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Comparative Study of Phytonutrient Content and Antioxidant Activity of the Fruit Juices of Watermelon (*Citrullus lanatus*) and Horned melon (*Cucumis metuliferus*)

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ABSTRACT

This study was aimed at determining the vitamin, phytochemical compositions and the antioxidant properties of watermelon and horned melon fruit juices. The analyses were carried out using standard methods. The results obtained showed that the fruits contained vitamins C (0.13 mg/ml and 0.68 mg/ml), A (1.60 and 0.20 mg/ml), B_1 (0.0011 and 0.00009 mg/ml), B_2 (0.0005 and 0.00011 mg/ml), B_3 (6.62 and 0.00007 mg/ml), B_9 (0.12 and 0.0021 mg/ml) and E (3.48 and 0.00042 mg/ml) for fruit juice of watermelon and horned melon respectively. From the phytochemical analysis, phytate (0.33%), alkaloids (0.48 mg/ml), Oxalate (3.24 mg/ml) and tannins (108.79 mg TAE/g) were detected only in the fruit juice of horned melon while flavonoid (3.45 mg/ml) and terpenoids (0.12 mg/ml) were detected only in the fruit juice of watermelon. Others were 0.17 and 0.06 mg/ml phenol, 0.21 and 0.46 mg/ml cardiac glycosides, 0.09 and 0.19 mg/ml saponin for fruit juices of watermelon and horned melon respectively. Horned melon juice showed no antioxidant activity up to a concentration of 100% while the fruit juice of watermelon showed varying activity at different concentrations. The EC_{50} for scavenging ability on DPPH and inhibition of lipid peroxidation were 40.0 and 76.0% respectively while $OD_{0.5}$ for reducing power was 45.0 %. These results showed that watermelon juice is richer in phenols and flavonoids than horned melon juice. Therefore, watermelon juice could be used in pharmaceutical preparations.

Keywords: *Cucumis metuliferus*, *Citrullus lanatus*, horned melon, watermelon, juice, vitamin, phytochemical, antioxidant

Introduction

There has been a great increase in consumption of fruits due to the numerous health benefits derived from them. Fruits supply vitamins, minerals, fiber and digestible carbohydrate.[1] Most fruits have low calorific value and are beneficial for management of weight. Fruits can be eaten raw and whole or processed into different forms such as smoothie or the expressed juice. The smoothie form contains the whole fruits which includes the fiber as well as the juice and in some cases the seeds are crushed together with the fruit. The juice however is mainly the liquid content only. Fruit juices are refreshing and are rich in various phytonutrients serving both nutritional and therapeutic purposes. Fruit juice could be obtained from numerous fruits but among other plants, melon hold good promise as potential harmless sources for obtaining natural antioxidants.[2] The abundant locally consumed melon plants in Nigeria may, therefore, be potential rich reservoirs of antioxidants to be harnessed if studied and established.

Watermelon (*Citrullus lanatus*) and *Cucumis metuliferus* (horned melon) are both tropical exotic fruits belonging to the same melon family (*Cucubitacaea*).[3] The *Curcubitaceae* are mostly postrate or climbing herbaceous annual crops comprising about 90 genera and 700 species.[4] Both fruits are said to have originated from South Africa and are sprawling annual vines grown around the world.

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Both fruits are high in water content and possess succulent sweet flesh with seeds embedded within and a hard rind. The ripe pulp of the fruits are consumed raw in their fresh forms and can equally be processed into fruit juice. From studies, it has been shown that watermelon and horned melon fruits are rich in minerals, phytochemicals, vitamins and amino acids.[5,6,7] Their various therapeutic effects through antioxidant, anti-viral and anti-bacterial activities has also been reported.[7,8,9] This work therefore, studied and compared the phytochemical and vitamin composition as well as the antioxidant properties of the fruit juices of watermelon and horned melon. The findings from this study will create more awareness on the health benefits associated with consumption of these juices and encourage the use.

Materials and Methods

Collection and Identification of plant materials

Watermelon fruit was bought from Ogbete market in Enugu state (6°26'8" N 7°29'5" E) while horned melon fruit was bought from local market in Gboko, Benue State (7.3368° N, 9.0018° E) in October 2021. Watermelon was authenticated by a taxonomist, Mr Iroka Chisom of Botany Department in Nnamdi Azikiwe University, Awka (Voucher number NAUH-189A) while horned melon was authenticated by Prof. C.E. Eze in the Department of Applied Biology and Biotechnology in Enugu State University of Science and Technology.

