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Phytochemical Composition and Antioxidant Potential of *Citrullus lanatus* Fruit Juice for Goat Semen Preservation

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Abstract
Watermelon is a natural fruit containing various bioactive compounds, including lycopene, carotenoids, and vitamins, which may contribute to its antioxidant properties. This study assessed the phytochemical composition and antioxidant potential of *Citrullus lanatus* (watermelon) fruit juice as an external antioxidant source for preserving goat semen. The Sugar Baby variety was cultivated at the National Horticultural Research Institute, Ibadan, during the second cropping season (August–December). Fully ripened watermelons was harvested, washed, and the flesh was separated from the rind before juice extraction using a mechanical juice extractor. The extracted juice was then centrifuged at 3000 rpm, and the upper phase was used for further analysis. The study determined both qualitative and quantitative phytochemical components, including lycopene, free radical scavenging ability, minerals, and vitamins. The proximate composition of the watermelon flesh was analyzed using standard methods, revealing the following composition: moisture (93.50±1.00%), crude protein (7.35±0.35%), crude fiber (1.06±0.06%), ether extract (6.14±0.02%), ash (4.57±0.43%), and nitrogen-free extract (80.88±0.12%). The qualitative phytochemical screening confirmed the presence of phenolics, terpenoids, beta-carotenes, flavonoids, lycopene, and anthocyanins in the watermelon juice. The quantitative analysis showed the levels of terpenoids (93.33±2.36 mg/100g), beta-carotene (958.3±10.27 µg/100g), anthocyanins (26.66±6.24 mg/100g), and flavonoids (945±20.41 mg/100g). The mineral composition included iron (9.1±0.08 mg/100g), zinc (0.6±0.00 mg/100g), calcium (76.67±2.36 mg/100g), sodium (265.23±10.80 mg/100g), potassium (23.33±2.36 mg/100g), and phosphorus (88.33±6.23 mg/100g). The vitamin analysis revealed ascorbic acid (15.33±0.47 mg/100g), thiamine (0.066±0.00 mg/100g), and niacin (0.163±0.01 mg/100g). The study also recorded total phenolic content (24.43±0.17 GAE/g), lycopene concentration (53.33±2.36 mg/100g), and antioxidant activity (free radical scavenging ability) at 32.267±0.26%. These findings highlight that watermelon is a valuable source of lycopene, demonstrating notable antioxidant activity and containing significant amounts of phytochemicals, vitamins, and minerals. The high lycopene content in watermelon juice contributes to its effectiveness in neutralizing free radicals.

Keywords: Water Melon, Antioxidants, Phytochemicals, Goat Semen Preservation, Free Radical Scavenging Activity

Introduction
Free radicals are a type of reactive oxygen species (ROS), consisting of highly reactive oxygen-containing molecules such as hydroxyl radicals, superoxide anion radicals, and hydrogen peroxide (Kohen & Gati, 2000). In fresh goat semen, antioxidant enzymes and small-molecule antioxidants secreted by the reproductive system provide protection against ROS. However, when semen is diluted with an extender, these protective mechanisms weaken, leading to possible sperm quality deterioration due to lipid peroxidation caused by ROS during storage. Therefore, identifying factors that influence ROS levels in both fresh and extended buck semen is crucial. Oxidative stress has been recognized as a key factor contributing to fertility issues in animals (Makkar et al., 2009). When ROS levels exceed antioxidant defenses, sperm cells can suffer damage and structural abnormalities, ultimately leading to male infertility. ROS impair sperm function primarily by attacking lipids in

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the sperm membrane, initiating lipid peroxidation (LPO). Mammalian spermatozoa are particularly susceptible to external oxidative damage, resulting in a decline in motility due to lipid peroxidation (Agarwal et al., 2003).

