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# Antioxidant Activity, Antibacterial Screening, Proximate Composition and GC-Mass Spectrometry Analysis of Cantaloupe Seeds

**Ola A. Wahdan ; Neamat I. Bassuony ; Zeinab M. Abd El-Ghany and Ghadir A. El-Chaghaby**
**Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt**



## ABSTRACT

Cantaloupe refers to the *Cucumis melo* species in the Cucurbitaceae family. Seeds are solid by-product generated in large quantity. Regulation of agricultural by-products as a source of bioactive compounds could minimize environmental hazard. For that reason, cantaloupe seeds were evaluated for its antioxidant and antibacterial activities. The current study included the proximate analysis of the seeds that had protein content 20.8%, crude fiber 33.1%, moisture 8% and fat 24.6%. Characterization of bioactive constituents by GC-MS analysis revealed the existence of methionine (60.17%), 4-aminoheptanedioic acid (4.75%), 9-cis-retinoic acid (34.12%) and stearic acid allyl ester (0.96%). Data revealed that total phenolic and flavonoid contents of cantaloupe seeds had range of 50.5 mg GAE/100 g dry weight and 6.43 mg QE/100 g dry weight, respectively. Total antioxidant activity valued 272.6 mg AAE/100 g dry weight. Ferric reducing antioxidant power increased relative to increase the extract concentration which indicated high reducing ability of cantaloupe seeds. Ethanolic extract of cantaloupe seeds had mild inhibition activity against *E. coli* and *Salmonella sp.*, while *Staphylococcus aureus* was resistant to the ethanolic extract. Aqueous extract did not show any antagonistic effect against the three pathogenic bacterial strains. Inclusion of phytochemical bioactive compounds and methionine as sulphur-containing amino acid in cantaloupe seeds may contribute to the apparent antibacterial activity against Gram-negative bacteria. Results ensured that cantaloupe seeds possessed nutritional, antibacterial and antioxidant properties.

**Keywords:** Cantaloupe seeds, proximate analysis, total phenol content, flavonoids, total antioxidant activity, ferric reducing antioxidant power (FRAP), antibacterial activity, GC-MS technique.

## INTRODUCTION

Cantaloupes, commonly known as muskmelons, mush melons, rock melons and persian melons, are members of the botanical family Cucurbitaceae. Egypt is one of the top producers of cantaloupe with production of 1 to 1.7 million tons per year (FAO STAT, 2013). Seeds with high potential antioxidant activity could be utilized for commercial purposes. Plants are regarded as the natural pharmaceutical factories for drug synthesis (Jerlin *et al*., 2014).

Investigation of plants´ structural composition and activity is important to validate their therapeutic uses (Nair and Chanda, 2006). *In-vitro* characterization method provided the needed preliminary information of plant constituents that could be directed for subsequent pharmacological studies (Mathekaga and Meyer, 1998).

Plant-derived products contain diverse phytochemicals that possess antibacterial, anticarcinogenic and vasodilatory activities (Bidlack *et al*., 2000).

Neamat (2015) postulated that ethanolic extracts of the peels of banana and lemon seeds had an inhibitory effect against Gram-positive and Gram-negative bacterial strains.

Siddhuraju and Becker (2007) established that polypenolic constituents of legume seeds had potential antioxidant medicinal properties.

The medical potency of plants was correlated to the antioxidants, phenolic compounds and free radical quenchers' property (Ademiluyi and Oboh, 2008). Many plant constituents were effective as remedy of several diseases in western pharmacopoeia, especially taxol and artemisinin (Aderogba *et al*., 2004).

The present work tends to identify the antioxidant and antibacterial properties of cantaloupe seeds, as well as the proximate analysis of the seeds will be involved with GC-MS identification of the bioactive phytochemicals in an attempt to introduce cantaloupe seeds as a prime natural source. This trial points to proper handling of fruit by-products for use in the food industry and to get rid of waste in a good manner for eliminating ecological pollution.

