Current Research in Microbial Sciences 6 (2024) 100243

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Valorisation of raw mango pickle industry waste into antimicrobial agent against postharvest fungal pathogens

Gouthami Shivaswamy [a], Shalini Gaur Rudra [a,*], Lham Dorjee [b], Aditi Kundu [c], Robin Gogoi [b], Anupama Singh [c]

[a] Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India
[b] Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India
[c] Division of Agricultural Chemicals, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

ARTICLE INFO

Keywords:
Mango kernel phenolic extract
Mangiferin
Quercetin
Rhamnetin
Pickling industry waste

ABSTRACT

In mango pickle industry, a significant quantity of mango seed kernels is discarded as solid wastes. These seed kernels can be an ideal source for obtaining extracts rich in bioactive polyphenolic compounds with good antioxidant properties. The potential of mango kernel phenolic extract (MKPE) was investigated as a natural and effective antimicrobial agent for controlling major postharvest fungal pathogen infections, a significant threat to global food supply chains. Fungal pathogens contribute to the deterioration of fruits, vegetables, and grains during storage and transportation, leading to economic losses and compromised food safety. MKPE was obtained from pickling variety 'Ramkela' raw mango kernels, and its phenolic composition was characterized using LC–MS. The *in vitro* antifungal activity of MKPE against *Botrytis cinerea, Colletotrichum gloeosporoides*, and *Rhizopus stolonifer* was evaluated in vitro. A concentration-dependent inhibition of fungal radial growth against all three pathogens was observed, exhibiting the potential of MKPE as a valuable natural resource for addressing postharvest losses caused by fungal pathogens. The extraction process yielded a total phenolic content of 2128 mg GAE/100 g. Major polyphenolic bioactive compounds present were mangiferin, quercetin, and rhamnetin. The *in-vitro* antimicrobial assay showed reduction in the radial growth and inhibition percent of the pathogens. EC_{50} values of MKPE for *B. cineria, C. gloeosporoides*, and *R. stolonifer* was found to 364.17, 963.8 and 926 ppm, respectively. Our results demonstrate an economical, sustainable, and eco-friendly approach to manage postharvest diseases rendered by fungi using mango MKPE from pickling industry waste.

Introduction

The mango pickling industry is a vast market in India with a 7.9 billion USD market. In the pickle industry mature but raw mangoes of Ramkela cultivar are preferred because of their acidity, fibrosity and tangy taste. A significant 25–45 % of mango seed kernels are discarded as solid wastes. These seed kernels can prove to be ideal raw materials for extracting bioactive polyphenolic compounds with antioxidant and other beneficial properties. The conversion of these mango kernel wastes into utilizable food ingredients would immensely help in reducing environmental problems associated with mango processing waste disposal.

Natural phenolic compounds exhibit notable antioxidant properties and contain at least one aromatic ring connected to hydroxyl substituents. Their effectiveness as antioxidants rises from their capability to neutralize and stabilize free radicals by integrating them into their aromatic ring and absorbing UV light (Kalogianni et al., 2020). In contrast to synthetic phenolic antioxidants (with toxicity and carcinogenicity issues), use of natural phenolic compounds is an eco-friendly approach.

Fungal pathogens are major contributors to the deterioration of fruits, vegetables, and grains during postharvest storage and transportation (Davies *et al.*, 2021). These lead to economic setbacks to the global food supply chain. Fungal infections result in spoilage, decay, and production of mycotoxins, further exacerbating the challenges associated with food security and safety. This emphasizes the intricate need for innovative strategies, advanced sustainable technologies, and a holistic approach to mitigating factors contributing to these losses.

Colletotrichum stands out as a widespread and significant genus of plant-pathogenic fungi. Nearly every crop cultivated globally is

* Corresponding author.
E-mail addresses: gaurshalini@gmail.com, shalini.rudra@icar.gov.in (S.G. Rudra).

https://doi.org/10.1016/j.crmicr.2024.100243

Available online 26 May 2024

vulnerable to one or more species of *Colletotrichum* as it causes damage in both pre- and post-harvest conditions resulting in the loss of upto 100% of stored fruit if care is not taken (Talhinhas and Baroncelli, 2021). *R. stolonifer* causes soft rot in fruits and vegetables. Its infection becomes evident post-storage, especially in market conditions or within the consumer's home when the storage temperature of produce exceeds 5 °C (Li *et al.*, 2023). Additionally, when fruit reaches maturity or is stored at room temperature, the spread of soft rot from infected to healthy fruit occurs very rapidly leading to loss of whole lot of fruits. Controlling *Rhizopus* soft rot primarily relies on the application of synthetic fungicides. Existing fungicides registered for use are also not highly effective in controlling this pathogen (Zhou *et al.*, 2018). *B. cinerea* is recognized as the second most important plant pathogen affecting fresh fruits and vegetables, leading to annual global economic losses surpassing $10 to 100 billion (Papoutsis and Edelenbos, 2021). This necrotrophic fungal pathogen is responsible for inducing grey mold rot in over 500 plant species and has shown devastating economic consequences on a range of economically significant crops like grapes, strawberries, tomatoes etc. (Bi *et al.*, 2023). Controlling *B. cinerea* poses a serious challenge due to its wide range of host plants, diverse modes of attack and presence of both asexual and sexual stages, allowing it to survive in various conditions. Currently, synthetic fungicides are primarily used to manage grey mold rot caused by *B. cinerea*, constituting around 8 percent of the global fungicide market, with annual global expenditures exceeding €1 billion. Despite their widespread usage, their effectiveness against *B. cinerea* is limited due to the fungus's genomic plasticity and capable of developing drug-resistant genes (Chaouachi *et al.*, 2021). However the persistent use of these fungicides can lead to issues such as pesticide residues, environmental risks, and the development of strains resistant to fungicides, posing a significant threat to human health (Dorjee *et al.*, 2023). Moreover, fungicides pose safety concerns for both human health and the environment. Hence exploring chemical-free treatments and eco-friendly alternatives is the need of the hour to control the damage of fungal pathogen attacks.

Since phenolics have been known to demonstrate antimicrobial activity, this research investigated the potential of mango kernel phenolic extract (MKPE) from raw pickling cultivar mango as a natural and effective antimicrobial agent for controlling postharvest fungal infections. The rich phenolics composition of MKPE positions it as a promising and sustainable solution for reducing postharvest losses in the agricultural supply chain. Firstly, the phenolic compounds were extracted from the raw mango kernels and characeterized. Subsequently, *in-vitro* antifungal activity of MKPE against *B. cinerea, C. gloeosporoides* and *R. stolonifer* were evaluated at nine different concentration dosages thereby offering a comprehensive insight into the antimicrobial potential of MKPE against the three main postharvest pathogens.

This study aims to expand the knowledge base concerning the phenolic compositions and antimicrobial attributes of phenolic extracts from mango kernels of pickling variety and their potential role in mitigating postharvest diseases. As the demand for chemical-free treatments rises, exploring eco-friendly alternatives from by-products like mango kernels becomes imperative for the development of effective and sustainable agricultural practices. Several researchers have demonstrated the antimicrobial activity of mango kernel extracts against *Colletotrichum* brevisporum (100 % inhibition at 3 g/L, Gómez-Maldonado et al., 2020); *Rhizoctonia solani* (92.9 % at 5 % level; Moorthy et al., 2023). However, it is the very first-time phenols extracted from raw pickling mango kernels are analysed and explored for controlling the growth of major postharvest pathogens. No data regarding the same exists in scientific literature so far.

