Antimicrobial activity of decoction extracts of residual parts (seed and peels) of *Mangifera indica* L. var. Kesar against pathogenic and food spoilage microorganism

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Food borne diseases and emergence of epidemic drug resistant microbes, provoked by food pathogenic bacteria and fungi, are of major concern in developing as well as developed countries. Therefore galvanized interest and research in developing new natural based drugs is needed and in fact it is an urgent task. The aim of this study was to investigate the efficacy of natural antimicrobics from ripe peel, unripe peel, ripe seed and unripe seed of *Mangifera indica* L. (Anacardiaceae). The extraction was done by decoction method. Antimicrobial efficacy was carried out by agar well diffusion method against 10 Gram positive bacteria, 10 Gram negative bacteria and 5 fungi. The extracts which showed >15 mm zone of inhibition were further screened for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC); MIC and MBC of the extracts was determined using a 96-well micro titer plate method. The extractive yield was highest in ripe peel. The Gram negative bacteria were more susceptible than Gram positive bacteria. None of the extracts showed activity against fungi. *M. flavus* was the more susceptible Gram positive bacterium towards the ripe seed extract followed by ripe peel and unripe seed. Ripe seed showed highest zone of inhibition against *P. vulgaris* followed by *P. morganii*. Ripe seed extract showed lowest MIC and MBC values i.e. 156 and 625 µg ml^{-1} respectively against *M. flavus*. Seed extracts showed remarkable antimicrobial activity to control food spoilage and food borne pathogens. It can serve as a potential source of antimicrobics for use in the preparation of food ingredients and pharmaceutical products.

Keywords *Mangifera indica*; Peel; Seed; food spoilage; *Micrococcus flavus*; MIC; MBC; MIC index

1. Introduction

Food borne diseases have emerged as an important public health menace and evoked many economic problems in many countries during the last decade [1]. Many microbial strains cause predominately food borne diseases. Hence, it is obligatory for the food industry to periodically check the contamination levels of the microbial pathogens in the processing units. Further, to enhance the shelf life of food, there needs to be control measures that can effectively inhibit microbial growth. The application of synthetic chemicals as preservatives has greatly contributed to enhancing shelf life of food items by preventing microbial contamination but cause many undesirable side effects. Therefore several industries especially pharmaceutical companies are in search of alternative preservatives from natural sources, which have gradually provided impetus to eliminating synthetic preservatives in food due to their side effects on human health [2]. As opposed to synthetic compounds, natural preservatives obtained from medicinal plants are rich in phenolic compounds and can enhance overall quality through decrease in lipid oxidation and microbial growth [3].

Recently, the advent of antibiotic resistance of certain food borne pathogens and the reluctance of consumers towards consumption of chemically treated goods have encouraged the development of safe and natural antimicrobials for food products and treatment of various ailments. Substances obtained from plants are preferred over synthetic biocides as they have been used in traditional medicine for a long time [4]. In this context, antimicrobial properties from medicinal plants have increased interest in the possibility of forming an alternative for responding to consumer demands, considered to be safe for them and are not known to cause harm to the environment for the use of natural additives in foods [5].

It has been recognized that natural compounds play an important role in modern pharmaceutical care. Almost all the medicinal plants available in the world are great potential sources for discovery as well as production of new drugs benefit to mankind. Presently, there are many approaches available to search for new biologically active ingredients in the medicinal plants for the preparation of safe drugs. The plant crude extracts are of particular interest because of their wide acceptance by consumers for their potential multi purpose uses [6].

For the last decade, efforts have been made to improve methods and ways for proper utilization of fruits and vegetables wastes. The food and agricultural industries produce large volumes of wastes annually worldwide, causing a serious disposal problem. Till now, agro industrial waste is mostly utilized as feed or fertilizer. But using this agro waste therapeutically is a new idea which is slowly gaining popularity. The reutilization of biological wastes is of great interest since, due to legislation and environmental reasons, the industry is increasingly being forced to find an alternative use for its residual matter i.e. seed and peels [7]. One of the agro-wastes currently causing pollution problems is the peels and seeds of the mango fruit.

