



Journal of Applied Sciences

ISSN 1812-5654

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Research Article

Evaluation of the Phytonutrient Content and Antioxidant Activity of Watermelon Juice

¹Onuabuchi Nnenna Ani, ²Kelechi Kingsley Asogwa and ¹Ebere Immaculata Akpata

¹Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

²Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Abstract

Background and Objective: Watermelon is a fruit grown in tropical regions and widely consumed for its refreshing, sweet and juicy taste. This study evaluated the vitamin and phytochemical compositions as well as the antioxidant activity of its juice.

Materials and Methods: Watermelon fruit was purchased from Ogbete market in Enugu State, Nigeria and the various analyses were carried out using standard methods. The antioxidant activity was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, reducing power and inhibition of lipid peroxidation assays. **Results:** From the result of the vitamin analysis, vitamin B3 was the highest in concentration ($6.62 \pm 3.54 \text{ mg mL}^{-1}$) while folic acid was the least ($0.12 \pm 0.04 \mu \text{ mL}^{-1}$). The phytochemical analysis showed the presence of flavonoid ($3.45 \pm 0.04 \text{ mg mL}^{-1}$), cardiac glycoside ($0.21 \pm 0.02 \text{ mg mL}^{-1}$), phenol ($0.17 \pm 0.02 \text{ mg mL}^{-1}$), terpenoids ($0.12 \pm 0.02 \text{ mg mL}^{-1}$) and saponin ($0.09 \pm 0.01 \text{ mg mL}^{-1}$) while oxalate, phytate and alkaloids were not detected. The antioxidant activities were concentration-dependent. The IC_{50} of watermelon juice for scavenging ability on DPPH and inhibition of lipid peroxidation were 40.0 and 76.0 mL, respectively while $\text{OD}_{0.5}$ for reducing power was 45.0 mL. Ascorbic acid was used as standard and exhibited better antioxidant ability for DPPH radical scavenging and inhibition of lipid peroxidation with IC_{50} of 12.0 and 46.0 mL, respectively. However, the reducing power ability of the standard ($\text{OD}_{0.5}$ of 52.0) was lower than that of watermelon juice. **Conclusion:** This study showed that watermelon juice is a good source of nutrients and the presence of these phytochemicals and the antioxidant potential shows that it may be useful for therapeutic purposes.

Key words: Antioxidant, phytochemical, watermelon, juice, vitamin

Citation: Ani, O.N., K.K. Asogwa and E.I. Akpata, 2022. Evaluation of the phytonutrient content and antioxidant activity of watermelon juice. J. Appl. Sci., 22: 196-206.

Corresponding Author: Onuabuchi Nnenna Ani, Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

Copyright: © 2022 Onuabuchi Nnenna Ani *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Various plants, fruits and herbs have been exploited by man for therapeutic and medicinal values¹. Fruits in particular play both nutritional and therapeutic roles in the body. These roles result from various active metabolites which generate physiological actions in the body. The active metabolites and constituents are mostly the vitamins and phytochemicals which also are antioxidants². Vitamins have dual functions in that they are involved in different biochemical reactions and act as antioxidants. Such biochemical functions include hormones (vitamin D), regulations of metabolism (the B complex vitamins) as well as in cell and tissue growth and differentiation (vitamin A). Phytochemicals act in synergy in contributing to the health and nutritional benefits of foods either separately, or in combination³.

Antioxidants are compounds in low amounts that hinder greatly the oxidation of materials vulnerable to oxidation⁴. Antioxidants are responsible for the defence mechanisms of the organism against the pathological effect caused by the attack of free radicals. Antioxidants abort these processes by eliminating the free radicals and hindering the oxidative processes⁵.

Studies have shown that there is a positive association between the intake of vegetables and fruits and a reduction in the incidence of cancer and cardiovascular diseases^{6,7}. Fruits and vegetables contain several secondary metabolites which are responsible for these therapeutic effects. Thus, the intake of plant-derived antioxidants is involved in the prevention of these degenerative diseases caused by free radicals^{8,9}. Flavonoids and phenols the largest group of phytochemicals account for most of the antioxidant activity in plants. They exert their antioxidant effects within the body by mopping up cell-damaging free radicals. The uncontrolled and excessive production of free radicals is implicated in the onset of numerous debilitating diseases like cancer and the degenerative process associated with ageing, Parkinson's and Alzheimer's diseases^{10,11}. These phytochemicals slow or prevent the oxidation of molecules.

Watermelon is an important horticultural crop mostly grown in the warm region for its sweet and juicy taste. It belongs to the cucumber family (Cucurbitaceae)¹². Water in this fruit constitutes about 92% of the total fruit weight and it is of great importance in Africa due to its quenching ability during a water shortage. The crop is mainly harvested for juice and its juice concentrates are an excellent source of vitamins, such as vitamin A and C¹³. The botanists refer to it as a 'pepo' which is a fruit having a thick ring and fleshy center¹⁴.

Watermelon fruit is widely eaten as refreshing fruit during summer, valued for its revitalizing capability, alluring colour, intricate taste and high water content. It is approximately 55.3% juice, 31.5% rind and 0.4% pomace¹⁵. Watermelon has a high content of carotenoids and exerts a decreasing effect on the risk of myocardial infarction, anticancer properties and supports a healthy eye¹⁶. Lycopene is the major carotenoid of red-fleshed watermelons having positive health effects. Prostate cancer, Coronary Heart Disease (CHD) and lung cancer may be prevented by intake of watermelon¹⁷. The sucrose, fructose and glucose content is responsible for its sweetness. A ripe watermelon contains about 20-40% sucrose and 30-50% fructose. It has been reported that it can be used to effectively manage weight loss due to its low sodium, saturated fat and cholesterol level¹⁸. As a result, the consumption of watermelon has been associated with various health benefits such as lowering the risk of developing heart disease, age-related degenerative pathologies and some kinds of cancer¹⁹.

