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MULTIPLE ANTIBACTERIAL AND PHYTOCHEMICAL ANALYSIS OF MANGO KERNEL EXTRACTS ON AQUATIC AND ANIMAL PATHOGENS

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ABSTRACT

The antibacterial activities of hexane, ethyl acetate, acetone, ethanolic, methanolic and aqueous extracts of *Mangifera indica* kernel were studied *in vitro* against aquatic pathogens viz. *Aeromonas hydrophila* (six strains), *Pseudomonas putida* (three strains), *P. aeruginosa* (three strains), *P. fluorescens* (two strains), *Flavobacterium columnare* (three strains), *Vibrio parahaemolyticus* (two strains), *V. alginolyticus* (two strains), *V. fluvialis*, *V. harveyi*, *Edwardsiella tarda* (two strains) and animal pathogen, *Escherichia coli* (five strains). The ethanolic extract showed higher antibacterial activities against almost all tested microorganisms with minimum inhibitory concentration (MIC) ranged from 200-300µg. The highest zone size were exhibited in acetone extract (22.16mm/400µg) against one strain of *A. hydrophila* and ethanolic extract (19mm/400µg) against three strains of *A. hydrophila* and one strain of *P. aeruginosa.* The MIC of aqueous extract was 250-400µg with zone size ranged of 7.66-13.5mm. In phytochemical analysis, ethanolic, methanolic and aqueous extract showed the presence of saponin, flavonoid, alkaloid, tannin and sterol. The study showed that various extracts of mango kernel have potentialities in using against aquatic bacterial pathogens.

KEYWORDS: ***Mangifera indica*, antibacterial activity, phytochemicals, aquatic pathogens**

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INTRODUCTION

Mangifera indica L. (family, Anacardiaceae) commonly known as mango is a tropical and perennial indigenous species distributed on a large scale in Asia, Africa and America[1]. India is the biggest mango producer in the world, accounting for about 52 percent of world production[2]. Mango kernel represents 20% to 60% of the whole fruit weight, depending on the mango variety[3]. Different parts of mango such as leaves[4], peel[5], kernel[6,7], bark[8], shoot[9], flower[10], root[11] showed antimicrobial activity on different human and other pathogens. Mango kernels reported to be a good source of polyphenols, phytosterols, campesterol, beta-sitosterol and tocopherols[12].Bacterial diseases have been reported as a major limiting factor in the production of both culture and capture species[13]. Intensification of aquaculture with more supply of protein feed, high stocking density, leads unhygienic water quality that lead to fish, susceptible to several diseases, which ultimately elevated mortality rates and high economic losses[14]. Due to the development of resistant pathogens and other undesirable side effects of many of the antibiotics and other synthetic drugs in biological system, use of natural products is an alternative solution in overcome the problem[15]. Botanical plants possess natural antimicrobial agents which has no residual effect and bio-accumulating properties. The immunostimulatory activity of the traditional mango plant has been found effective to fish[16]. The present study is directed to find out the antimicrobial properties of different crude and fractionated products of mango kernel to a wide number of aquatic pathogens which can be further incorporated in the aquaculture practices for controlling microbial diseases.

MATERIALS AND METHODS

(i) Collection and extraction of mango kernel

The mango kernel was collected locally from Bhubaneswar, Odisha. They were washed and air dried. The kernels and kernel sheathes were removed manually from the seeds. Fresh kernel seeds were cut into small pieces and dried at hot air over at 50^0C for two to three days to remove the moisture. The dried kernel was grinded and powdered. The dried mango kernel powder was sequentially extracted using various solvents (hexane, ethyl acetate, chloroform, acetone, ethanol and methanol) and distilled water according to their polarity[17].Dry mango powder (200 g) was added to 600 ml hexane (Hex) and incubated for 24 h at room temperature. After incubation, it was filtered using Whatman filter No. 40, and further made solvent free by using rotavapour (Buchii-11). Same procedure was repeated thrice. The filtrate was then added to 600 ml of ethyl acetate (EA) and incubated for 24 h at room temperature with continuous stirring. Previous procedure was followed for the acetone (A), ethanolic (EtOH), methanolic (MeOH) and aqueous: alcohol (3:1) (Aq) extraction. All the crude extracts were concentrated under vacuum at a temperature (<40^0C). Percent extractive values were calculated by the following formula

