

Phytochemical Profiling and Antimicrobial Potential of Mango (*Mangifera indica* L.) Seed Waste: A Waste-to-Value Approach

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Abstract: Mango (*Mangifera indica* L.) seed waste is an important by-product of fruit processing industry and a rich source of bioactive compounds with potential pharmaceutical applications. The present study was carried out to determine the phytochemical constituents and antimicrobial activity of mango seed and seed kernel extracts against selected bacterial and fungal pathogens. Phytochemical screening revealed the presence of some secondary metabolites such as phenolics, flavonoids, tannins, alkaloids and glycosides which are known for their antimicrobial properties. The antimicrobial activity was tested by agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. The extracts showed concentration dependent antibacterial activity against *S. aureus* with maximum zone of inhibition was 16 mm at 10,000 µg and no inhibitory activity was observed for *E. coli*. Antifungal testing showed moderate activity against *C. albicans* with maximum zone of inhibition of 15mm at the highest concentration tested. No activity was detected against *A. niger*. *S. aureus* and *C. albicans* showed a higher susceptibility, which could be related to the effect of phenolic and flavonoid compounds present in the extracts. The results indicate that mango seed waste is a potential source of natural antimicrobial agents, which can be utilised in pharmaceutical, nutraceutical and food preservation applications as a sustainable source.

Keywords: Phytochemicals, Antimicrobial Activity, Phenolics, Flavonoids, Natural Antimicrobial Agents.

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Introduction

The rising antimicrobial resistance has heightened the concern for the search of novel antimicrobial agents from the natural sources. Plant secondary metabolites are well studied for their broad spectrum of biological activities and less toxicity. *Mangifera indica* L. (Mango) is one of these important medicinal plants as the leaves, bark, peel, pulp and especially seed kernels (Masibo and He, 2008) contain lots of bioactive compounds. The mango seed accounts for 10–25 % of the total fruit weight and is normally regarded as an agro-industrial waste. However, several studies reported that mango seed kernel is rich in phenolic compounds, tannins, flavonoids, alkaloids, saponins and xanthenes such as mangiferin with significant antioxidant and antimicrobial properties (Abdalla *et al.*, 2007; Barreto *et al.*, 2008).

Antimicrobial activity against *Escherichia coli*

There are many reports on antibacterial activity of mango seed extracts against Gram positive as well as Gram negative bacteria. The authors reported the efficacy of ethanolic extracts of mango seed kernels to inhibit the growth of *Escherichia coli* and they attributed the activity to the high content of polyphenolic compounds (Abdalla *et al.*, 2007). In the same way, Dorta *et al.* (2014) showed the high antimicrobial activity of mango by-products extracts against foodborne pathogens as *E. coli*. Phenolics exert their antibacterial activity by disrupting bacterial cell membranes, denaturing proteins and interfering with cellular metabolism (Cowan, 1999). *E. coli* and other Gram negative bacteria have an outer lipopolysaccharide membrane that often provides resistance to phytochemicals, but at sufficiently high

concentrations the polyphenols of mango can penetrate this barrier (Nikaido, 2003).

Antibacterial activity against *Staphylococcus aureus*

Staphylococcus aureus is the most reported bacterial pathogen which is highly sensitive to phytochemicals of mango. The methanolic extracts of mango seed kernel have been reported to show significant inhibitory activity against *S. aureus* (Bshabshe *et al.*, 2020). Mango polyphenols were reported by Engels *et al.* (2011) to be effective in inhibiting the growth of Gram positive bacteria. The greater susceptibility of *S. aureus* is usually attributed to the less impenetrable peptidoglycan-rich cell wall and the absence of outer membrane that permits the penetration of antimicrobial compounds (Cushnie and Lamb, 2005).

Antifungal activity against *Candida albicans*

Several investigators have described antifungal activity of extracts of mango seeds against pathogenic yeasts. Rich mangiferin extracts inhibit the growth of *Candida albicans* by damaging the membrane integrity, and by inhibiting the fungal enzyme system (Sánchez *et al.*, 2000). Engels *et al.* (2011) also showed that mango polyphenols possess significant antifungal activity against different *Candida* spp. Phenolic compounds and tannins have been reported to bind to fungal cell wall proteins, inhibit ergosterol biosynthesis and disrupt fungal metabolism (Cowan, 1999).