MATERIALS AND METHODS

Study area: The study was carried out between April to June, 2021 and the analyses were performed at Natural Products Research and Development Laboratory, Awka, Anambra State, Nigeria.

Collection and identification of sample: Watermelon fruit was bought from Ogbete market in Enugu State and authenticated in the Department of Applied Biology and Biotechnology in Enugu State University of Science and Technology.

Sample preparation: The watermelon fruit was washed under a running tap and cut into medium pieces using kitchen knife. From the freshly cut watermelon, the juice was extracted by mechanical squeezing and filtered using a muslin cloth.

Vitamin analysis

Determination of vitamin A: The vitamin A content of the watermelon juice was analyzed using the method of Rutkowski and Grzegorzczak²⁰. One millilitre of the sample was measured out. The vitamin A content was extracted using 10 mL of ethanol. This was centrifuged at 1500 rpm for 10 min and the supernatant was used as the extract for the analyses. One millilitre of the sample extracts was placed in a test tube (centrifugal) with a tight stopper and 1 mL of 0.1 M

ethanolic KOH solution was added, the tube was plugged and shaken vigorously for 1 min. It was then heated in a water bath at 60°C for 20 min, then cooled down in cold water. 1 mL of xylene was added and the tube was plugged and shaken vigorously for 1 min. The tube was centrifuged at 1500 rpm for 10 min. The upper layer was collected in a test tube and the absorbance was measured at 335 nm in a UV recording Spectrophotometer. This was recorded as A1. The tube was irradiated in UV light for 30 min and the absorbance was recorded again as A2.

The concentration of Vitamin A (Cx) in µM was calculated using the formula:

$$Cx (\mu M) = (A1-A2) \times 22.23$$

22.23 multiplier received on basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette about thickness = 1 cm.

Determination of vitamin E: The vitamin E content of the watermelon juice was analyzed using the method of Rutkowski and Grzegorzczak²⁰. One mL of the sample was measured out. The vitamin E content was extracted using 10 mL of ethanol. This was centrifuged at 1500 rpm for 10 min and the supernatant was used as the extract for the analyses. An aliquot (0.5 mL) of the sample extract was mixed with 0.5 mL of anhydrous ethanol and shaken for 1 min. This was followed by the addition of 3 mL of xylene. The tube was shaken again for 1 min and the content of the tube was centrifuged for 10 min at 1500 rpm. About 1.5 mL of the upper layer was mixed with 0.25 mL of 6.02 mM bathophenanthroline. This was followed by the addition of 0.25 mL of 0.98 mM Ferric chloride solution. Phosphoric acid (0.25 mL, 40 mM) was added and mixed well. The absorbance was measured at 539 nm. Tocopherol was used as the standard (23.2 µM).

The concentration of tocopherol in µM was calculated using the formula:

$$\text{Tocopherol } (\mu M) = \frac{Ax}{As \times Cs}$$

Where:

Ax = Absorbance of Sample

As = Absorbance of Standard

Cs = Concentration of standard

Determination of ascorbic acid: Ascorbic acid content of the watermelon juice was determined according to the method

described by Achikanu *et al.*²¹ with some modifications. The sample (0.2 mL) was extracted with 10 mL of 1% metaphosphoric acid. It was allowed to stand for 45 mins at a temperature of 28°C (laboratory temperature) after which it was filtered through Whatman No. 4 filter paper. The filtrate (1 mL) was mixed with 9 mL of 50 µM 2,6-dichlorophenolindophenol sodium salt hydrate and the absorbance was measured at 515 nm using a UV-Vis spectrophotometer after 30 min. Ascorbic acid content was calculated from the calibration curve of authentic L-ascorbic acid and the result was expressed as mg ascorbic acid equivalent per gram (mgAE mL⁻¹) of watermelon juice.

Determination of vitamin B3 (Nicotinamide): Vitamin B3 was determined according to the method described by Achikanu *et al.*²¹. The sample (5 mL) was diluted using 20 mL anhydrous glacial acetic acid and warmed slightly. Acetic anhydride (5 mL) was added and mixed. Two drops of crystal violet solution were added as the indicator and titrated with 0.1 M perchloric acid to a greenish-blue colour.

Calculation:

$$\text{Vitamin B3 (mg \%)} = \frac{\text{Titre value} \times 0.0122}{0.1}$$

Folic acid determination: The folic acid Vitamin B9 content of the watermelon juice was determined according to the method of Padmarajaiah *et al.*²². This is based on the reaction between diazotization of the p-amino benzoyl glutamic acid obtained after reduction of folic acid and 3-aminophenol. The sample (1 mL) was measured out. The folic acid content was extracted using 10 mL of distilled water. This was centrifuged at 1500 rpm for 10 min and the supernatant was used as the extract for the analyses. The sample solution (1.0 mL) was mixed with 1.0 mL of 4 mol L⁻¹ hydrochloric acid, 1.0 mL of 1% (w/v) sodium nitrite, 1.0 mL of 1% (w/v) sulfamic acid and 1.0 mL of 1% (w/v) 3-aminophenol which resulted in an orange-yellow complex. The absorption of complexation was measured at 460 nm using a UV-Visible spectrophotometer. A standard curve was prepared using standard folic acid and the concentration of folic acid in the sample was extrapolated from the graph.