$$\text{Percent Extracts} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

(ii) Test organisms

Antibacterial sensitivity was tested against the pathogenic Gram-negative strains of *Aeromonas hydrophila* (Ah1, Ah2, Ah3, Ah4, Ah MTCC 646, Ah ATCC 49140), *Pseudomonas putida* (PP1, PP2, PP3), *Pseudomonas aeruginosa* (PA1, PA2, ATCC 35032), *Pseudomonas fluorescens* (PF1, PF2), *Vibrio*

alginolyticus (VA, VA ATCC 17749), *Vibrio parahaemolyticus* (VP, VP ATCC 17802), *Vibrio fluvialis* (VF), *Vibrio harveyi* (VH), *Edwardsiella tarda* (ED, ED ATCC 15947), *E. coli* (O115, O1, O156, O164, O111 & O109) and *Flavobactrium columnare* (M1, M41, M43). The above pathogens maintained in the Fish Health Management Division, Central Institute of Freshwater Aquaculture, Bhubaneswar used for the antibacterial activity study. Brain heart infusion (BHI) (Hi-media, Mumbai, India) broth was used for broth culture of these pure cultured bacterial strains except *Vibrio parahaemolyticus* which was maintained in BHI broth supplemented with 2.5% NaCl and incubated at 37° C for 18 h and subsequently used for antibacterial assay

(iii) Inhibitory effect using disc diffusion method

Antibacterial sensitive test of different crude extracts of mango kernel was done using disc diffusion method as described by Chabbert[18]. All bacteria were grown in BHI broth incubated at 37°C for 24 h and plated using a sterile swab, on to petridishes containing Antibiotic Assay Medium adjusting the bacterial count to 10^7 CFU mL^{-1}. Each extract of 400 µg 10 μL^{-1} concentrations was applied to sterile filter paper discs and put on to inoculated plates and incubated at 37°C. Discs with solvent (10 µL) used for dissolution were taken as control after evaporation of the solvent. Activity of the different extracts was determined after 24 h at 37°C by measuring the diameter of the halo around the discs (average of three experiments)[19]. The antibacterial activities of mango kernel extracts were compared with inhibition zones around 4 commercial antibacterial discs i.e. Cotrimazole (Co), Tetracycline (T), Furazolidone (Fu) and Cephalaxin (Cp) that were used as references. Zone size in mm were analysed using one way analysis of variance (ANOVA) and significant difference among three crude extracts of mango kernel means were compared using Duncan's multiple range test (DMRT)[20] at 5% significant level.

(iv) Determination of minimal inhibitory concentration (MIC)

The extracts were serially diluted in nutrient broth. Equal amount (2 mL) of bacterial suspension corresponding to 10^7 CFU mL^{-1} of the test organism was added to each of the test tube and incubated at over night. The highest dilution of the different extracts of mango kernel in which there was no growth of the organism on the nutrient broth was observed to assess the susceptibility of the growth of the pathogen and to explain the lethality of the toxins present in the mango kernel extracts[21].

(v) Preliminary phytochemical screening

The phytochemical screening of the crude extracts of *M. indica* was carried out by using standard methods of Sofowora[22] and Trease and Evans[23].

RESULTS

(i) Quantitative estimation

The preliminary phyto–profiling for the mango kernel was cited in Table 1 and found that sticky extracts in high polar solvent and aqueous extract and the low polar solvent extracts was found to be non-sticky and oily in nature. The yield (%) of the extracts was also analyzed where in the highest yields was recorded for aqueous extract (12.64%) followed by hexane (11.21%), acetone (7.15%), ethanol (6.73%), methanol (3.71%), ethyl acetate (2.89%).