Material and methods

Experimental materials and chemicals

'Ramkela' cultivar mango kernels used in this research were procured from Sri Krishna Pickles Pvt Ltd, South West Delhi. The chemicals and reagents necessary for the study were sourced from Sigma Aldrich Chemicals Pvt. Ltd. Bangalore (India) and Merck India Pvt. Ltd. Mumbai (India).

Preparation of mango kernel powder (MKP)

Raw mango kernels of Ramkela cv. were procured from the pickle industry within 6 h of slicing for pickling. Kernels were washed with excess water to remove any adhering material, then dipped in 0.001 % sodium hypochlorite solution for 5 min and again washed thoroughly. After cutting and drying in a hot air dryer at 50 °C for 36 h, mango kernels were ground in a hammer mill to fine powder (MKP).

Extraction of mango kernel phenolic extract (MKPE)

MKP was suspended in 50 % ethanol solution in a 1:10 ratio. The starch slurry was centrifuged at 3500 rpm for 45 min, and filtered using Whatman filter paper 1. The filtrate was concentrated in a rotary evaporator at 40 °C to almost 10 times. MKPE obtained was stored in amber coloured glass container at 4°C until further use.

Total phenolics content

Total phenolic content (TPC) of the obtained extracts was conducted using the Folin–Ciocalteu reagent with some modifications to Buelvas-Puello *et al.* (2021). 100 μL of MKPE was combined with 100 μL FC reagent, mixed, and allowed to stand for 15 min in a dark location. Subsequently, 300 μL of 20 % sodium carbonate solution was added, vortexed, and left to stand for 30 min in the dark to allow the reaction to proceed. The absorbance of the resulting blue-coloured solution was measured at 765 nm using UV–Vis spectrophotometer (Genesys 50, ThermoFisher Scientific). Gallic acid (GA) was employed to establish a standard curve for quantification. The total phenolic content was expressed as milligrams of GA equivalents per 100 g of extract (mg GAE. 100 g^{-1}).

Characterization of MKPE

MKPE was characterized using Liquid chromatography-mass spectrometry (Thermo Electron Corporation, USA). Ethanolic extract of mango kernel phenol was subjected to LC–MS/MS analysis using Waters SYNAPT G2 with 2D nano ACQUITY System.

Phytopathogenic fungi

The fungi used in this study were *C. gloeosporoides* (ITCC Accession number 6157), *B. cinerea* (ITCC Accession number 6011) and *R. stolonifer* (ITCC Accession number 5100) acquired from the Indian Type Culture Collection (ITCC), ICAR-IARI, New Delhi. These pathogens were sub-cultured on potato dextrose agar (PDA, Merck, USA) under aseptic laminar conditions at 27 ± 2°C. Spores were purified by the single spore method and were identified based on morphological characteristics (Ziqin *et al.*, 2021).

Examination for identification of fungi

The fungal morphology was studied macroscopically by observing the colony features (colour, shape and size) and microscopically by a compound microscope (Olympus microscope connected to CMOS HDMI Truechrome HD, Radical, Japan) with a digital camera.

In-vitro anti-fungal assay

The assessment of MKPE efficacy on the radial growth (mm) and percentage inhibition of mycelial growth followed the *in-vitro* poison food technique method (Gómez-Maldonado *et al.*, 2020) with slight modifications. The *in-vitro* study was done under the aseptic laminar condition. MKPE at various concentrations were added to 60 mL sterilized molten PDA media in a conical flask at 40 °C to achieve concentrations of 25, 100, 175, 250, 500, 750, 1000, 1250 and 1500 ppm MKPE. 20 mL of MKPE-PDA media was poured to petri plates using pour plate technique. A mycelium disc with a diameter of 5 mm, obtained from the actively growing culture fungus colony on the margin of the petri dish was placed at the centre of the sterile MKPE-PDA media described above and incubated at 27 ± 2 °C for 7 days (for *C. gloeosporoides* and *B. cinerea*) and for 5 days (for *R. stolonifer*). Data was recorded in terms of radial growth (cm) after the control plate without MKPE amendment attained full growth for *Colletotrichum* and *Botrytis* on 5th day and *Rhizopus* on 3rd day. Triplicates were maintained for each treatment. The inhibition of mycelial growth (%) and radial growth (mm) was employed to evaluate the efficiency of the MKPE concentrations. Untreated PDA culture and PDA with 1000 ppm carbendazim were used as control and negative control, respectively. The obtained results were subsequently compared with those of the positive control. The experiment was replicated three times, and the mean of the three readings was used for subsequent calculations. Minimum Inhibition Concentration (MIC) of the MKPE was determined as the lowest concentration of the plant extract that resulted in more than 50 % growth inhibition compared to the control.

The inhibition percent of mycelial growth was calculated using the following formula

$$\text{Mycelial growth inhibition } (\%) = \frac{(AC - AE)}{AC} \times 100$$

Where, AC is mean colony area for control plates; AE is mean colony area for the treatment sets. To quantify the radial growth, the mean of both the horizontal and vertical diameters of mycelial growth was measured and expressed in mm.

Statistical analysis

The experiments were performed using a completely randomized design (CRD) with 3 replications per each treatment. One-way ANOVA analysis was undertaken for data of all the experiments. Significance was determined at a probability level of ($p \leq 0.05$). Differences with a p-value of less than 0.05 were considered statistically significant and denoted by different letters. All statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The means comparisons were made using Duncan's Multiple range tests. Probit analysis was employed to establish curves and determine the EC_{50} values.

Results

Yield of concentrated phenolic extract procured from 2 kg of mango kernels was 9.4 %. As expected, the TPC of ethanolic extract of mango kernel cv. Ramkela was very high *i.e.*, 2210 mg GAE/100 g.

Characterisation of MKPE by LC–MS analysis

Total ion chromatogram (TIC) of MKPE (Fig. 1) showed 33 diverse polyphenolic bioactive compounds. The major peaks identified by LC-MS analysis are presented in Table 1. Most of the compounds identified in the MKPE extract were flavonoids, xanthones, galloyl esters and phenolic glycosides. phenolic acids, hydroxybenzoic acid derivatives, hydroxycinnamic acid and gallotannins were present in trace quantities. Major polyphenolic compounds identified according to area units were mangiferin (12.4 %), quercetin (11.6 %), rhamnetin (8%), rhamnetin hexoside (7.6 %), quercetin glucoside (7.4 %) and galloyl ethylgallate (7.2 %). Minor compounds identified were apigenin-7-glucoside (6.35 %), ferulic acid (6.3 %), quercetin 3-o-rutinoside (5.3 %), epicatechin (3.98 %), ethyl trigallate (3.5 %), rhamnetin-3-[butenoil-hexoside], iriflophenone glucoside, ellagic acid, maclurin-c-(o-galloyl)-glucoside, tetra-o-galloyl-glucoside, quercetin-3-o-alactoside, pentagalloyl glucose, maclurin(o-galloyl)-glucoside, methyl gallate, p-hydroxybenzoic acid, galloyl methylgallate, digallic acid, galloylquinic acid, theogallin, maclurin-3-c-β-d-glucoside, galloyl diglucoside, mangiferin gallate, gallic acid, homomangiferin, protocatechuic acid, hexagalloyl glucose and galloyl glucose. Total gallate structures accounted for 15.3 % of composition while quercetin conjugates and rhamnetin conjugates accounted for 25.81 % and 19 % by mass, respectively.