## MATERIALS AND METHODS

**Chemicals**

Gallic acid, Folin-Ciocalteu and ascorbic acid were supplied from Sigma Aldrich (St. Louis, MO, USA). All reagents and solvents were of analytical grade.

**Plant material**

Cantaloupe seeds were assembled from restaurants in Cairo and Giza governorates during the year 2014. The collected seeds were washed, air dried for three days and pulverized into fine powder.

**Proximate analysis**

Moisture, crude protein, fat and crude fiber were determined according to the methods described by AOAC (2012). All tests were done in duplicate and averaged.

**Bacterial species**

Three pathogenic bacterial strains were chosen namely: *Escherichia coli*, *Salmonella sp*. and *Staphylococcus aureus*. The bacteria were developed in peptone water (buffered, pH 7.0) and incubated at 37°C for 24 h to obtain viable cell count of $1\times 10^8$ cfu/ml (Rene, 2003).

**Preparation of ethanolic extract**

The powdered cantaloupe seeds were soaked in ethanol 90%, stirred for four hrs. The extract was subjected to filtration and the solvent was then evaporated. The remainders were then gathered and kept refrigerated at 4 °C until used.

**Preparation of aqueous extract**

Aqueous extract was carried out by decoction procedure described by Johnson *et al*. (2011). Twenty five gram of the dried powder was mixed with 125 ml of hot distilled water and boiled for 15 min. The extract was filtered and stored at 4˚C in a clean sterilized container till used. Author

**Antibacterial activity of ethanolic and aqueous extracts**

The antibacterial activities of the ethanolic and aqueous extracts as well as negative control ethanol (90%) were assessed using the agar well diffusion method (William, 1989). The test plates were prepared by using Mueller-Hinton agar media. The plates were seeded with 100 µl of the microbial suspension ($10^8$ cfu/ml). Wells were prepared in the plates, and then fifty to five hundred micro liters of the each extract were placed in the agar holes inoculated with the tested bacteria. At the end of incubation period (48 h at 37°C) the diameters of the zones of inhibition (mm) were measured (Masih *et al*., 2012 and Liviu *et al*., 2010). All results were determined in triplicate and the average values were calculated.

**Gas chromatography analysis (GC/MS)**

The chemical constituents of cantaloupe seeds were identified using GC (Agilent Tecnologies 7890A) connected to a mass-selective detector (MSD, Agilent 7000). The flow of helium used as carrier gas was retained at 1 ml/min during the run (Patricia *et al*., 2013). The components were confirmed by coordinating their mass spectra and retention time with the database of National Institute of Standard and Technology (NIST) library. The names, molecular weights and chemical structure of each of the components of the test materials were determined.

**Evaluation of total antioxidant capacity (TAC)**

The antioxidant activity of the extract was evaluated using the phosphomolebdenum assay described by Prieto *et al* (1999). The assay is based on the reduction of Mo $^{(VI)}$ to Mo $^{(V)}$ by the sample analyte. In the assay 1 ml of sample solution was added to 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. After cooling, the absorbance was measured at 695 nm. Total antioxidant activity was expressed as mg AAE/100g dry weight. Values were presented as mean of triplicate measurements.

**Determination of total flavonoids (TF)**

Total flavonoids content was determined as described by the method of Willet (2002). Aqueous ethanolic extract (0.5 ml), 10% aluminium chloride (0.1 ml), 1 M potassium acetate (0.1 ml), and distilled water (4.3 ml) were mixed. After incubation at room temperature for 30 min., the absorbance was measured at 415 nm using Spectro D 250 plus Analytik Jena AG, Germany.

Total flavonoids content was carried out in triplicate and results were reported as mg QE/100g dry weight.

**Determination of total phenol content (TPC)**

The concentration of phenolics in cantaloupe seeds was determined using spectrophotometric method described by Singleton *et al*. (1999). Ethanolic extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of ethanolic seeds extract; 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$, incubated at 45°C for 45 min. The absorbance was measured using spectrophotometer at 765 nm. Total phenol content was expressed as mg GAE/100g dry weight.