The production of reactive oxygen species (ROS) is a natural physiological process that occurs during spermatogenesis, semen collection, and storage. While endogenous antioxidants typically regulate ROS levels, excess ROS can cause oxidative stress in seminal plasma, which is rich in polyunsaturated fatty acids. This oxidative stress compromises sperm integrity, ultimately leading to infertility. Research indicates that lipid peroxidation-induced damage to the sperm cell plasma membrane plays a significant role in male infertility (Khosrowbygi & Zarghami, 2007; Privadharshni et al., 2012). Consuming natural antioxidants from plant-based foods can help neutralize these harmful free radicals, promoting overall health (Krishnamoorthy et al., 2011).Watermelon is a rich source of phytochemicals, particularly lycopene, which is the primary carotenoid found in watermelon products. Lycopene is known for its strong antioxidant properties, particularly its ability to neutralize singlet oxygen more efficiently than other carotenoids (Di Mascio et al., 1989). Additionally, it plays a protective role by shielding blood lymphocytes from nitrogen dioxide (NO_2) radicals (Bohm, 1995). As a naturally occurring red pigment, lycopene is produced by plants and microorganisms (Agarwal & Rao, 2000) and is considered one of the most powerful antioxidants (Di Mascio et al., 1989; Miller et al., 1996). It exhibits a singlet oxygen-quenching ability that is ten times greater than α-tocopherol (Di Mascio et al., 1989). Research has shown that dietary supplementation with α-tocopherol, a key lipid-soluble antioxidant in cell membranes, can reduce lipid peroxidation while enhancing semen quality and fertility (Brezezinska-Slebozinska et al., 1995; Geva et al., 1996; Kessopoulou et al., 1995). Given these properties, this study aimed to evaluate the phytochemical composition and antioxidant capacity of *Citrullus lanatus* (watermelon) fruit juice as an external antioxidant source for preserving goat semen.

Materials and Methods

The cultivation of the Sugar Baby variety of watermelon was carried out in polythene bags in a nursery before transplanting the seedlings to the field after three weeks. It flourished in well-drained sandy loam soil. The soil preparation involved ploughing, harrowing, and bed formation. At the time of planting, NPK fertilizer (20:10:10) was mixed into the beds, and urea (46% nitrogen) was applied as a top dressing on the surface of the beds four weeks after transplanting. Weeding and pest and disease management were also carried out. Harvesting occurred between 85 to 100 days when the fruits were physiologically mature.

Extraction of Watermelon Juice and Preparation of Extender: Fresh, mature Sugar Baby watermelon fruits (*Citrullus lanatus*) were sourced from the demonstration farm of the National Horticultural Research Institute in Ibadan during the second cropping season (August to December). The flesh was separated from the rind, and the juice was extracted using the juice extractor method (Ambreen et al., 2013). The extracted juice was then spun at 3000 rpm to obtain the supernatant, which was utilized for further experiments. This watermelon juice (the supernatant) was mixed with normal saline diluent in various proportions, while a skimmed milk-glucose solution was prepared to serve as a negative control.

Proximate Analysis of Watermelon Flesh

Physicochemical Analysis: The watermelon flesh was examined for moisture content, crude protein, crude fat, crude fiber, ash, and nitrogen-free extract using duplicate samples.

Moisture Content: The moisture content of the watermelon was measured by oven-drying a weighed flesh sample at 105±5°C until a constant weight was attained, in accordance with the AOAC (2006) method.

Crude Protein: Crude protein levels were assessed using a Kjeltech apparatus, in accordance with AOAC (2006) guidelines.

Crude Fat: The determination of crude fat was conducted using a Soxhlet apparatus, as specified in AOAC (2006).

Crude Fiber: Crude fiber content was determined by digesting a fat-free sample for 30 minutes with a 1.25% H_2SO_4 solution, followed by digestion with a 1.25% NaOH solution, in accordance with AOAC (2006) protocols.

Ash: The ash content of the dried sample was measured by charring, followed by direct incineration at 550°C in a muffle furnace until a grayish-white residue was obtained, as outlined by AOAC (2006).

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Nitrogen-Free Extract (NFE): The NFE was calculated using the formula: NFE (%) = 100 - (CP + Crude Fat + Crude Fiber + Ash)%, where CP represents crude protein.