Antifungal Activity of *Aspergillus niger*

Unlike the activity against *Candida* species, the few studies on the activity of mango extracts against filamentous fungi such as

Aspergillus niger have reported limited or variable efficacy. Resistance of *Aspergillus* spp. to a number of natural antimicrobials has been linked to complex cell wall architecture and melanin (Lalgé, 2010). Some researchers reported moderate inhibition of *A. niger* by concentrated mango seed extracts (Dorta *et al.*, 2014) while others observed little or no activity depending on extraction methods and solvent systems. Thus, lack of inhibition of *A. niger* in this study is in agreement with previous reports which show that filamentous fungi are, in general, more resistant to antimicrobial compounds of plant origin than yeast species.

Bioactive compounds with antimicrobial properties

The antimicrobial activity of mango seed kernels is due to the higher amount of phenolics, flavonoids, tannins, gallotannins and mangiferin. Various mechanisms such as membrane destruction, enzyme inhibition, metal ion chelation, induction of oxidative stress and interference with microbial DNA replication have been proposed for the antimicrobial activity of these compounds (Cowan, 1999; Masibo & He, 2008). Also, it has been recently reported that extracts from mango seed kernel exhibit a broad spectrum of antimicrobial activity and can serve as potential substitutes for synthetic antimicrobial agents in pharmaceutical, food preservation and nutraceutical applications (Dorta *et al.*, 2014). Hence, mango seed waste has attracted more and more attention as a sustainable source of bioactive compounds with high therapeutic potential.

Materials and Methods

Plant Material and Extraction Method

Mango (*Mangifera indica* L.) seeds and seed kernels were collected, cleaned, shade dried and powdered. The powdered material was extracted with a suitable organic solvent and the extract was concentrated under reduced pressure and stored at 4°C until further use.

Microorganisms to be tested

Antimicrobial activity of extracts was determined against two bacterial strains, *Escherichia coli* and *Staphylococcus aureus* (MTCC 96) and two fungal strains, *Candida albicans* (MT 854)

and *Aspergillus niger* (MTCC 281). Pure cultures were kept on nutrient agar (bacteria) and potato dextrose agar (fungi) under standard laboratory conditions.

Anti Microbial Assay:

The agar well diffusion method was used to determine the antibacterial and antifungal activities following standard procedures (Perez *et al.*, 1990; Balouiri *et al.*, 2016). Standardised microbial suspensions were inoculated on sterile agar plates. Different concentrations of extract (625, 1250, 2500, 5000 and 10,000 µg) were aseptically loaded into wells. Positive control for the antibacterial assays was Ciprofloxacin and positive control for the antifungal assays was standard antifungal agent. The solvent used in the preparation of the extract was used as a negative control.

Incubation & Measurement

Bacterial plates were incubated at 37°C for 24 h and fungal plates at 28°C for 48-72 h. Antimicrobial activity was determined by measuring the diameter of the inhibition zone (mm) around each well. All experiments were performed in triplicate (Plates A, B and C).

Statistical Analyses

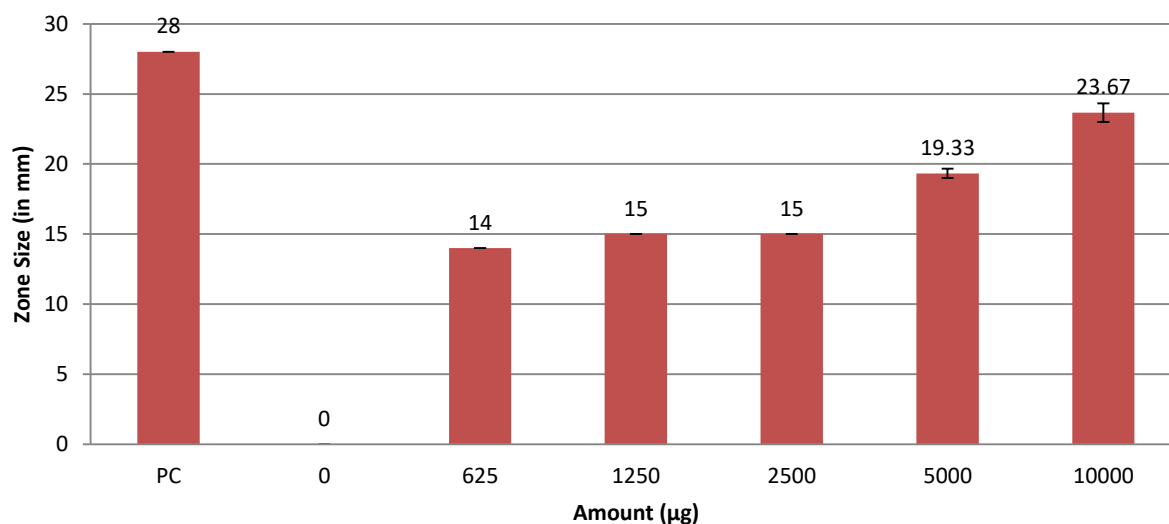
Results were expressed as mean inhibition zone ± standard deviation (SD) and standard error of mean (SEM). Statistical parameters were calculated from three independent replicates for evaluation of reproducibility and consistency of the assay.

