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Estimation of nutritional, phytochemical and antioxidant activity of seeds of musk melon (*Cucumis melo*) and water melon (*Citrullus lanatus*) and nutritional analysis of their respective oils

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Abstract
The dried seeds of musk melon (*Cucumis melo*) and water melon (*Citrullus lanatus)* were analyzed for different characteristics such as nutritional, phytochemical and antioxidant activity to understand its potential benefits. The nutritional analysis shows that the seeds are a good source of energy, carbohydrates, fat, proteins and minerals such as calcium, iron, magnesium, phosphorus and potassium. Phytochemical analysis reveals that the seeds are a good source of flavonoids, phenolics, saponins, alkaloids and other secondary metabolites.
The oil of seeds was extracted by cold pressing and analyzed for various parameters such as iodine value, saponification value and fatty acid profile. The results revealed the high iodine value and presence of polyunsaturated fatty acids such as omega- 6 (linoleic acid), monounsaturated fatty acids such as omega-9 (oleic acid). It also consists of saturated fatty acids such as palmitic acid and stearic acid. The seeds provide opportunities to develop as value added products, dietary supplements and medicines.

Keywords*:* Musk Melon seeds, Water melon seeds, Nutritional Analysis, Phytochemical Analysis, Antioxidant Activity, Iodine Value, Saponification Value, Fatty Acids

1. Introduction
Fruits and vegetables are soft, fleshy, edible plant products and, because of their high moisture content, are relatively perishable in the freshly harvested state Desai BB *et al.* [1]. The term "vegetable" refers to all the soft, edible plant products that are usually eaten with meat, fish, or other savoury dish, either fresh or cooked Desai BB *et al.* [1]
Fruits and vegetables are of particular interest for their content in phytochemicals, antioxidants, vitamins, minerals and dietary fiber. All these substances are related to lower the risk for the development of health problems such as certain types of cancer, cardiovascular diseases, type 2 diabetes, obesity and constipation Aranceta J. [2]. Low intake of fruits and vegetables can lead to increased risk of lung cancer and nutritional deficiencies.
A **melon** belongs to the family Cucurbitaceae with an edible fruit. Melons have their origin in Africa and southwest Asia, but they later started appearing in Europe at the end of the Roman Empire. Melons are a nourishing food. Its seeds are used to treat tuberculosis. They have high levels of potassium. Melons are considered diuretics due to their high water content. It has been researched that melons possess the ability to lower the risk of cancer. They contain lycopene, an antioxidant found in some fruits and vegetables.
The **Muskmelon** (*Cucumis melo*) is a species of melon, including varieties such as Crenshaw, Casaba and Honeydew which have smooth skin, and Cantaloupe, Santa Claus or Christmas Melon and Persian melon which have netted skin. Cantaloupe melons are an excellent source of Vitamin A, Vitamin C, Potassium and Magnesium. It has been shown to possess useful medicinal properties such as analgesic, anti-inflammatory, anti-oxidant, anti-ulcer, anti-cancer, anti-microbial, diuretic, anti-diabetic, and anti-fertility activity Parle M *et al* [3].
The **Watermelon** (*Citrullus lanatus)* is a member of the family Cucurbitaceae. It is a vine-like flowering plant. Its fruit is also called watermelon. It is referred as a pepo by botanists, which is a berry having a thick rind (exocarp) and fleshy center (mesocarp and endocarp). Watermelons are a good source of Vitamin C. These have also been investigated for their potential as significant diuretic agents Gul S *et al.* [4]. A recent study has concluded that *Citrullus lanatus* seed extracts possess antioxidant activity and the potency of antioxidant activity depends on the type of extract. The n-hexane extract of *Citrullus lanatus* seeds possessed the highest antioxidant activity Rahman H *et al.* [5].

2. Materials and Methods

2.1 Collection of Samples

The Musk melon and the Water Melon seeds were collected from a local market of Chandni Chowk, New Delhi, India.
The seeds were ground and then stored in an airtight container at room temperature to prevent any gain in moisture. Some portion of the seeds was used to extract oil. The extract of the seeds was prepared using methanol as a solvent.