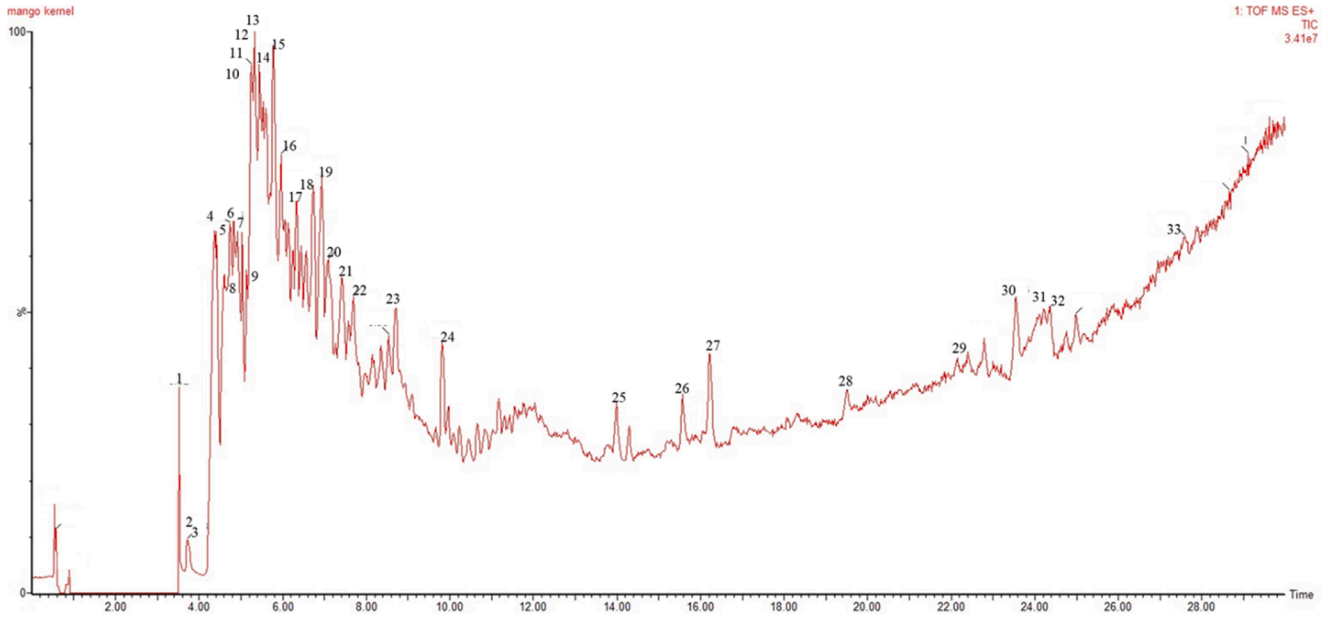

Fig 1. Total ion chromatogram (TIC) of the phenolic compounds identified in Mango Kernel Phenolic Extract (MKPE) by LC-MS.

Table 1
Major phenolic bioactive compounds in mango kernel extract cv. Ramkela identified by LC-MS.

Peak no	Rt (min.)	Tentative identified compound	Molecular formula	Neutral mass (Da)	[a] $[M+H]^+$	Error (ppm)	Area%
1	3.71	Protocatechuic acid	$C_7H_6O_4$	153.0193	154.028	2.6	0.04
2	4.31	Methyl gallate	$C_8H_8O_5$	183.0299	184.037	-4.35	0.91
3	4.33	p-Hydroxybenzoic acid	$C_7H_6O_3$	137.0244	138.032	-1.45	0.90
4	4.38	Gallic acid	$C_7H_6O_5$	169.0142	170.022	-1.76	0.19
5	5.06	Maclurin-3-C-β-d-glucoside	$C_{19}H_{20}O_{11}$	423.0945	424.101	-2.83	0.46
6	5.34	Digallic acid	$C_{14}H_{10}O_9$	321.0252	322.032	-1.86	0.57
7	5.46	Galloyl ethylgallate	$C_{16}H_{14}O_9$	349.0565	350.064	-1.43	7.21
8	5.49	Galloylquinic aicd	$C_{14}H_6O_{10}$	343.0671	344.075	1.16	0.55
9	5.52	Apigenin-7-glucoside	$C_{21}H_{20}O_{10}$	432	433.007	-1.62	6.39
10	5.63	Mangiferin	$C_{19}H_{18}O_{11}$	421.0776	422.086	1.66	12.43
11	5.7	Iriflophenone glucoside	$C_{19}H_{20}O_{10}$	407.0984	408.106	-1.23	2.41
12	5.71	Epicatechin	$C_{15}H_{14}O_6$	289.0718	290.08	1.72	3.98
13	5.78	Homomangiferin	$C_{20}H_{20}O_{11}$	435.0933	436.102	1.08	0.18
14	6.19	Mangiferin gallate	$C_{26}H_{22}O_{15}$	573.0886	574.097	0.52	0.34
15	6.27	Galloyl diglucoside	$C_{13}H_{16}O_{10}$	331.0671	332.074	-1.51	0.36
16	6.28	Quercetin-3-O-rutinoside	$C_{27}H_{30}O_{16}$	609.1813	610.19	2.13	5.26
17	6.81	Quercetin	$C_{15}H_{10}O_7$	301.0354	302.043	-1.99	11.63
18	6.91	Maclurin (O-galloyl)-glucoside	$C_{33}H_{28}O_{19}$	727.1152	727.121	-2.2	1.79
19	6.96	Ferulic acid	$C_{10}H_{10}O_4$	193.0506	194.058	-0.52	6.31
20	7.31	Tetra-O-galloyl-glucoside	$C_{34}H_{28}O_{22}$	787.0999	788.105	-3.05	1.51
21	8.03	Ethyl trigallate	$C_{23}H_{18}O_{13}$	501.0675	502.075	-1	3.50
22	8.3	Theogallin	$C_{14}H_{16}O_{10}$	343.059	344.067	-0.29	0.52
23	8.35	Hexagalloyl glucose	$C_{48}H_{36}O_{30}$	1091.122	1092.13	1.28	trace amount
24	11.03	Rhamnetin-3-[butenoil-hexoside]	$C_{26}H_{16}O_{13}$	645.0583	646.066	-0.46	3.30
25	15.58	Pentagalloyl glucose	$C_{41}H_{32}O_{26}$	939.1109	940.121	2.77	1.21
26	16.24	Galloyl glucose	$C_{13}H_{16}O_{10}$	331.0671	332.075	-0.9	trace amount
27	18.94	Galloyl methylgallate	$C_{15}H_{12}O_9$	335.0409	336.048	-1.19	0.62
28	20.5	Rhamnetin hexoside	$C_{22}H_{22}O_{12}$	477.1038	478.111	-2.09	7.64
29	22.71	Ellagic acid	$C_{14}H_6O_8$	300.999	302.006	-2.32	1.89
30	24.18	Rhamnetin	$C_{26}H_{16}O_{13}$	545.0583	546.065	-2.38	8.06
31	24.27	Quercetin glucoside	$C_{21}H_{20}O_{12}$	463.0882	464.097	1.51	7.43
32	24.35	Quercetin-3-O-alactoside	$C_{21}H_{20}O_{12}$	463.0882	464.096	0.65	1.49
33	28.37	Maclurin(O-galloyl)-glucoside	$C_{26}H_{24}O_{15}$	575.1042	576.113	2.08	0.95

Examination for identification of fungi

All the three pathogens were examined under microscope for identification. Plate 1A shows the microscopic images of *B. cinerea*, with their characteristic conidiophores. The conidia of *B. cinerea* were single-celled, hyaline (translucent) and ellipsoidal or oval in shape. They are often formed in chains along the conidiophores. The hyphae of *B. cinerea* are septate (divided into cells by cross-walls), and they form a dense network of mycelium. Identification of *Botrytis* was supported by Abdelhalim *et al.* (2023). Plate 1B presents the microscopic images of *C. gloeosporioides* with its hyaline (translucent) and one-celled conidia. They are typically cylindrical or slightly curved, often with a rounded end (Wang *et al.*, 2022). *R. stolonifer* produces globose shaped erect structures called sporangiophores (Plate 1C). Sporangia are the structures that contain the spores and are typically dark-coloured with varying shape and it contains numerous round shaped asexual spores called sporangiospores. These are asexual spores produced within the sporangia and were often black and numerous. *Rhizopus* hyphae were coenocytic, meaning they lacked septa (cross-walls) in their hyphae. Identification results were in line with the identification reports given by Parthasarathy (2024).

