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Exploration of Two *Cucurbitaceae* Fruit (Muskmelon and Watermelon) Seeds for Presence of Phytochemicals, and Antioxidant and Antimicrobial Activities

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Cucurbitaceae family fruits, especially melons, offers significant quantities of minerals carotenoids and phenolic compounds, contributing to their antioxidant activity. However, seeds of these fruits are usually discarded as waste by products. In current study, seeds of watermelon (*Citrullus lanatus*) and muskmelon (*Cucumis melo*) were separated, dried, grounded and extracted, with 70% ethanol, to investigate total phenolic content (TPC), flavonoid content (TFC), carotenoid content (TC) content, and total antioxidant activity (TAA). Further, antimicrobial activities of these extracts were tested against selected bacterial and fungus strains. Results showed that extracts of both *cucurbits* presented significant amounts of phytochemicals, with higher quantities presented by watermelon seeds. In watermelon seeds, TPC were found 156.50 mg/GAE 100 g, TFC 56.78 mg CE/100 g, TC 36.65 mg/100 g, and TAA 71%, and these amounts were significantly higher than those found in muskmelon seeds. Antimicrobial study results showed that extracts of both seeds exhibited significant zone of inhibitions against three bacterial and three fungal species, and these values were very comparable to the reference antimicrobial drug used, Ciprofloxacin. Findings of current research work provided significant grounds for presence of phytochemical bioactives in two melon fruits seeds, providing the basis for extraction and utilization of these bioactives, through processing and fortification different pharma foods.

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Introduction

Numerous natural substances that are found in plants are used to treat a variety of diseases. The usage of synthetic pharmaceuticals has become more prevalent in modern society, but they always have negative effects on humans. Fruits and vegetables in particular play a significant impact in promoting health and lowering the risk of disease (Mala and Kurian, 2016). By preventing cell oxidation and scavenging the free radicals generated in the body, antioxidants play a critical role in the body of life during various types of chronic diseases. In a live organism, antioxidants prevent oxidizing chain reactions. Antioxidants can be either synthetic or natural. Due to their carcinogenic consequences, the use of synthetic antioxidants is restricted (Skandrani et al., 2010). Human diseases are brought on by bacteria, viruses, fungi, and other parasites; as a result, millions of people have died, been disabled, and experienced social and economic difficulties. Despite the fact that many diseases may be treated with safe and effective medications, many people do not have affordable, safe, or healthy access to the resources they need to prevent or treat these illnesses. Seeds of the *cucurbit* fruits are a good source of antibacterial compounds (Hammer et al., 1999).

Melons are quite popular internationally, because of their delicate and delicious flavor, but seeds of these fruits, loaded with phytochemicals and sustainable ingredients for novel food formulations, go away as waste streams (Hussain et al., 2022b). Food waste is a major concern for the entire planet. Several fruits, including watermelon, muskmelon, and others, are accessible in the summer.

Fruits like muskmelons are eaten all around the world. Approximately 32% of muskmelon, which comprises 5% of the seeds and 27% of the peel by weight, is wasted. Muskmelons' seeds offer excellent nutritional value. They contain a huge variety of bioactive substances, including phenolics, flavonoids, and carotenoids. Muskmelon seeds can be used to cure or prevent a wide range of illnesses since they include antibacterial, antioxidant, and antidiabetic qualities. Thus, the seeds can be used as a meal that serves a purpose (Kumar et al., 2022). Watermelon seeds, a by-product of watermelon juice extraction, has high potential for recovery of value-added bioactive compounds (Rico et al., 2020).

Among *Cucurbitaceae* fruit crops that are commonly grown throughout the world, are the melons. In actuality, melons play a significant role in global fruit and vegetable production. The sweetness, pleasant flavour, and high nutritious content of melon are only a few of the qualities that characterize a melon's quality (Zhao et al., 2023). *Cucurbitaceae* fruits have significant quantities of carotenoids and phenolic compounds as well as excellent antioxidant activity, making them a rich source of phytochemicals. It has also shown that the various fruit components from this family responded differently in terms of antioxidant activity. At the immature stage, the majority of the antioxidant activity was discovered (Kubola and Siriamornpun, 2011). Due to presence of biochemical constituents in *cucurbits* and their role as pharma foods, these fruits have been widely consumed around the globe, as each part of these fruits have loads of bioactives (Hussain et al., 2023).