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Mango (*Mangifera indica* L.) is a tropical fruit that originated from Southeast Asia and has been cultivated for at least 4000 years [8]. Recognized for its attractive color, delicious taste and exotic flavour, mango is a rich source of carotenoids and provides high content of ascorbic acid and phenolic compounds, and has been recognized as 'king of the fruit'. Plant parts have been widely used to treat various ailments in traditional medicines and various parts of the plant has been scientifically proved to possess medicinal properties such as antidiabetic [9], antioxidant [10], antimicrobial [11], Antimicrobial and Radicial scavenging [12], Pharmacognostic study [13], Anti-inflammatory [14] and so on. Therefore, the objective of the present study was to investigate the antimicrobial activity from ripe peel, unripe peel, ripe seed and unripe seed of *Mangifera indica* L. against 25 food borne pathogens.

2. Materials and Methods

2.1. Collection of the plant materials

Mangifera indica L. var. Kesar fruits were purchased from local market of the Rajkot, Gujarat, India in the month of May 2010. The parts were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles.

2.2. Decoction extraction method

For the decoction method [15, 16], 5 grams of dried powder was extracted with 100 ml of deionized water at 100°C for 30 min in a water bath. It was filtered through eight layers of muslin cloth and centrifuged at 5,000 rpm in centrifuge (Remi Centrifuge, India) for 15 min. The supernatant was collected and water was evaporated using rotary vacuum evaporator (Equitron, India) to dryness. The residue was weighed to obtain the extractive yield and it was stored in air-tight bottles at 4°C.

2.3. Antimicrobial activity

2.3.1. Microorganisms tested

Ten Gram positive bacteria [*Bacillus cereus* ATCC11778 (*BC*); *B. megaterium* ATCC9885 (*BM*); *B. subtilis* ATCC6633 (*BS*); *Corynebacterium rubrum* ATCC14898 (*CR*); *Listeria monocytogenes* ATCC19112 (*LM*); *Micrococcus flavus* ATCC10240 (MF); *Staphylococcus albus* NCIM2178 (*SAL*); *S. aureus* ATCC25923 (*SA1*) and ATCC29737 (*SA2*); *S. epidermidis* ATCC12228 (*SE*)], 10 Gram negative bacteria [*Citrobacter frundii* NCIM2489 (*CF*); *Enterobacter aerogenes* ATCC13048 (*EA*); *Escherichia coli* NCIM2931 (*EC*); *Klebsiella aerogenes* NCIM2098 (*KA*); *K. pneumoniae* NCIM2719 (*KP*); *Proteus mirabilis* NCIM2241 (*PM*); *P. morganii* NCIM2040 (*PMO*); *P. vulgaris* NCIM2857 (*PV*); *Salmonella typhimurium* ATCC23564 (*ST*); *Pseudomonas aeruginosa* ATCC27853 (*PA*)] and 5 fungi [*Candida albicans* ATCC2091 (*CA*); *C. neoformans* NCIM3542 (*CN*); *C. glabrata* NCIM3448 (*CG*); *C. epicola* NCIM3367 (*CE*); *Trichosporon beigelii* NCIM3404 (*TB*)] were obtained from National Chemical Laboratory (NCL), Pune, India. The bacteria and fungi were maintained on nutrient agar and MGYP medium (Hi Media, India) respectively while *L. monocytogenes* and *E. coli* were maintained on Brain heart infusion agar and Luria medium (Hi Media, India) respectively at 4°C and sub cultured before use. The microorganisms studied are clinically important ones causing several infections, food borne diseases, spoilages, skin infection and it is essential to overcome them through some active therapeutic agents.

2.3.2. Antibiotics used in present study

The antibiotics Ampicillin ($AMP^{10\mu g/disc}$) and Amoxyclav ($AC^{30\mu g/disc}$) were used for antimicrobial susceptibility test. Antibiotics were purchased from Hi-Media Laboratory Pvt. Ltd., (Mumbai, India).