Results and Discussion

The agar well diffusion assay was used to test the antibacterial activity of *Mangifera indica* seed and seed kernel extract against *Escherichia coli*. The extract exhibited definite concentration dependent inhibitory effect on the test organism (Table 1, Graph 1 and Figure 1). The absence of inhibition zone in negative control (0 µg) shows that the solvent used for extraction did not contribute any antibacterial activity. The positive control showed a maximum zone of inhibition of 28 mm confirming the efficiency and reliability of the assay.

Table 1. Results of Antibacterial Activity of *E.coli* on Plate A,B & C

Amount (µg)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	28	28	28	28	0	0
0	0	0	0	0	0	0
625	14	14	14	14	0	0
1250	15	15	15	15	0	0
2500	15	15	15	15	0	0
5000	20	19	19	19.33	0.57735027	0.33333
10000	25	23	23	23.67	1.15470054	0.66667



Graph 1. Antibacterial Activity of STD and sample

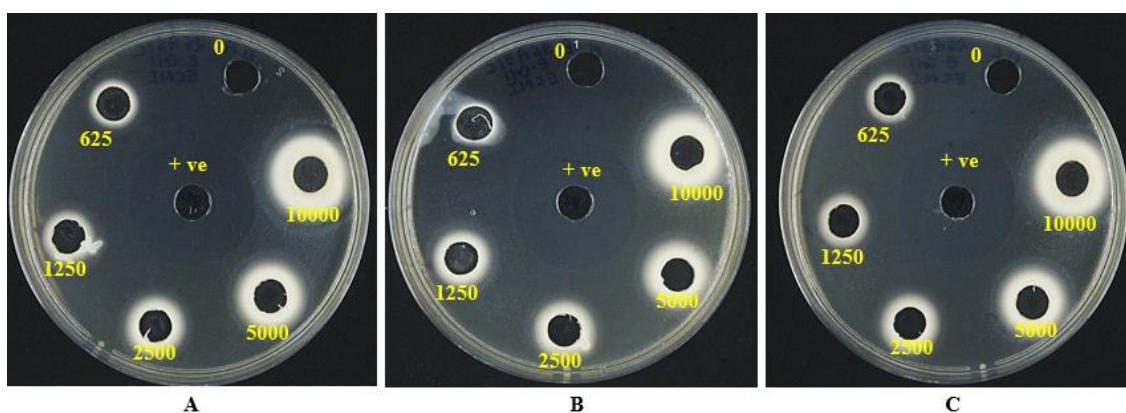
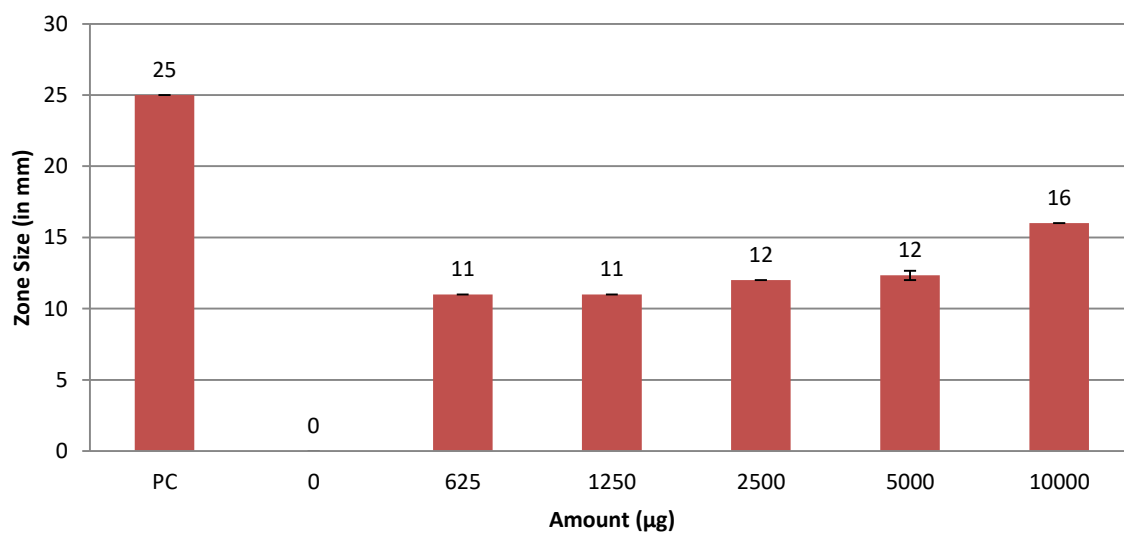


