol and water were taken as negative control and three commercial antibiotics Rifampicin, Gentamycin and Apoxicillin were taken as positive control. The inoculated plates were kept for 24 hrs incubation at 37°C. Zone of inhibition around the disc were recorded to evaluate the antimicrobial activity (Bhattacharyya *et al.*, 2013).

Calculation of "Relative percentage inhibition"(RPI):

RPI index of different extracts from different cultivar were calculated with respect to reference antibiotics as follows

$$\text{RPI of Test extract} = \frac{100 \times (a-b)}{(c-b)}$$

Where,

a: "Total area of inhibition of the test extract"

b: "Total area of inhibition of the solvent" (control)

c: "Total area of inhibition of the standard drug"

Results and Discussion:

For the study of antimicrobial activity, an amount of 400gm of *Mangifera indica* air-dried kernel and two extraction solvents, methanol and water were used. Methanolic and aqueous extracts of three varieties Banganapalle, Himsagar and Dasherri mango seed kernel were used for this study. After incubation of the extracts inoculated bacterial plates at 37°C overnight, antibacterial activity was determined in terms of zone of inhibition diameter (mm) around the disc.

The present study on the antimicrobial activity of different solvent extracts of *Mangifera indica* kernel was tested against *S.aureus*. In the antimicrobial study, it was observed that the methanolic extract from sample 'a' showed maximum inhibitory zone around the Disk against test organism followed by the aqueous extract of sample 'b' (Table-1). Methanolic extract of sample 'b' has shown moderate antimicrobial activity against the bacteria. Methanolic extract of sample 'c' exhibited the lowest microbial activity while the aqueous extract exhibited no antimicrobial activity against the *S.aureus* (Fig.-1). It was clear from the present results, that methanolic kernel extract of the variety Banganapalle exhibited pronounced activity against the tested bacteria.

This study proves that the methanolic extract of kernel of different mango varieties has high activities against *S.aureus*. The aqueous extract of the variety 'Banganapalle' and 'Dasherri' exhibited lower inhibitory activity as compared with the other methanolic extracts whereas aqueous extracts of variety Himsagar shows a relative higher zone of inhibition as compared to its methanolic extracts. The present results contradict the finding of Abdalla *et al.*, (2007) who reported less antimicrobial activity was exhibited by aqueous extracts than organic extracts. It was found that aqueous extract of variety Himsagar shows little more inhibitory effect than its methanolic extract, whereas organic extracts of variety Banganapalle shows more

effective inhibition zone compare to other extracts.

Among the 3 different antibiotics used as control, Rifampicin and Gentamicin shows inhibition zone but amoxicillin cannot inhibit the growth of *S. aureus*. It seems that the target *S. aureus* strains is Amox[®] (Table-1) resistant. More inhibitory effect have been found and exhibited by crude methanolic extract against *S. aureus*. The uses of organic solvents provide more efficiency in extracting antimicrobial activity (Kaur *et al.*, 2010; Omotubga *et al.*, 2012). These mango varieties exhibit antibacterial activity which makes them interesting wastes for screening natural products. So, further study needed to be taken so that mango seed kernel wastes can be utilized for manufacturing natural antibiotics (Jane *et al.*, 2017; Litz, 2009). Therefore, it is important to pay more attention to develop antibiotics that would fight against causative organisms of many dreadful diseases (Sowmiya *et al.*, 2009).

Methanolic extracts of a variety Himsagar has more antibacterial properties to Rifampicin than Gentamycin, against the *S. aureus* Amox[®]. This experiment shows that the antibacterial activity of mango kernel extracts varies with solvent type and variety dependent manner. The capacity of inhibition of different extracts is met banganapalle>aqhimsagar>met himsagar>aqbanganapalle> met Dasher. Aqueous extracts of variety 'c' (Dasher) does not show any antimicrobial properties against *S. aureus*. Higher antibacterial activity towards *Staphylococcus* resistance to methicillin was shown by crude methanolic seed kernel extract of Banganapalle mango. This supports the finding of Mutua *et al.*, (2017). Ahmed *et al.*, (2005), also reported that the organic solvent extract was active on most microbial growth having a maximum effect against *P.aeruginosa*, *S.aureus*, etc. In vitro antibacterial activity mango seed kernel alcoholic extract against *S.aureus* was observed may be due to the presence of the phenolic compound, methyl gallate, gallate, and pentagalloyl glucopyranose etc. (Kittiphoom & Sutasinee, 2013).

