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PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF MANGO (*MAGNIFERA INDICA* L.) SEED KERNEL AND PEEL OILS

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ABSTRACT

The peel and kernels mango (*Mangifera indica* L.) processing by products can be used as a source of valuable products. Therefore, the present study was attempted to study physicochemical properties, antioxidant and antimicrobial activities of mango seed kernel and peel wastes. The result of physicochemical properties indicated that significantly higher oil yield (38.75±1.77), specific gravity (0.86±0.04), acid value (2.66±0.20) and free fatty acid value (1.34±0.12); and higher DPPH (16.70±0.70) antioxidant activities were recorded for mango seed oil extract. However, significantly higher hydrogen peroxide scavenging activity (HPSA, 31.10±1.70) and ascorbic acid (43.00±2.73) were recorded for fruit peel oil extract. Stronger antibacterial activity with maximum zone of inhibition (16.50 mm), minimum inhibitory concentration MIC (0.10µl/ml) and corresponding minimum bactericidal concentration MBC (0.20 µl/ml) was recorded for seed oil extract against *S. aureus*. Stronger antifungal activity with maximum zone of inhibition (16.47 mm), MIC (0.05 µl/ml, the least value) and MFC (0.10 µl/ml) for seed oil extract against *C. albicans*. It can be concluded from the results of present study that seed oil extract was found to be more effective antioxidant and antimicrobial potential than peel oil extract in mango *(M. indica* L.*)*

Keywords: Antibacterial potential, Diameter of zone inhibition, Free radical scavenging activities, MBC, MFC, MIC

INTRODUCTION

Mango (*Magnifera indica* L.) belonging to Anacardiaceae family, is known for its attractive color, delicious taste, good flavors **(Pott *et al.*, 2003)**. During the processing of mango for pulp, the stone containing seed kernel contributing 15-20 % of total fruit weight is generated as by-product. The mango seed kernel contains protein, carbohydrates, high oleic lipids, minerals, crude fiber, ash, various bioactive compounds. The mango seed kernel contains almost 15% edible oil **(Akinyemi *et al.*, 2015)**. The variation in oil content can be due to cultivar differences, soil type, ripening stage, the harvesting time and the extraction method of oil used **(Arogba, 1997; Yamoneka *et al.*, 2015)** and difference in climatic conditions of their geographical locations **(Sani, 2014)**. Mango oil is good for baby creams, suncare balms, cosmetic, soap industry and within other moisturizing products **(Kittiphoom and Sutasinee, 2010)**. Depending on the cultivar, the mango seed represents from 10 % to 25 % of the whole fruit weight **(Ahmad *et al.*, 2007)**. The kernel inside the seed accounts for 45 % to7 5% of the seed and about 20 % of the whole fruit. Domestic consumption and industrial processing of the fruits produce huge amount of mango seed kernel and peels by products. The processing of such byproduct wastes into valuable products helps in resolving the problems of raw materials, energy, environmental pollution, and drug resistances.
Therefore, the present study was attempted to study physicochemical properties, antioxidant and antimicrobial activities of mango seed kernel and peel wastes.

MATERIALS AND METHODS

Plant material and extract preparation

The experiment was conducted in Molecular Biology and Biotechnology Laboratory, Haramaya university. The mango fruit sample was collected from Home Garden, Bahirdar district, Ethiopia. The fruit samples were manually washed with distilled water and residual moisture was evaporated at room temperature. Thereafter, the seeds and peel samples were chopped and ground in a grinder for 2 min, the process was stopped for 15 sec to avoid heating of sample. The oil extraction was done in Soxhelt apparatus using hexane as a solvent. Then, physicochemical properties of the oil were done based on determination of oil content, specific gravity, acid value, free fatty acid and peroxide value, and the antioxidant activities were based on DPPH and hydrogen peroxide free radical scavenging activities, and ascorbic acid as per the standard procedure described by **AOAC (1990)**.