Figure 1. Test Organism- *E. coli* (ECMI) on Plate A,B & C

Table 2. Results of Antibacterial Activity of *S. aureus* on Plate A,B & C

Amount (µg)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	25	25	25	25	0	0
0	0	0	0	0	0	0
625	11	11	11	11	0	0
1250	11	11	11	11	0	0
2500	12	12	12	12	0	0
5000	12	13	12	12	0.57735	0.33333
10000	16	16	16	16	0	0



Graph 2. Antibacterial Activity STD & Sample

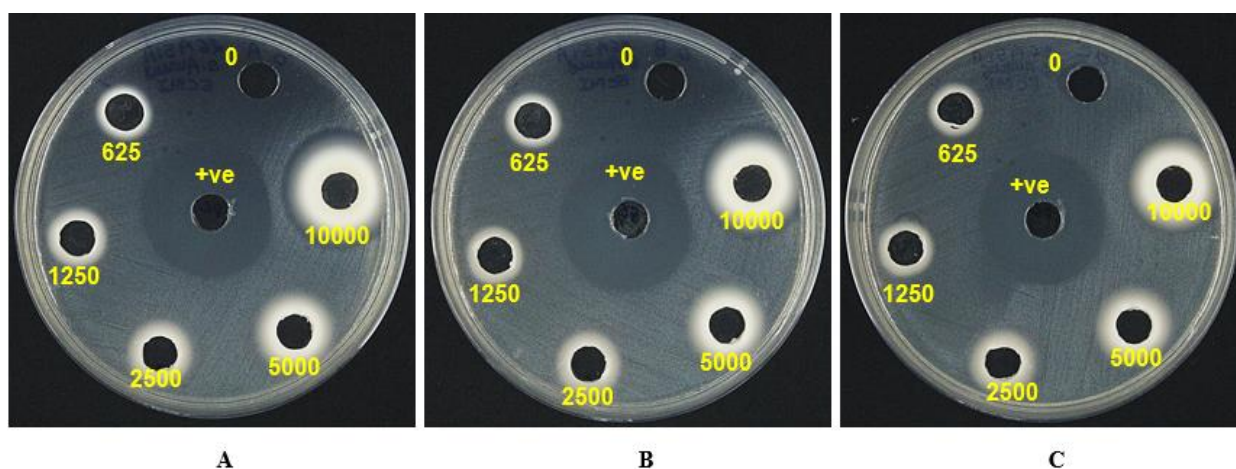
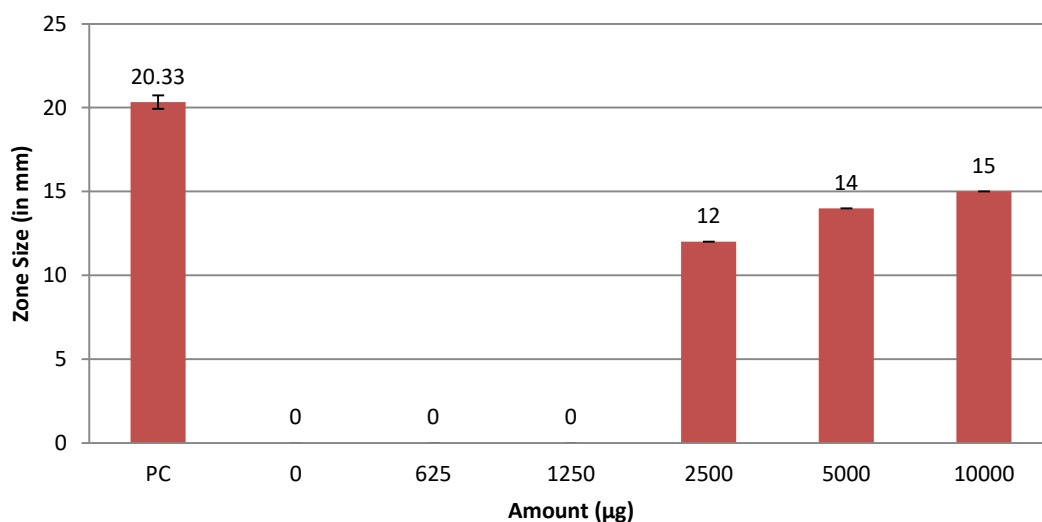