Vitamins B1 and B2: Vitamin B1 and B2 were determined according to a method described by Nora²³ with some modifications. This was based on the selective absorption of the vitamins in the UV region at 262 and 242 nm, respectively.

One millilitre (1 mL) of watermelon juice was diluted with 100ml of deionized water. This was placed in a Stuart shaker and heated for 5 min and allowed to cool and filter. The filtrate was centrifuged at 4000 rpm for 10 min and an aliquot, the supernatant (2 mL) was poured into a quartz glass cuvette and the respective wavelengths for the vitamins were set to read the absorbance using a spectrophotometer.

- Vitamin B1 was measured at 261 nm
- Vitamin B2 was measured at 242 nm

Calculations:

$$\text{Concentration (mg \%)} = \frac{A \times DF \times \text{Volume of cuvette}}{E}$$

Where:

A = Absorbance

DF = Dilution factor

E = Molar extinction coefficient of vitamin B1/B2

Beta carotene and lycopene contents: These were determined by the method of Barros *et al.*²⁴. The quantity of 0.1 mL of sample extract was vigorously shaken with 6 mL acetone-hexane mixture in the ratio of (4: 6) for 1 min and filtered using Whatman No. 4 filter paper. The absorbance of the filtrate was read at 453, 505 and 663 nm, respectively. The contents of lycopene and β -carotene were calculated according to the following Eq.:

$$\text{Lycopene (mg/100 mL)} = -0.0458A_{663} + 0.372A_{505} + 0.0806A_{453}$$

$$\beta\text{-carotene (mg/100 mL)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

Phytochemical analysis

Qualitative phytochemical analysis: The qualitative phytochemical screening of the juice was carried out according to the method described by Edeoga *et al.*²⁵ and Oluduro²⁶.

Quantitative phytochemical analysis

Total phenolic content determination: The phenol content was determined by using the slightly modified colourimetry method described by Barros *et al.*²⁴. The juice (1 mL) was mixed with folin and ciocalteu 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.

Saponin content: The saponin content of the juice was determined using the method of Obadoni and Ochuko²⁷. Five mL of the juice was placed into a conical flask and 200 mL of 20% aqueous ethanol was added to extract the saponin. The sample was left for 3 hrs with intermittent shaking. The mixture was filtered and the filtrate was reduced to 10 mL over a water bath at 90°C. 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. The 15 mL of n-butanol was added to the extracts and washed twice with 2.5 mL of 5% aqueous sodium chloride. The remaining solution was discharged into a reweighed evaporating dish and was heated in a water bath to dry. The evaporating dish was dried in an oven to a constant weight and the percentage saponin content was calculated as follows:

$$\text{Percentage saponin} = \frac{W_2 - W_1}{W_0} \times 100$$

Where:

W_0 = Weight of the sample

W_1 = Weight of evaporating dish

W_2 = Weight of evaporating dish+dried extract

Total flavonoids: The flavonoid content was determined by using the slightly modified colourimetry method described by Barros *et al.*²⁴. An aliquot (0.5 mL) 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 min 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 min. 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 mL of sample (mg CE mL⁻¹).

Determination of cyanogenic glycoside content: The cardiac glycoside content of the sample was determined by the method described by Ejikeme *et al.*²⁸. In this method, 200 mL

of distilled water was added to 0.1 mL of the juice in triplicate in an 800 mL capacity distillation flask. The flask was fitted for distillation and allowed to stand for 2 hrs, for autolysis to take place. An antifoaming agent (silicon oil) was then added. Steam distillation was carried on and 150 mL of the distillate was collected into a 250 mL capacity conical flask containing 20 mL of 2.5% sodium hydroxide then diluted to mark with distilled water. To 100 mL of a diluted distillate containing the cyanogenic glycoside, 8.0 cm³ of 6 N NH₄OH solution and 2.0 mL of 5% potassium iodide were added. This was titrated against 0.02 N silver nitrate (AgNO₃) solution using a burette. The end-point was noted as permanent turbidity against a black background. Titre values were obtained and used to calculate cyanide contents, using the formula:

$$\text{Cyanogenic glycoside mg/100g} = \frac{\text{Tv} \times 108 \times \text{Ev}}{\text{Sm} \times \text{Al}} \times 100$$

Where:

Tv = Titre value (mL)

Ev = Extract volume (mL)

Sm = Sample mass (g)

Al = Aliquot (mL) used

Note: 1cm³ of 0.02N AgNO₃ = 1.8 mg HCN

Determination of terpenoids: The terpenoids content was determined according to the method of Oluduro²⁶. The 1 mL of 5% phosphomolybdic acid solution was added to 0.1 mL of the juice and shaken. Gradually 1 mL of concentrated H₂SO₄ was added. The mixture was left to stand for 30 min. Ethanol (2 mL) was added and absorbance was measured at 700 nm.

$$\text{Concentration of terpenoids} = \frac{\text{Abs} \times \text{Path length}}{100 \times \text{Weight of the sample}}$$

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 Ebrahimzadeh *et al.*²⁹. An aliquot of the juice solution (0.3 mL) of different concentrations (0-100%) was mixed with 2.7 mL of methanolic solution of DPPH (100 µM) in test tubes. The mixture was vortexed and kept in dark for 60 min. The absorbance was taken at a wavelength of 517 nm using a spectrophotometer. Vitamin C was used as a standard. The percentage scavenging activity was calculated using the formula:

$$\text{RSA (\%)} = \frac{\text{ADPPH} - \text{As}}{\text{ADPPH}} \times 100$$

where, A is the absorbance of the test solution with the sample and ADPPH is the absorbance of the DPPH solution. The IC₅₀ (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.*²⁴. This method is based on the principle of an 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 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 2 min. Trichloroacetic acid (2.5 mL, 10%) was added and the mixture was centrifuged at 1000 rpm for 8 min. 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 a standard antioxidant.