By utilizing the most recent methods, bioactive chemicals found in *Cucurbitaceae* seeds can be extracted to their fullest potential (Massa et al., 2019). According to Jukic et al. (2019), flour made from *cucurbit* seed oil press cake can be utilized to make functional and nutritious food ingredients. Functional food products could be developed by incorporation of seeds and peels of *cucurbit* fruits (Hussain et al., 2022c).

Although several research investigations have been done on different members of *Cucurbitaceae* family, but limited data could be found upon phytochemistry of the seeds of these fruits. Keeping in view the problems created as a result of waste generated due to consumption and processing of different melon fruits and vegetables, the objectives of current investigations were to explore seeds of two melons for presence of phytochemicals, and antioxidant and antimicrobial activities.

Materials and methods

Procurement of Fruits, Reagents, Chemicals and Microbial Strains for Study

Watermelon and muskmelon, for investigations of their seeds, were manually harvested from the fields, when they were fully developed and mature, and were brought to the Department of Food Science and Nutrition, University of Sargodha, where botanical identification of the fruits was carried out with the help of experts from the botany department. During the harvesting, uniformity of size, color, shape and weight was considered, and the harvested samples were precooled to remove field heat, and analyzed after being kept in a dark place at temperature between 20 and 25°C. All the reagents and chemicals used for this study were purchased from Tech Chemicals Ltd. Located in the Liberty market of Lahore, Pakistan. All these chemicals were of Sigma Aldrich, Germany. However, strains of bacteria and fungus were acquired from the Biochemistry Department, University of engineering and Technology, Lahore. Reference antimicrobial drug was purchased from Clinix pharmacy, Lahore, Pakistan.

Preparation of Muskmelon and Watermelon Seeds Powders

Muskmelon and watermelon seeds were rinsed with distilled water after being cleaned with tap water. To preserve the sample, the fruit seeds were immersed in 0.2% sodium metabisulphite for a period of fifteen minutes. Then, these seeds were dried in a hot air oven (model 400/D200°C, New Ethics®, São Paulo, Brazil) for 72 hours at 150°C, by following the procedure earlier adopted by Hussain et al. (2021a). The dried seeds were then ground into powder using a stainless-steel grinder, sieved, and stored in an airtight container in the laboratory shelf.

Development of Ethanolic Extracts From Muskmelon and Watermelon Seeds

For development of ethanolic extracts of the seeds, procedure from the studies of Hussain et al. (2021a) was followed with required adjustments. Briefly explaining, 200 g of each powder was well mixed with solvent having 70% ethanol and 30% water, and this solution was placed in a shaker for 48 hours after that filtration was carried out using muslin cloth. Then the extracts were further concentrated at 40°C in a rotary evaporator to find out the final crude extracts. Yield of extracts was found out by below given formula;

$$\text{Yield (\%)} = \frac{\text{Solvent free extract weight (g)}}{\text{Weight of dried extract (g)}} \times 100$$

Determination of Total Phenolic Contents (TPC) In Ethanolic Extracts of Muskmelon and Watermelon Seeds

The Folin-Ciocalteu colorimetric method, as described by Hussain et al. (2021a), was used to determine the TPC of the fruits seeds samples. In a nutshell, 20 mL of sample were combined with 1.57 mL of water, 100 mL of Folin-Ciocalteu reagent, and 300 mL of 7% Na_2CO_3 were added to the mixture, after 6 to 8 minutes. For two hours, the mixes were maintained at room temperature in the dark. A spectrophotometer (Spectronic, model 4001/4, ThermoFisher Scientific, Waltham, MA, USA) was then used to measure the absorbance at 765 nm. The results are presented as mg GAE/100 g of the dry weight of seed.

Determination of Total Flavonoid Contents (TFC) In Ethanolic Extracts of Muskmelon and Watermelon Seeds

For TFC determination in two melon seed powders, the protocols were followed from the procedure already adopted by Hussain et al. (2022d), with some modifications. Explaining briefly, 0.5 mL diluted solution was taken and 2 mL distilled water was added and then 5% $NaNO_2$, 0.15 mL was also added. Then, 1 mL of 1 M NaOH was added after 5 minutes. This final solution was thoroughly mixed for some time and using a spectrophotometer the absorbance at 510 nm was measured, in triplicate and results were presented as mg of quercetin equivalent per 100 g of dried weight.