Sample Preparation

The fruits of watermelon and horned melon were washed with distilled water and cut into medium pieces using knife. From the freshly cut fruit, the juice was extracted by mechanical squeezing with hand and filtered using muslin cloth.

Phytochemical Screening
Qualitative phytochemical screening
Qualitative phytochemical screening was done to determine the various natural bioactive compounds in the juice. Saponin, glycoside, terpenoid and steroid were determined according to the method of Sofowara.[10] Tannin, flavonoid and phenol were determined according to the method of Trease and Evans [11] while alkaloid was determined according to the method of Harbone.[12]

Quantitative phytochemical screening
Quantitative phytochemical screening was done using standard methods. Thus;

Determination of total phenolic content
The slightly modified colorimetry method of Barros et al[13] was used in determination of the phenol content. The juice (1 ml) each was mixed with folin and ciocalteu's phenol reagent (1 ml). After 3 min, 1 ml of saturated sodium carbonate was added and adjusted to 10 ml with distilled water and was kept in the dark for 90 min, after which the absorbance was read at 725 nm.
Gallic acid was used to calculate the standard curve and results were expressed as mg of Gallic acid equivalent per ml of extract.

Determination of Saponin content
The saponin content was determined using the method of Obadoni and Ochuko.[14]
Five milliliter (5 ml) of the samples were each placed into a conical flask and 200 ml of 20% aqueous ethanol was added to extract the saponin. The samples were left for 3 h with intermittent shaking. The mixture was filtered and the filtrate was reduced to 10 ml over water bath at 90ºC. The concentrate was transferred into a 250 ml separating funnel and 5 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 15 ml of n-butanol was added to the samples and washed twice with 2.5 ml of 5% aqueous sodium chloride. The remaining solution was discharge into a pre-weighed evaporating dish and was heated in a water bath to dryness. The evaporating dish was dried in an oven to a constant weight and the percentage saponin content was calculated as follows.
Percentage Saponin = (W2-W1)/W0 x 100
Where: W0 = weight of sample
W1 = Weight of evaporating dish
W2 = weight of evaporating dish + dried extract.

Determination of flavonoids
The flavonoid content was determined by using the slightly modified colorimetry method described by Barros *et al*.[13] An aliquot, 0.5 ml of each of the juice was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % NaNO2 solution. After 6 min, 0.15 ml of 10% AlCl3 solution was added and allowed to stand for 6 minutes, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. The absorbance of the mixture was read at 510 nm versus reagent blank with reference standard prepared with catechin concentrations. The analyses were performed in duplicate. The result was expressed as mg Catechin equivalents per gram of sample (mg CE/ml).

Determination of tannin
Tannin content was determined according to method of AOAC.[15] An aliquot (0.5 ml) of each the juice was mixed with 4.5 ml of distilled water followed by the addition of $FeCl_3$ (0.1M, 0.5 ml) and 0.3 ml of 0.1M potassium ferrocyanate. Six milliliter (6 ml) of distilled water was added to the test tubes and the absorbance taken at 720 nm. Tannic acid was used as the standard and the results obtained were reported as mg tannic acid equivalent (TAE) per ml of sample (mgTAE/ml).

Determination of phytate content
The phytate content was determined using the method of Young and Greaves.[16] Aliquot (2 ml) of each of the juice was measured into 250 ml conical flask. It was mixed with 100 ml of 2% concentrated HCl for 3 h. The samples were then filtered using Whatman filter paper (No. 4). The filtrate (25 ml) was placed in 250 ml beaker and 50 ml distilled water added to each. 5 ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195g iron per ml. The percentage phytic acid was calculated using the formula:
Phytic acid (%) = (Titre Value x 0.00195 x 1.195)/2 x 100

Determination of alkaloids
The alkaloid content of the juice was determined using the method of Harbone.[17] Five mililitre (5 ml) of each of the juice was measured into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 h at 25^0C. This was filtered with Whatman filter paper no. 4 and the filtrate was concentrated to one quarter of the original volume by boiling. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the samples until the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). It was then filtered with pre-weighed filter paper. The residue on the filter paper was the alkaloid, which was dried in the oven at 80^0C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample.