(ii) Antibacterial sensitivity of different extracts of mango kernel against aquatic pathogens:

Aeromonas hydrophila

High antibacterial sensitivity of kernel extract was found to all the strains of *A. hydrophila* except MS:Hex (Fig. 1). The maximum zone of inhibition was observed in acetone extract and ethyl acetate extract of mango kernel against Ah1 and Ah MTCC 646. The ethanolic and methanolic extract of mango kernel showed zone of inhibition 14.33mm-19.66 mm to all strains of *A. hydrophila*.

Pseudomonas spp.

It was noticed that all the strains of *Pseudomonas* spp. showed high sensitivity to all the crude ethyl acetate, acetone, ethanolic, methanolic and aqueous extracts except MS:Aq to PP1, PA1, PF2 which showed moderate and MS:Aq to PP2 resistant activity. The highest zone of inhibition (19.83 mm) was observed in the ethanolic extract of mango kernel to *P. putida* (PP2) and *P. aeruginosa* (PA2), whereas the hexane extract of mango kernel were resistant against *Pseudomonas* spp. (Fig. 2).

Vibrio sp. and Edwardsiella tarda

Ethyl acetate, acetone, methanolic, ethanolic and aqueous extracts of mango kernel showed moderate to high antibacterial sensitivity to all the selected species of *Vibrio* and *E. tarda* except *V. fischeri* (VF) to MS:A, *Vibrio harveyi* (VH) and *E. tarda* ATCC 15947 to MS:MeOH. The maximum zone of inhibition (20.66 mm) was found in *V. parahaemolyticus* (VP) to ethanolic extracts (MS:EtOH) of mango kernel (Fig. 3)

Escherichia coli* and *Flavobacterium columnare

The ethyl acetate, acetone, ethanolic, methanolic and aqueous extract of mango kernel showed higher zone of inhibition against all the serotype of *E. coli* and *F. columnare* except O164 serotype to MS:MeOH, which showed resistant and M43 to MS:Aq showed moderate sensitivity. The maximum zone of inhibition (20.0 mm and 20.5 mm) was found in MS:A and MS:EtOH extracts on M1(Fig. 4). Again, the antibacterial sensitivity of different extracts of mango kernel compared with the standard antibiotic discs i.e. Cotrimazole (Co), Tetracyclins (T), Furazolidone (Fu) and Cephalaxin (Cp) and found to be similar zone of inhibitions (Table 2).

(iii) MIC value of different extracts of mango kernel against different bacteria

Ethanolic extract showed the lowest MIC value of 200 µg against three strains of *A. hydrophila* (Ah1, Ah2, Ah3), two strains of *Pseudomonas* (PA2, PF1), one strains each of *E. coli* (O1), *V. parahaemolyticus* (VP) and *F. columnare* (M1). Aqueous extracts of mango kernel showed MIC within a range of 250-400 µg with a lowest MIC of 250 µg found against Ah1 (Table 3).

(iv) Phytochemical study

The phytochemical screening showed that the presence of flavonoid and tannin in all extracts expect hexane extract of mango kernel (MS:Hex). Similarly sterol was present in all the extracts of mango kernel. Ethanolic, methanolic and aqueous extracts of mango kernel there was presence of alkaloid and saponin (Table 4).

Table 1
Solvents used for extraction of active antibacterial from mango kernel

Crude Extracts	Solvents used	Nature of the product	Colour	% of extracts
MS:Hex	Hexane	Oily	Yellowish-white	11.21
MS:EA	Ethyl acetate	Oily	Greenish-white	2.89
MS:A	Acetone	Sticky	Brownish	7.15
MS:EtOH	Ethanol	Sticky	Brownish	6.73
MS:MeOH	Methanol	Sticky	Brownish	3.71
MS:Aq	Distilled water:Ethanol(3:1)	Sticky	Brownish	(2.64

Table 2
Antibacterial sensitivity of aquatic pathogens to antibiotics

Antibiotics	Disc potency	Ah1	Ah MTCC 646	PP1	PA1	PF1	M1
		Zone of Inhibition (mm)					
Cotrimazole	10 µg	26	0	10	9	9	9
Tetracycline	20 µg	29	33	19	15	19	21
Furazolidone	50 µg	23	24	18	14	15	ND
Cephalaxin	30 µg	12	13	13	11	10	23