Antimicrobial activity of the oil extracts

The antimicrobial experiment was arranged as 2 x 1 x 4 [2 source extracts: seed and peel oil extracts of Mango (*Mangifera indica* L.) at three concentration levels, 1 solvent system i.e. hexane, 4 test organisms (2 bacteria: *E. coli* (gram negative), and *Staphyllococcus aureus* (gram positive), and; two fungi (*Aspergillus niger* and *C. albicans*)] completely randomized factorial design in three replications. The test pathogens were obtained from Ethiopian Institute of Food and Health, Addis Ababa, Ethiopia. The fungal and bacterial pathogens were subcultured and maintained on Potato Dextrose Agar (PDA) and Nutrient Agar, respectively. Thenceforth, the fungal and bacterial cultures were incubated for 72 h at 27 ºC and for 18-24 h at 37 ºC, respectively.

Media Preparation and Standardization of Inoculum

Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Muller Hinton agar (MHA) was used for sub-culturing of bacterial test organism, fungal test organism, and determination of antimicrobial activities, respectively. These media were prepared and sterilized using an autoclave according to the manufacturers' instructions. Two to three bacterial colonies on the plate were picked up with a sterile inoculating loop and transferred into a test tube containing sterile normal saline and vortexes thoroughly. The spores of the test fungi were harvested by washing the surface of the fungal colony using 5mL of sterile saline solution. This procedure repeated until the turbidity of each bacterial and fungal spore suspension matched the turbidity of 0.5 McFarland Standards as described by the Clinical Laboratory Standards Institute **(CLSI, 2012)**. The resulting suspension was used as inoculums for the test pathogen in the antimicrobial susceptibility test.

Disc diffusion Method

The discs of 6 mm diameter were prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and then impregnated each of them was impregnated with 0.01 ml of the prepared test extract ethyl acetate solution. The extract impregnated discs were placed onto MHA plates evenly inoculated with test pathogens **(Abdel Wahab and Gismalla, 2017)**. Following this step, the impregnated discs were dispensed onto the surface of the inoculated agar plates using sterile forceps **(CLSI, 2015)**. Discs of commercial ampicillin (1µl/disc) and ketoconazole (1µl/disc) were used as positive controls for bacterial and fungal pathogens, respectively and distilled water impregnated discs were used as negative controls. Then the MHA plates were sealed with parafilm and incubated at 37 ºC for 24 h and 27 ºC for 72 h for bacterial and fungal pathogens, respectively. The diameters of the zone of inhibition around each disc were measured to the nearest millimeter along two axes (i.e. 90° to each other) using a transparent ruler and the means of the two readings were be recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The oil extracts that showed significant antimicrobial activity in the antimicrobial activity tests were selected for determination of MIC based on broth dilution method followed by **Mousavi *et al.* (2015)** with slight

modifications. In broth dilution method, two milliliters of nutrient broth and potato dextrose broth for bacteria and fungi respectively were added into all test tubes and 0.1 ml of the prepared concentration of each oil extract were mixed with the nutrient broth and potato dextrose. Thereafter, standardized inoculums of 0.1ml of the respective test pathogens were dispensed into the test tubes containing the suspensions of the broth and the oil extract. Then, all test tubes were properly corked and incubated at 37 °C for 24 h for bacteria and 27 °C for 72 h for fungi. After that, they were observed for absence or presence of visible growth. The lowest concentration at which no visible growth of organisms was regarded as the MIC. The experiment was carried out for each test organism in triplicates.

Determination of minimum bactericidal (MBC) and fungicidal concentrations (MFC)

For the determination of the MBC and MFC, fresh nutrient agar and potato dextrose agar plates were inoculated with a loop taken from each of the broth cultures that showed no growth in the MIC tubes. That is MBC/MFC values were determined by subculturing from respective MIC values. Since antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC **(CLSI, 2015)**. MBC/MFC is the amount of the extract that kills microbial growth. While MBC assay plates were incubated for 48 h, MFC assay plates were incubated for 3 days. After the incubation periods, the lowest concentration of the extract that did not allow any bacterial or fungal growth on solid medium was regarded as MBC and MFC for the extract **(CLSI, 2012)**.

Statistical analysis

The experimental data were analyzed using SAS version 9.2. **(SAS, 2011)** to investigate statistical significance between the different oil quality parameters. Differences between means were considered statistically significant at *P<0.05*.

RESULTS AND DISCUSSION

Physicochemical properties of mango seed and peel oil extracts

The physicochemical properties of mango seed and peel oil extracts were assessed based on physicochemical parameters like oil content, specific gravity, acid value, free fatty acids, and peroxide values as in Table 1.