Oxalate determination by titration method
This was determined according to method of Osagie.[18] Two milliliters (2 ml) of each of the juice was mixed with 190 ml of distilled water in a 250 ml volumetric flask, 10 ml of 6M HCl was added, and the suspension digested at 100°c for 1 hour. It was cooled and then made up to 250 ml mark before filtration. Duplicate portions of 125 ml of the filtrate were measured into beakers and four drops of methyl red indicator added. This was followed by the addition of NH_4OH solution (dropwise) until the test solution changed from salmon pink colour to a faint yellow colour (PH 4-4.5). Each portion was then heated to 90ºC, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90ºC and 10 ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and then centrifuged at 2500rpm for 5 min. The supernatant was decanted and then precipitated completely and dissolved in 10 ml of 20% (v/v) H_2SO_4 solution. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized $KMNO_4$ solution to a faint pink colour which persisted for 30s. The calcium oxalate content was calculated using the formula,

$$\frac{T \times (vme)(Df) \times 105\ (mg/ml)}{(ME) \times Mf}$$

Where T is the titre of $KMnO_4$ (ml), Vme is the volume – mass equivalent (i.e. 1ml of 0.05M $KMnO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid). Df is the dilution factor Vt/A (2.4 where Vt is the total volume of titrate (300ml) and A is the aliquot used (125ml), ME is the molar equivalent of $KMnO_4$ in oxalate (KMO_4 redox reaction) and Mf is the mass of sample used.

Determination of Cardiac Glycoside Content
The cardiac glycoside content of the juice was determined by the alkaline titration method of the AOAC.[15] In this method 200 ml of distilled water was added to 5 ml of each of the juice in triplicate in an 800 ml capacity distillation flask. The flask was fitted for distillation and allowed to stand for 2 hours, for autolysis to take place. An antifoaming agent (silicon oil) was then added. Steam distillation was carried on and 150 ml of the distillate collected into 250 ml capacity conical flask containing 20 ml of 2.5% sodium hydroxide then diluted to mark with distilled water. To 100 ml of diluted distillate containing the cyanogenic glycoside, 8.0cm3 of 6N NH4OH solution and 2.0 ml of 5% potassium iodide were added. This was titrated against 0.02N silver nitrate ($AgNO_3$) solution using a burette. The end-point was noted as a permanent turbidity against a black background. Titre values were obtained and used to calculate cyanide contents, using the formula:

Cyanogenic glycoside mg/ml = (Tv x 1,08 x EV)/(SM x Al) x 100
TV= Titre value (ml);
EV= extract vol(ml)

SM= sample mass (g)
AL= alioquot (ml) used
Note: 1cm3 of 0.02N AgNO3 = 1.8 mg HCN.

Vitamin analysis
Vitamins A, C and E was determined by the calorimetric method of Kirk and Sawyer.[19] Vitamins B1, B2, B3 and B9 were determined spectrophotometrically according to the standard method of AOAC.[15]

Antioxidant Assay
DPPH Scavenging Activity Assay
The stable 2,2-diphenyl- 1- picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the sample. This was assayed using the method of Ebrahimzadem *et al*.[20] An aliquot of the juice solution (0.3 ml) of different concentrations (0-100%) were mixed with 2.7 ml of methanolic solution of DPPH (100 µM) in test tubes. The mixture was votexed and kept in dark for 60 mins. The absorbance was taken at a wavelength of 517 nM using spectrophotometer. Vitamin C was used as standard. The percentage scavenging activity was calculated using the formula:
%RSA = [(ADPPH – As)/ ADPPH] x 100
Where A is the absorbance of the test solution with the sample and ADPPH is the absorbance of DPPH solution. The EC50 (concentration of the sample at 50% RSA) was calculated from the graph of %RSA against the sample concentration.

Reducing Power Capacity
The reducing power capacity was determined according to the method of Barros *et al*.[13] This method is based on the principle of increase in the absorbance of the reaction mixture. 2.5 ml of various concentrations of the sample (0-100%) was mixed with 2.5 ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 2 mins. Trichloroacetic acid (2.5ml, 10%) was added and the mixture centrifuged at1000rpm for 8 mins. The upper layer (5 ml) was mixed with 5 ml of deionized water followed by the addition of 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nM. The graph of the absorbance at 700 nM against the sample concentrations was plotted. Ascorbic acid was used as standard antioxidant.