Table 3
MIC value (µg) of different crude extract of mango kernel against aquatic and animal pathogens

	A. hydrophila					
	Ah1	Ah2	Ah3	Ah4	Ah MTCC646	Ah ATCC49140
MS:Hex	-	-	-	-	-	-
MS:EA	225	250	250	275	200	250
MS:A	200	225	225	250	225	275
MS:EtOH	200	200	200	225	225	250
MS:MeOH	200	200	225	225	250	250
MS:Aq	250	275	275	300	275	300

	P. putida			***P. aeruginosa***			***P. fluorescens***	
	PP1	PP2	PP3	PA1	PA2	PA ATCC 35672	PF1	PF2
MS:Hex	-	-	-	-	-	-	-	-
MS:EA	250	200	275	275	200	300	300	300
MS:Aceton	250	200	275	250	200	300	275	300
MS:EtOH	225	225	250	225	200	225	200	225
MS:MeOH	225	200	225	225	200	250	225	250
MS:Aq	400	-	300	375	300	325	300	375

	***Vibrio* sp.**						***E. tarda***	
	VA	VA ATCC 17749	VP	VP ATCC 17802	VH	VF	*E. tarda*	*E.tarda* ATCC 15947
MS:Hex	-	-	-	-	-	-	-	-
MS:EA	275	350	325	400	-	400	400	-
MS:A	300	300	325	400	400	-	-	-
MS:EtOH	225	250	200	225	225	225	250	300
MS:MeOH	275	250	250	300	-	275	300	-
MS:Aq	400	350	300	325	325	325	300	400

	E. coli					***F. columnare***		
	O1	0115	0156	0164	0111	M1	M41	M43
MS:Hex	-	-	-	-	-	-	-	-
MS:EA	250	275	275	275	275	250	300	300
MS:A	275	275	275	275	275	200	275	275
MS:EtOH	200	225	225	225	225	200	250	250
MS:MeOH	225	225	225	-	250	200	225	250
MS:Aq	300	325	300	275	300	325	325	350

Note: MS:Hex, MS:EA, MS:A, MS:EtOH, MS:MeOH and MS:Aq- hexane, ethyl acetate, acetone, ethanolic, methanolic and aqueous extract of mango kernel.

Table 4
Phytochemical analysis of different extracts of mango kernel

Extracts	Alkaloid		Flavonoid	Tannin	Sterol	Saponin
	Dragendroff's reagent	Mayer's reagent				
MS:Hex	NA	NA	-ve	-ve	+ve	-ve
MS:EA	-ve	-ve	+ve	+ve	+ve	-ve
MS:A	NA	NA	+ve	+ve	+ve	-ve
MS:EtOH	+ve	+ve	+ve	+ve	+ve	+ve
MS:MeOH	+ve	+ve	+ve	+ve	+ve	+ve
MS:Aq	+ve	+ve	+ve	+ve	+ve	+ve

NB. +ve- present, -ve- absent

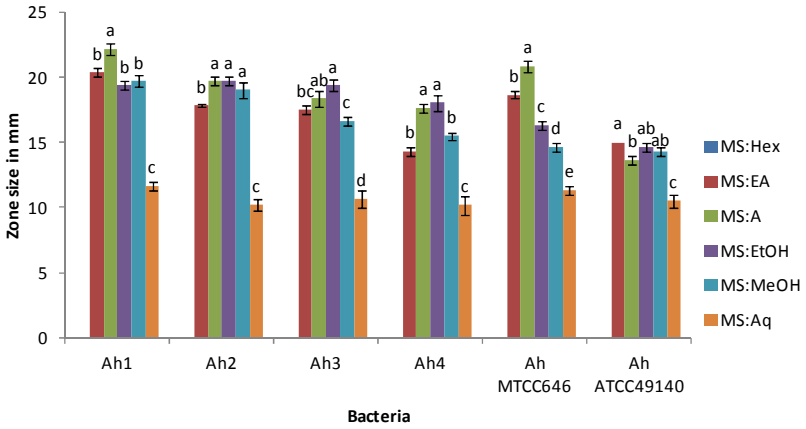

Figure 1
Antibacterial activity of various extracts of Mango kernel against Aeromonas hydrophila