Inhibition of lipid peroxidation activity assay: This was determined by the method of Barros *et al.*²⁴. Determination of the extent of inhibition of lipid peroxidation was carried out using homogenate of the brain of a goat. The brain of the goat used was purchased from Kwata Slaughter at Awka from a goat of approximately 70 kg. The brain was dissected and homogenized with pestle and mortar in an ice-cold Tris-HCl buffer (pH 7.4 20 mM) to produce 50% w/v brain homogenate which was centrifuged at 300 g for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with 0.2 mL of the sample at various concentrations (0-100%), in the presence of 0.1 mL of 10 µM ferric sulphate and 0.1 mL of 0.1 mM 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 min. After centrifugation at 3000 rpm for 10 min to remove the precipitated protein, the colour 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:

$$\text{Inhibition ratio (\%)} = \frac{\text{A} - \text{B}}{\text{A}} \times 100$$

Where A and B were the absorbance of the control and the compound solution, respectively. The sample concentration providing 50% lipid peroxidation inhibition (IC_{50}) was calculated from the graph of antioxidant activity percentage against the sample concentrations. Ascorbic acid was used as the standard.

Statistical analysis: The data were analyzed using ANOVA and values were expressed as Means $\pm$ SD.

RESULTS

Vitamin composition: The vitamin compositions of the watermelon juice are shown in Table 1. The juice contains 6.62 ± 3.54 mg mL⁻¹ vitamin B3 which was the highest in composition while folic acid was the least at a concentration of 0.12 ± 0.04 μ g mL⁻¹.

Phytochemical composition: The result of the qualitative and quantitative phytochemical analysis of watermelon juice is shown in Table 2. The result of the phytochemicals screened showed the presence of phenol (0.17 ± 0.02 mg g⁻¹), flavonoids (3.45 ± 0.04 mg g⁻¹), cardiac glycosides (0.21 ± 0.02 mg g⁻¹), Saponin (0.09 ± 0.01 mg g⁻¹) and Terpenoids (0.12 ± 0.02 mg g⁻¹). Oxalate, phytate and alkaloids were not detected.

Antioxidant activity

DPPH scavenging activity: The result of the DPPH scavenging activity of watermelon juice compared with the standard; vitamin C is presented in Fig. 1. The activity was concentration-dependent. At the highest concentration of 100%, the RSA (%) for the sample was 49.11% while that of vitamin C was 70.58%. Vitamin C gave a lower IC_{50} of 12.0 than the sample (40.0) as shown in Table 3.

Reducing power capacity: The reducing power capacity of watermelon juice with that of the standard ascorbic acid is shown in Fig. 2. At the highest sample concentration of 100%, the absorbance of the sample was 0.21 and that of liquid ascorbic acid was 1.53. The watermelon juice gave a lower $OD_{0.5}$ of 45.0 than the liquid ascorbic acid 52.0 as shown in Table 4.

Inhibition of lipid peroxidation activity: The inhibition of lipid peroxidation of watermelon juice in comparison with the standard ascorbic acid is shown in Fig. 3. At the highest sample concentration of 100%, the inhibition (%) of lipid

peroxidation of the sample was 41.49% and that ascorbic acid was 71.39%. The IC_{50} of the Ascorbic acid was lower (46.0) than that of the sample (76.0) as shown in Table 5.

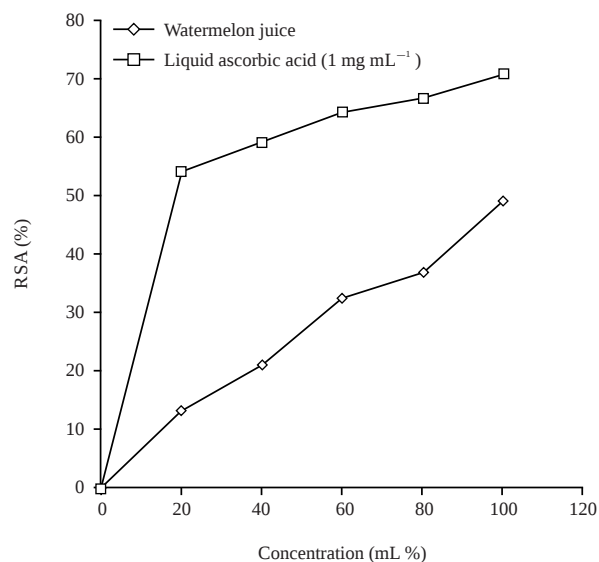


Fig 1: RSA (%) of the sample compared with the standard Vitamin C

Table 1: Vitamin composition of watermelon juice

Vitamins (mg mL ⁻¹)	Concentration
Vitamin B1 (mg mL ⁻¹)	0.0011 $\pm$ 0.00014
Vitamin B2 (mg mL ⁻¹)	0.0005 $\pm$ 0.001
Vitamin C (mg mL ⁻¹)	0.13 $\pm$ 0.003
Vitamin B3 (mg mL ⁻¹)	6.62 $\pm$ 3.54
Folic acid (μ g mL ⁻¹)	0.12 $\pm$ 0.04
Tocopherol (μ g mL ⁻¹)	3.48 $\pm$ 0.65
Vitamin A (mg mL ⁻¹)	1.60 $\pm$ 0.04
Beta carotene (mg mL ⁻¹)	1.42 $\pm$ 0.19
Lycopene (mg mL ⁻¹)	0.05 $\pm$ 0.01