Determination of Total Carotenoid Content (TC) In Ethanolic Extracts of Muskmelon and Watermelon Seeds

For total carotenoid determination, the spectrophotometric technique was modified per the one introduced by De Carvalho et al. (2009), with necessary changes. The melon seeds extracts were analyzed for total carotenoid content using a spectrophotometer (10S UV/visible spectrophotometer, TSG, Australia). The samples were examined by the spectrophotometer in the visible spectrum between 190 and 1100 nm. Then 3 mL of the diluted samples was utilized for analysis at wavelengths of 450, 470, and 502 nm after the samples were diluted with ethanol. The blank was comprised of ethanol. The spectrophotometer was constructed with a quartz cuvette. The absorbance coefficient for ethanol was determined using the extinction coefficient of 1% and the coefficient of A1%, respectively.

Determination of Total Antioxidant Activity (TAA) In Ethanolic Extracts of Muskmelon and Watermelon Seeds

According to the methodology adopted by of Aryal et al. (2019) with certain adjustments, the total antioxidant activity (TAA) of melon seeds powders was assessed using the DPPH method. Based on an estimation of the stable 1,1-diphenyl-2-picrylhydrazyl radicals' ability to scavenge free radicals, the antioxidant capacity of the samples was assessed. By detecting the drop in absorbance at 517 nm, the DPPH scavenging ability was assessed spectrophotometrically using spectrophotometer (Biochrom, Libra S22, and England). Each reaction was performed in triplicate to find out the means values of TAA, which were presented as mg trolox/100 g dry weight.

Determination of Antimicrobial Activities of Muskmelon and Watermelon Seeds Extracts

Antibacterial assay

Antibacterial activity of ethanolic extracts of two melon seeds was tested against three different microbial pathogens (*Bacillus cereus*, *Escherichia Coli* and *Streptococcus aureus*). Nutrient agar media was used for standardization of bacterial pathogens. Seeds ethanolic extract prepared were tested for the antimicrobial activity through well diffusion method. In each well 100 micro liters of ethanolic seed extract was added. Using sterile swabs, bacterial strain cultures were plated on Mueller Hinton agar and adjusted to a final concentration of 108 CFU/mL using a 0.5 McFarland standard. 40 L were pipetted into the different wells for each 500 mg/mL extract. As a standard, Ampicillin was utilized at a final concentration of 30 g/mL. Afterward, the plates were incubated for 24 hours at 37°C by following the guidelines of Neglo et al. (2021). Mean values of zone of growth inhibitions (mm) were calculated.

Antifungal assay

Pure cultures of three fungal species (*Candida albicans*, *Mucor meihi* and *Aspergillus niger*) were collected in same way as bacterial. Nutrient agar was used to provide the growth medium for the antifungal test, as was reported by Barbero-Lopez, (2020). Following an autoclave (120°C, 15 min), 15 mL of each growth medium was cast in a petri dish and cooled under sterile circumstances. As a control, a growth medium containing 4% malt, 2% agar, and milli-Q water was created. Using a plug measuring 0.28 cm^2, the fungus was inoculated under sterile circumstances. Following that, the petri dishes were subsequently maintained at 25°C and 65% relative humidity in a growth chamber. Depending on the fungal strain and replicates, it took between 8 and 14 days for the fungus to spread across the entire petri dish when they were growing in the control samples. Zone of inhibition in mm was calculated for the antifungal activity of the muskmelon and watermelon seed extracts.

Statistical Analyses of The Obtained Results

The findings of each analysis were produced in triplicate, and they were presented as means and standard deviations. The statistical analysis was conducted using the one-way ANOVA approach. Using the Duncan's multiple-range test, the mean values were separated. For statistical analysis, guidelines from Steel et al. (1980) procedures were used.

Results and Discussion

Extracts Yield of Muskmelon and Watermelon Seeds

Yield of ethanolic extracts of both watermelon and muskmelon seeds has been given in Table 1, from where it was evident that among the seeds of the two melons, watermelon seeds had the highest percentage of extract yield and muskmelon seeds had the lowest percentage of extract yield. Waste by products of melon and watermelon are cheap and sustainable sources of valuable bioactive compounds, utilization of their extracts in food industry could be proved useful in development of pharma foods, capable of lowering economic potential on populations (Rico et al., 2020).

Singh et al. (2016) extracted various *cucurbit* fruit components using various solvents, and their findings were generally consistent with those of the present, leading them to the conclusion that the seeds of these fruits contain valuable bioactives in their extracts. In a related investigation, Xanthopoulou et al. (2009) extracted four different types of *cucurbit* seeds groups using four different types of solvents and calculated the yield as g of extract per 100 g of seed. The acquired results were sufficient to support the findings of the current study. Similar findings were also reported by Hussain et al. (2021a), during extraction of *cucurbit s*eeds using 70% ethanol.