Inhibition of Lipid Peroxidation Activity Assay
This was determined by the method of Barros *et al*.[13] Determination of the extent of inhibition of lipid peroxidation was carried out using homogenate of brain of a goat. The brain of the goat used was purchased from Kwata Slaughter at Awka from a goat of approximately 70kg. The brain was dissected and homogenized with pestle and mortar in an ice cold Tris-HCL buffer (pH 7.4 20 nM) to produce 50% w/v brain homogenate which was centrifuged at 300g for 10mins. An aliquot (0.1 ml) of the supernatant was incubated with 0.2ml of the sample at various concentrations (0-100%), in the presence of 0.1 ml of 10 uM ferrosulphate and 0.1 ml of 0.1 nM ascorbic acid at 37°C for 1 hr. The reaction was stopped by the addition of 0.5 ml of 28% TCA followed by the addition of 0.38 ml of 2% TBA. The mixture was then heated at 80°C for 20 mins. After centrifugation at 3000rpm for 10 mins to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nM. The inhibition ratio (%) was calculated using the following formula:
Inhibition ratio (%) = [(A-B)/A] x 100%
Where A and B were the absorbances of the control and the compound solution respectively. The sample concentration providing 50% lipid peroxidation inhibition (EC50) was calculated from the graph of antioxidant activity percentage against the sample concentrations. Ascorbic acid was used as the standard.

Statistical Analysis
Data analysis was done using the Statistical Package for Social Sciences (SPSS) software. All the data were expressed as Mean ± SD.

Results and Discussion

Phytochemical Composition of watermelon and horned melon.
The results of the phytochemical analysis of both juice are presented in Table 1. Watermelon had higher concentrations of phenol and flavonoid while horned melon had higher concentrations of phytate, alkaloids, saponin, cardiac glycoside, oxalate and tannins. These phytochemicals are known biologically active substances with antioxidant ability. Flavonoids are known radical quenchers with redox properties which can act as reducing agents, hydrogen donors and singlet oxygen quenchers.[21] Horned melon juice contained higher concentrations of phytate, alkaloids, saponins, cardiac glycosides, oxalate and tannins. These phytochemicals are known as anti-nutrients with medicinal values and health implications.

Vitamin Composition
Table 2 shows the vitamin composition of the juice of watermelon and horned melon. Horned melon juice had higher concentration of only vitamin C. Others were higher in watermelon juice. The B vitamins are well-known for their roles in macromolecule metabolism. They also play major roles in functioning of the immune system.[22] Vitamin A plays an important role in vision and in lymphocyte function as well as antibody response to infections.[23] Vitamin A is also needed for the normal development of many types of blood cells, including lymphocytes. Deficiency of vitamin A can lead to alteration of networks of cytokines that influence immune responses and modification of antibody responses to antigens.[24] Vitamins A and E also have antioxidant properties. Vitamin E enhances chondrocyte growth and exhibits anti-inflammatory activity, as well as plays an important role in the prevention of cartilage degeneration.[25]

Antioxidant Activity
Three *in-vitro* assay methods were used for evaluation of the antioxidant ability of the two fruit juices. While watermelon juice exhibited varied activities at different concentrations measured, horned melon juice showed no activity up to a concentration of 100%. This is understandable owing to the low concentration of key biologically active constituents which are known as antioxidants in the juice. The complete absence of phytochemical such as flavonoids and low concentration of other phytochemicals including the antioxidant vitamins correlates to the inactivity. The high concentration of the anti-nutrients could also be responsible. However, watermelon juice exhibited varied antioxidant activities in all the assays conducted and was comparable with the standard antioxidant (ascorbic acid) used. The different antioxidant activities of watermelon juice are outlined thus:

DPPH radical Scavenging Activity
The DPPH scavenging activity of watermelon juice and that of the standard; ascorbic acid is presented in Figure 1. The activity was concentration-dependent. Highest %RSA for watermelon juice was 49.11% with EC_{50} of 40.0 while that of ascorbic acid was 70.58% with EC_{50} of 12.0 (Table 3).

Table 1: Phytochemical Composition of the juice of Watermelon and horned melon.

Phytochemicals	Watermelon	Horned melon
Total Phenols (mg GAE/ml)	0.17 ±0.02	0.06 ± 0.01
Flavonoid	3.45 ± 0.04	ND
Phytate (%)	ND	0.33 ± 0.03
Alkaloids (%)	ND	0.48 ± 0.06
Saponins (%)	0.09 ± 0.01	0.19 ± 0.04
Cardiac glycosides (%)	0.21 ± 0.02	0.46± 0.16
Terpenoids	0.12 ± 0.02	ND
Oxalate (mg/ml)	ND	3.24 ± 0.38
Tannins (mg TAE/ml)	ND	108.79 ± 5.16

Values are mean ± standard deviation (n=3).