Table 1 Physicochemical properties of mango seed and peel oil extracts

Oil extract	Oil yield	Spgr	ACV	FFA	PV
Seed	38.75±1.77^{a}	0.86±0.04^{a}	2.66±0.20^{a}	1.34±0.12^{a}	1.10±0.25^{b}
Peel	31.00±1.41^{b}	0.69±0.03^{b}	1.54±0.01^{b}	0.78±0.10^{b}	2.20±0.28^{a}

Legend: Means followed by same letter within a column were not significantly different at *P<0.05* probability level based on LSD (Least Significance difference) test. Small letters: significance within column; Spgr: specific gravity; ACV: acid value; FFA: free fatty acids; PV: peroxide value

It was observed that significantly higher oil yield (38.75±1.77), specific gravity (0.86±0.04), acid value (2.66±0.20) and free fatty acid value (1.34±0.12) for mango seed oil extract. However, significantly higher peroxide value (2.20±0.28) was recorded for peel oil extract. Similar study was conducted by Abdelaziz (2018) who reported the characteristics of mango kernel meal oil The Specific gravity at 24° C, Reflective Index, and Iodine value (g/100 g oil) were 0.89, 1.58 and 46.0, respectively.

The peroxide values for mango seed (1.10) and peel (2.20) of mango oil extract obtained in the present study were lower than that expected of rancid oil which ranges from 20.00-40.00 mg/g oil **(Ishida *et al.*, 2000)**. Generally, in the fresh oil, the peroxide value should be less than 10 mg/g oil **(Kittiphoom and Sutasinee, 2013)**. High peroxide values are associated with higher rate of rancidity **(Sani, 2014)**. Acid value for mango seed oil (2.66) and peel oil (1.54mg KOH/g), indicates that the oils were edible because it falls within the recommended codex of 0.6 and 10 for virgin and non-virgin edible fats and oil, respectively **(Olajumoke, 2013)**.

Antioxidant activities of oil extracts of mango seed and peel oil extracts

The antioxidant activities of mango seed and peel oil extracts was evaluated based on DPPH and hydrogen peroxide free radical scavenging activities and ascorbic acid content as in Table 2. There was no significance difference in DPPH between kernel seed and peel oil extracts even though, it was higher for seed oil extract (16.70±0.70). Significantly higher hydrogen peroxide scavenging activity (HPSA, 31.10±1.70) and ascorbic acid (43.00±2.73) were recorded for mango fruit peel oil extract. The higher DPPH value indicates higher antioxidant activities and the presence of higher essential omega-3 fatty acids in mango seed oil extract. The DPPH radical scavenging activity of hexane extract was higher than that of ethanol extract because hexane more extracting other antioxidant components soluble in oil, such as β-carotene and vitamin E (tocopherols and tocotrienols) **(Masud *et al.*, 2018).**

Table 2 Antioxidant activities of mango seed and peel oil extracts

Oil extract	DPPH	HPSA	AA
Mango seed	16.70±0.70^{a}	12.65±0.92^{b}	14.74±2.98^{b}
Mango peel	14.05±1.20^{a}	31.10±1.70^{a}	43.00±2.73^{a}

Legend: Means followed by same letter within a column were not significantly different at *P<0.05* probability level based on LSD (Least Significance difference) test. Small letters: significance within column; DPPH: 2, 2- diphenyl-1-picrylhydrazyl; HPSA: hydrogen peroxide scavenging activity; AA: ascorbic acid

Antimicrobial Activities of Mango (Mangifera indica L.) seed and peel oil extracts

The antimicrobial activities based on diameter of inhibition zone for *Mangifera indica* L. seed and fruit peel oil extracts were shown in Table 3. Significance differences were recorded for both seed and peel oil extracts at different concentration levels. The mean zone of inhibition at highest concentration (3 µl/ml) against bacterial test pathogens ranged from 13.90±0.36 mm to16.50±0.45 mm, while 12.67±0.40mm to 16.47±0.50 mm against fungal test pathogens. Stronger antibacterial activity with maximum zone of inhibition (16.50 mm) at highest dose (3 µl/ml) was recorded for seed oil extract against *S. aureus* while the weaker antibacterial activity (13.90 mm) was observed for peel oil extract against *E. coli* indicating that *S. aureus* (gram positive) was more susceptible to the oil extract than *E. coli* (gram negative). Thus, seed oil has exhibited more antibacterial potential than peel oil in mango (*Mangifera indica* L.).