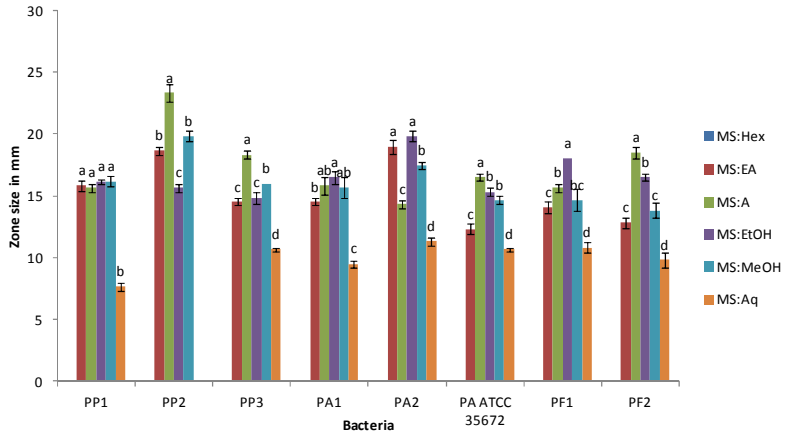

Figure 2
Antibacterial activity of various extracts of Mango kernel against different strains Pseudomonas spp

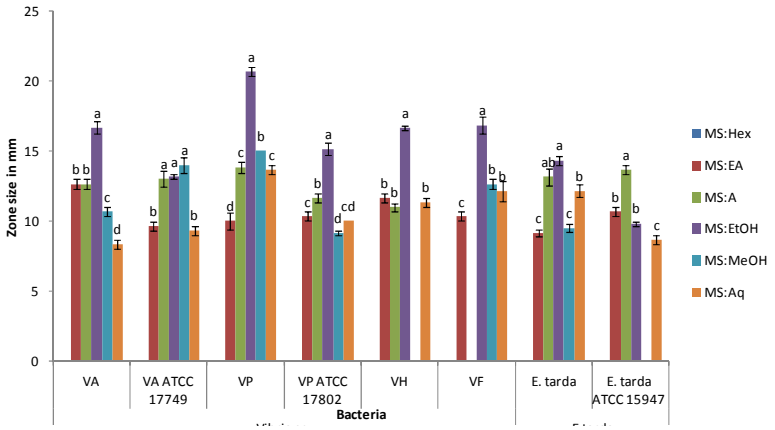

Figure 3
Antibacterial activity of various extracts of Mango kernel against Vibrio and Edwardsiella tarda

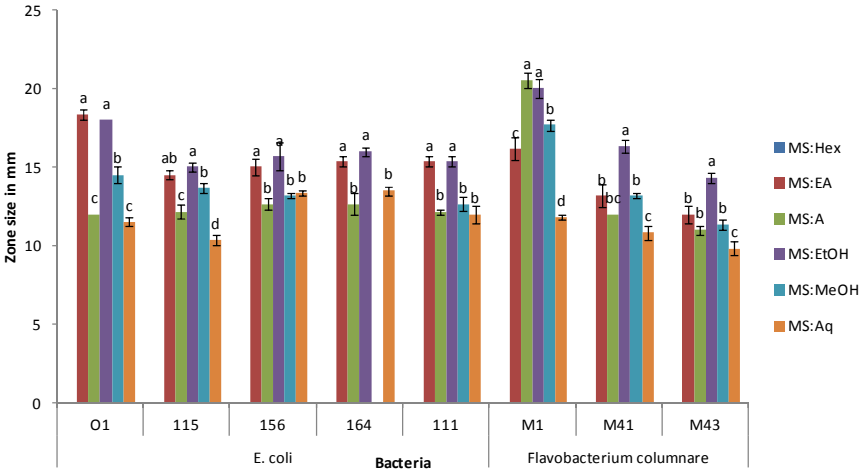

Figure 4
Antibacterial activity of various extracts of Mango kernel against Escherichia coli and Flavobactrerium columnare