Table-1: Zone of inhibition (ZoI) exhibited by different kernel extracts and antibiotics

Antibiotics	ZoI (mm)	Antibiotics	ZoI (mm)
Amoxicillin	5.28±0.6	Rifampicin	18.85±0.32
Gentamicin	24±1.2	Aqueous a	9.±0.45
Methanolic a	13.5±0.33	Aqueous b	12.33±0.66
Methanolic b	11.2±0.33	Aqueous c	2.86±0.22
Methanolic c	5.42±0.33		

Conclusively, the study clearly suggests the presence of phytochemicals in variety 'a' and variety 'b' could act as an alternative source of medicine for the control of pathogenic *S. aureus* to avoid synthetic drugs and their unintended side effects. As the results, showed positive assessment, there is an intense possibility for the phytochemical compounds of mango kernel extracts to be utilized as alternate sources of

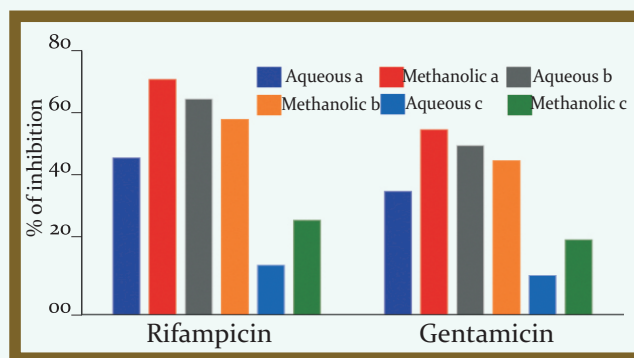


Figure-1: RPI of kernel extract of different Mango cultivar with respect to Rifampicin and Gentamicin

antimicrobial agents for the treatment of infection (abscesses, bacteremia, endocarditis, osteomyelitis etc.) caused by the test pathogen. Further research is needed to identify the active phytochemicals in the extracts showing a better inhibition zone. This organic waste can be utilized as a better agent in medical and pharmaceutical research in future.

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References:

- Abdalla, A.E.M., Darwish, S.M., Ayad, E.H.E. & El-Hamahmy, R.M.(2007): Egyptian mango byproduct 1. Compositional quality of mango seed kernel. *J. Food Chem.*, 103(4): 1134-1140.
- Abdullah, A.H., Mirghani, M.E.S. & Jamal, P. (2011): Antibacterial activity of Malaysian mango kernel. *Af. J. Biotech.*, 10(81): 18739-18748.
- Ahmed, I.S., Tohami, S.M., Almagboul, Z.A. & Verpoorte, R. (2005): Characterization of anti-microbial compounds isolated from *Mangifera indica* L seed kernel Univ. Af. J. Sci., 2: 77-91.
- Ashoush, I.S. & Gadallah, M.G.E. (2011): Utilization of mango peels and seed kernels powders as sources of phytochemicals in Biscuit. *Wor. J. Dairy Food Sci.*, 6: 35-42.
- Bhattacharyya, S., Deep, P.R., Nayak, B., Panigrahi, M., Mohapatra, B. (2013): Antimicrobial activity of two diazotropic cyanobacteria against *Staphylococcus aureus*. *Int. J. Med. Aromat. Plants*, 3(2):283-292.
- Deep P.R. & Bhattacharyya S., (2020): Antibacterial activity of kernal extracts from three mango cultivars 'Totapuri', 'Langra' And 'Sundri'. *Biosci. Biotechn. Res., Comm.*, 13 (12):171-174
- Dorta, E.M., Lobo, G. & Gonzalez, M. (2012): Reutilization of mango by products: study of the effect of extraction solvent and temperature on their antioxidant properties. *J. Food Sci.*, 77: 80-88.
- Ahmed E.F. (2015): Antimicrobial and Antibiofilm Activity of Mango Seeds Extract. *Iraqi J. Sci.*, 56(4B):3121-3129.
- Gulcin, L., Uguz, M.T., Oktay, M., Beydemir, S. & Kufrevioglu, O.L. (2004): Evaluation of the antioxidant and antimicrobial activities of Clary Sage (*Slaviasclarea* L.). *Tur. J. Agri. Forest.*, 28: 25-33.