Table 2: Vitamin composition of watermelon and horned melon juice

Vitamins	Watermelon	Horned melon
Vitamin B1 (mg/ml)	1.10±0.10	0.00009
Vitamin B2 (µg/ml)	0.5±0.10	0.11±0.02
Vitamin B3 (mg/ml)	6.62±1.00	0.00007±0.00002
Vitamin B9 (µg/ml)	0.12±0.03	0.0021±0.0001
Vitamin A (mg/ml)	1.60±0.20	0.20±0.02
Vitamin C (mg/ml)	0.13± 0.03	0.68±0.04
Vitamin E (µg/ml)	3.48±1.00	0.00042±0.0002

Values are mean ± standard deviation (n=3)

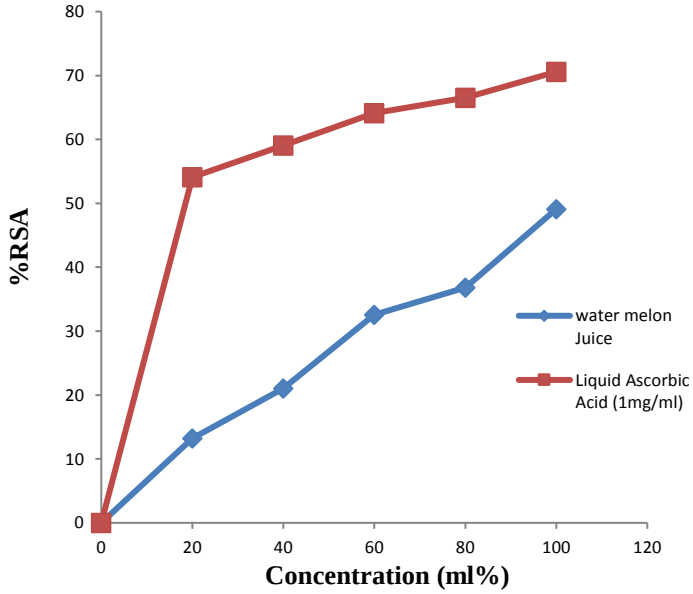

Figure 1: Percentage RSA of watermelon juice and the standard; ascorbic acid.

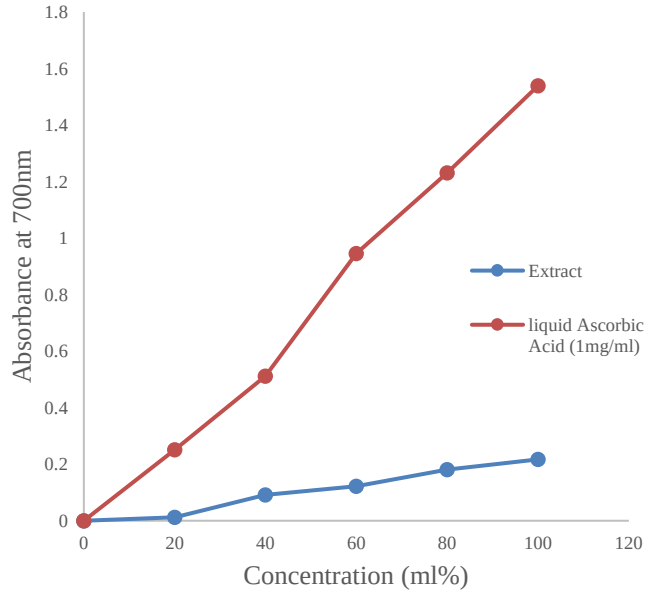

Figure 2: Reducing Power of Watermelon Juice and the standard Ascorbic acid.

Table 3: EC_{50} values of the Sample and Vitamin C for DPPH radical scavenging ability

Sample	IC_{50}
Watermelon juice	40.0
Ascorbic acid	12.0

Overall, watermelon juice displayed moderate DPPH radical scavenging ability when compared with the standard. This ability could be attributed to the presence of phenol and flavonoid which are known antioxidants.[26] These antioxidants in the watermelon juice were able to reduce the stable radical DPPH to the yellow coloured diphednyl-picryhydrazine.

