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Phytochemical composition and free radical scavenging activity of *Cucurbita maxima* fruit juice

Research Article

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Abstract

Phytochemical constituents and antioxidant potentials of *Cucurbita maxima* fruit juice was evaluated using standard methods. The result obtained showed that *Cucurbita maxima* fruit juice contains some phytochemicals such as flavonoids and tannins in high concentration, phenols, and saponins in moderate concentration and terpenoids in low concentration. Alkaloids and steroids were not detected. The percentage inhibition of DPPH radical by *Cucurbita maxima* fruit juice was concentration dependent with IC_{50} of 50.54±2.22 µg/ml relative to the standard (ascorbic acid) with IC_{50} of 26.88±0.57 µg/ml. The nitric oxide radical scavenging activity of the juice was dose dependent with IC_{50} of 79.54±1.27 µg/ml relative to the standard with IC_{50} of 32.1 ±3.79 µg/ml. The ferric reducing power of the juice was also concentration dependent with a half maximal inhibitory concentration (IC_{50}) of 83.58 ± 5.37 µg/ml relative to standard (gallic acid) with an IC_{50} of 60.64 ± 2.81µg/ml. Our result suggests that the use of *Cucurbita maxma* in traditional medicine could be attributed to its phytochemical constituents. We therefore conclude that *Cucurbita maxima* fruit juice could serve as component of a promising nutritional and therapeutic preparation.

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Public Interest Statement:

Cucurbita maxima fruit pulp is commonly consumed as vegetable in eastern Nigeria. Most people enjoy it as a component of yam porridge. There is also a claim that the fruit juice possesses some medicinal properties and thus accounts for its use in traditional medicine. This study therefore, evaluated the phytochemistry and antioxidant profile of the plant to ascertain its role in human nutrition and disease as well as provide, as much as possible, scientific explanation for its use in traditional medicine.

1. Introduction

Every human cell generates reactive oxygen species during its metabolic activities. Efficient defensive mechanisms exist to cushion the damaging effects of these reactive species on cellular components. Oxidative stress results when the amount of the reactive oxygen species generated during cellular metabolism outweighs the defensive mechanisms (Wood *et al*., 2003). Several pathologies ranging from cancer to other age-related diseases are known to be related to oxidative stress (Savas, 2012).

Antioxidants are molecules that possess the ability to scavenge, mop-up and neutralize the reactive oxygen species (ROS). Plants have been reported to possess such effect due their rich arsenal of phenolic components like flavonoids, phenolic acids and phenolic diterpenes (Kwee and Niemeyer, 2011). These phenolic compounds, through their inherent ability to donate hydrogen ion to the unstable radicals, convert these radicals to stable non-radical products.

The use of medicinal plants in health care delivery has gained a considerable attention recently due to the level of application in human health (Ayoola*et al*., 2008). According to Madueke and Anosike (2017), among the host of other research reports, the medicinal properties of plant components are attributed to their phytochemical constituents. *Cucurbita maxima*, winter squash, or pumpkin is a species in the gourd family, *Cucurbitaceae* (Burrows and Tyrl, 2013). It is one of such plant that is frequently used as food as well as traditional medicine. Both of its fruits and the aerial parts are commonly consumed as vegetable. The plant has shown high levels of proteins, polysaccharides, sterols, fixed oils and paraamino-benzoic acids in all plant parts (Velioglu*et al*., 1998; Caili*et al*., 2006). It is therefore, necessary to evaluate the phytochemical composition of *Cucurbita maxima* as well as its antioxidant potential to ascertain its role in human nutrition and health.

2. Materials and Methods

2.1 Plant Materials

The fruits of *Curcubita maxima* were used for the study. Fruit samples were collected from Odoru, Nsukka Local Government Area of Enugu state and identified by a taxonomist from department of

plant science and biotechnology, university of Nigeria, Nsukka. The fruit was washed under running water and homogenized (daily before administration) and extracted using Crown Star 7-in-1 Juice extractor and used without further dilution.

2.2 Qualitative Phytochemical Analysis of *Cucurbita maxima* fruit juice

Phytochemical analysis of *Cucurbita maxima* fruit juice was carried out following the methods of Harborne (1998) and Trease and Evans (2002).