Table 2: Qualitative and quantitative phytochemical composition

Phytochemical	Qualitative concentration	Quantitative concentration (mg g ⁻¹)
Total Phenol	+	0.17 $\pm$ 0.02
Flavonoid	++	3.45 $\pm$ 0.04
Cardiac glycoside	+	0.21 $\pm$ 0.02
Saponin	+	0.09 $\pm$ 0.01
Terpenoids	+	0.12 $\pm$ 0.02
Oxalate	ND	ND
Phytate	ND	ND
Alkaloid	ND	ND

KEY: + = Slightly present, ++ = Moderately present and ND = Not detected

Table 3: IC_{50} values of the sample and vitamin C for DPPH radical scavenging ability

Samples	IC_{50}
Watermelon juice	40.0
Vitamin C	12.0

Table 4: OD_{0.5} values of the sample and vitamin C

Samples	OD _{0.5}
Watermelon juice	45.0
Liquid ascorbic acid	52.0

Table 5: IC₅₀ values of the sample and ascorbic acid

Samples	IC ₅₀
Watermelon juice	76.0
Ascorbic acid	46.0

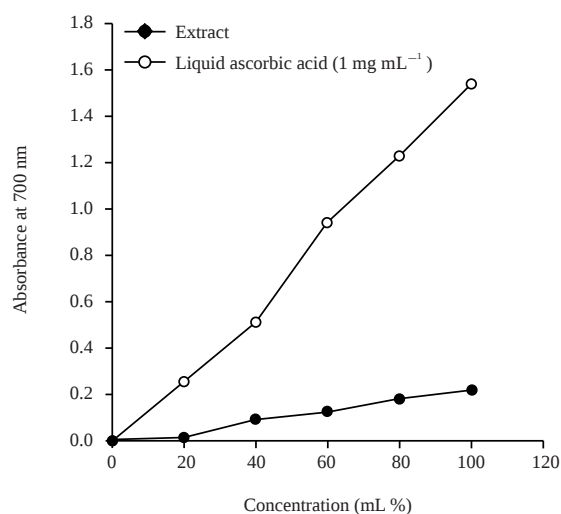


Fig. 2: Reducing power of watermelon juice compared with the standard ascorbic acid

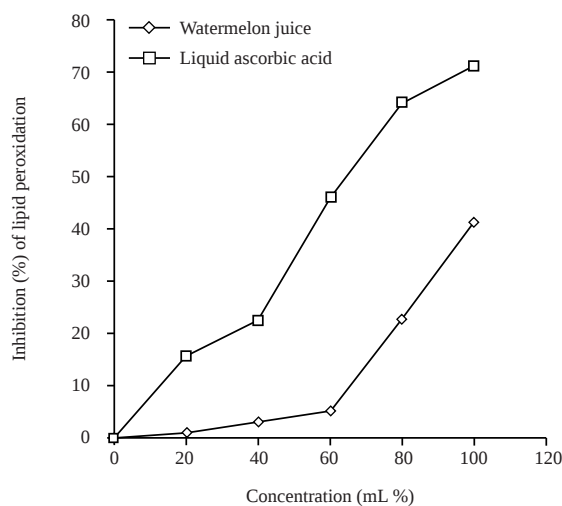


Fig. 3: Percentage inhibition of lipid peroxidation activity compared with standard vitamin C

DISCUSSION

From the results of this study, it was shown that watermelon juice has nutritive and therapeutic values. Table 1 shows the vitamin content. Vitamins are required in

small amounts and they play protective roles in the body. Among the analyzed vitamins, vitamin B3 was the highest in concentration (6.62 mg mL⁻¹). This is though not up to the daily recommended dose of 16 mg per day for an adult but will go a long way in contributing to the required dose when in combination with other sources in food. Vitamin B3 (niacin) called pellagra preventive factor, aids in the break down of carbohydrates, fats and proteins for the production of energy in the body, functions in the liver and gland, removes toxic chemicals from the liver, has a healing effect on migraines, circulatory system issues, light-headedness and diarrhoea resulting from cholera while its deficiency results to depression, pellagra, canker sores, poor circulation and fatigue³⁰. Vitamins B1 and B2 were also present although in small amounts and lower than the daily recommended doses of 1.2 and 1.3 mg per day, respectively³¹. These vitamins function as coenzymes in the metabolism of macronutrients. Vitamin B1 functions as the coenzyme thiamin pyrophosphate (TPP) in the metabolism of carbohydrates and branched-chain amino acids³². This implies that its deficiency will cause an overall decrease in carbohydrate metabolism and its inter-connection with amino acid metabolism have severe consequences, such as a decrease in the formation of acetylcholine for neural function. Vitamin B2 functions in oxidative phosphorylation and coenzyme formation³³. Vitamin B9 (Folic acid) was also found in the low amount of 0.12 µg mL⁻¹. Folic acid plays a role in the synthesis of purines and pyrimidines which are required for erythropoiesis and DNA production³⁴. Vitamin C content was 0.13 mg mL⁻¹ and lower than the daily recommended allowance of 45 mg per day for an adult³⁵. It is generally involved in protein metabolism and collagen synthesis³¹ and plays a protective role as an antioxidant. The antioxidant properties of vitamin C aid in the stabilisation of folate in plasma and food and causes increased excretion of oxidized folate derivatives in human scurvy³¹. Other vitamins present include tocopherol (vitamin E), vitamin A, beta carotene and lycopene. Vitamin E also possesses antioxidant properties, vitamin A plays a role in vision, immune health and possesses anti-cancer property³⁶.