2.2 Reagents and Chemicals

All analytical grade solvents, chemicals and acids used in the present study were obtained from different sources. The different reagents and chemicals used were: Aluminum Chloride (Fisher), Acetone, Ascorbic Acid (SRL), Dimethyl Sulfoxide (SRL), Ethanol, Ethyl acetate, Folin-Ciocalteu's Phenol reagent (SRL), Ferrous Chloride (Thomas Baker), Gallic acid (HiMedia), ICP Multielement Standard (Qualigens), Methanol (Thomas Baker), Petroleum Ether (LobaChemie), Sodium Hydroxide (SRL), TPTZ (Fluka).

2.3 Extract preparation

Solvent extraction with methanol was performed. 50 grams of the powdered sample of musk melon seeds were weighed and soaked in 35 ml of the solvent. Then, the mixture was incubated in an incubator shaker at 40 °C with 140 rpm for 48 hours. The mixture was filtered through a whatman filter paper 1 and the filtrate obtained was evaporated, concentrated at room temperature and stored at 4 °C. The extract obtained after double extraction with solvent was dark yellowish brown in color.

2.4 Nutritional analysis

The moisture and the total ash contents were evaluated using gravimetrical methods at 105 °C and at ≤ 525 °C by AOAC method Ref. 942.05 respectively. The total nitrogen content was estimated using a Kjeldahl method Ref. 976.05 Arlington VA [6]. The crude fat content was examined by extracting the sample in petroleum ether and the total carbohydrate content was also measured Arlington VA [6]. A gravimetric method was used for estimation of the dietary fiber content after the enzymatic digestion of starch and protein in fat and moisture free sample Arlington VA [6].
Minerals, trace elements and heavy metals in the examined material were determined by using Optima 2100 DV ICP-OES (Perkin-Elmer, USA), as per Ref 956.52 (AOAC, 2005).

2.5 Phytochemical analysis

2.5.1 Total phenolics

The total phenolic content was measured using the Folin Ciocalteu reagent McDonald S *et al.* [7]. An aliquot of the extract (100 µl) was mixed with 250 µl of Folin Ciocalteu's reagent and incubated at room temperature for 5 minutes. 1.5 ml of 20 % sodium bicarbonate was added to the mixture and incubated again at room temperature for two hours. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The results were expressed in terms of µg gallic acid equivalents (GAE)/ mg dry extract Soni N *et al.* [10].

2.5.2 Total flavonoids

The total flavonoids were measured using the Aluminium Chloride colorimetric method Chang C *et al.* [8] and the results were expressed in terms of µg catechin equivalents (CE)/g of dry extract. Catechin was used as a standard. The sample extract (250 µl) was added to 4.5 ml distilled water, followed by 5 % $NaNO_2$ (0.03 ml). After an incubation of 5 minutes, $AlCl_3$ (0.03 ml, 10 %) was added at 25 °C. At the sixth minute, the reaction mixture was treated with 2 ml of 1M NaOH. The reaction mixture was then diluted to 10 ml using distilled water and absorbance was measured at 510 nm.

2.5.3 Saponins

The saponins content was estimated as per the method described by Obadoni *et al.* [9]. Five grams of the powdered sample were mixed with 50 ml of 20% aqueous ethanol and heated over a hot water bath with continuous stirring for 4 hours at 55 °C. The mixture was then filtered and the residue was re-extracted with 50 ml of 20% ethanol. The combined extracts were reduced to a final volume of 10 ml over a water bath at 90 °C. The concentrate was transferred into a separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered. 15 ml of n-butanol was then added and the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated over the water bath. The samples were dried in an oven to a constant weight and the saponins content was calculated as percentage Soni N *et al* [10].

2.5.4 Crude alkaloids determination

The crude alkaloids content was estimated gravimetrically Herborne JB. [11]. 2.5 grams of the sample were taken and 100 ml of 10% acetic acid in ethanol was added to it. It was then incubated for four hours at room temperature. The solution was filtered and concentrated up to one-fourth of the original volume using a water bath. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The solution was allowed to settle and the precipitates collected were washed using dilute ammonium hydroxide and filtered. The crude alkaloid was weighed and calculated.

2.6 Antioxidant activity

The antioxidant potential of the samples was measured by evaluating their ability to reduce compounds by donating electrons using the FRAP assay Soni N *et al* [10].