In - vitro antifungal assay

Radial growth (mm) and percentage inhibition

a *B. cinerea*

B. cinerea belongs to family sclerotiniaceae of the division

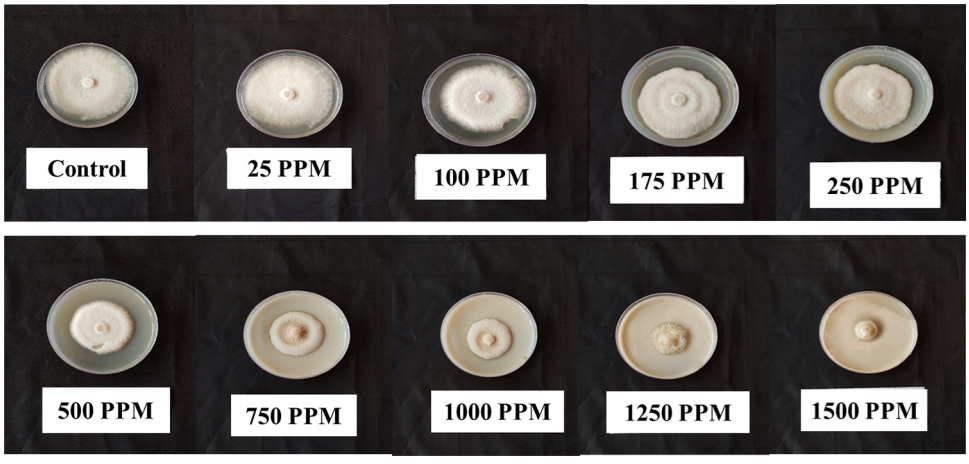

Fig 2. Effect of MKPE on growth of *B. cinerea*.

Ascomycota and classified as a necrotrophy. It is recognized as the predominant post-harvest fungal pathogen responsible for substantial losses in fresh fruits, vegetables and ornamental plants. A notable decline in the radial growth of the *Botrytis* fungus with increasing concentration of MKPE was evident across all the treatments (Fig. 2). The MIC and EC_{50} values of MKPE for *B. cineria* under *in vitro* conditions were 25 ppm and 364.17 ppm. After 7 days of *Botrytis* inoculation, control treatment showed full growth of *Botrytis* in the Petri plates (9 mm) whereas T_{10} (1500 ppm) showed the least growth of 1.53 mm followed by T_9 (1250 ppm) with 3.23 mm growth. 1500 ppm MKPE demonstrated the highest inhibition (82.96 %), followed by 1250 ppm (64.07%; Table 2). In contrast, the control treatment showed no inhibition (Table 3). EC_{90} of *B. cinerea* was found to be 4789.49 ppm.

b *C. gloeosporioides*

Colletotrichum, a genus known for causing detrimental diseases in a diverse range of fruit crops, earned recognition in 2012 as one of the top 10 fungal plant pathogens. This acknowledgement stems from its extensive host range, its impact on crucial crops, and its significance as a postharvest pathogen. *C. gloeosporioides*, a member of the family Phyllachoraceae within the division Ascomycota, is an asexual facultative parasite. The influence of MKPE on the growth of *C. gloeosporioides* is presented in Fig. 3, revealing a significant reduction in the radial growth of the *Colletotrichum* across all treatments. The in vitro MIC and EC_{50} values of MKPE against *C. gloeosporioides* were determined to be 25 ppm and 963.80 ppm, respectively. Seven days after inoculation, the control treatment exhibited full fungus growth in the Petri plates (9 mm), while T_{10} with a concentration of 1500 ppm displayed the least growth of 3.40 mm, followed by T_9 with a concentration of 1250 ppm, which showed 4.10 mm of growth (Table 2).

1500 ppm MKPE displayed the highest inhibition percentage of 62.22 % followed by 1250 ppm which exhibited a substantial inhibition of 54.44 % as compared to control (Fig. 3). EC_{50} and EC_{90} for *C. gloeosporioides* were found to be 963.80 ppm and 4929.20 ppm, respectively.

c *R. stolonifer*

R. stolonifer, belongs to the Zygomycota phylum and is recognized as the most significant species within the *Rhizopus* genus. It is responsible for inducing soft rot in plums, nectarines, and grapes, resulting in substantial losses for peaches, tomatoes, strawberries etc. It is the most prevalent postharvest pathogen leading to major post harvest losses in horticultural crops. Control treatment without MKPE took 5 days to show full growth of fungus in Petri plates. The impact of MKPE is elucidated in Table 2, demonstrating a significant decrease in the radial growth of the fungus across all treatments. The concentration of MKPE played a pivotal role in hindering the growth of the *Rhizopus* fungal colony, with a discernible effect as the concentration increased (Fig. 4). The determined *in vitro* MIC and EC_{50} values of MKPE against *R. stolonifer* were 25 ppm and 925.58 ppm, respectively. 1500 ppm MKPE showed least growth of 2.80 mm, followed by 1250 ppm concentration, with 3.17 mm growth by the end of the 5-day period (Table 2).

The highest inhibition percentage was understandably at 1500 ppm with an impressive 89.67 % inhibition while 1250 ppm MKPE demonstrated 81.62 % inhibition. EC_{90} was found to be 5724.37 ppm.

Table 2
Effect of mango kernel phenolic extract on radial growth of *B. cinerea, C. gloeosporoides* and *R. stolonifer*.

Treatments	*B. cinerea*	*C. gloeosporoides*	*R. stolonifer*
Control	9.00 ± 0.00^{a}	9.00 ± 0.00^{a}	9.00 ± 0.00^{a}
25 ppm	6.27 ± 0.03^{b}	7.77 ± 0.12^{b}	7.64 ± 0.07^{b}
100 ppm	5.83 ± 0.03^{c}	7.10 ± 0.17^{c}	7.01 ± 0.03^{c}
175 ppm	5.57 ± 0.03^{d}	6.27 ± 0.15^{d}	6.93 ± 0.03^{c}
250 ppm	5.17 ± 0.03^{e}	5.73 ± 0.13^{e}	5.98 ± 0.02^{d}
500 ppm	4.73 ± 0.03^{f}	5.47 ± 0.03^{ef}	5.13 ± 0.02^{e}
750 ppm	4.37 ± 0.03^{g}	5.20 ± 0.20^{f}	4.89 ± 0.03^{f}
1000 ppm	4.07 ± 0.03^{h}	4.60 ± 0.10^{g}	3.85 ± 0.06^{g}
1250 ppm	3.23 ± 0.03^{i}	4.10 ± 0.06^{h}	3.17 ± 0.02^{h}
1500 ppm	1.53 ± 0.03^{j}	3.40 ± 0.10^{i}	2.80 ± 0.01^{i}

Means with different superscripts indicate difference at 5 % significance level.

Table 3
Effect of mango kernel phenolic extract on percentage inhibition of *B. cinerea, C. gloeosporoides* and *R. stolonifer*.