Determination of Vitamins:
Thiamine (Vitamin B1) Analysis
The method described in AOAC (2006) was followed for determining thiamine levels. One gram of watermelon juice was mixed with 50% methanol (50 mL) and 17% sodium carbonate (50 mL) in equal proportions. The mixture underwent filtration using Whatman No. 1 filter paper. Folin-Denis reagent was then introduced to the filtrate, and the solution was cooled until a blue tint developed. The absorbance was measured at 415 nm with a spectrophotometer.

Preparation of Standard Curve: Tannic acid was utilized to create a standard curve within a 0–10 ppm range, and its absorbance values were obtained spectrophotometrically for thiamine quantification.

Ascorbic Acid (Vitamin C) Estimation: Ascorbic acid is susceptible to temperature fluctuations and pH changes, particularly in alkaline settings. Due to its strong reducing power, it reacts with 2:6-dichlorophenolindophenol, a blue dye, leading to decolorization.

To prepare the standard solution, 1 mg of ascorbic acid was dissolved in 100 mL of 5% acetic acid. Separately, 0.1 g of 2:4-dichlorophenolindophenol was dissolved in distilled water, which had been previously boiled, and the volume was adjusted to 1 liter.

Procedure
A 1 g watermelon juice sample was mixed with glacial acetic acid and transferred into a 50 mL volumetric flask, which was then topped up with distilled water. The mixture was swiftly filtered, and 10 mL of the filtrate was placed in a conical flask. After adding one drop of diluted acetic acid, titration was performed using 2:6-dichlorophenolindophenol. The procedure was repeated with a 1 mg/100 mL standard solution of vitamin C, and the concentration per 100 g of watermelon was calculated accordingly.

Niacin (Vitamin B3) Quantification
The niacin content was extracted using calcium hydroxide and heat treatment and analyzed colorimetrically using cyanogen bromide.

A 1 g sample of watermelon juice was blended with 0.75 g of calcium hydroxide and 20 mL of deionized water inside a centrifuge tube. The mixture was autoclaved for 30 minutes at 121°C, ensuring that the tube caps were slightly loosened. After cooling, the solution was diluted to 50 mL, mixed, and centrifuged at 2500 rpm (0°C) for 15 minutes.A 15 mL portion of the supernatant was adjusted to pH 7 using aqueous oxalic acid (10%) and diluted to 25 mL with water in a vial. After a second centrifugation at 2500 rpm for 10 minutes, calcium oxalate precipitated. The estimation process included blank samples and 1–10 ppm standard niacin solutions. To each, 1 mL of cyanogen bromide was added, and absorbance was recorded at 650 nm. Niacin concentration was extrapolated using a standard graph.

Mineral Composition Assessment
Wet Digestion: Mineral content analysis followed the guidelines established by AOAC (2006).
Lycopene Measurement via Spectrophotometry

Lycopene levels were analyzed following the AOAC (2006) method. A 0.2 mL sample of watermelon juice was placed in a screw-cap tube, along with blank samples. To each, 8.0 mL of a solvent mixture (hexane: ethanol: acetone in a 2:1:1 ratio) was added. The tubes were tightly sealed, vortexed, and shielded from direct light. After 10 minutes, 1.0 mL of water was introduced, followed by another vortexing step. The samples were then allowed to separate for 10 minutes, removing air bubbles. A spectrophotometer was calibrated at 503 nm using the upper layer of a blank sample. The lycopene content was then calculated using the formula: Lycopene (mg/kg) = (A503 × 171.7) / W where W is the fruit weight in grams.

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Phytochemical Quantification

Terpenoids

The terpenoid content was evaluated following the Marcano and Hasenawa (1991) protocol. One gram of watermelon juice was combined with 10 mL of petroleum ether and left to extract for 15 minutes. The solution was filtered, and its absorbance was measured at 420 nm using a spectrophotometer.

Flavonoids

A 1 g sample was extracted using 10 mL of 80% methanol for 2 hours. The extract was filtered into a pre-weighed petri dish, oven-dried at 40°C, and reweighed after reaching a constant mass.

Total Flavonoid Content

The Harborne (1998) method was used to quantify flavonoids. A mixture containing 0.5 mL of 2% $AlCl_3$ in methanol and 0.05 mL of watermelon juice was prepared. After 1 hour at room temperature, absorbance was measured at 420 nm. A yellow color confirmed the presence of flavonoids, which were quantified as mg/g of quercetin based on a calibration curve.