2.6.1 Ferric reducing antioxidant power (FRAP)

The assay was based upon the methodology of Benzie and Strain [12]. The FRAP reagent consisted of 10 mM TPTZ in 40 mM HCl, 250 mM sodium acetate buffer (pH 3.6) and 20 mM $FeCl_3$. The reagent was freshly prepared by mixing TPTZ solution, $FeCl_3$ solution and acetate buffer in a ratio of 1:1:10. An extract solution (100 µl) was mixed with 900 µl of FRAP reagent. The mixture was incubated at 37 °C for 4 minutes and the absorbance was estimated at 593 nm. BHT was used as a standard. The results were expressed as µg BHT equivalent/mg sample.

2.7 Other secondary metabolites

An Agilent 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050). Volatiles and semi volatiles were screened using the automatic RTL screener software in combination with the Agilent NIST'05 library Soni N *et al* [10].

3. Results and Discussions

3.1 Nutritional Analysis of Musk Melon Seeds and Water Melon Seeds

The analysis of musk melon seeds and water melon seeds has shown their nutritional richness (Table 1). It has revealed that the musk melon and the water melon seeds are rich in protein content (32.80 % and 34.22 %, respectively) and fat content corresponds to 37.167 % and 31.999 % respectively. Proteins are essential nutrients for the body. Carbohydrates are generally available as an immediate energy source. The carbohydrate content of musk melon seeds and watermelon seeds was found to be 22.874 % and 26.57 %, respectively, and thus they have high energy value (557.199 Kcal/ 100g and 531.151 Kcal/ 100g respectively).

Dietary Fiber content in musk melon seeds and watermelon seeds (0.2 % and 0.1 % respectively) was low. Dietary Fiber aids in a healthy digestion. Musk melon seeds were found to contain moisture (2.358 %) and high ash content (4.801 %). Water melon seeds contain 3.575 % moisture and ash content of 3.636 %. The moisture content of the seeds should be low to ensure a long shelf life. The moisture content also affects the sensory qualities of the seeds. Moisture content more than 5 % enhances microbial spoilage and decrease the overall quality of the seeds. Ash is composed of the inorganic matter such as minerals. It includes iron, copper, calcium, magnesium, potassium, sodium, zinc etc.

Table 1: Nutritional profile of Musk melon and Water melon seeds

Sample	Energy (Kcal/100g)	Total Carbohydrate%	Protein%	Fat%	Dietary Fiber%	Moisture%	Ash%
Musk Melon seeds	557.199	22.874	32.80	37.167	0.2	2.358	4.801
Water Melon seeds	531.151	26.57	34.22	31.999	0.1	3.575	3.636

Mineral content was also analyzed using ICP-OES. The mineral analysis of musk melon seeds shows that they are rich in Calcium (2477 ppm), Magnesium (4496 ppm), Phosphorus (9249 ppm), Potassium (7599 ppm) and low in Iron (164 ppm), Sodium (74 ppm) and Zinc (75 ppm).

The mineral analysis of water melon seeds reveals that they are rich in Calcium (444 ppm), Potassium (6520 ppm), Magnesium (3090 ppm) and Phosphorus (6630 ppm) (Table 2).

Minerals offer a wide range of health benefits to the human body. Minerals such as calcium, phosphorus and magnesium provide structures for our bones. Sodium and potassium help in the maintenance of normal blood pressure. Iron is a part of haemoglobin and myoglobin. Copper, zinc and manganese play an important role in the breakdown of carbohydrates, fats and proteins into digestible forms and convert them into energy. Trace elements like copper (Cu), manganese (Mn) and zinc (Zn), are essential in bone metabolism as cofactors for specific enzymes Saltman PD *et al.* [13].

Table 2: Mineral content of Musk Melon and Water melon Seeds

S. No.	Analyte	Sample concentration unit (ppm) in Musk Melon seeds	Sample concentration unit (ppm) in Water Melon seeds
1	Ca	2477	444
2	Cd	0.2	Not detected
3	Zn	75	37
4	Cu	22.5	16.1
5	Fe	164	90
6	K	7599	6520
7	Mg	4496	3090
8	Mn	34.2	26.2
9	Na	74	39
10	Ni	1.2	3.6
11	P	9249	6630
12	Pb	0.9	Not detected
13	As	0.002	Not detected
14	Hg	0.002	Not detected

3.2 Phytochemical Analysis of Musk Melon Seeds and Water melon Seeds

The phytochemical analysis revealed the presence of phenolics, flavonoids, alkaloids and saponins in Musk melon and Water melon seeds (Table 3).