Treatments	*B. cinerea*	*C. gloeosporoides*	*R. stolonifer*
Control	0.00 ± 0.00^{j}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}
25 ppm	30.37 ± 0.37^{i}	13.70 ± 1.34^{h}	14.80 ± 2.47^{h}
100 ppm	35.19 ± 0.37^{h}	21.11 ± 1.93^{g}	27.62 ± 2.49^{g}
175 ppm	38.15 ± 0.37^{g}	30.37 ± 1.62^{f}	32.45 ± 2.46^{g}
250 ppm	42.59 ± 0.37^{f}	36.30 ± 1.48^{e}	38.11 ± 1.89^{f}
500 ppm	47.41 ± 0.37^{e}	32.96 ± 0.37^{de}	42.93 ± 1.58^{e}
750 ppm	51.48 ± 0.37^{d}	42.22 ± 2.22^{d}	46.60 ± 1.75^{d}
1000 ppm	54.81 ± 0.37^{c}	48.89 ± 1.11^{c}	52.37 ± 1.61^{c}
1250 ppm	64.07 ± 0.37^{b}	54.44 ± 0.64^{b}	63.61 ± 0.63^{b}
1500 ppm	82.96 ± 0.37^{a}	62.22 ± 1.11^{a}	71.67 ± 0.29^{a}

Means with different superscripts indicate difference at 5 % significance level.

Discussion

In recent times, researchers have shown particular interest in mango kernel due to its nutritional content and potential health benefits. It serves as a valuable reservoir of various phenolic compounds that hold significant antioxidative, nutraceutical and pharmaceutical importance. TPC found in our MKPE was much higher in phenolics than methanolic extract of ripe mango kernel extracts reported by Abdel-Aty et al. (2018; 174 mg GAE/g); and in ethanolic mango kernel extracts as reported by Lim et al. (2019; 18.19 to 101.68 mg GAE/g). Results of our study proved that TPC of unripe mango kernel cv. Ramkela had much higher TPC than ripe mango kernels. Mango seed kernel exhibits greatest antioxidant activity followed by tamarind, longan, avocado and jackfruit seeds (Mwaurah et al., 2020). It contains abundant phenolic compounds like mangiferin, quercetin, gallic acid, ellagic acid, ferulic acid, cinnamic acids, tannins, vanillin, coumarin, anthocyanins, catechins, gallic acid, ellagic acid, alkylresorcinol, gallotannins, benzophenone derivatives and other constituents showcasing antioxidant properties (Mounica and Subbiah, 2014). These phenolic compounds play a significant role in determining the antioxidant capabilities and making them a natural reservoir of antioxidants. Thus, MKPE represents a highly promising natural antioxidant source. Hence it can be advocated for the management of postharvest spoilage as there is a growing demand for treatments that are free from chemicals. Addressing postharvest fungal infections is imperative for preserving food quality, minimizing economic losses, and fostering sustainable practices within the realm of global agriculture. To address the issue of postharvest losses due to postharvest fungal pathogens, there is an increasing trend globally to adopt sustainable and chemical-free methods for the preservation and protection of fresh produce. Today, the control of postharvest diseases with naturally available bioactive extracts from plant source has been significantly noticed as a novel trend in biological preservation.

Characterization of MKPE

LC–MS stands as a potent analytical method employed for the separation, identification, and quantification of both known and unknown compounds. Additionally, it serves to unveil the structure and chemical properties of various molecules. By LC-MS data, depicted in Table 1, it can be deciphered that mangiferin (12.43 %), quercetin (11.63 %) and rhamnetin (8.06 %) are the major phenolic compounds present in our MKPE. Mangiferin is informally referred as "super antioxidant" due to its

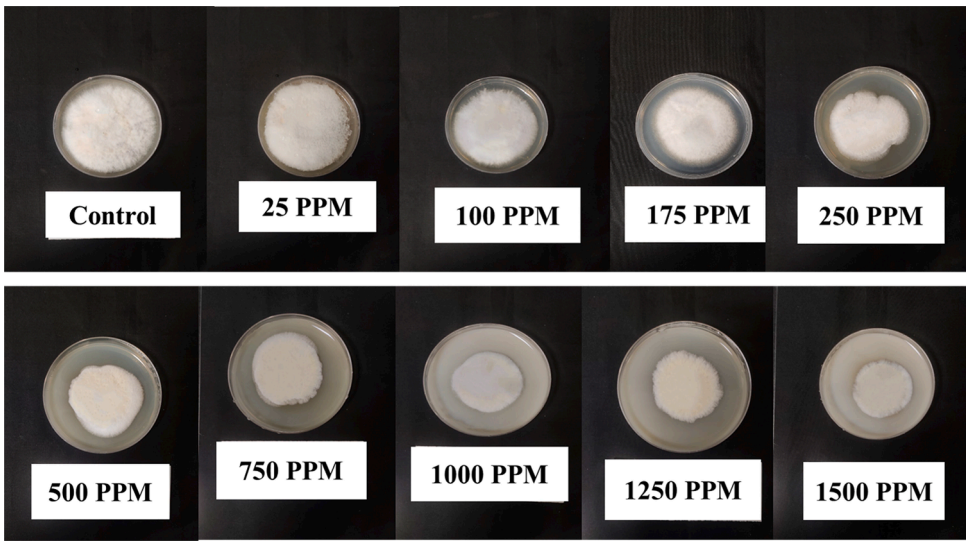

Fig 3. Effect of MKPE on growth of *C. gloeosporioides*.

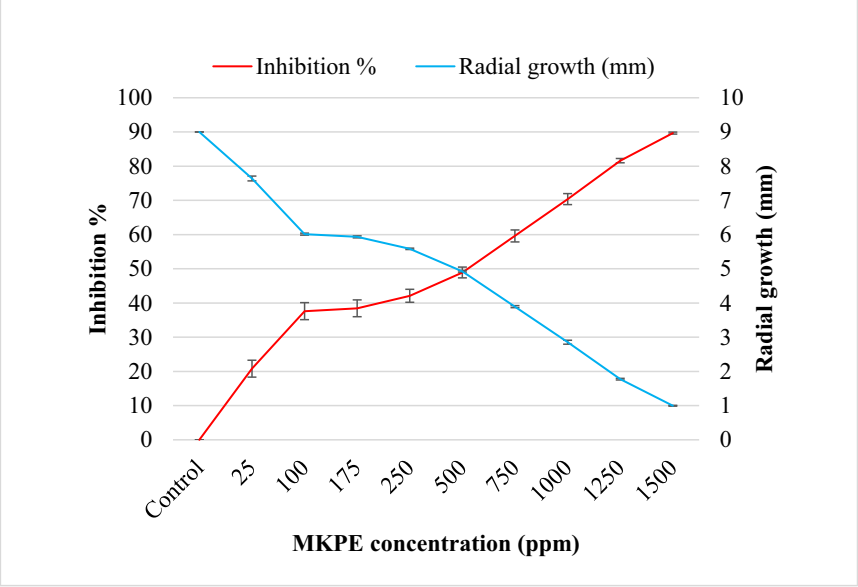

Fig 4. Effect of MKPE on radial growth and percentage inhibition of *R. stolonifer*.

exceptional qualities and it is categorized as a xanthone, exhibits radical scavenging abilities comparable to those of natural antioxidants like vitamin C and vitamin E (Abdalla et al., 2007). Moreover, it possesses high heat stability in comparison to other compounds. Many reports have shown the antifungal properties of mango extracts. Singh et al. (2009) showed that mangiferin isolated from ethanolic extracts of the stem bark of *Mangifera indica*, demonstrated strong antifungal activity against *Candida albicans* and *Aspergillus niger*. In a recent publication Sudheeran *et al.* (2020) reported that substantial accumulation of flavanols and anthocyanins was identified in the skin of red mango fruit. These compounds demonstrated direct antifungal activity against fungal pathogens such as *Colletotrichum*. Interestingly, during fungal attacks, the fungi secrete β-glucosidase, leading to the formation of aglycon flavonoids that exhibit higher toxicity to the fungi. Two other major flavanol compounds identified were Quercetin and Rhamnetin. Quercetin is present in plants as glycosides. In mango kernel, the commonly identified quercetin glycosides include quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-arabinoside and quercetin aglycon. Although flavanol glycoside is also present, it is in trace amounts (Asif et al., 2016). Rhamnetin is the other major flavonoid compound present and it is usually present in the form of Rhamnetin-3-(butenoil-hexoside) and Rhamnetin hexoside (Torres-León et al., 2017).