Reducing power capacity.

Figure 2 shows the reducing power capacity of watermelon juice with that of the standard; ascorbic acid. The highest absorbance observed for watermelon juice was 0.21 while ascorbic acid was 1.53 at 100% concentration. The $OD_{0.5}$ for watermelon was 45.0 and that of ascorbic acid was 52.0 (Table 4). The reducing power capacity was assayed based on its ability to reduce Fe^{3+} to Fe^{2+}. Fe^{3+} reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action.[26] The reducing power ability was determined based on the principle of increase in absorbance. The reducing power ability observed shows the medicinal importance and usefulness of watermelon juice. Antioxidants are strong reducing agents and this is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure. Watermelon juice displayed a lower EC_{50} value than that of the standard (Table 4). This implies that the reducing power potency of watermelon juice is higher compared to the standard. It means that watermelon juice contains reductones which exert antioxidant activity by hydrogen or electron donation; and has demonstrated its antioxidant potential as the reducing capacity of compounds indicates its potential antioxidant properties.[27,28]

Inhibition of Lipid Peroxidation Activity

The ability to inhibit lipid peroxidation by watermelon juice and the standard; ascorbic acid is shown in Fig. 3. The highest % inhibition of lipid peroxidation by watermelon juice was 41.49% with EC50 of 76.0 while that of ascorbic acid was 71.39% with EC_{50} of 46.0 (Table 5). Lipid peroxidation is a type of oxidative degradation of biomolecules, where a peroxide is formed from a lipid substrate.[29] The oxygen atoms in peroxides are in the oxidative state -1, a less common and less stable form of oxygen.[30] Lipid peroxides (LPO) in the cells result in the degradation of the lipid bilayer composing cell membranes. Due to LPO, a number of compounds are formed such as alkanes, isoprotanes and malanoaldehyde. These compounds are used as markers in lipid peroxidation assay and have been implicated in many diseases such as neurogenerative diseases, ischemic reperfusion, injury, diabetes. [31] In the inhibition of lipid peroxidation assay, watermelon juice inhibited the process of lipid peroxidation in a concentration-dependent manner. However, the standard exhibited more potent inhibition activity with a lower EC_{50} value. Therefore, it can be said that watermelon juice has the ability of inhibiting the process of lipid peroxidation.

Conclusion

The analysis of fruit juices of watermelon and horned melon revealed the presence of important biologically active substances which have significant therapeutic application. The high phytochemical and vitamin concentration of watermelon juice over horned melon juice makes it a better potential source of therapeutic compound.

Conflict of Interest

The authors declare no conflict of interest.

Table 5: EC_{50} values of watermelon juice and ascorbic acid

Sample	IC_{50}
Watermelon juice	76.0
Ascorbic acid	46.0

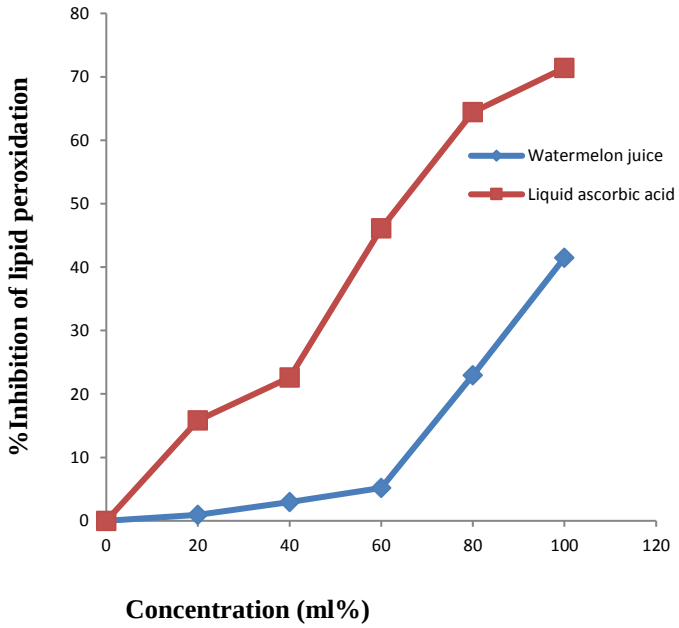

Figure 3: Percentage Inhibition of Lipid Peroxidation Activity and the standard; ascorbic acid.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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