Active metabolites in plants are intensively screened to supplement synthetic drugs with minimal or no side effects. Phytochemicals are secondary metabolites that are useful in therapeutics. The qualitative and quantitative phytochemical analysis of watermelon juice revealed the presence of phytochemicals such as phenols, flavonoids, saponin, cardiac glycoside and terpenoids as shown in Table 2. Flavonoid (3.45 mg g⁻¹) was the most abundant followed by phenol (0.17 mg g⁻¹) while the least was saponin (0.09 mg g⁻¹).

Flavonoids and phenols possess antioxidant properties and so their presence in watermelon juice suggests that the juice can scavenge free radicals. The antioxidant ability of flavonoid compounds is attributed to their redox properties which can act as reducing agents, hydrogen donors and singlet oxygen quenchers³⁷. From studies, it has been shown that coronary heart disease is opposed by dietary flavonoids³⁸. This study also revealed the presence of cardiac glycosides. Cardiac glycosides belong to a group of organic compounds that inhibit the cell's sodium-potassium ATPase pump by increasing the heart's output force and decreasing the rate of heart contractions³⁹. Cardiac glycosides are the most useful and a group of secondary metabolites in therapeutics. They are used for the treatment of heart failure and arrhythmias⁴⁰. They are said to also possess anticancer activity⁴¹. Saponin was detected in the juice in a very low amount. This is an advantage as saponin is an anti-nutrient that reduces the uptake of nutrients and cholesterol at the gut⁴². Saponin has hypercholesterolemic effect and is useful in the human diet in controlling cholesterol levels⁴³. Terpenoids were also detected in the watermelon juice. Terpenoids have several medicinal properties such as anti-cancer, antimalarial, anti-ulcer, antimicrobial or diuretic activity⁴⁴. Volatile terpenes are produced by plants to attract specific insects for pollination and also act as a defence mechanism against preys⁴⁵. The presence of terpenoids in watermelon juice suggests that it may be useful in the treatment and management of malaria, ulcers and cancer.

The antioxidant assay carried out on watermelon juice shows that watermelon juice possesses antioxidant activity. The DPPH free radical scavenging ability of the juice shows the ability of juice to produce an antioxidant activity that prevents the production of free radicals as well as neutralize and scavenge free radicals in the body. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability^{46,47}. DPPH is a stable nitrogen-centred free radical which gets reduced through contact with reducing agent by donation of the electron, loses colour stoichiometrically and turns from violet to yellow⁴⁸. Such reactivity has been widely used to test the ability of compounds/plant extracts to act as free radical scavengers⁴⁹. Substances capable of performing this reaction are considered antioxidants and therefore, radical scavengers⁵⁰. From the study, watermelon juice displayed moderate DPPH radical scavenging ability. This ability could be attributed to the presence of phenol and flavonoid which are known antioxidants⁵¹. In the DPPH assay, these antioxidants in the watermelon juice were able to reduce the stable radical DPPH to the yellow coloured diphenyl-picryl hydrazine.

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 action⁵¹. The reducing power capacity was determined according to the principle of increase in absorbance. The reducing power ability observed in this work explains 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. The reductive potency as shown in Table 4 shows a lower IC_{50} value for watermelon juice than that of the standard. This implies that the reducing power potency of watermelon juice is higher compared to the standard. This also shows 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^{52,53}.

Lipid peroxidation is a process by which free radicals attack lipids, especially polyunsaturated fatty acids (PUFAs)⁵⁴. The oxygen atoms in peroxides are in the oxidative state -1, a less common and less stable form of oxygen. Lipid peroxidation cells result in the degradation of the lipid bilayer composing cell membranes. Lipid peroxidation results in the formation of many compounds such as alkanes, isoprostanes and malonaldehyde. These compounds serve as indicators in lipid peroxidation assay and have been implicated in many toxic tissue injuries and pathological processes⁵⁴. In this assay, watermelon juice inhibited the generation of Fe^{2+} ascorbate in the brain homogenate. The inhibition activity increased with an increase in concentration as with the standard. However, the standard exhibited more potent inhibition activity with a lower IC_{50} value. Therefore, it can be said that watermelon juice can inhibit the process of lipid peroxidation. This ability could be attributed to the presence of antioxidants such as flavonoids and phenol.

CONCLUSION

Watermelon juice is one of the versatile fruit drinks used for mood stability when modulating stress as it has about 90% water content enabling a good bowel movement. From this study, it has been shown that it has important nutritional and therapeutic properties. The assays in this study revealed that it possesses antioxidants that are of medicinal importance. Therefore, it could be inferred that the juice will

be useful for medicinal purposes and there could be the possibility of utilizing the fruit as nutraceuticals in the prevention or management of some debilitating diseases arising from free radicals.

SIGNIFICANCE STATEMENT

This study discovered the various vitamins and phytochemicals present in watermelon juice that can be beneficial for the maintenance of good health and therapeutic purposes. This study will help the researchers to uncover the critical areas of drug formulations based on their antioxidant potentials. Thus, this study is a stepping stone in the research, development and drug discovery from natural products around us.