**Ferric reducing antioxidant power assay (FRAP)**

The reducing power of cantaloupe seeds was determined according to the method of Oyaizu (1986). In this procedure 0.5 ml of crude plant extract and isolated compounds were added to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min. 2.5 ml of trichloroacetic acid (10%) was added to the mixture, centrifuged at 1000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of freshly prepared $FeCl_3$ solution (0.1%). The absorbance was measured at 700 nm. The increase in absorbance of the reaction mixture indicated increased reducing power.

## RESULTS

Proximate analysis and quantitative phytochemical evaluation of cantaloupe seeds are presented in Tables (1 and 2). Data showed that seeds can be regarded as a good source of protein, as well as being natural antioxidant.

**Table 1. Proximate analysis of cantaloupe seeds**

| Parameters | Protein (%) | Moisture (%) | Fat (%) | Fiber (%) |
|---|---|---|---|---|
| Cantaloupe seeds | 20.8 | 8.0 | 24.6 | 33.1 |

**Results were expressed as mean of duplicate determinations**

**Table 2. Total antioxidant activity (TAA), Total phenol (TPC) and Total flavonoid contents (TFC) of cantaloupe seeds**

| | TPC (mg GAE/100 g) | TFC (mg QE/100 g) | TAA (mg AAE/100 g) |
|---|---|---|---|
| Cantaloupe seeds | 50.5±1.59 | 6.43±0.02 | 272.6±12.51 |

**All parameters were determined in triplicate and represented as mean±SD**

Ferric reducing antioxidant power (FRAP) of the seeds is shown in Fig. (1), this assay measured the ability of antioxidants to reduce ferric iron.

**Fig. 1. Ferric reducing antioxidant power (FRAP) of cantaloupe seeds**

**Antibacterial activity of ethanolic and aqueous extracts**

Volumes of 50-500 µl of both extracts were used throughout the study. The application of 500 µl of ethanolic extract was found to give the best results and exhibited mild inhibitory action against Gram-negative bacteria. Whereas, the aqueous extract did not exhibit any activity against the tested bacterial strains. The antibacterial activity of ethanolic extract is shown in Table (3). The inhibition zone diameters of ethanolic extract were found to be of eight millimeters and nine millimeters when applied to the bacterial stains *E. coli* and *Salmonella sp.*, respectively. It is also worthy to note that *Staphylococcus aureus* was resistant to both extracts. On the other hand no inhibition effect was recorded by the ethanolic solution (90%) used as negative control.

**Table 3. Antibacterial activity of ethanolic extract of cantaloupe seeds against the pathogenic bacterial strains**

| Bacterial strain | Inhibition zone diameter (mm) | | |
|---|---|---|---|
| | *E. Coli* | *Salmonella sp.* | *Staphylococcus aureus* |
| Control (ethanol 90%) | 0 | 0 | 0 |
| Ethanolic extract | 8 | 9 | 0 |
| Aqueous extract | 0 | 0 | 0 |

**Gas chromatography analysis (GC/MS)**

GC-MS identification of the various compounds present in the seeds powder is illustrated in Fig. (2) and Table (4).

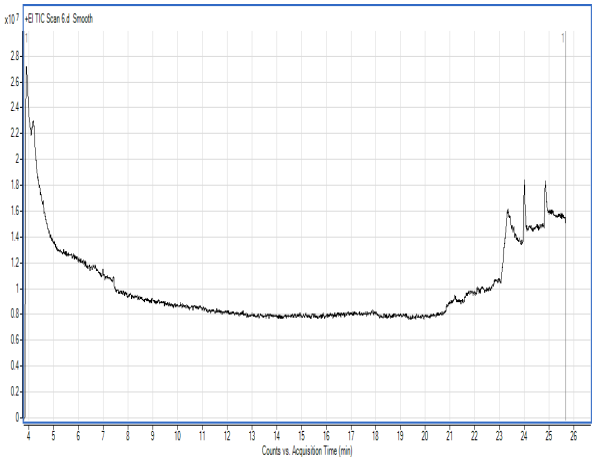
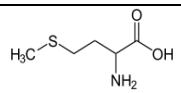
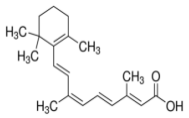


