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Physicochemical Properties, Antioxidant and Antimicrobial Activities of Pumpkin (*Cucurbit* sp.) Seed and Fruit Pulp Oil Extracts

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Abstract: The consumption of pumpkin seeds in the oil form or roasted pumpkin seeds is proved to exhibit several positive health effects. Since, the content of particular nutrients in the pumpkin seed may vary considerably, depending on soil conditions, climate and genetic factors. The aim of the present study was to examine physicochemical properties, antioxidant and antimicrobial activities of pumpkin (*Cucurbit* sp.) seed and pulp oils. The oil extraction was done in Soxhelt apparatus using hexane as a solvent. The result of physicochemical properties of pumpkin (*Cucurbit* sp.) seed and pulp oils indicated Seed oil has recorded significantly higher oil yield (17.5%), specific gravity (0.91), acid value (1.82) and free fatty acid value (0.92%). Significantly higher antioxidant activities with respect to ascorbic acid content (38.94±2.68), DPPH (32.30±1.27) and hydrogen peroxide (11.85±0.64) free radical scavenging activities were obtained for pumpkin seed oil. The stronger antibacterial activity with maximum zone of inhibition (15.50 mm), minimum inhibitory concentration (MIC, 0.19 µL/mL) and minimum bactericidal concentration (MBC, 0.25 µL/mL) were recorded for fruit pulp oil extract against *S. aureus.* On the other hand, the stronger antifungal activity with maximum zone of inhibition (15.00 mm) with MIC (0.125 µL/mL) and minimum fungicidal concentration (MFC, 0.19 µL/mL) were observed for seed oil against *A. niger*. The result of this study indicated that seed oil presented superior in antioxidant and antifungal potentials but lower in antibacterial activity than fruit pulp oil in pumpkin.

INTRODUCTION

Pumpkin (*Cucurbita spp.*) belong to *Cucurbitaceae,* is a fruit vegetable[1]. Pumpkin fruit is one of the widely grown vegetables incredibly rich in antioxidants such as carotenoids that are used in synthesis of vitamin Afor most of people living in developing countries[2]. Pumpkin seeds have almost always been discarded as waste in spite

of having a great nutritive value. After harvesting, the seeds are often used as animal feed, ground up for fertilizer or even discarded[3]. Pumpkin seed contribute significantly to the nutrition of human population in many parts of the world[2]. The chief nutritional components of pumpkin seed are proteins (30-51%) and oil (up to 40%), carbohydrates (up to 10%) and micronutrients (between 4 and 5%)[4]. The total unsaturated fatty acid content ranged from 73-81% and the predominance of linoleic, oleic, palmitic and stearic acid was observed in pumpkin seed oil[5]. The major fatty acids in the pumpkin seeds were palmitic, stearic, oleic and linoleic acids[6]. Differences in the chemical composition of pumpkin seed between *Cucurbita* species and cultivars from different parts of the world might be related to growth and fertilization conditions and also to the harvest time[4].

Water extracts of pumpkin seed are used in the treatment of heterophyiasis, preventing and alleviating prostate and bladder problems, lowering cholesterol levels, lipid-associated disorders such as atherosclerosis[4]. Diets rich in pumpkin seed oil have also been associated with lower level of gastric, breast, lung and colorectal cancer[7]. Pumpkin seed oil is not widely used commercially even though it has characteristics that are well suited for industrial application and can contribute to healthy human diets[8]. Since, the content of particular nutrients in the pumpkin seed may vary considerably, depending on soil conditions, climate and genetic factors. The aim of the present study was to examine physicochemical properties, antioxidant and antimicrobial activities of pumpkin (*Cucurbit* sp.) seed and pulp oils.

MATERIALS AND METHODS

Oil extraction, determination of physicochemical and antioxidant activities: The study was conducted in Molecular Biology and Biotechnology Laboratory, Haramaya University. The Pumpkins (*Cucurbit* sp.) fruit sample was collected from Bedeno District, East Hararghe, Ethiopia. The fruit samples were manually washed with distilled water and residual moisture was evaporated at room temperature. Afterwards, the pulp and seed samples were freeze dried and milled to fine powders 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. The physicochemical properties of the oil extracts were done based on determination of oil content, specific gravity, acid value, percent free fatty acid and peroxide values. The antioxidant activity was investigated based on determination of ascorbic acid content, DPPH and hydrogen peroxide free radical scavenging activities.

Antimicrobial activity of the oil extracts: The antimicrobial experiment was arranged as 2×1×4 (2 source extracts (seed and pulp oil extracts from pumpkins (*Cucurbit* sp.) at three concentration levels), 1 solvent system i.e. hexane, 4 test organisms (2 bacteria: *Escherichia coli* (gram negative), *Staphylococcus aureus* (gram positive), two fungi (*Aspergillus versicolor* and *A. Niger*) completely randomized factorial design in three replications. The test pathogens including were obtained from Plant Pathology Laboratory, Haramaya University. The fungal and bacterial pathogens were subcultured and maintained on Potato Dextrose Agar (PDA) and Nutrient Agar, respectively. Then, the fungal and bacterial cultures were incubated for 72 h at 27°C and for 18-24 h at 37°C, respectively.