REFERENCES

1. Assareh, M.H., M. Sedaghati, K. Kiarostami and A.G. Zare, 2010. Seasonal changes of essential oil composition of eucalyptus maculata hook. Iran. J. Medic. Aromat. Plants, 25: 581-588.
2. Okwu, D.E. and O. Ekeke, 2003. Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. Global J. Pure Appl. Sci., 9: 235-238.
3. Okogeri, O. and R. Onu, 2016. Nutritional and phytochemical profiles of three neglected fruit seeds from Ebonyi state. Int. J. Food Sci. Nutr., 1: 42-44.
4. Halliwell, B. and J.M. Gutteridge, 2015. Free Radicals In Biology And Medicine. 5th Edn., Oxford University Press, Oxford, England, USA., ISBN: 9780198717485, Pages: 905.
5. Shenoy, K. and A. Shirwaiker, 2002. Anti inflammatory and free radical scavenging studies of Hyptis suaveolens (Labiatae). Indian Drug, 39: 574-577.
6. Hu, F.B., 2003. Plant-based foods and prevention of cardiovascular disease: An overview. Am. J. Clin. Nutr., 78: 544S-551S.
7. Ikram, E.H.K., K.H. Eng, A.M.M. Jalil, A. Ismail and S. Idris *et al*, 2009. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. J. Food Compos. Anal., 22: 388-393.
8. Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.
9. Pisoschi, A.M. and G.P. Negulescu, 2011. Methods for total antioxidant activity determination: A review. Biochem. Anal. Biochem., 1: 106-115.
10. Ali, S.S., N. Kasoju, A. Luthra, A. Singh, H. Sharanabasava, A. Sahu and U. Bora, 2008. Indian medicinal herbs as sources of antioxidants. Food Res. Int., 41: 1-15.
11. di Matteo, V. and E. Esposito, 2003. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Curr. Drug Targets-CNS Neurol. Disord., 2: 95-107.
12. Kyriacou, M.C., D.I. Leskovar, G. Colla and Y. Rouphael, 2018. Watermelon and melon fruit quality: The genotypic and agro-environmental factors implicated. Sci. Horti., 234: 393-408.
13. Edwards, A.J., B.T. Vinyard, E.R. Wiley, E.D. Brown and J.K. Collins *et al*, 2003. Consumption of watermelon juice increases plasma concentrations of lycopene and β -carotene in humans. J. Nutr., 133: 1043-1050.
14. Mehra, M., V. Pasricha and R.K. Gupta, 2015. 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. J. Pharmacogn. Phytochem., 3: 98-102.
15. Romdhane, M.B., A. Haddar, I. Ghazala, K.B. Jeddou, C.B. Helbert and S. Ellouz-Chaabouni, 2017. Optimization of polysaccharides extraction from watermelon rinds: Structure, functional and biological activities. Food Chem., 216: 355-364.
16. Davis, A.R., J. Collins, W.W. Fish, Y. Tadmor, C.L. Webber and P. Perkins-Weazie, 2007. Rapid method for total carotenoid detection in canary yellow-fleshed watermelon. J. Food Sci., 72: S319-S323.
17. Tadmor, Y., S. King, A. Levi, A. Davis and A. Meir *et al*, 2005. Comparative fruit colouration in watermelon and tomato. Food Res. Int., 38: 837-841.
18. Lum, T., M. Connolly, A. Marx, J. Beidler and S. Hooshmand *et al*, 2019. Effects of fresh watermelon consumption on the acute satiety response and cardiometabolic risk factors in overweight and obese adults. Nutrients, 10: 3390/nu11030595
19. Choudhary, B.R., S.M. Haldhar, S.K. Maheshwari, R. Bhargava and S.K. Sharma, 2015. Phytochemicals and antioxidants in watermelon (*Citrullus lanatus*) genotypes under hot arid region. Indian J. Agric. Sci., 85: 414-417.
20. Rutkowski, M. and K. Grzegorz, 2007. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. Acta Sci. Pol., Technol. Aliment., 6: 17-28.
21. Achikanu, C.E., P.E. Eze-Steven, C.M. Ude and O.C. Ugwuokolie, 2013. Determination of the vitamin and mineral composition of common leafy vegetables in South Eastern Nigeria. Int. J. Curr. Microbiol. Appl. Sci., 2: 347-353.
22. Nagaraja, P., R.A. Vasantha and H.S. Yathirajan, 2002. Spectrophotometric determination of folic acid in pharmaceutical preparations by coupling reactions with iminodibenzyl or 3-aminophenol or sodium molybdate-pyrocatechol. Anal. Biochem., 307: 316-321.