Differences in phenolic compounds and their content discrepancies are associated with various factors such as variety, genetics, environment, ripening stage, and agricultural practices. The potent antifungal activity demonstrated by MKPE may be attributed to the presence of secondary metabolites, including phenolic compounds, flavonoids, and tannins, which exhibit a broad spectrum of biological activities. This underscores the multifaceted nature of MKPE, with its diverse array of secondary metabolites contributing to its robust antifungal properties, thereby enhancing its potential as a natural and effective antimicrobial agent (Gómez-Maldonado et al., 2020).

In-vitro anti-fungal assay

The outcomes of the in-vitro antimicrobial assay revealed a significant inhibitory effect of the MKPE on the growth of *R. stolonifer, C. gloeosporioides* and *B. cinerea*. The presence of major polyphenolic

compounds in MKPE, such as mangiferin, quercetin and rhamnetin, is believed to be responsible for the observed antifungal effects on the pathogens. Additionally, the documented antifungal and antimicrobial properties of these identified compounds in our study, namely mangiferin (Sudheeran et al., 2020), quercetin (Matrose et al., 2022) and rhamnetin (Omoruyi et al., 2023), have been substantiated by previous researchers. This information enhances our understanding of the potential mechanisms underlying the observed antimicrobial effects of MKPE, providing valuable insights for future applications in controlling fungal pathogens.

Similar reports have shown that liquid polyphenols extracted from olive mill waste rich in flavonoid Quercetin, Caffeic and ferulic acids were able to control the growth of *B. cinerea* in vitro (Leontopoulos et al., 2015). Ethanolic extract derived from mango kernel exhibited inhibitory effects on the growth of *C. brevisporum* spores (Gómez-Maldonado et al., 2020). Quercetin aglycones strongly inhibited conidial germination and exhibited good antifungal activity against *C. gloeosporioides* (Oliveira et al., 2016). In another study, Roy *et al.* (2018), showed that amongst different phenolic compounds and flavonoids investigated on the growth of *Colletotrichum* spp., only trans-cinnamic acid, ferulic acid and quercetin inhibited fungal growth. Mikulic-Petkovsek *et al.* (2013) showed that quercetin glycosides, luteolin glycosides, and chlorogenic acid were responsible for defence response of pepper fruits to *C. coccodes*. Similarfindings for control of *R. stolonifer* have been reported by López *et al.* (2007).

Synthetic fungicides are routinely used to control *B. cinerea* and EC_{90} values of 5 ppm Tebuconazole, 3 ppm Iprodione and more than 3000 ppm Pyrimethanil, Boscalid, Fenpyrazamine were found to be effective to control growth of *B. cinerea in-vitro* (Kim *et al.*, 2016). Since synthetic fungicides have lingering toxic effects and noticeable residues, only a limited number of fungicides have received approval for managing postharvest diseases. Hence using natural phenolic substances is found to be a suitable and effective eco-friendly approach.

Major synthetic fungicides used to control *C. gloeosporioides* are Mancozeb, Chlorothalonil, Triazoles etc. At concentration of 3000 ppm, Chlorothalonil 75 % WP, Propineb 50 % WP, at concentration of 1500 ppm, Difenconazole 25 % EC, Propiconazole 25 % EC, Tebuconazole 25 % EC, Tricyclazole 75 % WP, at concentration of 1000 ppm, Mancozeb 75 % WP, Zineb 75 % WP and Hexaconazole 5 EC at concentration of 250 ppm, recorded cent percent (100 %) mycelial inhibition of *C. gloeosporioides* (Vandana et al., 2023). When compared to chemical fungicides, 50 μg ml^{-1} (50 PPM) benomyl was used to completely inhibit the growth of *C. gloeosporioides* and 25 μg ml^{-1} (25 PPM) reduced the colony diameter by 89 % (Almada-Ruiz et al., 2003). But continued use of the fungicides can result in significant issues such as the emergence of resistance in pathogen populations, elevated residues on fruits, posing a substantial risk to human health, and contributing to environmental pollution. Synthetic fungicides like Triazoles, Imazalil, Iprodione, Thiocarbamates etc., are majorly used to control *Rhizopus* (Bautista-Baños et al., 2014). Minimum inhibition concentration for *Rhizopus* was found at concentration of 1 PPM of Tebuconazole (Park et al., 2020), at concentration >500 PPM carbendazim, complete inhibition was found (Thomidis et al., 2009) and 600 PPM Iprodione significantly reduced the mycelium growth and germination of *Rhizopus* (Al-Masri et al., 2015). Because of negative impacts of synthetic fungicides on environment and health, utilization of natural plant extracts for managing postharvest diseases and prolonging the storage shelf life of fresh produce has garnered attention as a viable and environmentally friendly approach now-a-days. In this context, MKPE has shown promising results in controlling three major postharvest pathogens *i.e.*, *B. cinerea C. gloeosporioides* and *R. stolonifer under in-vitro* condition.

Similar studies on antimicrobial property of mango kernel extracts in increasing inhibition percent has been given by Moorthy *et al.* (2023), they showed that at a concentration of 5 % (50,000 ppm) methanol mango kernel crude extract inhibited the growth of *Rhizoctonia solani* by 92.90 ± 0.31 %. As per Gómez-Maldonado et al. (2020), complete mycelial growth of *C. brevisporum* inhibition was achieved with MKE at 2 g/L (2000 ppm). On the other hand, the EC_{90} values for Ramkela cv MKPE were around 5000 ppm, *i.e.*, 0.5 % which is 4 times more effective than the reported values by previous researchers. Hence, it is possible that raw mango kernels can serve as a better source of phenolics for antimicrobial use.

The utilization of mango kernel waste from pickling industries contributes to addressing Sustainable Development Goal (SDG) 12 through a two-pronged approach. Firstly, it involves the effective utilization of waste, by repurposing mango kernel waste, it reduces overall environmental impact associated with disposal and aligns with the SDG 12 target that focuses on responsible consumption and production. Secondly, this approach aids in reducing food spoilage. Mango kernel waste can potentially be harnessed for various purposes, such as, extraction of valuable compounds like phenolic compounds, starch or as a source of bioenergy. By integrating this waste into different value chains, we can minimize the environmental burden of disposal and simultaneously contribute to sustainable practices, aligning with SDG 12′s objectives of promoting sustainable consumption and production patterns.

Mechanism of action of mango polyphenolic bioactive compounds on pathogens

Phytochemicals present in MKPE demonstrate their antimicrobial efficacy by inhibiting the synthesis of microbial enzymes. Flavonols are flavonoids with a ketone group and they play a crucial role in the constitutive defence response of plants (Sudheeran et al., 2020). Mangiferin and quercetin belong to flavonoid groups. Notably, a high accumulation of flavonols has been observed to exhibit direct antifungal activity against various fungal pathogens. The bioactivity of mangiferin is premised in its ability to safeguard cells by functioning as both an antioxidant and a radical captodative agent. It forms a stable complex with iron metal ions in the substrate and it regulates polymer chain reactions by interacting with reactive oxygen, producing caged oxygen radicals (Masibo and He, 2008).