2.3 DPPH Radical Scavenging Activity

The free radical scavenging capacity of *Curcubita maxima* fruit juice was determined following the method of Gyamfi *et al.* (1999). DPPH (200µM) solution was prepared in 95% methanol. Fresh juice, 20, 40, 60, 80, and 100µg/ ml were taken in five test tubes. Freshly prepared DPPH solution (0.5ml) was incubated with test drug for 10 minutes and absorbance read at 517 nm. Standard ascorbic acid was used as reference. Calculation of % scavenging of the DPPH free radical was evaluated as;

DPPH Scavenging activity (%) = $\frac{control-test}{control}$ x 100

2.4 Nitric Oxide Radical Scavenging Activity

The Nitric Oxide scavenging activity of *Curcubita maxima* fruit juice was determined according to the method described by Vijayakumar **(**2015**).** About 5 mM of Sodium nitroprusside in standard phosphate buffer solution was incubated with different concentrations (200-1000µg/ml) of the fruit juice dissolved in phosphate buffer (0.025 M, pH 7.4) at 25 ^{0}C for 5 hours. Control tube was maintained in an identical manner. After 5 hours, 0.5ml of the incubated solution was removed and diluted with 0.5ml of Griess reagent (1% sulfanilic acid, 5 % phosphoric acid, and 0.1 % naphthylethylenediaminedihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite ions with suphanilic acid and its subsequent coupling with Napthylethylenediamine was read at 546 nm.

NO_2 Scavenging activity was calculated thus;

NO_2 Scavenging activity (%) = $\frac{control-test}{control}$ x 100

2.5 Ferric Reducing Antioxidant Power Assay

The reducing power was determined according to the method used by Vijayakumar *et al.* (2015). A quantity (1 ml) of varying concentrations (20-100 µg/ml) of the fruit juice was mixed with 2.5 ml phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Aliquots of 2.5 ml of trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10min. The upper layer of the solution (2.5 ml) was mixed with equal

volume of distilled water. To this, 0.5ml of freshly prepared 1% ferric chloride solution was added and the absorbance was measured at 700 nm against a blank. Gallic acid was used as a standard for comparison of the activity. Increased absorbance of the reaction mixture indicated an increase of reduction capability.

$$(\%)\ \text{increase in reducing power} = \frac{\text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

2.6 Statistical Analysis

Data are expressed as mean ± SD of triplicate experiments. Duncan multiple test range was used to compare the means obtained from each sample.

3. Results

3.1 Qualitative and Quantitative Phytochemical Composition of *Cucurbita maxima* Fruit Juice

The result of the phytochemical analysis shows that *Cucurbita maxima* fruit juice contains some phytochemicals such as flavonoids and tannins in high concentration, phenols, and saponins in moderate concentration and terpenoids in low concentration. Alkaloids and steroids were not detected (Table 1).

Table 1: Qualitative and Quantitative Phytochemical Composition of *Cucurbita maxima* Fruit Juice

Phytochemical	Inference	Concentration(mg/ml)
Flavonoids	+++	11.96±0.04
Phenols	++	5.06±0.31
Alkaloids	ND	ND
Terpenoids	+	2.35±0.58
Steroids	ND	ND
Saponins	++	4.07±1.01
Tannins	+++	11.30±1.09

Key:+Low Concentration; ++ Moderate Concentration;+++ High Concentration; ND Not detected

3.2 DPPH radical scavenging activity of *Cucurbita maxima* fruit Juice

The *Cucurbita maxima* fruit juice showed a substantial dose-dependent antioxidant activity and the activity was comparable to that of ascorbic acid, which was used as a control antioxidant. The IC_{50} value represents the concentration of the juice or standard that caused 50% inhibition of the radical.

Table 2: DPPH radical scavenging activity of *Cucurbita maxima* fruitjuice.

Concentration (µg/ml)	%Inhibition by the juice	% Inhibition by the Standard
20	33.08 ± 2.5	48.11 ± 1.14
40	44.07 ±1.33	58.64 ± 1.58
60	56.06 ±1.37	61.74 ± 2.69
80	66.74 ± 0.50	86.74 ± 0.58
100	75.12 ± 0.88	89.37 ± 2.53
IC50 (µg/ml)	50.54 ± 2.22	26.88 ± 0.57

Values are expressed as mean ± standard deviation (n=3)

3.3 Nitric Oxide Radical Scavenging Activity

The result showed a concentration dependent nitric oxide radical scavenging activity of the juice with a half maximal inhibitory concentration (IC_{50}) of 79.54 ±1.27 µg/ml compared to the standard (ascorbic acid) with IC_{50} of 32.16 ± 3.79µg/ml.

Table 3: Nitric Oxide Radical Scavenging Activity.

Concentration (µg/ml)	% Inhibition by Juice	% Inhibition by the Standard
20	13.10 ± 1.38	35.43 ± 2.81
40	21.84±1.44	57.93 ± 1.24
60	37.24± 4.19	71.72 ± 2.12
80	53.10± 2.07	76.55 ± 1.42
100	61.38± 1.83	84.21 ± 0.14
IC50 (µg/ml)	79.54 ±1.27	32.16 ± 3.79

Values are expressed as mean ± standard deviation (n=3)

3.4 Ferric Reducing Antioxidant Power

The result shows a concentration dependent ferric reducing power of the plant with IC_{50} of 83.58 ±5.37 µg/ml compared to the standard (gallic acid) with IC_{50} of 60.64 ± 2.81µg/ml.