- Gupta, P., Ray, J., Aggarwal, B.K. & Goyal, P. (2015): Food processing residue analysis and its functional components as related to human health: recent developments. *Aust. J. Nutri. Food Sci.*, 3:1068-1081.
- Jane, K., Mutua, S.I. & Willis, O. (2017): Evaluation of the proximate composition, antioxidant potential and antimicrobial activity of mango seed kernel extracts. *Food Sci. Nutri.*, 5(2):349-357.
- Jeeva, S. (2009): Horticultural potential of wild edible fruits used by the Khasi tribes of Meghalaya. *J. Horticul. Forest.*, 1(9):182-192.
- Jiamboonsri, P., Pithayanukul, P., Bavovada, R., & Chomnawang, M.T. (2011): The inhibitory potential of Thai mango seed kernel extract against methicillin-resistant *Staphylococcus aureus*. *Molecules*, 16(8):6255-6270.
- Kaur, J., Rathinam, X., Kasi, M., MiewLeng, K., Ayyalu, R., Kathiresan, S. & Subramaniam, S. (2010): Preliminary investigation on the antibacterial activity of mango (*Mangifera indica* L: Anacardiaceae) seed kernel. *Asian Pac. J. Trop. Med.*, 3(9): 707-710.
- Kittiphoom, S., & Sutasinee, S. (2013): Mango seed kernel oil and its physicochemical properties. *Int. Food Res. J.*, 20: 1145-1149.
- Litz, R.E. (2009): **The Mango Botany, Production and Uses**, 2nd ed. Pub. by: CABI Publishing, Wallingford, UK.
- Maisuthisakul, P. & Gordon, M.H. (2009): Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. *Food Chem.*, 117:332-341.
- Mutua, J. M., Gitao, C. G., Bebor, L. C., & Mutua, F. K. (2017). Antimicrobial resistance profiles of bacteria isolated from the nasal cavity of camels in Samburu, Nakuru, and Isiolo Counties of Kenya. *J. Veterin. Med.*, 1-6.
- Nithitanakool, S., Pithayanukul, P., Bavovada, R., & Saparpakorn, P. (2009): Molecular docking studies and anti-tyrosinase activity of Thai mango seed kernel extract. *Molecules*, 14(1): 257-265.
- Omotubga, S.K., Kehinde, A.S. & Olayinka, O.O. (2012): Proximate evaluation of nutritional value of mango (*Mangifera indica*). *Int. J. Res. Chem. Environ.*, 2(4): 244-245.
- Orijajogun, J.O.L., Batari, M. & Aguzue, O.C. (2014): Chemical composition and phytochemical properties of mango (*Mangifera indica*) seed kernel. *Int. J. Adv. Chem.*, 2:185-187.
- Shah, K.A., Patel, M.B., Patel, R.J. & Parmar, P.K. (2010): *Mangifera indica* (Mango). *Pharmacognosy Rev.*, 4(7): 42-48.
- Soong, Y. & Barlow, P.J. (2004): Antioxidant activity and phenolic content of selected fruit seeds. *Food Chem.*, 88(3): 411-417.
- Sowmiya, S., Soundarapandian, P. & Rajan, S. (2009): Bioactive studies of *Mangifera indica* against bacteria isolated from urine samples. *Current Res. J. Biol. Sci.*, 1(3):139-143.