Saponins possess detergent properties since they contain both water-soluble and fat-soluble components. Because of their surfactant properties, they are useful industrially in preparation of emulsions for photographic films, and extensively in cosmetics, such as lipsticks and shampoos. The blood cholesterol-lowering properties of dietary saponins are of particular interest in human nutrition but sometimes saponins can irritate the immune system.

Phenols, also known as phenolics, are one of the main secondary metabolites present in the plant kingdom. They are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. They are essential for the plant growth and reproduction, and are produced as a response for defending injured plant against pathogens Saltman PD *et al.* [13].

Flavonoids are the most common group of polyphenolic compounds that are found ubiquitously in plants. They are important in the plants for normal growth, development and defense against infection and injury. These plant secondary metabolites also show anti-allergic, anti-inflammatory, anti-microbial and anticancer activities Khatiwora E *et al.* [14]. Flavonoids are a class of secondary plant metabolites with significant antioxidant and chelating properties Heim K *et al.* [15].

Table 3: Phytochemicals present in Musk melon and Water melon seeds

Analyte	Content in Musk Melon seeds	Content in Water Melon seeds
Total Phenolics (µg GAE/ mg sample)	2.415	4.222
Crude Alkaloid Content (%)	8.63	15.688
Saponins Content (%)	3.201	0.041%
Total Flavonoids (µg CE/ mg sample)	401.288	3.066

3.3 GC-MS Analysis of other secondary metabolites

Some other secondary metabolites present in the extract were analyzed through GC-MS. The Musk melon seeds were observed to be rich in Hexadecanoic Acid, Heptadecanoic

Acid, Octadecanoic Acid, Oleic Acid, Pentadecanoic Acid, Squalene, Tetradecanoic Acid, γ - Tocopherol and β - Tocopherol (Table 4) while Water melon seeds were found to be rich in γ - Sitosterol, β - Sitosterol, Vitamin E and Lupeol (Table 5). γ - tocopherol has been found to reduce inflammation and regulate factors that guard against certain cancers. Oleic acid found in the seeds is used as an emollient.

Table 4: Other secondary metabolites in musk melon seeds

S. No.	Compound Detected	CAS No.	Retention Time	% Area
1	Pentadecanoic Acid	001002-84-2	19.181	0.06
2	Hexadecanoic Acid, methyl ester	000112-39-0	19.854	1.91
3	Hexadecanoic Acid, Z-11	002416-20-8	20.033	0.21
4	9- Hexadecanoic Acid	002091-29-4	20.033	0.21
5	n- Hexadecanoic Acid	000057-10-3	20.325	13.03
6	Octadecanoic Acid	000057-11-4	20.516	0.46
7	Hexadecanoic Acid, ethyl ester	000628-97-7	20.516	0.46
8	Heptadecanoic Acid	000506-12-7	21.166	0.09
9	9, 12- Octadecadienoic Acid (Z, Z) - methyl ester	000112-63-0	21.514	8.08
10	10, 13- Octadecadienoic Acid, methyl ester	056554-62-2	21.514	8.08
11	Octadecanoic Acid, methyl ester	000112-61-8	21.772	1.15
12	9, 12- Octadecadienoic Acid (Z, Z)	000060-33-3	22.063	42.01
13	9, 17- Octadecadienal, (Z)	056554-35-9	22.063	42.01
14	Octadecanoic Acid	000057-11-4	22.220	7.07
15	9, 12- Octadecadienoic Acid (Z, Z)	000060-33-3	22.355	1.30
16	9, 17- Octadecadienal, (Z)	056554-35-9	22.355	1.30
17	9, 12- Octadecadienoic Acid (Z, Z)	000060-33-3	22.669	0.34
18	2- Chloroethyl linoleate	025525-76-2	22.669	0.34
19	1, 13- Tetradecadiene	021964-49-8	22.669	0.34
20	E, Z-1, 3, 12-Nonadecatriene	1000131-11-3	22.792	0.31
21	E, E-10, 12-Hexadecadien- 1-ol acetat	1000130-87-6	23.106	0.32
22	2-Methyl-Z, Z-3, 13-octadecadienol	1000130-90-5	23.263	0.27
23	Bicyclo [10.1.0] tridec- 1-ene	054766-91-5	23.476	0.18
24	1,2-15,16 Diepoxyhexadecane	1000192-65-0	23.555	0.43
25	Oleic Acid	000112-80-1	23.813	0.41
26	E, Z-1, 3, 12-Nonadecatriene	1000131-11-3	24.340	0.07
27	Hexadecanoic Acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester	023470-00-0	25.035	2.23
28	2- Chloroethyl linoleate	025525-76-2	26.448	5.15
29	Tetracosanoic Acid, methyl ester	002442-49-1	26.684	0.60
30	Squalene	007683-64-9	27.604	0.21
31	γ - Tocopherol	007616-22-0	30.307	0.11
32	β - Tocopherol	000148-03-8	30.307	0.11