Media preparation and standardization of inoculums: nutrient Agar (NA), Potato Dextrose Agar (PDA) and Muller Hinton agar (MHA) were 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 manufacturer's 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 5 mL 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[9]. The resulting suspension was used as inoculums for the pathogens in the antimicrobial susceptibility test.

Disc diffusion method: Discs of 6 mm diameter was prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and then each of them impregnated with 0.2 mL of the prepared test extract. The extract impregnated discs were placed onto MHA plates evenly inoculated with test pathogens[10]. Discs of commercial amoxicillin (1 µL/disc) and ketokonazole (1 µL/disc) were used as positive controls for bacterial and fungal pathogens, respectively and the pure solvent (hexane) impregnated discs were used as negative controls. Then the MHA plates were sealed with parafilm and incubated at 37°C for 24 h and 27°C 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. Two milliliter 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 was mixed with the nutrient broth and potato dextrose. Thereafter, standardized inoculums of 0.2 mL 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 growths of organisms were 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 one loop full of culture taken from each of the broth cultures that showed no growth in the MIC tubes. That is MBC/MFC values were determined by sub culturing from respective MIC values. Since, antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC[9]. 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[9]. This observation was matched with the MIC test tube that did not show evidence of growth after 48 h of incubation for bacteria or spore germination after 3 days of incubation for fungi. The experimental data were analyzed using SAS version 9.2.[11] 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 and antioxidant activities of pumpkins (*Cucurbit* sp.) seed and pulp oils: Seed oil has recorded significantly higher oil yield (17.5%), specific gravity (0.91), acid value (1.82) and free fatty acid value (0.92%) (Table 1). This finding was in agreement with Stevenson *et al.*(2007) who reported that oil content pumpkin seed fell in the range reported for different species of *cucurbita* (9.8-52.1%) and different varieties of *C. pepo* (31.2-51.0%).

The antioxidant activities of oil extracted from pumpkins (*Cucurbit* sp.) seed and pulp evaluated based on ascorbic acid content, DPPH and hydrogen peroxide free radical scavenging activities as in Table 2. Significantly higher antioxidant activities with respect to ascorbic acid content (38.94±2.68), DPPH (32.30±1.27) and hydrogen peroxide (11.85±0.64) free radical scavenging activities were obtained for pumpkin seed oil than for pulp oil. The higher DPPH value (32.30±1.27) indicates higher antioxidant activities and the presence of higher essential omega-3 fatty acids in pumpkin seed oil. The antioxidant activities of seed oil was found to be significantly higher than pulp oil extract indicating that seed oil might possess better biological activities, oil quality and pharmacological applications. This finding was in agreement with previous researchers[12, 13] who reported an antioxidant activity ranging between 5.44 and 17.75% and between 0.19 and 11.75% in *Cucubita pepo* seed genotypes. The analysis of the scavenging activity on DPPH radicals provides adequate information to determine the antiradical activity of pumpkin seed oil[14].

Antimicrobial Activities of pumpkin (*Cucurbit* sp.) seed and pulp oils: The disk diffusion method was used to measure diameter of inhibition zone. The diameter of inhibition zone for pumpkin seed and fruit pulp oils as in Table 3. Significance differences were recorded for both seed and pulp oil extracts at different concentration levels. Considerable antimicrobial activities were obtained for both seed and pulp oil extracts against tested bacteria and fungi. The mean zone of inhibition at highest concentration (3 µL/mL) against bacterial test pathogens ranged from 13.83±0.60-15.50±0.50 mm while 12.00±0.50-15.00±0.50 mm against fungal test pathogens.

Stronger antibacterial activity with maximum zone of inhibition (15.50 mm) at highest concentration (3 µL/mL)of the oil was recorded for pulp oil extract against *S. aureus* while the weaker antibacterial activity (13.83 mm) was observed for pumpkin seed oil against *E. coli* indicating that *S. aureus* was more susceptible than *E. coli*. Hence pulp oil has exhibited more antibacterial potential than seed oil in pumpkin. On the other hand, the stronger antifungal activity with maximum zone of inhibition (15.00 mm) was recorded for seed oil against *A. niger* as the weaker antifungal activity with minimum zone of inhibition (12.00 mm) was observed for pulp oil against *A. versicolor* suggesting seed oil extract might be more effective antifungal potential than pulp oil extract pumpkin.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), Minimum Fungicidal Concentration (MFC) of oils from pumpkin (*Cucurbit* sp.) seed and fruit pulp: The effectiveness of pumpkin (*Cucurbit* sp.) seed and fruit