2.3.3. Agar well diffusion method for antibacterial and antifungal activity

In vitro antimicrobial activity of the different solvent extracts was studied against pathogenic microbial strains by the agar well diffusion method [11, 17]. Mueller Hinton No. 2 / Sabouraud dextrose agar (Hi-media) was used for the antibacterial and antifungal susceptibility test respectively. The different solvent extracts were diluted in 100% DMSO to give a concentration of 20 mg ml^{-1}. The Mueller Hinton agar / Sabouraud dextrose agar was melted and cooled to 48-50°C and a standardized inoculum (1.5×10^8 CFU ml^{-1}, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes; wells (8.5 mm) were prepared in the seeded agar plates. The test compound (100 µl) was introduced into the well. The plates were incubated over night at 37°C and 28°C for 24 h and 48 h respectively, for bacteria and fungi. DMSO was used as negative control. The microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented with ± S.E.M.

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2.3.4. Preparation of the extracts and/or antibiotics for MIC and MBC study

The extracts dissolved in 100% of DMSO were first diluted to highest concentration (1250 µg ml^{-1}) to be tested, and then serial two fold dilution was made in a concentration range (39 to 1250 µg ml^{-1}). Chloramphenicol (CH) was used as a positive control (1 to 32 µg ml^{-1}).

2.3.4.1. Preparation of bacterial inocula for MIC and MBC study

The inocula of the test organisms were prepared using the colony suspension method [18]. Colonies picked from 24 h old cultures grown on nutrient agar were used to make suspension of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by transfer of 0.1 ml of the bacterial suspension to 9.9 ml of sterile Mueller Hinton broth before use to yield 6×10^5 CFU ml^{-1}.

2.3.4.2. Determination of the minimum inhibitory concentrations (MIC)

The MIC was determined by the micro well dilution method [19] with some modifications. This test was performed in sterile flat bottom 96 well micro test plates (Tarsons Products Pvt. Ltd.). 150 µl volume of Mueller Hinton broth was dispensed into each well and 20 µl of various concentrations of the extract was added in decreasing order along with 30 µl of the test organism suspension. The final volume in each well was 200 µl (150 µl Mueller Hinton broth, 30 µl of the test organism suspension, and 20 µl plant extract/antibiotic). Three control wells were maintained for each test batch; the positive control (CH, MHB and test organism), sterility control (MHB and DMSO) and organism control (MHB, test organism and DMSO). Plates were then incubated at 37°C for 24 h**.** Experiments were carried out in duplicate. After incubation, 40 µl of INT (2-(4-Iodo phenyl)-3-(4-nitro phenyl)-5-phenyltetrazolium chloride) solution (200 µg ml^{-1}) dissolved in sterile distilled water was added to each well [20]. The plates were incubated for further 30 min and estimated visually for change in color to pink indicating reduction of the dye due to bacterial growth. The highest dilution (lowest concentration) that remained clear corresponded to the MIC.

2.3.4.3. Determination of the minimum bactericidal concentration (MBC)

MBC was determined from all wells showing no growth as well as from the lowest concentration showing growth in the MIC assay for all the samples. Bacterial cells from the MIC test plate were sub cultured on freshly prepared solid nutrient agar plates by making streaks on the surface of the agar. The plates were incubated at 37°C for 24 h overnight. Plates that did not show growth were considered to be the MBC for the extract or drug used [21]. The experiment was carried out in duplicate.

2.3.4.4. Determination of MIC index

The MIC index (MBC/MIC) was calculated for extracts to determine whether an extract had bactericidal effect (MBC/MIC≤4) or bacteriostatic effect (>4 MBC/MIC<32) on growth of bacteria [22, 23].

2.4. Statistical analysis

Each sample was analyzed individually in triplicate and the results are expressed as the mean value (n = 3) ± Standard Error of Mean (S.E.M.).

