Original Research Article

Phytochemical and vitamin composition of *Cucumis metuliferus* juice

ABSTRACT

The pursuit of healthy lifestyle has led to increased demand for fruits and vegetables. Fruit juice in particular, is increasingly receiving attention because of the therapeutic benefits derived from its consumption. The bioactive compounds in fruit juice have various pharmacological applications. *Cucumis metuliferus* is a plant of the *Cucurbitaceae* family. The fruit has a high water content and sought after to quench thirst. The ethno-medicinal properties of the fruit requires investigation of all the parts in order to maximally utilize it. Therefore, in this study, the phytochemical and vitamin composition of the juice were investigated. The assays were conducted using standard methods. From the result, the phytochemicals present were phytate (0.33 ± 0.03%), alkaloids (0.48 ± 0.06%), saponins (0.19 ± 0.04%), cardiac glycosides (0.46 ± 0.16%), Oxalate (3.24 ± 0.38 mg/ml), tannins (108.79 ± 5.16 mg TAE/g) and phenols (0.06 ± 0.01 mg GAE/ml). The vitamin present were vitamin A (198.51 mg/kg), vitamin B_1 (0.09 mg/ml), Vitamin B_2 (0.11 mg/ml), Vitamin B_3 (0.07 mg/ml), vitamin B_6 (835.0 mg/ml), vitamin B_9 (2.089 mg/ml), vitamin B_{12} (0.1 mg/ml), Vitamin C (682.0 mg/ml), vitamin D (5.28 mg/ml), vitamin E (4.42 mg/ml) and vitamin K (2.4336 mg/ml). These results have shown that *Cucumis metuliferus* juice have pharmacological properties and could also be utilized as an alternative source of vitamins.

Keywords: *Cucumis, metuliferus*, juice, phytochemical, vitamin, fruit.

INTRODUCTION

Fruits and their juices are said to be among the most important foods for human, as their consumption maintains good health and replaces nutrient losses by the body [1]. Fruit juice is highly nutritious and plays an important role in a healthy diet as it offers a variety of micro-nutrients found on earth [2]. All over the world, especially in rural areas where fruits are mostly available, fresh fruits are seasonally consumed or in most areas throughout the year. In Nigeria, some fruits are seasonal while others are available all year round. These fruits are also carried from rural to urban areas or imported from other countries during the periods of abundance. Fruits are consumed in several forms such as fresh slices, added to dishes and beverages as well as processed as natural juice. Some fruits are grossly underused due to lack of information about their nutritional and medicinal values. Fruits are sources of vitamins, minerals as well as fibre and digestible carbohydrate [3]. In fact, most fruits have low energy density and are recommended for weight management. Studies show that fruits contain about 85% of water, very small varying amount of fats and protein, and a fair proportion of carbohydrate present as cellulose, starch in small quantity, vitamins and sugar [4]. The high fiber content of fruits and vegetables have beneficial effects on blood cholesterol and they play a crucial role in the prevention of large bowel diseases. Therefore, people who consume diet rich in fruits and vegetables are said to have significantly lower rates of many types of cancers [5].

Cucumis metuliferus is a member of *Cucurbitaceae* family [6]; commonly called African horned cucumber, jelly melon or Kiwano. In Nigeria it is called 'bùuràr zaàki', 'nòònòn-kuùraà' 'gautar kaji' in Hausa [7, 8]. It is mostly found in tropical Africa south of the Sahara down to Senegal, Nigeria, Namibia, Botswana, South Africa and Swaziland where it naturally grows [9]. The plant grows at an altitude of between 210 m and 1800 m above sea level. It flowers from January and bears fruits from February to July. The fruit is bright orange when ripe with a bright green, gelatinous flesh and tastes like cucumber and banana combined. It is eaten as snack in raw form as well as used in cooking [10]. The fruits occur in the bitter and non-bitter forms. The bitter forms are wild-growing plants and inedible, considered poisonous if eaten. This bitter form contains cucurbitacins, a highly toxic compound [11] known to have cytotoxic, anti-tumour and anti-inflammatory effects [12]. The non-bitter form which are sweet, less toxic and cultivated widely [13, 14]. This sweet form has been used in treatment of HIV /AIDS patients [6]. The fruit is highly sought after as a source of water from people of the Kalahari Desert [15] and a good source of energy with low caloric value. From studies it was shown that the juice contain essential minerals and amino acids [16]. Therefore, in addition to studies done so far, the phytochemical and vitamin content of the juice were investigated in order to ascertain its therapeutic and nutritional usefulness.

MATERIALS AND METHODS

Sample collection and identification

Fresh fruits of *Cucumis metuliferus* were purchased from a market in Gboko, Benue state, Nigeria and authenticated in the department of Applied Biochemistry, Enugu State University of Science and Technology, Enugu. Nigeria.

Sample preparation

The fruits of Cucumis metuliferus were rinsed thoroughly with distilled water and were cut into halves using kitchen knife. The pulp was scooped into a muslin cloth and the juice was squeezed out and used for the analysis.

PHYTOCHEMICAL SCREENING

Qualitative phytochemical screening

Quantitative phytochemical screening was carried out to investigate the various classes of natural compounds present in the extract. This was carried out according to the method of Sofowora [17, 18, 19].