Phenolic Compounds

Phenol estimation was performed following Harborne (1998). A 2 mL juice sample was mixed with 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 20% sodium carbonate solution. The solution was stirred for 15 seconds and incubated at 40°C for 30 minutes. Absorbance was recorded at 765 nm, with results expressed as Gallic Acid Equivalent (GAE/g).

Carotenoids

Carotenoid content was assessed based on the Harborne (1998) method. A 1 g juice sample was mixed with 20 mL of acetone and allowed to extract for 1 hour before filtration. 10 mL of water was added to the filtrate, which was then transferred to a separating funnel. After introducing 5 mL of petroleum ether, the lower layer was discarded. Absorbance was recorded at 440 nm, using a standard curve for quantification.

Free Radical Scavenging Potential

The DPPH assay, as outlined by Patel and Patel (2011), was employed to assess antioxidant activity. A 0.2 mL juice sample was combined with 2.8 mL of a freshly prepared 20 mg/dm³ DPPH solution in methanol and incubated for 20 minutes at room temperature. The absorbance reduction at 517 nm was measured, and the radical scavenging capacity (**RSC %) was computed using: RSC % = (1 - Asample/Ablank) × 100%,whereAblank is the control absorbance, and Asample is the juice sample absorbance

Results

The proximate composition and phytochemical profile of watermelon juice are presented in Table 1. The proximate analysis revealed that the moisture content was 93.50±1.00%, while the levels of crude protein, crude fiber, ether extract, ash, and nitrogen-free extract (NFE) were recorded as 7.35±0.35%, 1.06±0.06%, 6.14±0.02%, 4.57±0.43%, and 80.88±0.12%, respectively. The phytochemical screening confirmed the presence of phenolics, terpenoids, beta-carotenes, flavonoids, lycopene, and anthocyanins in the juice.

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Table 1. Proximate composition and qualitative phytochemical analysis of watermelon (*Citrullus lanatus*) flesh.

Parameters	Percentage (%)
Crude protein	7.35±0.35
Ash	4.57±0.43
Crude fibre	1.06±0.06
Ether extract	6.14±0.02
Moisture	93.50±1.00
Dry matter	6.50±0.20
Nitrogen free extract	80.88±0.12
Qualitative phytochemical analysis	
Parameters	Presence
Phenolic (GAE/g)	+ve
Terpenoids (mg/100g)	+ve
B-carotenes (ug/100g)	+ve
Anthocyanin (mg/100g)	+ve
Flavonoids (mg/100g)	+ve
Lycopene (mg/100g)	+ve

+ve: Positive

The quantitative analysis of phytochemicals in watermelon juice is shown in Table 2. The observed values for terpenoids, beta-carotene, anthocyanins, and flavonoids were 93.33±2.36 mg/100g, 958.3±10.27 µg/100g, 26.66±6.24 mg/100g, and 945±20.41 mg/100g, respectively. The levels of iron, zinc, calcium, sodium, potassium, and phosphorus were measured at 9.1±0.08 mg/100g, 0.6±0.00 mg/100g, 76.67±2.36 mg/100g, 265.23±10.80 mg/100g, 23.33±2.36 mg/100g, and 88.33±6.23 mg/100g, respectively. Additionally, the ascorbic acid, thiamine, and niacin content were found to be 15.33±0.47 mg/100g, 0.066±0.00 mg/100g, and 0.163±0.01 mg/100g, respectively. The total phenolic content was 24.43±0.17 GAE/g, while the lycopene level was 53.33±2.36 mg/100g. The antioxidant activity, measured as free radical scavenging ability, was 32.267±0.26%

Table 2. Quantitative phytochemical analysis of water melon juice.