Phenolic compounds induce harm to the microbial cell membrane through their interaction with microbial enzymes. The process involves the adsorption of phytochemicals onto the cell membrane, causing a change in the pH and electrical potential of the microbial cell membrane. This adsorption of phenolic compounds results in the alteration of both pH and electrical potential, leading to damage in the microbial cell membrane. Consequently, this damage causes the leakage of cytoplasmic material and ultimately leads to cell death (Pandey et al., 2021). Conjugates of mangiferin and quercetin are present in higher quantities in MKPE. Mangiferin activates an antifungal defence mechanism that involves the synthesis of jasmonic acid, ethylene and phenylpropanoid compounds. An intriguing aspect of this defence response is the secretion of β-glucosidase by fungi during attack, leading to the formation of aglycon flavonoids. These aglycon flavonoids demonstrate increased toxicity to the fungi, resulting in the death of fungal cells. This intricate interplay between flavonoids and the fungal defence mechanisms highlights the sophisticated nature of plant-pathogen interactions and the multifaceted role of flavonoids in mounting an effective antifungal response (Sudheeran et al., 2020). Quercetin action against fungal pathogens seems to involve the formation of complexes with protein kinases produced by the fungus. These flavonoids have the ability to bind to the produced protein kinases, inducing alterations in the regulation or signal transduction processes. This interaction ultimately showcases antimicrobial properties (Santos Júnior *et al.*, 2014). This elucidation underscores the intricate molecular interactions between flavonoids and fungal components, shedding light on the specific mechanisms through which these compounds exert their antimicrobial effects.

Conclusion

In conclusion, MKPE doses at 1500 ppm proved to be a potential, natural, sustainable, chemical-free and effective antimicrobial agent for controlling postharvest fungal infections. Although, EC_{50} values for *B. cinerea, C. gloeosporioides* and *R. stolonifer* were found to be 364.17 ppm, 963.80 ppm and 925.58 ppm, respectively, showing *B. cinerea* was more susceptible to MKPE. Quercetin, mangiferin, rhamnetin, gallic acid and their conjugates were determined to be the predominant phenolics present in MKPE which are responsible for antimicrobial property as per LC-MS data. Finally, 1500 ppm of MKPE has shown great promise in controlling the pathogens *in-vitro* by hindering the growth of fungal colony and showing more than 85 % inhibition rate.

Future thrust

The study provides valuable insights into the potential application of MKPE as a natural, sustainable, chemical-free and effective antimicrobial agent for controlling postharvest fungal infections. This approach aligns with the growing demand for chemical-free treatments, emphasizing the importance of sustainable practices in agriculture. So, in future, by evaluating the effect of MKPE on naturally infected fruits and vegetables, we can reduce the dependency on the use of agricultural chemicals for infection control, which might otherwise seep into surrounding ecosystems causing harmful effects, and subsequently lessening adverse environmental impact of chemicals on agriculture. The rich phenolic composition of MKPE, particularly mangiferin, quercetin, and rhamnetin, positions it as a promising candidate for further exploration in the development of eco-friendly solutions for postharvest pathogen control. This research contributes to the broader goal of minimizing postharvest losses, promoting sustainable eco-friendly agricultural practices and thereby aligning with SDG 12.

CRediT authorship contribution statement

Gouthami Shivaswamy: Data curation, Writing – original draft, Investigation. **Shalini Gaur Rudra:** Conceptualization, Methodology, Visualization, Supervision, Writing – review & editing. **Lham Dorjee:** Investigation, Methodology, Writing – original draft. **Aditi Kundu:** Methodology, Resources. **Robin Gogoi:** Investigation, Supervision, Resources. **Anupama Singh:** Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2024.100243.

References

Abdalla, A.E., Darwish, S.M., Ayad, E.H., El-Hamahmy, R.M., 2007. Egyptian mango by-product 2: antioxidant and antimicrobial activities of extract and oil from mango seed kernel. Food Chem. 103 (4), 1141–1152.

Abdel-Aty, A.M., Salama, W.H., Hamed, M.B., Fahmy, A.S., Mohamed, S.A., 2018. Phenolic-antioxidant capacity of mango seed kernels: therapeutic effect against viper venoms. Revista Brasileira de Farmacognosia 28, 594–601.

Abdelhalim, A., El-Kot, G.A., &Elzaawely, A.A, 2023. Isolation, morphological characterization, and phylogenetic identification of botrytis cinerea associated with postharvest gray mold of apple fruits. J. Sustain. Agric. Environ. Sci. 2 (4), 91–103.

Almada-Ruiz, E., Martínez-Téllez, M.Á., Hernández-Álamos, M.M., Vallejo, S., Primo-Yúfera, E., Vargas-Arispuro, I., 2003. Fungicidal potential of methoxylated flavones from citrus for in vitro control of Colletotrichum gloeosporioides, causal agent of anthracnose disease in tropical fruits. Pest Manage. Sci. Formerly Pesticide Sci. 59 (11), 1245–1249.

Al-Masri, M.I., Sharawi, S.M., Barakat, R.M., 2015. Effect of Clammy Inula (Inula viscose) plant extract in combination with a low dose of the fungicide iprodione on Botrytis cinerea in vitro and in vivo. Am. J. Plant Sci. 6 (09), 1519.

Asif, M., Zafar, M., Saleem, M., Saadullah, M., Khalid, S.H., Khan, M.S.S., Iqbal, Z., Khan, I.U., Hussain, L., Yaseen, H.S., Zubair, H.M., 2016. Evaluation of antidiabetic and wound healing properties of ethanol extract of *Hedera nepalensis* in alloxan-induced diabetic rats. S. Afr. J. Botany 146, 118–126.

Bautista-Baños, S., Bosquez-Molina, E., Barrera-Necha, L.L., 2014. *Rhizopus stolonifer* (soft rot). Postharvest Decay. Academic Press, pp. 1–44.

Bi, K., Liang, Y., Mengiste, T., Sharon, A., 2023. Killing softly: a roadmap of Botrytis cinerea pathogenicity. Trends Plant Sci. 28 (2), 211–222.

Buelvas-Puello, L.M., Franco-Arnedo, G., Martínez-Correa, H.A., Ballesteros-Vivas, D., Sánchez-Camargo, A.D.P., Miranda-Lasprilla, D., Narváez-Cuenca, C.E., Parada-Alfonso, F., 2021. Supercritical fluid extraction of phenolic compounds from mango (*Mangifera indica* L.) seed kernels and their application as an antioxidant in an edible oil. Molecules 26 (24), 7516.

Chaouachi, M., Marzouk, T., Jallouli, S., Elkahoui, S., Gentzbittel, L., Ben, C., Djébali, N., 2021. Activity assessment of tomato endophytic bacteria bioactive compounds for the postharvest biocontrol of Botrytis cinerea. Postharvest Biol. Technol. 172, 111389.

Davies, C.R., Wohlgemuth, F., Young, T., Violet, J., Dickinson, M., Sanders, J.W., Vallieres, C., Avery, S.V., 2021. Evolving challenges and strategies for fungal control in the food supply chain. Fungal Biol. Rev. 36, 15–26.

Dorjee, L., Gogoi, R., Kamil, D., Kumar, R., Verma, A., 2023. Copper nanoparticles hold promise in the effective management of maize diseases without impairing environmental health. Phytoparasitica 1–27.

Gómez-Maldonado, D., Lobato-Calleros, C., Aguirre-Mandujano, E., Leyva-Mir, S.G., Robles-Yerena, L., Vernon-Carter, E.J., 2020. Antifungal activity of mango kernel polyphenols on mango fruit infected by anthracnose. LWT 126, 109337.