**Fig. 2. GC-MS chromatogram of dried cantaloupe seeds**

**Table 4. Bioactive compounds of cantaloupe seeds**

| Retention time (min) | Compound | Area (%) |
|---|---|---|
| 4.18 | Methionine | 60.17 |
| 22.6 | 4-Aminoheptanedioic acid | 4.75 |
| 23.06 | 9-cis-Retinoic acid | 34.12 |
| 23.6 | Stearic acid allyl ester | 0.96 |

GS-MS analysis ensured that methionine is the predominant compound (60.17%), followed by 9-cis-retinoic acid (34.12%).

| NAME | STRUCTURE | MOLECULAR FORMULA | MOLECULAR WEIGHT | CLASSIFICATION |
|---|---|---|---|---|
| Methionine |  | $C_5H_{11}NO_2S$ | 149.21 g/mol | Essential amino acid |
| 9-cis-Retinoic acid |  | $C_{20}H_{28}O_2$ | 300.442 g/mol | Metabolite of vitamin A |

## DISCUSSION

In the present study, cantaloupe seeds showed a remarkable antioxidant activity which is attributed to their content of phenolic compounds and flavonoids. Our findings are in accordance with Borneo *et al.* (2008) and Qader *et al.* (2011) who confirmed a direct relationship between phenolic content and antioxidant activity. Our results were also in agreement with Siddhuraju and Becker (2007) who mentioned that polyphenolic constituents of seeds had prospective therapeutic properties, including antioxidant activities.

Natural antioxidants acted as reducing agents that inactivated oxidants through redox-reaction (Siddhuraju and Becker 2007).

The ferric reducing antioxidant power (FRAP) of cantaloupe seeds increased by increasing the extract concentration indicating high reducing capability and antioxidant capacities, possibly due to accumulation of phytochemicals. Our results support the findings of Kunradi *et al.* (2009) who mentioned that fruit residues that possess high phenolic and antioxidant compounds can be considered as natural source of phytochemicals.

Parekh and Chanda (2007) reported that alkaloids, flavonoids, tannins, terpenes, amino acids, phenolic compounds, carboxylic acids and inorganic acids were the most important bioactive constituents of plants.

GS-MS analysis ensured that methionine and 9-cis-retinoic acid were the predominant compounds in cantaloupe seeds. Plant-based foods are good sources of methionine. Methionine is the only sulphur-containing essential amino acid, it plays an important role in the synthesis of proteins, and serves as powerful antioxidant (Finkelstein, 1990).

Retinoids refer to a class of chemicals that are structurally or functionally similar to retinol, or vitamin A (Tang and Gudas, 2011) that is derived only from food, and cannot be made in the body of any animal. Retinoids are involved in cellular growth, immune response, and epithelial growth (Mora *et al.*, 2008 and Pino-Lagos *et al.*, 2008) through the interaction with the nuclear receptors, retinoic acid receptor (RAR) and retinoid X receptor (RXR).

The type of extracting solvent plays an important role in the extraction of antioxidants from plant

material. In the present study, ethanol was selected as a solvent having good polarity to extract polar compounds such as phenolic and flavonoids compounds.

Ethanolic cantaloupe seeds extract exhibited mild inhibitory action against Gram-negative bacteria. This finding is strongly correlated with the findings of Xu *et al*. (2010) who stated that flavonoids possessed antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic activities. The antibacterial activity of cantaloupe seeds could also be related to the presence of the amino acid methionine. Our results are in accordance with Seokwon *et al*. (2006) who cited that sulfur-containing compounds had antibacterial and antifungal activity.