Figure 2. Test Organism- *S. aureus* (SAMI) on Plate A,B & C

Table 3. Results of Anti-Fungal Activity of *Candida albicans* Plate A,B & C

Amount (µg)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	21	20	20	20.33	0.5773503	0.40825
0	0	0	0	0	0	0
625	0	0	0	0	0	0
1250	0	0	0	0	0	0
2500	12	12	12	12	0	0
5000	14	14	14	14	0	0
10000	15	15	15	15	0	0



Graph 3. Antibacterial Activity STD & Sample

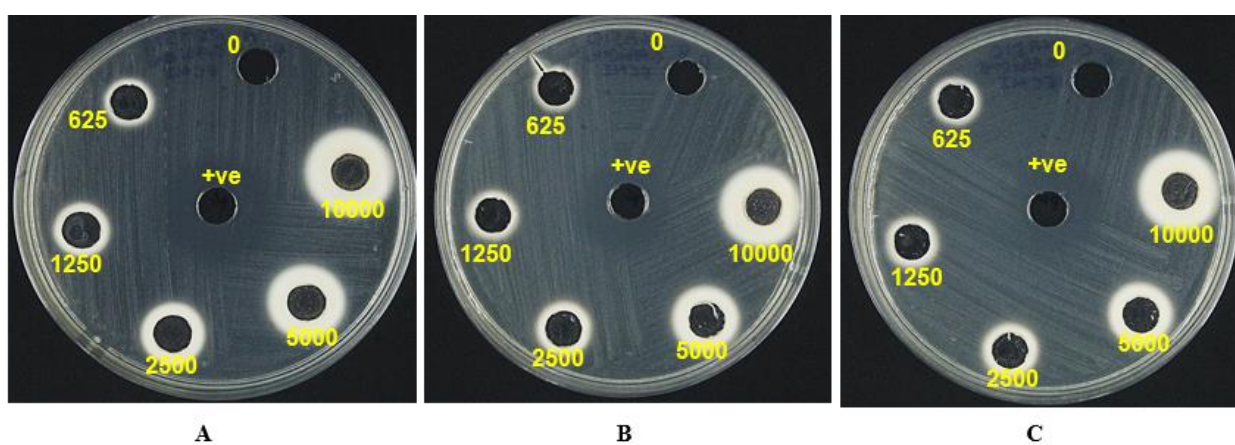
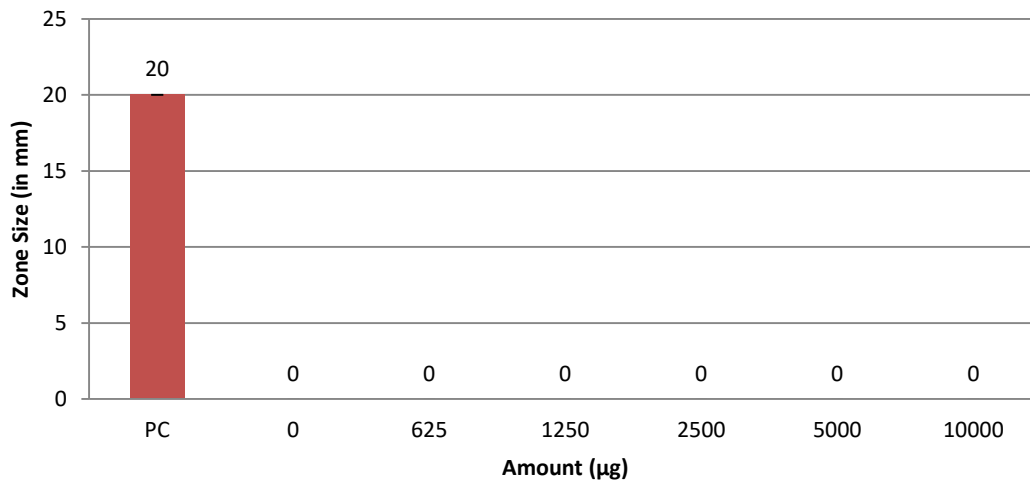