Since all volatile and non-volatile components are extracted by the mixing of two types of solvents, extracts of two melon seeds were made using the most environmentally friendly and productive solvents, ethanol and water in a 70:30 ratio. Because seeds are sites of higher metabolism, a higher output of extracts from them may reflect the presence of more active ingredients (Asif et al., 2017).

Table 1. Extracts yield of muskmelon and watermelon seeds

Melon seeds	Extracts yield (%)
Muskmelon seeds	$9.13{\pm}0.15^{b}$
Watermelon seeds	$11.05{\pm}0.1^{a}$

Values are presented as means of triplicate analysis along with standard deviation. Whereas, different alphabetical letters in a column represent significant results (P>0.05).

Table 2. TPC, TFC, TC and TAA of watermelon and muskmelon seeds extracts

Treatments	Total Phenolic Contents (mg GAE/100 g)	Total Flavonoid Contents (mg CE/100 g)	Total Carotenoids (mg/100 g)	Total Antioxidant Activity (%)
Muskmelon seeds extracts	105.25±0.25[b]	34.60±0.10[b]	22.90±0.20[b]	48.56±0.15b
Watermelon seeds extracts	156.50±0.30[a]	56.78±0.25[a]	36.65±0.15a	71.0±0.10[a]

Different alphabetical letters in a column represent significant results (P>0.05). (P< 0.05), TPC; total phenolic contents, TFC; total flavonoid contents, TC; total carotenoid content, TAA; total antioxidant activity, GAE; gallic acid equivalent, CE, catechin equivalent

TPC, TFC, TC and TAA of Watermelon And Muskmelon Seeds Extracts

Results presented in Table 2 showed that extracts of both *cucurbits* presented significant amounts of phytochemicals, with higher quantities presented by watermelon seeds. In watermelon seeds, TPC were found 156.50 mg/GAE 100 g, TFC 56.78 mg CE/100 g, TC 36.65 mg/100 g and TAA 71%, and these amounts were significantly higher than those found in muskmelon seeds. These results provided strong evidences of presence of phytochemicals in seeds of these two melon varieties, due to which these seeds have huge potential to be used as medicinal ingredients and to develop pharma foods that could promote health.

Among the significant phytochemical classes, carotenoids, flavonoids, and phenols are recognized for their ability to promote health. Bioactive chemicals are crucial for human nutrition and health since they prevent the start of numerous diseases (Algarni, 2020). For the purpose of evaluating the antibacterial and antioxidant activity of meat products, Boeira et al. (2018) isolated bioactive components from herbal plants and added them. They claimed that the main bioactives responsible for these therapeutic activities are phenolics and flavonoids. Lemon grass extracts may be used with chemotherapeutics to decrease the risk of drug-related toxicity and boost the efficacy of the therapy.

The values of antioxidant activity for seeds were consistent with the current findings when Singh et al. (2016) extracted different portions of *cucurbit* fruits with various solvents to assess their antioxidant activity by DPPH free radical scavenging technique. Alkaloids, saponins, flavonoids, and steroids are the phenolic compounds that have been discovered to be effective antioxidants and are abundant in pumpkin fruit sections, particularly the seeds and pulp (Mala and Kurian, 2016). According to Dissanayake et al.'s (2018) analysis of the antioxidant properties of the skin, seeds, and leaves of *cucurbit* fruits, seeds exhibit substantial antioxidant properties because they contain significant amounts of phenolic and flavonoid chemicals.

In their earlier investigations Hussain et al. (2022a) observed that seeds of *cucurbit* fruits have great potential to be used as antioxidants sources. Findings of Amin et al. (2018) were also in line, reporting antioxidant activities of muskmelon seed extracts. Similarly, presence of bioactives in watermelon seeds, leading towards antioxidant properties was reported by Lopusiewicz, (2018). Presence of significant amounts of carotenoids in melon seeds was reported by Fundo et al. (2018), supporting the current results regarding carotenoid contents in muskmelon and watermelon seeds extracts.

Antimicrobial Activities Of Melon Seeds Ethanolic Extracts

Table 3 presents the results of the antimicrobial activities of ethanolic extracts of two melon seeds extracts against bacterial and fungal strains, compared to the standard antibiotic ampicillin. The standard antibiotic ampicillin exhibited the highest zone of inhibition against all tested bacterial and fungal strains, indicating its potent antimicrobial activity, whereas antimicrobial activities of two melon seed extracts were also very comparable to the reference drug. Melons seeds extracts have the potential to be used as natural sources of antibacterial chemicals, as evidenced by the antimicrobial properties reported in this study. Incorporating these extracts into food products or developing topical applications could offer alternative strategies for microbial control and preservation.