3. Results and Discussion

Natural phenolic compounds are secondary plant metabolites which posses an aromatic ring bearing one or more hydroxyl groups. The main interest of antimicrobial compounds comes from the properties of natural phenols as food preservatives in order to avoid pathogenic microbes, rancidity and oxidation of lipids [24]. Food borne diseases pose a considerable threat to human health. Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of food borne diseases. The method of microbial growth inhibition in food is the use of food preservatives. Increasing use of chemical preservatives can effectively prevent the growth of most food borne microorganisms, but safety problems related to their use are receiving more attention and thus natural preservatives for foods have high potential in food industry. The substances that can inhibit pathogens, inexpensive, corrosion-free and have less toxicity to host cells could be considered candidates for developing new antimicrobial drugs/preservatives [25-27]. Therefore, in this study the antimicrobial activity of decoction extracts of ripe peel, unripe peel, ripe seed and unripe seed of *M. indica* was evaluated against 10 Gram positive, 10 Gram negative and 5 fungi strains by measuring the inhibition zone diameter and the determination of MIC and MBC values (Fig. 1; Table 1).

All extracts show varying degree of antibacterial activity against most of the Gram positive and all the Gram negative bacteria tested (Fig. 1). The studied decoction extracts of ripe peel, unripe peel, ripe seed and unripe seed of *M. indica* against Gram positive bacteria is shown in Fig. 1(a, b). The studied decoction extracts did not show activity against *B.*

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subtilis, B. megaterium and *L. monocytogenes* (Fig. 1a). All the extract showed activity against *C. rubrum, M. flavus, S. albus* and *S. aureus 2*. The highest activity was in ripe seed extract against *M. flavus* closely followed by unripe seed and ripe peel (Fig. 1b). *B. cereus* and *S. aureus 1* were susceptible to unripe peel, ripe seed and unripe seed extracts. *S. epidermidis* was susceptible to ripe peel, ripe seed and unripe seed extracts. All the extracts showed better activity against *M. flavus* while antibiotics did not show any activity.