Figure 3. Test Organism- *Candida albicans* (CAMI) on Plate A,B & C

Table 4. Results of Anti-Fungal Activity of *Aspergillus niger* Plate A,B & C

Amount (µg)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	20	20	20	20	0	0
0	0	0	0	0	0	0
625	0	0	0	0	0	0
1250	0	0	0	0	0	0
2500	0	0	0	0	0	0
5000	0	0	0	0	0	0
10000	0	0	0	0	0	0



Graph 4. Anti-Fungal Activity STD & Sample

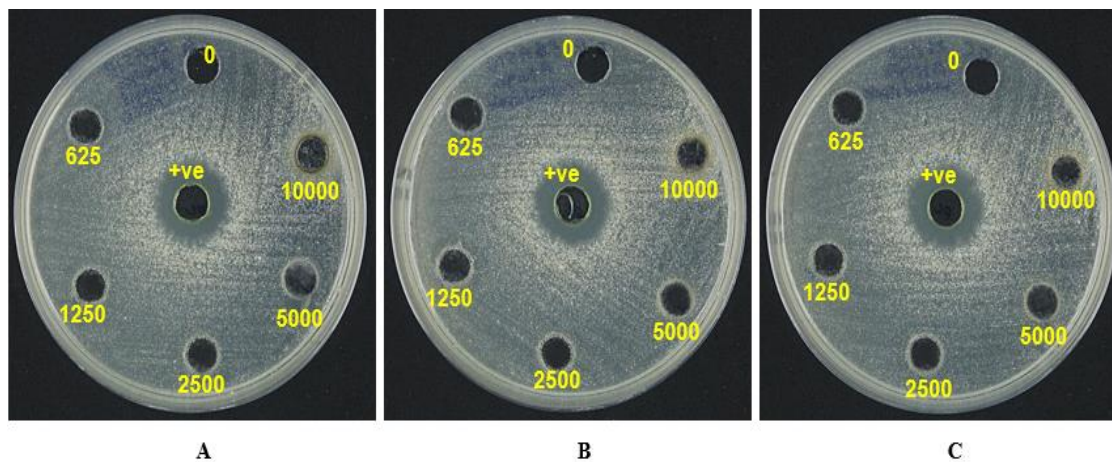


Figure 4. Test Organism- *Aspergillus niger* (ANMI) on Plate A, B & C

The lowest concentration of the extract showing inhibition zone was 625µg, with inhibition zone of 14mm. Inhibition at 1250 µg was slightly better with 15 mm and same at 2500 µg . At high concentration, the antibacterial activity was significantly higher with inhibition zones of 19.33 ± 0.58 mm and 23.67 ± 1.15 mm at 5,000 µg and 10,000 µg respectively. The inhibition zone showed a gradual increase in diameter indicating dose dependent antibacterial activity of the extract. The low values of SD and SEM at all concentration points show reproducibility and consistency of triplicate observations. The inhibition zone at 10000 µg similar to positive control, which shows the significant antibacterial effect of mango seed extract on *E. coli*. The antibacterial activity observed could be attributed to the presence of phenolic compounds, flavonoids, tannins, alkaloids and xanthone derivatives like mangiferin which have been widely reported in mango seeds and kernels . These phytochemicals demonstrated antimicrobial activity by disintegrating the bacterial cell membrane, obstructing the synthesis of nucleic acid, altering the permeability of the membrane and affecting the vital metabolic enzymes (Cowan, 1999; Cushnie and Lamb, 2005). Mangiferin is one of the major bioactive compounds of mango seed kernels and has been reported to have significant antimicrobial and antioxidant properties.