Cucurbitaceae fruit sections contain phytochemicals such saponins, tannins, flavonoids, alkaloids, and steroids that may have been acting as antibacterial agents (Chonoko and Rufai 2011). In a related study, Dissanayake et al. (2018) used three bacterial and fungal strains to test the antibacterial efficacy of extracts from *cucurbit* skin, seeds, and leaves using three different types of solvents. They discovered that the seeds showed a prominent zone of inhibition. Melon seed extracts are effective antifungal agents, according to a subsequent investigation by Pandey et al. (2010). Three different fungi strains were used as test subjects, and volatile components from the essential oils of *cucurbit* seeds were discovered to be important in regulating the fungus' growth. Findings of Amin et al. (2018) were also in line, reporting high antimicrobial activities of muskmelon seed extracts. Studies of Lopusiewicz, (2018), also provided scientific evidences for strong antimicrobial potential of watermelon seeds.

Extracts of different plant-based household wastes, including those of melon, pumpkin, squash and watermelon, have potential to counter the growth of a range microbes, possibly due to presence of antimicrobial substances in their wastes (Barbero-Lopez, 2020). Experiment by Neglo et al. (2021) provided scientific results regarding peel, skin, rind and seeds of watermelon extracts for antibacterial activities, and zone of inhibitions against all bacterial species were prominent from seed extracts as compared to peel and pulp extracts. Further phytochemical analysis of watermelon seed extracts revealed the presence of phenolics, flavonoids, alkaloids, saponins, and tannins. Sovljanski et al. (2022) investigated the nutritional, antimicrobial, and phytochemical properties of five samples of peel, pulp, and seed extracts using spectrophotometric methods of *in vitro* antioxidant potential as well as in vitro antimicrobial testing methods.

Table 3. Antimicrobial activities of ethanolic extracts of muskmelon and watermelon seeds

Melon seeds extracts	Antimicrobial activities (Zone of inhibition mm)					
	Bacterial strains			Fungal strains		
	Bacillus cereus	*Escherichia coli*	*Streptococcus aureus*	*Candida albicans*	*Aspergillus niger*	*Mucor meihi*
Watermelon seed extract	15.20±0.1[b]	14.30±0.1[b]	17.40±0.2[b]	12.10±0.1[b]	14.20±0.2b	12.30±0.3[b]
Muskmelon seed extract	11.20±0.3[c]	12.10±0.2[c]	15.10±0.3[c]	10.10±0.2[c]	13.70±0.3c	11.20±0.2[c]
Ampicillin (Reference)	23.50±0.5[a]	21.30±0.4[a]	20.60±0.3[a]	16.20±0.10[a]	15.30±0.3a	17.50±0.2[a]

Values in a row or column with similar alphabetic letter are statistically non-significant, whereas with different alphabetic letters are significant ($P< 0.05$)

They came to the conclusion that the phytochemicals present in seed extracts, which affect antioxidant and antibacterial activity, are the most promising.

The researchers have received a variety of broad spectrum anti-microbial components from *Cucurbitaceae* seeds. Numerous bacterial and fungal species are inhibited by the oil from *Cucurbitaceae* seeds (Hammer et al., 1999). According to Caili et al. (2006), the antimicrobial qualities of melon seed extracts are correlated with antibacterial proteins, phenolic chemicals, and organic acids.

Conclusion

Muskmelon and watermelon are both valuable fruits contributing towards the healthy food with adequate nutritional contents. However, seeds of these fruits, which are considered as waste, have also strong potential of utilization as bioactive source. Current study provided scientific evidence of presence of useful bioactive components in muskmelon and watermelon seeds, and watermelon seeds ethanolic extracts presented significantly high amounts of TPC, TFC and TC, due to which antioxidant and antimicrobial activities of watermelon seed extracts were also significantly higher than those of watermelon seed extracts. Therefore, the use of these seeds for various food and pharmaceutical purpose could prove the useful valorization of these seeds.

Recommendations

It should be emphasized that the seeds of various melon fruits can be taken into consideration as a possible source of diverse antioxidant components, which are not currently utilized but may find use in many industrial fields. Fruit seeds are frequently waste products, thus using them again as antimicrobial and antioxidant source could result in quantifiable economic gains and help reduce environmental pollution caused by the fruit and vegetable sectors. Further *in vivo* and *in vitro* trials could be conducted to investigate the biological activities of extracts of melon seeds to correlate them with the possible medicinal activities.

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