Table 4: Ferric Reducing Antioxidant Power

Concentration (µg/ml)	% Inhibition by juice	% Inhibition by Standard
20	25.19± 0.32	39.28 ± 1.21
40	31.86±0.53	43.01 ± 2.12
60	40.58± 1.61	47.98 ± 1.01
80	51.07± 4.13	56.90 ± 0.23
100	55.04± 0.53	62.02 ± 1.21
IC50 (µg/ml)	83.58 ±5.37	60.64 ± 2.81

Values are expressed as mean ± standard deviation (n=3)

4. Discussion

The phytochemical analysis revealed the presence of flavonoids, saponins, phenols, terpenoids, and tannins. The antioxidant potentials of flavonoids are well known. They have been reported to possess several biological activities such as anti-microbial, cytotoxic and anti-tumor activities (Shirsat *et al.*, 2012; Teiten *et al.*, 2013). Saponins possess inhibitory effects against inflammatory mediators like serotonin and histamine in addition to its antioxidant activities. (Sayyah *et al.,* 2004; Tatiya *et al.,* 2007). The antioxidant potentials of phenolic compounds occur due to their ability to inhibit lipoxygenases thereby retarding the peroxidation of membrane lipids (Dai and Mumper, 2010). The phytochemical screening result of this study is contrary to the report of Peninah *et al.* (2018) who reported the absence of phenolics, tannins and saponins but reported the presence of terpenoids and flavonoids and the absence of glycosides and alkaloids in the pulp of *Cucurbita maxima* which is in line with the result of the present study. Our data suggests that the use of Cucurbita maxima in traditional medicine could be attributed to its reservoir of the various bioactive compounds as revealed by our analysis.

The antioxidant potentials of experimental samples could be routinely determined by the ability of the samples to scavenge DPPH *in vitro*. The free radical, DPPH, reacts with the antioxidant compounds in the sample due to the inherent ability of the antioxidant compounds present in the sample to donate hydrogen atom to DPPH leading to a color change, whose intensity is calorimetrically measured. There exists a direct relationship between the color intensity and the inhibition of DPPH. According to our data, as the concentration of our fruit juice increased, DPPH inhibition also increased with a maximum inhibition recorded at the concentration of 100 µg / ml. This observation points to the fact that the fruit pulp could possess some health benefits as a component of human diet.

A free radical produced from sodium nitroprusside, nitric oxide combines with oxygen to form nitrite. Our study measured the activity of our sample in inhibition of nitrite formation from nitric oxide. From our results, *Cucurbita maxima* fruit juice demonstrated significant inhibitory

activity against nitrite formation from nitric oxide. An explanation to this could be due to the ability of our sample components to react with oxygen and other compounds of nitrogen thus, inhibiting the oxidation of nitric oxide (Mathew and Abraham, 2006).

The reducing power gives an insight into the electron transfer potentials of the experimental sample. According to Meir *et al.* (1995), reducing power assay is used to determine ability of our sample components to reduce Fe^{3+} to Fe^{2+}. Our data show that *Cucurbita maxima* fruit juice reduced Iron (III) to Iron (II) in a concentration dependent fashion. This observation demonstrate the potentials of our sample in not only reducing Fe^{3+} in to Fe^{2+} but also minimize the oxidative damage to cellular components. This effect is particularly desirable of a fruit that is well consumed by rural population that is home to everyday stress.

5. Conclusion

Our results revealed that *Cucurbita maxima* fruit juice contains a considerable number of phytochemicals and demonstrated a promising DPPH radical scavenging activity, ferric reducing power and nitric oxide inhibition. We therefore conclude that *Cucurbita maxima* fruit juice could serve as component of a promising nutritional and therapeutic preparation.

6. Recommendation

Isolation and purification of various fractions of the fruit juice is hereby recommended as this could sponsor the identification of specific compounds with possible industrial and therapeutic applications.

Competing Interest: We declare no competing interests.

Bionote:

Anosike Chioma Asumpta is a senior lecturer in the department of biochemistry, university of Nigeria Nsukka. She is a researcher with several publications in both local and international journals. She is also a reviewer and has several undergraduate and postgraduate students supervised over the years. Her research areas include pharmacological biochemistry, nutritional biochemistry and drug delivery.

Madueke Augustine Chidi is a research and teaching assistant in the department of biochemistry, university of Nigeria Nsukka. He holds an MSc in pharmacological biochemistry with research interest in the areas of drug delivery, medicinal chemistry, nutritional biochemistry and molecular biology. He has published papers in local and international journals.

Ozioko Sylvester Chiedozie is a postgraduate student of biochemistry, university of Nigeria, Nsukka. His research interest is in nutritional biochemistry and medical biochemistry. He is a young researcher, passionate about unraveling the nutritional basis of disease management.

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