DISCUSSION

From ancient, plant extracts have been used as curing agent against bacteria, but in the last three decades it has been studied in more intensified way. During this period, a lot of herbal plants for antimicrobial screening were evaluated for their natural drug[24]. The sequential extraction has used by various workers[25,26] for extraction of different plant parts also used in this study. In our present study, aqueous extraction of mango kernel produced the highest yields as compared to all the solvents used. In general, the percentage yields for water extracts were more than the percentage yields for organic solvents. This is due to the fact that water is very polar than organic solvents hence it is able to extract more compounds from a plant material. Korir *et al*.[26] also found that water extracts produced the highest yields as compared to organic solvents. Vaghasiya and Chanda[25] extracted mango kernel by different solvent such as petroleum ether, chloroform, ethyl acetate, acetone and

methanol and got an extractive yield of 13, 2.9, 0.8, 1.8 and 5% respectively. According to them maximum yield was in petroleum ether while minimum was in ethyl acetate. Our findings corroborate the findings of Vaghasiya and Chanda[25]. The antibacterial activity of various extracts of mango kernel was tested against different strains of aquatic and animal pathogens. From the screening, it was noticed that, all the extracts of mango kernel showed moderate to high zone of inhibition against most of the selected pathogens except hexane extract. Ahmed *et al*.[27] studied the antimicrobial activity of chloroform, methanol and aqueous extracts of *M. indica* seeds kernel against *Bacillus subtilis, Staphylococcus aureus*, *E. coli*, *Proteus vulgaris* and *P. aeruginosa* reported that high activity against all tested organisms. In our present study, a similar result was found in aqueous extract of mango kernel in which the zone of inhibition on *E. coli.* According to Sowmiya *et al*.[6] antibacterial activity for aqueous and ethanolic extracts of mango seed kernel showed best activity against *S. aureus* at 800 µg concentration and to *E. coli* and *S. pyrogens.* Latha *et al*.[11] opined that ethanolic and ethyl acetate extract of *M. indica* L. var. Rasapuri root possessed the MIC at 2 mg/ml and 4 mg/ml respectively on *B. substilis*, *E. coli* and *K. pneumonia* where as Rajan *et al*.[28] found the MIC of methanolic fraction of mango kernel against *Shigella dysenteriae* was 95±11.8 µg/mL. In our present study, the MIC value of different extracts of mango kernel was found at a range of 200-400 µg with minimum inhibitory concentration of 200 µg which are much more lower than the bacteria selected by Latha *et al*.,[11] and nearly equal as reported by Rajan *et al*.[28].Phytochemical compounds are key factor to perform biological activities like antibacterial, antioxidant etc[3,4,6]. In our present phytochemical study, it was found that tannin, saponin, sterols, flavonoid and alkaloid was found in ethanoilc, methanoilc and aqueous extract of mango kernel. This result was supported by Sowmiya *et al*.[6] who found that tannin, flavonoid, saponin was present in ethanolic and aqueous extract of mango kernel. Abdalla *et al*.[29] studied the phenolic compound present in mango kernel extract and found that tannin and vanillin was present in highest amount whereas garlic acid, caffeic acid and mangiferin are lower amount. The presence of polyphenolic compound might have showed antibacterial activities[30]. The mango seed kernel extracts contain Phellandrene, α-pinene, ambolic acid, ascorbic acid, β-carotene, gallic acid, gallotannic acid, mangifelic acid, mangiferol peroxidase, phenyl alanin and proline as reported by Kabuki *et al*.[31] and gallotannins reported by Engels *et al.*[32] which showed antimicrobial activity to both Gram positive and Gram negative bacteria. Flavonoids are containing one carbonyl group complexes with extracellular and soluble protein and with bacterial cell wall[33], thus exhibits antibacterial activity through these complexes. In our study though we have not specifically purified individual compounds, however a group of compounds present in the crude extracts might have contributed to the antimicrobial properties.It was concluded that addition to the antibacterial activity to human pathogens as stated by many authors and enhancing immunity and disease resistant to the fish and shell fish[16,34] all the crude extracts except hexane showed antibacterial activity to most of the selected aquatic pathogens. However, the bioactive compounds from ethanolic extract of mango kernel have strong antibacterial activity on aquatic and animal pathogens. Further studies have taken to isolation of pure compound of biological active for disease free in aquaculture.