Vaara (1993) reported that Gram-negative bacteria are resistant to antibiotics and chemotherapeutic agents than are Gram-positive bacteria. Antibiotics of natural origin lacked activity against *Escherichia coli*, although they were active against Gram-positive bacteria. The outer membrane of Gram-negative bacteria contributes to this intrinsic resistance by limiting the penetration of hydrophilic solutes (Ple´siat and Nikaido, 1992). In contrast to the above-mentioned data, the current results highlighted the unusual beneficial effect of cantaloupe seeds ethanolic extract exerting activity against the resistant *Escherichia coli* bacteria.

As a result, cantaloupe seeds can be classified as a natural unique source of protein, packed with phenolic and flavonoids compounds possessing antioxidant and antibacterial properties.

## CONCLUSION

The present study was directed to identify the medicinal active components of cantaloupe seeds in order to introduce a natural product of high value. Seeds had antibacterial and antioxidant activities that could be exploited in food industries and cosmetics. Moreover, high protein content of cantaloupe seeds increased its benefit as a reverent added product. Further studies on a large scale are needed to explore the perks of cantaloupe seeds.

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## النشاط المضاد للاكسدة و للبكتيريا بالاضافة للتحليل الكيميائى و الكروماتوجرافي الغازى لبذور الكانتالوب

**علا علي وهدان ، نعمات ابراهيم بسيونى ، زينب محمد عبد الغنى و غدير علي الشغبى**
**المركز الاقليمى للاغذية و الاعلاف - مركز البحوث الزراعية - جيزة – مصر**

تحتوى المخلفات الزراعية على العديد من المركبات الفعالة التى يمكن توجيه استخدامها لانتاج منتجات عالية الجودة. تم اجراء التحليل الكيميائى لبذور الكانتالوب و كان محتوى البروتين ٢٠,٨٪ ، الالياف ٣٣,١٪ ، الرطوبة ٨٪ و الدهن ٢٤,٦٪. واكد تحليل كروماتوجرافيا الغاز وجود مركبات فعالة مثل: الميثيونين وهو حامض امينى اساسى (٦٠,١٧٪)، حامض الريتينويك (٣٤,١٢٪) الذى له نشاط مضاد للسرطان بالاضافة الى حامض ٤- امينو هيبتا نيدويك و اليل استر الاستيارك. و قد تم تقييم النشاط المضاد للاكسدة لبذور الكانتالوب و اظهرت النتائج ان المحتوى الكلى للفينولات و الفلافونويد ٥٠,٥ مجم حمض الجاليك مكافىء/١٠٠ جرام وزن جاف و٦,٤٣ مجم كويرستين مكافىء/١٠٠ جرام وزن جاف ، على التوالى. كانت قيمة النشاط الكلى المضاد للاكسدة ٢٧٢,٦ مجم حمض اسكوربيك مكافىء/١٠٠جرام وزن جاف. اظهر اختبار FRAP زيادة النشاط المضاد للاكسدة بزيادة تركيز مستخلص البذور فى اشارة الى قدرة بذور الكانتالوب كعامل مختزل. تم تحضير المستخلصات المائية و الايثانولية من بذور الكانتالوب و اختبارهما كمضادات للبكتيريا باستخدام ثلاث سلالات بكتيرية: اثنين سالبة لجرام و هما الايشريشيا كولاى و السالمونيلا، وواحدة موجبة لجرام هى ستافيلوكوكاس اورياس. كان للمستخلص الايثانولي نشاط مثبط للبكتيريا سالبة لجرام الايشريشيا كولاى و السالمونيلا ، فى حين ان البكتيريا تاموجبة لجرام (ستافيلوكوكاس اورياس) كانت مقاومة للمستخلص. لم يسجل المستخلص المائي اى نشاط مضاد للبكتيريا سواء السالبة لجرام و الموجبة لجرام. ظهر جليا" ان بذور الكانتالوب ذات محتوى بروتينى عالى كما انها مصدر لمركبات فعالة لها نشاط مضاد للاكسدة و مضاد لبعض انواع البكتيريا.