Table 1: Physicochemical properties of pumpkin seed and pulp oils

Oil extract	Oil yield	Spgr	ACV	FFA (%)	PV (meq O2/kg oil)
Seed	17.50±0.71a	0.91±0.02a	1.82±0.20a	0.92±0.10a	1.10±0.14a
Pulp	14.38±0.18b	0.68±0.05b	0.70±0.19b	0.35±0.09b	0.50±0.15a

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

Table 2: Antioxidant activities of pumpkin (*Cucurbit* sp.) seed and pulp oils

Oil extract	DPPH	HPSA	AA
Seed	32.30±1.27a	11.85±0.64a	38.94±2.68a
Pulp	11.40±0.20b	7.55±0.50b	23.93±0.66b

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

Table 3: Antimicrobial Activities oil extracts from pumpkin (*Cucurbit* sp.) seed and fruit pulp as mean diameter of zone of inhibition against test pathogenic microbial spp.

		Concentrations of the oil extract (v/v)			
Test pathogens	Plant part	1 µL/mL	2 µL/mL	3 µL/mL	Amoxicillin (1 µL/mL)
E. coli	Seed	9.83±0.55aD	11.83±0.78aC	13.83±0.60bB	18.50±0.50aA
	Pulp	10.50±0.50aD	12.83±0.76aC	14.17±0.70bB	18.83±0.29aA
S. aureus	Seed	8.50±0.51bD	10.50±0.36bC	14.50±0.50abB	18.83±0.35aA
	Pulp	9.50±0.60abD	12.00±0.55aC	15.50±0.50aB	18.33±0.28aA
Ketokonazole (1 µL/mL)					
A. niger	Seed	9.50±0.40aD	11.50±0.76aC	15.00±0.50aB	17.83±0.45aA
	Pulp	8.50±0.50bD	10.00±0.45bC	13.50±0.55bB	17.33±0.76aA
A. versicolor	Seed	8.00±0.55bC	9.67±0.53bC	13.50±0.50bB	18.00±0.29aA
	Pulp	7.00±0.60cD	9.00±0.55bC	12.00±0.50cB	17.33±0.29aA

Means followed by same letter within a column were not significantly different at 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*

Table 4: MIC, MBC and MFC of pumpkin (*Cucurbit* sp.) seed and fruit pulp oils

Test pathogens	Plant part	MIC (µL/mL)	MBC/MFC (µL/mL)
E. coli	Seed	0.375	0.75
	Pulp	0.25	0.50
S. aureus	Seed	0.25	0.375
	Pulp	0.19	0.25
A. versicolor	Seed	0.375	0.75
	Pulp	0.75	1.25
A. niger	Seed	0.125	0.19
	Pulp	0.25	0.375

pulp oils against pathogenic microbes was evaluated by MIC, MBC and MFC as in Table 4. The oil extracts from pumpkin fruit pulp has exhibited strongest bactericidal activity with MIC (0.19 µL/mL) and MBC (0.25 µL/mL) against *S. aureus* while the weakest bactericidal activity with MIC (0.375 µL/mL, the largest value) and MBC (0.75 µL/mL) was recorded for seed oil against *E. coli* indicating that *S. aureus* is more susceptible to the oil extract than *E. coli* and also indicating fruit pulp oil possesses stronger antibacterial potential.

By contrast, pumpkinseed oil extract has demonstrated strongest antifungal activity with MIC (0.125 µL/mL, the least value) and MFC (0.19 µL/mL) against *A. niger* whereas the weakest antifungal activity with MIC (0.75 µL/mL) and MFC (1.25 µL/mL) was observed for the seed oil extract against *A. versiclor* showing that *A. niger* was more susceptible to the oil extract than *A. versicolor* and the seed oil was more effective antifungal potential than the fruit pulp oil in pumpkin.

CONCLUSION

The findings of the present study has demonstrated oil quality, antioxidant and antimicrobial activities of oils derived from pumpkin seed and fruit pulps suggesting that pumpkin seeds have the potential to be developed as novel value added product which are rich in nutrients and to exploit wastages of pumpkin seed. Seed oil presented superior in antioxidant and antifungal potentials but lower in antibacterial activity than fruit pulp oil in pumpkin. Further studies need to be conducted with various types of solvents used and other extraction methods, assess cultivar differences and the influence of environment on biological activities of the oil and synergistic or antagonistic effects of biological activities.

Authors' contribution: Zekeria Yusuf: initiation and design of the study, Lab experiment, data analysis; Doyzer Mohammed, Sewnet Mengistu, Sultan Seyida and Desta Dugasa: Lab experiment, data collection and write up of the document; Jemal Tenishu 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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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.

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