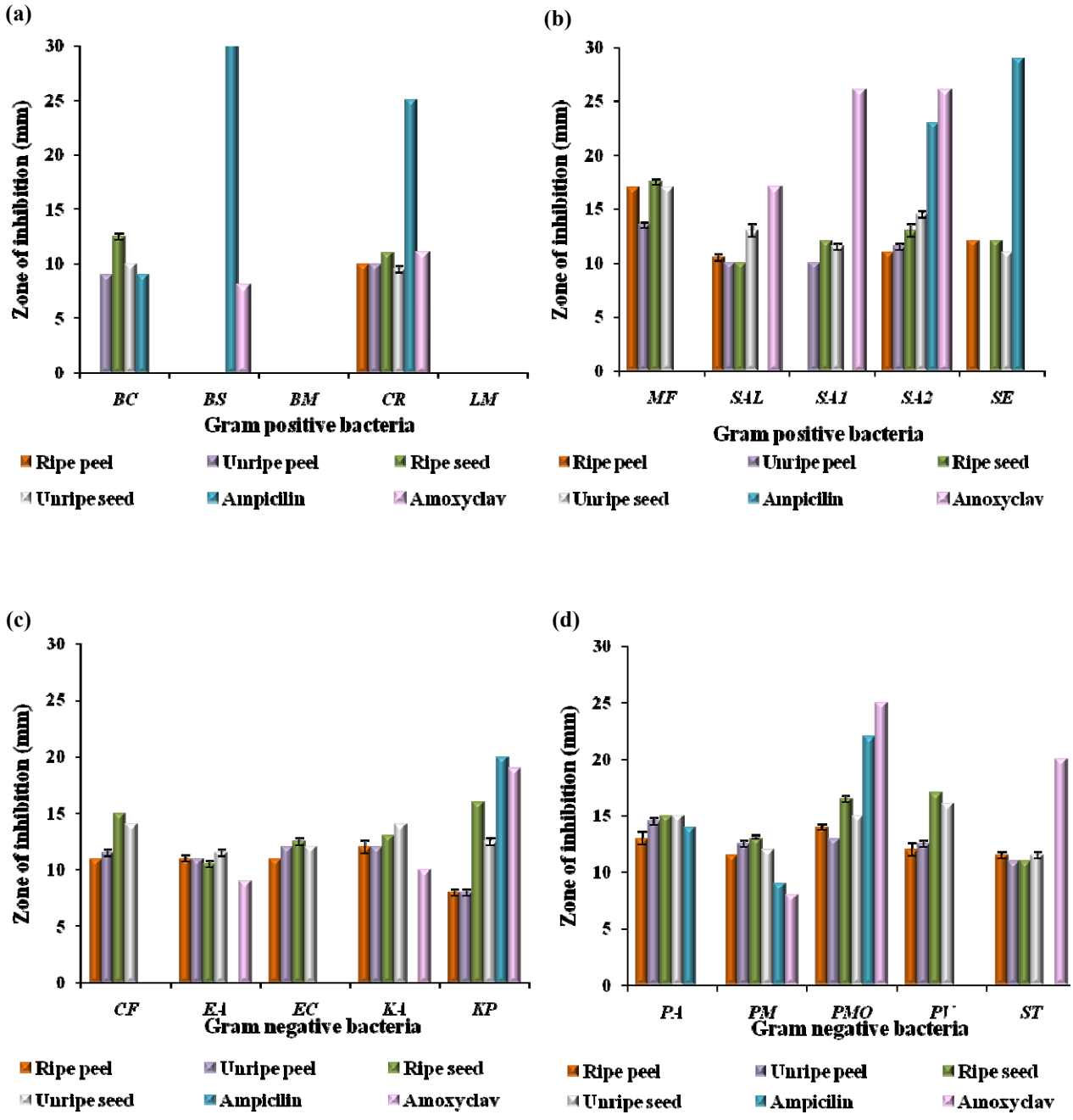

Fig. 1 Antimicrobial activity of ripe peel, unripe peel, ripe seed and unripe seed of *M. indica* and standard antibiotics.

The antibacterial activity of decoction extracts of ripe peel, unripe peel, ripe seed and unripe seed of *M. indica* against Gram negative bacteria is shown in Fig. 1(c, d). All the extracts showed activity against all the Gram negative bacteria. The ripe seed showed highest zone of inhibition against *P. vulgaris.* All the extracts showed activity against *C. freundii, E. coli, P. vulgaris* while antibiotics did not show any activity. Hence, it can be stated that in the present study, the decoction extracts displayed effective antibacterial effect against food spoilage and food borne pathogens.

None of the extracts showed activity against fungi, may be due to their cell structure. Similar result was reported in leaf extract of *Phyllantus muellerianus* by Ofokansi et al. [28]. The cell wall of fungi is poorly permeable and it possesses both cell membrane and cell wall. Resistance of fungi to plant extracts can be explained by chitinous structure

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of the cell wall, which does not allow easy penetration of bioactive substances [29]. This is in agreement with many earlier reports where plant extracts showed better antibacterial activity than antifungal activity [30-32].

Ripe peel extract inhibited 50% Gram positive bacteria and 100% Gram negative bacteria. Unripe peel extract inhibited 60% Gram positive bacteria and 100% Gram negative bacteria. Ripe and unripe seed extracts showed similar level of inhibition i.e. 70% Gram positive bacteria and 100% Gram negative bacteria. All the extracts of tested parts showed better antibacterial activity than antifungal activity; Gram negative bacteria were more susceptible than Gram positive bacteria. The differences in the antimicrobial effects between Gram positive and Gram negative bacteria are mainly due to their different cell wall structures. The cell wall of Gram-positive bacteria consists of a single layer, whereas the Gram-negative cell wall is a multilayered structure bounded by an outer cell membrane [33-35]. A single membrane structure has a poor buffering capacity against the localized protonation effects generated by phyto compounds. Therefore, the bacterial intra-cellular space can be easily hyper-acidified, causing functional disorder of bacterial energy metabolism. However, an external lipopolysaccharide layer and additional minor membrane components of the cell wall give more buffering capacity to Gram negative bacteria, functioning as a preventive barrier against hydrophobic compounds [36]. Consequently, these bacteria exhibit less sensitivity to the antimicrobial activities of phyto-compounds and many authors also supported the more susceptibility of the Gram positive bacteria towards phytoconstituents [37-41], but in the present study contradictory results are observed. Here, Gram negative bacteria were more susceptibility than Gram positive bacteria. This results match with the previous reports [42-44]. Compounds which can effectively diffuse the lipid bilayer and increase membrane fluidity might be more effective antibacterial agents. The ripe and unripe seed displayed a better and exerted considerable antibacterial effect against both Gram positive and Gram negative bacteria tested.