23. Al-Shaalan N.H., 2015. Smart spectrophotometric methods for the simultaneous determination of vitamin B1 and B2 concentrations in complex mixtures. *Orient. J. Chem.*, 31: 2343-2349.
24. Barros, L., S. Falcao, P. Baptista, C. Freire, M. Vilas-Boas and I.C.F.R. Ferreira, 2008. Antioxidant activity of *Agaricus* sp. mushrooms by chemical, biochemical and electrochemical assays. *Food Chem.*, 111: 61-66.
25. Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.*, 4: 685-688.
26. Oluduro, A.O., 2012. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria. *Malaysian J. Microbiol.*, 8: 59-67.
27. Obadoni, B.O. and P.O. Ochuko, 2002. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci.*, 8: 203-208.
28. Ejikeme, C.M., C.S. Ezeonu and A.N. Eboatu, 2014. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta area of Nigeria. *Eur. Sci. J.*, 10: 247-270.
29. Ebrahimzadeh, M.A., S.M. Nabavi, S.F. Nabavi, F. Bahramian and A.R. Bekhradnia, 2010. Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *Angustifolius*, *V. Odorata*, *B. Hyrcana* and *C. Speciosum*. *Pak. J. Pharm. Sci.*, 23: 29-34.
30. Prousky, J.E., 2010. Vitamin B3 for depression: Case report and review of the literature. *J. Orthomol. Med.*, 25: 137-147.
31. Vunchi, M.A., A.N. Umar, M.A. King, A.A. Liman, G. Jeremiah and C.O. Aigbe, 2011. Proximate, vitamins and mineral composition of *Vitex doniana* (black plum) fruit pulp. *Niger. J. Basic Appl. Sci.*, 19: 97-101.
32. Mander, L. and H.W. Liu, 2010. *Comprehensive Natural Products II: Chemistry and Biology*. Elsevier Science, Netherlands, ISBN: 9780080453828, Pages: 7388.
33. Nnenna, A.O., A. Okwudili, A.K. Kelechi, O.C. Kenekukwu, U.I. Izuchukwu and E.M. Mayer, 2020. Nutritional profile, bioactive compound content and antioxidant activity of ethanol leaf extract of eucalyptus tereticornis. *Eur. J. Biomed. Pharm. sci.*, 7: 61-73.
34. Lukaski, H.C., 2004. Vitamin and mineral status: Effects on physical performance. *Nutrition*, 20: 632-644.
35. Achikanu, C.E., O.N. Ani and E.I. Akpata, 2020. Proximate, vitamin and phytochemical composition of cucumis metuliferus seed. *Int. J. Food Sci. Nutr.*, 5: 20-24.
36. Jeon, Y.H., 2013. Vitamin E, an antioxidant, as a possible therapeutic agent for treating pain. *Korean J. Pain*, 26: 314-315.
37. Gulcin, I., M. Elmastas and H.Y. Aboul-Enein, 2007. Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies. *Phytother. Res.*, 21: 354-361.
38. Wadood, A., M. Ghufuran, S.B. Jamal, M. Naeem, A. Khan and R. Ghaffar, 2013. Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochem. Anal. Biochem.*, Vol. 2. 10.4172/2161-1009.1000144.
39. Patel, S., 2016. Plant-derived cardiac glycosides: Role in heart ailments and cancer management. *Biomed. Pharmacother.*, 84: 1036-1041.
40. Luciano, M., 2004. *Encyclopedia of Endocrine Diseases*. Elsevier Academic Press, Netherlands, ISBN: 9780124755734, Pages: 2400.
41. Riganti, C., I. Campia, J. Kopecka, E. Gazzano and S. Doublier *et al.*, 2011. Pleiotropic effects of cardioactive glycosides. *Curr. Medic. Chem.*, 18: 872-885.
42. Shi, J., K. Arunasalam, D. Yeung, Y. Kakuda, G. Mittal and Y. Jiang, 2004. Saponins from edible legumes: Chemistry, processing and health benefits. *J. Medic. Food*, 7: 67-78.
43. Owolabi, O.A., D.B. James, A.B. Ibrahim, O.F. Folorunsho, I. Bwalla and F. Akanta, 2010. Changes in lipid profile of aqueous and ethanolic extract of *Blighia sapida* in rats. *Asian J. Med. Sci.*, 2: 177-180.
44. Dudareva, N., E. Pichersky and J. Gershenzon, 2004. Biochemistry of plant volatiles. *Plant Physiol.*, 135: 1893-1902.
45. Degenhardt, J., J. Gershenzon, I.T. Baldwin and A. Kessler, 2003. Attracting friends to feast on foes: Engineering terpene emission to make crop plants more attractive to herbivore enemies. *Curr. Opin. Biotechnol.*, 14: 169-176.
46. Kedare, S.B. and R.P. Singh, 2011. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.*, 48: 412-422.
47. Ebrahimzadeh M.A., S.M. Nabavi, S.F. Nabavi and B. Eslami, 2009. Free radical scavenging ability of methanolic extract of hyoscyamus *Squarrosus* leaves. *Pharmacologyonline*, 2: 796-802.
48. Murthy, K.N.C., A. Vanitha, M.M. Swamy, G.A. Ravishankar, 2003. Antioxidant and antimicrobial activity of *Cissus quadrangularis* L. *J. Med. Food*, 6: 99-105.
49. Sharififar, F., G. Dehghn-Nudeh and M. Mirtajaldini, 2009. Major flavonoids with antioxidant activity from *Teucrium polium*. *Food Chem.*, 112: 885-888.
50. Sharma, S. and A.P. Vig, 2013. Evaluation of *in vitro* antioxidant properties of methanol and aqueous extracts of *Parkinsonia aculeata* L. leaves. *Sci. World J.*, 2013: 1-7.
51. Nabavi, S.M., M.A. Ebrahimzadeh, S.F. Nabavi, M. Fazelian and B. Eslami, 2009. *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Pharm. Mag.*, 5: 122-126.

52. Abbasi, M. A., H. Saleem, Aziz-ur-Rehman, T. Riaz and M. Ajaib, 2013. Determination of antioxidant activity and phytoconstituent screening of *Euphorbia heterophylla* Linn. Br. J. Pharmaceut. Res., 3: 202-216.
53. Ayala, A., M.F. Munoz and S. Arguelles, 2014. Lipid peroxidation: Production, metabolism and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-nonenal. Oxid. Med. Cell. Longevity, Vol. 2014. 10.1155/2014/360438
54. Yin, H., L. Xu and N.A. Porter, 2011. Free radical lipid peroxidation: Mechanisms and analysis. Chem. Rev., 111: 5944-5972.