Kalogianni, A.I., Lazou, T., Bossis, I., &Gelasakis, A.I, 2020. Natural phenolic compounds for the control of oxidation, bacterial spoilage, and foodborne pathogens in meat. Foods 9 (6), 794.

Kim, J.O., Shin, J.H., Gumilang, A., Chung, K., Choi, K.Y., Kim, K.S., 2016. Effectiveness of different classes of fungicides on Botrytis cinerea causing graymold on fruit and vegetables. Plant Pathol. J. 32 (6), 570.

Leontopoulos, S.V., Giavasis, I., Petrotos, K., Kokkora, M., Makridis, C., 2015. Effect of different formulations of polyphenolic compounds obtained from OMWW on the growth of several fungal plant and food borne pathogens. Studies *in vitro* and *in vivo*. Agric. Agric. Sci. Procedia 4, 327–337.

Li, Y., Ji, N., Zuo, X., Hou, Y., Zhang, J., Zou, Y., Jin, P., Zheng, Y., 2023. PpMYB308 is involved in Pichia guilliermondii-induced disease resistance against Rhizopus rot by activating the phenylpropanoid pathway in peach fruit. Postharvest. Biol. Technol. 195, 112–115.

Lim, K.J.A., Cabajar, A.A., Lobarbio, C.F.Y., Taboada, E.B., Lacks, D.J., 2019. Extraction of bioactive compounds from mango (*Mangifera indica* L. var. Carabao) seed kernel with ethanol–water binary solvent systems. J. Food Sci. Technol. 56, 2536–2544.

López, V., Akerreta, S., Casanova, E., García-Mina, J.M., Cavero, R.Y., Calvo, M.I., 2007. In vitro antioxidant and anti-rhizopus activities of Lamiaceae herbal extracts. Plant Foods Hum. Nutr. 62, 151–155.

Masibo, M., He, Q., 2008. Major mango polyphenols and their potential significance to human health. Compr. Rev. Food Sci. Food Saf. 7 (4), 309–319.

Matrose, N.A., Belay, Z.A., Obikeze, K., Mokwena, L., Caleb, O.J., 2022. Bioprospecting of helichrysum species: chemical profile, phytochemical properties, and antifungal efficacy against *Botrytis cinerea*. Plants 12 (1), 58.

Mikulic-Petkovsek, M., Schmitzer, V., Jakopic, J., Cunja, V., Veberic, R., Munda, A., Stampar, F., 2013. Phenolic compounds as defence response of pepper fruits to *Colletotrichum coccodes*. Physiol. Mol. Plant Pathol. 84, 138–145.

Moorthy, T.A., Saraswathi, M., Arunkumar, J., Perumal, K., 2023. Antibacterial, antifungal, antifeedent and larval mortality percent of solvent extraction of mango kernel (*Mangifera indica* L.) cv. Totapuri. S. Asian J. Experim. Biol. 13 (2).

Mounica, M.V., Subbiah, V., 2014. Extraction and Characterization of Mango seed kernel oil and its antimicrobial activity. Int. J. Adv. Inform. Sci. Technol. 21 (21), 25–29.

Mwaurah, P.W., Kumar, S., Kumar, N., Panghal, A., Attkan, A.K., Singh, V.K., Garg, M.K., 2020. Physicochemical characteristics, bioactive compounds and industrial applications of mango kernel and its products: a review. Compr. Rev. Food Sci. Food Saf. 19 (5), 2421–2446.

Oliveira, V.M., Carraro, E., Auler, M.E., Khalil, N.M., 2016. Quercetin and rutin as potential agents antifungal against *Cryptococcus* spp. Braz. J. Biol. 76, 1029–1034.

Omoruyi, B.E., Volschenk, H., Gelderblom, W.C., Lilly, M., 2023. Biocontrol of fusarium species utilizing indigenous rooibos and honeybush extracts. Microbiol. Spectr. 11 (3), e02742. -22.

Pandey, S.S., Ma, W., Pang, Z., Huang, X., Li, J., Xu, J., Wang, N, 2021. Citrus Huanglongbing is an immune-mediated disease that can be treated by mitigating reactive oxygen species triggered cell death of the phloem tissues caused by CandidatusLiberibacter asiaticus. bioRxiv, 2021-08.

Papoutsis, K., Edelenbos, M., 2021. Postharvest environmentally and human-friendly pre-treatments to minimize carrot waste in the supply chain caused by physiological disorders and fungi. Trends Food Sci. Technol. 112, 88–98.

Park, M.Y., Park, S.J., Kim, J.J., Lee, D.H., Kim, B.S., 2020. Inhibitory effect of Moriniafungin produced by Setosphaeria Rostrata F3736 on the development of Rhizopus Rot. Plant Pathol. J. 36 (6), 570.
Parthasarathy, S., 2024. Fundamentals of Plant Pathology. CRC Press.
Roy, S., Nuckles, E., Archbold, D.D., 2018. Effects of phenolic compounds on growth of Colletotrichum spp. in vitro. Curr. Microbiol. 75, 550–556.
Santos Júnior, J.R., Quelemes, P.V., Amorin, A., Primo, F.L., Gobo, G.G., Tedesco, A.C., Cardoso, V.S, 2014. Collagen-based silver nanoparticles for biological applications: synthesis and characterization. J. Nanobiotechnology 12, 1–9.
Singh, S.K., Sharma, V.K., Kumar, Y., Kumar, S.S., Sinha, S.K., 2009. Phytochemical and pharmacological investigations on mangiferin. Herba Polonica 55 (1), 126–139.
Sudheeran, P.K., Ovadia, R., Galsarker, O., Maoz, I., Sela, N., Maurer, D., Feygenberg, O., Oren Shamir, M., Alkan, N., 2020. Glycosylated flavonoids: fruit's concealed antifungal arsenal. New Phytologist 225 (4), 1788–1798.
Talhinhas, P., Baroncelli, R., 2021. Colletotrichum species and complexes: geographic distribution, host range and conservation status. Fungal Divers. 110 (1), 109–198.
Thomidis, T., Michailides, T., Exadaktylou, E., 2009. Contribution of pathogens to peach fruit Rot in northern Greece and their sensitivity to iprodione, carbendazim, thiophanate-methyl and tebuconazole fungicides. J. Phytopathol. 157 (3), 194–200.
Torres-León, C., Rojas, R., Serna-Cock, L., Belmares-Cerda, R., Aguilar, C.N., 2017. Extraction of antioxidants from mango seed kernel: optimization assisted by microwave. Food Bioprod. Process. 105, 188–196.
Vandana, V., Mesta, R.K., Kumar, K.C.K., Jhalegar, M.D.J., Thoke, S., 2023. Bio Efficacy of Fungicides and Bio Agents against *Colletotrichum gloeosporioides* causing Anthracnose in Pomegranate. Int. J. Curr. Microbiol. Appl. Sci. 10 (07), 457–473.
Wang, C.B., Jiang, N., Xue, H., Piao, C.G., Li, Y., 2022. *Colletotrichum chinense* sp. nov. from Yuccagloriosa and *C. quercicola* sp. nov. from Quercusvariabilis in China. MycoKeys 93, 1.
Zhou, D., Wang, Z., Li, M., Xing, M., Xian, T., Tu, K., 2018. Carvacrol and eugenol effectively inhibit *Rhizopus stolonifer* and control postharvest soft rot decay in peaches. J. Appl. Microbiol. 124 (1), 166–178.
Ziqin, L., Hui, Y., Qiang, Z., Xinwen, J., 2021. Isolation and Identification of Botrytis cinerea in Processing Tomato (*Solanum lycopersicum*). Plant Dis. Pests 12 (1).