Interestingly, the extract showed significant activity against *E. coli*, a Gram-negative bacterium which is generally more resistant to plant derived antimicrobial agents due to the outer lipopolysaccharide membrane that restricts the penetration of

bioactive molecules (Nikaido, 2003). Anti-microbial activity of mango seed extract against *E. coli* indicates the presence of potent anti-microbial constituents able to cross this permeability barrier. Similar antibacterial activity of mango seed kernel extracts against Gram-negative bacteria was also reported by Abdalla *et al.* (2007). This has been attributed to the high concentration of polyphenolic compounds present in the seed waste. The present findings are also in conformity with the work of Dorta *et al.* (2014) and Engels *et al.* (2011) who reported significant antibacterial activity of mango by-product extracts against food borne and clinical bacterial pathogens. The concentration dependent response observed in the present study further strengthens the potential of mango seed waste as a natural source of antimicrobial agents.

The total results indicate that extracts of *Mangifera indica* seed and seed kernel have promising antibacterial activity against *E. coli* and the activity was significantly higher at higher concentration. The results highlight the potential of mango processing waste as a source of added value bioactive compounds for pharmaceutical, nutraceutical and food preservation uses. The antifungal assay against *C. albicans* (plates A–C) showed a clear dose–response. The positive control (amphotericin B) produced a large inhibition zone (mean ≈ 20.33 mm, SD 0.58, SEM 0.41). At 0, 625, or 1250 µg extract no inhibition was observed. At 2500 µg, zones of ≈ 12 mm (n=3, SD=0) appeared, increasing to 14 mm at 5000 µg and 15 mm at 10000 µg (SD=0 for both). Negative controls (solvent only) yielded no zones. Thus antifungal activity

emerged only at the highest doses, rising modestly with concentration and remaining substantially below the positive control in efficacy overall. Indeed. The modest inhibition only at $\geq 2500 \mu\text{g}$ indicates low potency. By comparison, amphotericin B typically yields $\geq 15 \text{ mm}$ zones or $\text{MIC} \leq 1 \mu\text{g/mL}$ (Sytykiewicz et al. 2015), and potent plant extracts often have $\text{MICs} \ll 10 \mu\text{g/mL}$ (Esmaeili et al. 2025). Our extract's $\sim 15 \text{ mm}$ zone at $10000 \mu\text{g}$ is barely "moderate" by Sytykiewicz's criteria. Oves et al. (2024) similarly reported dose-dependent zone increases. Given that all strains in Sytykiewicz et al. were amphotericin-sensitive, our results imply only marginal extract activity at high dose. (Limitation: strain/incubation unspecified.)

Conclusion

In the present study, waste of mango (*Mangifera indica* L.) seeds with seed and seed kernel extract was confirmed as a rich source of bioactive compounds having good antimicrobial potential. Phytochemical analysis revealed the presence of important secondary metabolites such as phenolics, flavonoids, tannins, alkaloids and glycosides which are known to have antimicrobial properties. In anti-microbial assay, the extracts showed concentration dependent anti-bacterial activity against *Escherichia coli* and *Staphylococcus aureus* and the maximum inhibition zones were recorded at $10000 \mu\text{g}$. The extract exhibited higher activity against *E. coli* (Table 1) (23.67 mm) than *S. aureus* (Table 2) (16 mm) showing the broad spectrum anti-bacterial potential. On *Candida albicans* (Table 3) the extracts exhibited a moderate antifungal activity. Zone of inhibition was observed 12 mm at $2500 \mu\text{g}$ to 15 mm at $10000 \mu\text{g}$. No inhibition was observed in *Aspergillus niger* (Table 4) at any of the tested concentrations. The results showed that the antimicrobial activity of the mango seed waste could be due to the high content of phytochemicals especially polyphenolic compounds such as mangiferin. In conclusion, the present study demonstrated the potentiality of mango seed waste as a cheap, sustainable and environmentally friendly source of natural antimicrobial agents. This agro-industrial by-product could be exploited for waste valorisation and production of new bioactive compounds for pharmaceutical, nutraceutical and food preservation applications.

Future Perspective

Further studies should be directed towards the isolation and characterisation of individual bioactive compounds responsible for antimicrobial activity. Such findings may help to utilise mango seed waste for the development of natural therapeutics and food preservatives, determination of MIC, minimum bactericidal concentration (MBC), toxicity evaluation and formulation of antimicrobial product

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