REFERENCES

1. Ahmed D, Chaudhery R, Chaudhary MA, Ali Z, and Khan SR., Physicochemical, thin layer and gas-liquid chromatographic analysis of ungrafted desi mango flower oil and mineral estimation in its flowers.

African Journal of Biotechnology, 11 (41): 9844-9848, (2012).
2. Geethalakshmi R., MANGOes: new varieties to step up exports FACTS FOR YOU, April 2011 pp. 16-19, (2011).
3. Maisuthisakul P and Gordon MH., Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. *Food Chemistry,* 117: 332–341, (2009).
4. Kanwal Q, Hussain I, Siddiqui HL and Javaid A., Flavonoids from mango leaves with antibacterial activity *J Serb Chem Soc.,* 74 (12): 1389–1399, (2009).
5. de Oliveira SMS, Falcão-Silva VS, Siqueira-Junior JP, Costa MJdeC, Diniz MdeFFdeM. Modulation of drug resistance in *Staphylococcus aureus* by extract of mango (*Mangifera indica*) peel. *Brazilian Journal of Pharmacognosy,* 21 (1): 190-193, (2011).
6. Sowmiya S, Soundarapandian P and Rajan S., Bioactive studies of *Mangifera indica* against bacteria isolated from urine samples. *Current Research Journal of Biological Sciences,* 1(3): 139-143, (2009).
7. Abdullah SHA, Mirghani MES and Jamal P., Antibacterial activity of Malaysian mango kernel. *African Journal of Biotechnology*, 10 (81):18739-18748, (2011).
8. Mada SB, Garba A, Muhammad A, Mohammed A, Adekunle DO., Phytochemical screening and antimicrobial efficacy of aqueous and methanolic extract of *Mangifera indica* (Mango Stem Bark), *World J Life Sci. and Medical Research*, 2 (2):81-85, (2012).
9. Penecilla GL and Magno CP., Antibacterial activity of extracts of twelve common medicinal plants from the Philippines. *Journal of Medicinal Plants Research,* 5 (16):3975-3981, (2011).
10. Lima ZP, Severi JA, Pellizzo CH, Brito ARMS, Solis PN, C´aceres A, Gir´on LM, Vilegas W and Hiruma-Lima CA., Can the aqueous decoction of mango flowers be used as an antiulcer agent? *Journal of Ethnopharmacology*, 106 (1):29-37, (2006).
11. Latha MS, Latha KP, Vagdevi HMM, Virupaxappa BS, Nagashree AS., Phytochemical investigation and antibacterial activity of *Mangifera indica* L. Var. Rasapuri root extracts. *Int. J. Med. Aerom. Plants*, 1 (2): 45-47, (2011).
12. Soong YY, Barlow PJ and Perera CO., A cocktail of phytonutrients: identification of polyphenols, phytosterols and tocopherols from mango (*Mangifera indica* L.) seed kernel. In IFT annual meeting, July 12–16, Las Vegas, (2004).
13. Kumar D, Farkas J and Sinha VRP., Bacteria from diseased freshwater fish from India. *Aquaculture Hungarica*, 5:113-118, (1986).
14. Hatha M, Vivekanandhan AA and Joice GJC., Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish. *Int J Food Microbiol.*, 98: 131-134, (2005).
15. Castro SBR, Leal CAG, Freire FR, Carvalho DA, Oliveira DF and Figueiredo HCP., Antibacterial activity of plant extracts from Brazil against fish pathogenic bacteria. *Braz J Microbiol*., 39(4): 756-760, (2008).
16. Sahu S, Das BK, Pradhan J, Mohapatra BC, Mishra BK and Sarangi N., Effect of *Mangifera indica* as feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. *Fish and Shellfish Immunology,* 23: 109-118, (2007).
17. Patra JK, Rath SK, Jena K, Rathod VK and Thatoi HN., Valuation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) extract: A study on inhibition of Glutathione-Stransferase activity. *Turkish Journal of Biology*, 32: 119-125, (2008).
18. Chabbert YA., L'antibiogramme. Sensibilite et resistancedes bacteries auxantibiotiques. De la Tourelle, 257, (1963).
19. Izzo AA, DiCarlo G, Biscardi D and De Fusco R., Biological screening of Italian medicinal plants for antibacterial activity.