Table 3 Antimicrobial Activities oil extracts from mango seed and peel oil extracts as mean diameter of zone of inhibition against test pathogenic microorganism

Test microorganism	Oil extract	Concentration of the oil extract (v/v)			Ciprofloxacin (1µl/ml)
		1µl/ml	2µl/ml	3µl/ml	
E. coli	Seed	11.67±0.76aC	12.73±0.64aC	15.03±0.45bB	18.56±0.40aA
	Peel	11.00±0.50aBC	12.50±0.45aBC	13.90±0.36cB	18.60±0.40aA
S. aureus	Seed	11.17±0.29aC	12.30±0.26aC	16.50±0.45aB	18.60±0.36aA
	Peel	11.67±0.76aC	13.33±1.26aBC	14.83±0.76bcB	18.87±0.32aA
					Fluconazole (1µl/ml)
C. albicans	Seed	12.43±0.40aD	14.33±0.76aC	16.47±0.50aB	18.17±0.26bA
	Peel	11.83±0.76aC	13.83±0.57aB	14.87±0.71bB	19.00±0.50aA
A. niger	Seed	0.00±00cD	9.83±0.76cC	13.70±0.26cB	18.50±0.50abA
	Peel	10.73±0.25bC	12.00±0.50bB	12.67±0.40dB	18.83±0.29abA

Legend: Means followed by same letter within a column were not significantly different at *P<0.05* probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row. *E. coli*: *Escherichia coli*; *S.aureus*: *Staphylococcus aureus, C. albicans*: *Candida albicans, A. niger*: *Aspergillus niger*

By contrast, stronger antifungal activity with maximum zone of inhibition (16.47 mm) was recorded for seed oil against *C. albicans* as the weaker antifungal

activity with minimum zone of inhibition (12.67 mm) was observed for peel oil against *A. niger* suggesting seed oil extract is more effective antifungal potential than peel oil extract in *M. indica*. Similar study was conducted by **Vega-Vega *et al*. (2013)** who demonstrated a significantly higher total antioxidant capacity, phenolic content, and antimicrobial activity of mango byproducts (seed and peel) than of the edible portions.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) of oil from *Mangifera indica* L. seed and peel oil extracts

The efficacy of *Mangifera indica* L. seed and peel oil extracts against pathogenic microbes was evaluated by MIC, MBC and MFC as in Table 4. The oil extracts from seed kernel has exhibited strongest antibacterial activity with MIC (0.10 µl/ml) and corresponding MBC (0.20 µl/ml) against *S. aureus* while the weakest antibacterial activity with MIC (0.75 µl/ml, the largest value) and MBC (1.00 µl/ml) was recorded for peel oil against *E. coli* indicating that *S.aureus* is more susceptible to the oil extract than *E.coli*, and also indicating seed oil possesses stronger antibacterial potential than peel oil in mango fruit.

Table 4 MIC, MBC, and MFC of *Mangifera indica* L. seed and peel oil extracts

Test microorganism	Oil extract	MIC (µl/ml)	MBC/MFC (µl/ml)
E. coli	Seed	0.20	0.25
	Peel	0.75	1.00
S. aureus	Seed	0.10	0.20
	Peel	0.40	0.75
A. niger	Seed	0.25	0.50
	Peel	0.50	1.00
C. albicans	Seed	0.05	0.10
	Peel	0.20	0.25

CONCLUSION

Based on the results of physicochemical properties of mango seed kernel and peel oils, it could be concluded that the oil extract could be become valuable resource to produce high value of vegetable oil. The oil extracted with hexane has better quality. The results of present study provide useful information for edible oil and food industry, due to biological activities as antioxidant and antimicrobial activities.

Authors' contribution

Zekeria Yusuf: initiation and design of the study, Lab experiment, data analysis; Alemtsehay malede and Megersa Idris: Lab experiment, data collection, and write up of the document; Sultan Seyida and Mulugeta Desta: Analysis and interpretation of data. All authors contributed to drafting the article and revising it critically for important intellectual content.

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Availability of data and materials

The data materials will be available on request.

Ethics approval and consent to participate

The ethical approval is not applicable for this manuscript since it has no animal experiment according to Haramaya University's ethical committee.

Conflict of Interest

The authors declare no conflict of interest.

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