Table 5: Other secondary metabolites in water melon seeds

S.No.	Compound Detected	CAS No.	Retention Time	% Area
1	Pentadecanoic Acid, 14-methyl-, methyl ester	005129-60-2	19.854	1.56
2	Oxacyclohexadecan- 2-one, 16- methyl	004459-57-8	20.011	3.70
3	Cyclodecasiloxane, Eicosamethyl	018772-36-6	20.370	0.82
4	9, 12- Octadecadienoic acid (Z,Z)- , methyl ester	000112-63-0	21.503	4.60
5	Hexadecanoic Acid, methyl ester	000112-39-0	19.854	1.56
6	Hexadecanoic Acid, Z-11	002416-20-8	20.011	3.70
7	Z-7- Hexadecenoic Acid	1000130-90-8	20.011	3.70
8	n- Hexadecanoic Acid	000057-10-3	20.246	13.60
9	Octadecanoic Acid	000057-11-4	22.119	6.41
10	9-Octadecenoic Acid (Z)-, methyl ester	000112-62-9	21.547	1.35
11	8-Octadecenoic Acid, methyl ester	002345-29-1	21.547	1.35
12	8-Octadecenoic Acid, methyl ester, (E)	026528-50-7	21.547	1.35
13	Octadecanoic Acid, methyl ester	000112-61-8	21.772	1.04
14	Cis-7-Dodecen-1-yl acetate	014959-86-5	21.929	32.88
15	Cyclohexene, 4-pentyl-1- (4-propylcyclohexyl)	108067-17-0	22.288	1.07
16	Nonivamide	002444-46-4	22.445	8.41
17	γ - Sitosterol	000083-47-6	22.635	4.19
18	β -Sitosterol	000083-46-5	22.635	4.19
19	Cyclononasiloxane, octadecamethyl	000556-71-8	23.095	0.62
20	Lupeol	000545-47-1	25.888	1.49
21	Vitamin E	000059-02-9	30.407	0.46

3.4 Antioxidant activity

In the FRAP assay, ferric-tripyridyltriazine gets reduced to the ferrous complex, which forms an intense blue colour measured at a wavelength of 593 nm. The intensity of the color amounts to the antioxidants in the sample. FRAP activity was found to be moderate in musk melon seed extract and water melon seed extract (5.63 µg BE/ mg sample and 1.23 µg BE/ mg sample).

3.5 Nutritional analysis of musk melon seed oil and watermelon seed oil

The musk melon seed oil and water melon seed oil was analysed for various parameters such as fatty acid profile, iodine value and saponification value. (Table 6)

Table 6: Nutritional analysis of musk melon seed oil and water melon seed oil (Cold Pressed Oil)

Analyte	Contents of musk melon seeds oil	Contents of water melon seeds oil
Iodine value	115.11	117.13
Stearic acid % (C_{18}: 0)	9.564	9.151
Palmitic acid % (C_{16}: 0)	13.75	11.966
Oleic acid % (C_{18}: 1)	15.307	15.457
Linoleic acid % (C_{18}: 2)	61.378	63.426
Saponification value	197.17	196.95

4. Conclusion

The analysis of Musk melon and Water melon seeds for the nutritional composition has shown that they are potential sources of energy, protein, fat and carbohydrates. They were observed to be very good sources of Calcium, Magnesium, Phosphorus and Potassium. The musk melon and water melon seeds are rich sources of valuable bioactive compounds and can be explored as nutraceuticals. The seeds provide opportunities to develop as medicines, cosmetics, value added products and dietary supplements. The Musk melon seed oil and Water melon seed oil were found to be rich in various unsaturated fatty acids which possess potential health benefits.

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