Phytotherapy Research, 9: 281-286, (1995).
20. Duncan DB. Multiple range and multiple 'F' tests. *Biometrics*, 11: 1–42, (1955).
21. Alderman DJ and Smith P., Development of draft protocols of standard reference methods for antimicrobial agent susceptibility testing of bacteria associated with fish diseases. *Aquaculture* 196: 211–243, (2001).
22. Sofowora A., Medicinal plants and Traditional medicine in Africa: Spectrum Books Ltd, Ibadan, Ibadan, Nigeria, 289 (1993).
23. Trease GE and Evans WC., Pharmacognosy. 15th Ed. London: Saunders Publishers; pp. 42–44. 221–229, 246–249, 304–306, 331–332, 391–393, (2002).
24. Forestiere AM, Pizzimenti FC, Monforte TM and Bisignano G., Antibacterial activity of some African medicinal plants. *Pharmacological Research Communications,* 20(5): 33-36, (1988).
25. Vaghasiya Y and Chanda S., Antimicrobial and free radical scavenging activity of different extracts of *Mangifera indica* L. seeds. *Research Journal of Microbiology*, 5(12): 1207-1212 (2010).
26. Korir R K, Mutai C, Kiiyukia C and Bii C., Antimicrobial activity and safety of two medicinal plants traditionally used in Bomet District of Kenya. *Research journal of Medicinal plant,* 6(5): 370-382, (2012).
27. Ahmed SOM, Omer MAA, Almagboul AZ and Abdrabo AN., *In vitro* antimicrobial activity of Mangifera indica L. Gezira Journal of health sciences, 1(2): 82-91 (2005).
28. Rajan S, Thirunalasundari T, Jeeva S., Anti—enteric bacterial activity and phytochemical analysis of the seed kernel extract of *Mangifera indica* Linnaeus against *Shigella dysenteriae* (Shiga, corrig.) Castellani and Chalmers. *Asian Pasific Journal of Tropical Medicine,* 4(4): 294–300, (2011).
29. Abdalla AEM, Darwish SM, Ayad EHM and El-Hamahmy RM., Egyptian mango by-product 1. Compositional quality of mango seed kernel. *Food Chemistry*, 103(4): 1134-1140, (2007).
30. Cowan MM., Plant products as antimicrobial agents. *Clin Microbiol Rev*., 12: 564-582, (1999).
31. Kabuki T, Nakajima H, Arai M, Ueda S, Kuwabara Y and Dosako S., Characterization of novel antimicrobial compounds from mango (*Mangifera indica* L.) kernel seeds. *Food Chemistry,* 71: 61-66, (2000).
32. Engels C, Knõdler M, Zhao YY, Carle R, Gänzle MG and Schieber A., Antimicrobial activity of gallotannins isolated from mango (*Mangifera indica* L.) kernels. *J Agr Food Chem.,* 57: 7712-7718, (2009).
33. Adejuwon AO, Adejumoke M and Johnson B., Bioactive compounds and antimicrobial efficacy of the extracts of *Combretum pincianum* Hook. *Journal of Medicinal Plants Research* 5(15): 3561-3563, (2011).
34. Harikrishnan R, Kim J.-S. Balasundaram C, Jawahar S, Heo M.-S., Effect of *Magnifera indica* kernel–enriched feed on immune response of *Penaeus indicus* against white spot syndrome virus (WSSV) *Aquacult Int*, DOI 10.1007/s10499-012-9535-3 (2012).