Minimum inhibitory concentration refers to the lowest concentration of the antimicrobial agent which is required for the inhibition of visible growth of the tested microorganism [45]. The antimicrobial activity of an agent is usually quantified by determining the MIC values which serve as a guide for treatment of most infections. MIC values were calculated using INT dye on a 96 well micro titre plate. The MBC is interpreted as the lowest concentration that can completely remove the microorganisms.

The extracts which showed >15 mm zone of inhibition were further studied for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against selected bacterial strains. The values are presented in Table 1. The extracts exhibited concentration dependent inhibition of growth. In a negative control, DMSO had no inhibitory effect on the tested organisms. The MIC and MBC values of ripe peel extract were 1250 μg ml^{-1} and >1250 μg ml^{-1} respectively against *M. flavus.* The MIC and MBC values of ripe seed extract were 156 μg ml^{-1} and 625 μg ml^{-1} respectively against *M. flavus.*The ripe seed showed MIC values 78 μg ml^{-1} and 1250 μg ml^{-1} against *P. morganii* and *K. pneumoniae* respectively and their MBC value was >1250 μg ml^{-1}. The MIC and MBC values of unripe seed extract were 312 μg ml^{-1} and 1250 μg ml^{-1} respectively against *M. flavus.* The unripe seed showed MIC and MBC values were 1250 μg ml^{-1} >1250 μg ml^{-1} respectively against *P. vulgaris*. The ripe seed and unripe seed decoction extracts showed bactericidal activity against *M. Flavus*.

Table 1 MIC, MBC and MIC index of decoction extracts of ripe peel, ripe seed and unripe seed of *M. Indica*.

Microorganisms		Ripe peel		Ripe seed		Unripe seed		Chloramphenicol	
		MIC*	**MBC***	**MIC***	**MBC***	**MIC***	**MBC***	**MIC***	**MBC***
A	***MF***	1250	>1250	**156**	**625**	**312**	**1250**	**4**	**16**
B	***PMO***	NT	NT	78	>1250	NT	NT	4	>32
	KP	NT	NT	1250	>1250	NT	NT	4	32
	PV	NT	NT	NT	NT	1250	>1250	NT	NT

*: Values are expressed in μg ml^{-1}; NT: Not tested, **A**: Gram positive bacteria; **B**: Gram negative bacteria; **MBC/MIC ≤ 4**: Bactericidal effect, **> 4 MBC/MIC < 32**: Bacteriostatic effect

4. Concluding remarks

In conclusion, the contamination of food by microorganisms is a worldwide public health concern and is a leading cause of trade problems internationally. Plants parts can be used as preservatives during food processing to prevent the food borne microorganisms. Therefore, in the present study, the decoction extract was obtained from ripe and unripe peel and seed of mango. The antimicrobial activity of extracts were assessed by using agar well diffusion method in which ripe and unripe seed extracts showed more inhibition as compared to ripe and unripe peel. Moreover, Gram negative bacteria were more susceptible than Gram positive bacteria and fungi. According to MIC and MBC measurement, ripe and unripe seed showed lesser and better MIC, MBC values and bactericidal effects against *M. flavus*. According to these results, decoction extracts of mango could be considered as a natural preservative against food borne pathogens which could be considered as a natural alternative to traditional food preservatives and be used to enhance food safety and shelf life and suitable for applications in food industry and human health. Mango peels and seeds being waste materials,

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different profit products can be obtained, providing added value to these waste as a source antimicrobial compounds that can be used in pharmaceuticals, cosmetics or food industry and could improve the food nutritional value.

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