Parameters	Quantity
Lycopene (mg/100g)	53.33 ± 2.36
Phenolic (GAE/g)	24.43 ± 0.17
Terpenoids (mg/100g)	93.333±2.36
Beta Carotene (ug/100g)	958.333±10.27
Anthocyanin (mg/100g)	26.667±6.24
Flavonoids (mg/100g)	945±20.41
Antioxidants (% inhibition)	32.267±0.26
Iron (mg/100g)	9.1 ± 0.08
Zinc (mg/100g)	0.6 ± 0.00
Calcium(mg/100g)	76.67 ± 2.36
Sodium (mg/100g)	265±10.80
Potassium (mg/100g)	23.33±2.36
Phosphorus (mg/100g)	88.33±6.23
Ascorbic Acid (mg/100g)	15.33±0.47
Thiamine (mg/100g)	0.066 ± 0.00
Niacin (mg/100g)	0.163 ± 0.01

Discussion

The proximate analysis revealed the following percentages in the watermelon juice: moisture at 93.50±1.00%, crude protein at 7.35±0.35%, crude fiber at 1.06±0.06%, ether extract at 6.14±0.02%, ash at 4.57±0.43%, and nitrogen-free extract at 80.88±0.12%. Qualitative phytochemical tests confirmed the presence of phenolic compounds, terpenoids, beta-carotenes, flavonoids, lycopene, and anthocyanins in the watermelon juice. These

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findings align with those of Ambreen et al. (2013), who investigated the antioxidant properties of watermelon juice and lycopene extracts, confirming the existence of the same phytochemicals. The quantitative phytochemical analysis revealed that lycopene was present at 53.33 mg/100g, while the phenolic content measured 24.43 GAE/g. These findings align with those of Wenge et al. (2010), who reported lycopene levels in red-fleshed watermelon varieties ranging from 33.2 to 54.8 mg/kg, with an average of 44.1 mg/kg. The lycopene concentration in this study falls within this range, further emphasizing that watermelon juice is a rich source of lycopene.

The total phenolic content recorded (24.43 GAE/g) is comparable to the 23.63 GAE/g reported by Ambreen et al. (2013) for the Sugar Baby watermelon variety. The antioxidant activity, represented by 32.26% inhibition, slightly exceeded the 29.11% documented by Ambreen et al. (2013), confirming the juice's effectiveness in neutralizing free radicals. Additionally, the DPPH scavenging activity reported by Egydio et al. (2010) ranged between 18.90 and 49.7, aligning with the values obtained in this study. The concentrations of terpenoids, beta-carotene, anthocyanins, and flavonoids were 93.33 mg/100g, 958.3 µg/100g, 26.66 mg/100g, and 945 mg/100g, respectively, supporting the results of Reddy et al. (2010). The mineral analysis showed iron at 9.1 mg/100g, zinc at 0.6 mg/100g, calcium at 76.67 mg/100g, sodium at 265 mg/100g, potassium at 23.88 mg/100g, and phosphorus at 15 mg/100g. Additionally, the levels of ascorbic acid, thiamine, and niacin were recorded at 15.33 mg/100g, 0.066 mg/100g, and 0.163 mg/100g, respectively. Some of these values were slightly higher than those reported by Ambreen et al. (2013), who documented calcium at 5 mg/100g, sodium at 0.81 mg/100g, iron at 0.26 mg/100g, and zinc at 0.03 mg/100g. In conclusion, watermelon juice exhibits notable antioxidant properties and free radical scavenging activity, largely due to its high lycopene content.

Conclusion

Watermelon juice demonstrated significant free radical scavenging and antioxidant properties due to its high lycopene content. It effectively inhibited free radicals, confirming its role as a free radical quencher. This study clearly indicated that watermelon is rich in lycopene, possesses strong free radical scavenging abilities, and is abundant in phytochemicals, vitamins, and minerals.

Recommendations

1. Watermelon juice is recommended as one of the excellent sources of lycopene, Antioxidant, phytochemicals, vitamins and minerals which can be used to fortify goat semen diluents so as to prevent oxidative stress.
2. Water melon juice should be incorporated in daily meals and also in semen extension since it has the potential of neutralizing and attacking free radicals, reducing oxidative damage to sperm cells, increase antioxidant enzyme levels, reduces membrane lipid peroxidation during semen preservation, inhibits inflammatory factors, reduce male infertility thereby restoring male fertility

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