Quantitative phytochemical screening

Quantitative phytochemical screening was carried out using standard methods.

Determination of total phenolic content

The phenol content was determined by using the slightly modified colorimetry method described by Barros et al. [20]. The juice (1 ml) 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 of the juice was determined using the method of Obadoni and Ochuko [21].

Five milliliter (5 ml) of the sample 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 hour with intermittent shaking. The mixture was filtered and the filtrate was reduced to 10 ml over water bath at 90^{o}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. 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 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 the using the slightly modified colorimetry method described by Barros et al. [20]. An aliquot, 0.5 ml of the juice was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ 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 [22]). An aliquot (0.5 ml) of 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 tube and the absorbance taken at 720 nm. Tannic acid was used as the standard and the results obtained was 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 [23]. Aliquots (2 ml) of the juice was measured into 250 ml conical flask. It was mixed with 100 ml of 2% concentrated HCl for 3 h. The sample was 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 it. 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:

$$\text{Phytic acid (\%)} = \frac{\text{Titre Value x 0.00195 x 1.195}}{2} \text{ x } 100$$

Determination of alkaloids

The alkaloid content of the juice was determined using the method of Harbone [24]. Five mililitre (5 ml) 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 hours 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 extract until the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). 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 [25]. Two milliliters (2 ml) 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 100^0c for 1 hour. It was cooled and then made up to 250 ml mark before filtration.

Duplicate portions of 125 ml of the filtrate are 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 90^0c, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90^0c and 10 ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and then centrifuge 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,

$$\frac{T \times (vme)(Df) \times 10^5}{(ME) \times Mf} \quad \text{(mg/ml)}$$

Where T is the titre of $KMno_4$ (ml), Vme is the volume – mass equivalent (i.e. 1ml of 0.05M KMno4 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 KMno4 in oxalate (KMo4 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 [22]. In this method 200 ml of distilled water was added to 5 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 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 NH_4OH 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 = $\frac{Tv\ x\ 1{,}08\ x\ EV}{SM\ x\ Al}$ x 100

TV= Titre value (ml);

EV= extract vol(ml)

SM= sample mass (g)

AL= alioquot (ml) used

Note: 1cm3 of 0.02N $AgNO_3$ = 1.8 mg HCN.

VITAMIN ANALYSIS

Vitamin A was determined by the calorimetric method of Kirk and Sawyer [26]. Vitamins B1, B2, B3, B6, B9 and B12 were determined spectrophotometrically according to the standard method of AOAC [27]. Vitamin C was determined by the titrimetric method according to Kirk and Sawyer[26]. Vitamin E was determined by the futter-mayer colorimetric method with association of vitamin chemist‟s by Kirk and Sawyer [26]. Vitamins D and K were determined according to the method described by Zakara et al [28].

Statistical Analysis

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

RESULTS

Phytochemical composition of Cucumis metuliferus juice

Table 1 shows the qualitative and quantitative phytochemical composition of Cucumis metuliferus juice. It revealed that Tannins was highly present, Oxalate was found to be moderately present; while Saponins, Alkaloids, Phytate and Cardiac glycosides were slightly present.

Table 1: Phytochemical Composition of Cucumis metuliferus juice

Phytochemicals	Qualitative concentration	Quantitative concentration
Phytate (%)	+	0.33 ± 0.03
Alkaloids (%)	+	0.48 ± 0.06
Saponins (%)	+	0.19 ± 0.04
Cardiac glycosides (%)	+	0.46± 0.16
Oxalate (mg/ml)	++	3.24 ± 0.38
Tannins (mg TAE/ml)	+++	108.79 ± 5.16
Total Phenols (mg GAE/ml)	+	0.06 ± 0.01

+= Slightly present, ++= moderately present, +++= highly present.

Vitamin Composition

The result of the vitamin analysis revealed that *Cucumis metuliferus* juice contains high amount of Vitamins B6, C and A. Vitamins D, K and B9 were present in moderate amount while vitamins B1, B2, B3, B12 and E were present in trace amounts (Table 2).

Table 2: Vitamin Composition of Cucumis metuliferus juice.

Vitamins	Concentration (mg/ml)
Vitamin A	198.51
Vitamin B1	0.0912
Vitamin B2	0.11
Vitamin B3	0.0734
Vitamin B6	835.0
Vitamin B9	2.089
Vitamin B12	0.1
Vitamin C	682
Vitamin D	5.28
Vitamin E	0.42
Vitamin K	2.4336

DISCUSSION

Cucumis metuliferus fruit is a versatile fruit with numerous nutritional importance. This study on the juice revealed the presence of phytochemicals considered as active medicinal constituents. From the result it was shown that tannin was the most abundant phytochemical at the concentration of 108.79 ± 5.16 mg/ml. Tannins are oligomers of flavan-3-ols and flavan-3, 4-diols that are concentrated in the bran fraction of legumes [29]. Plant tannins are a major group of antioxidant polyphenols found in food with multifunctional properties to human health and have been reported to possess some medicinal properties [30]. Its wound healing properties which include anti-inflammatory and analgesic properties [31] have been reported, although they are anti-nutrients [32]. Tannins exhibit antinutritional properties by impairing the digestion of various nutrients and preventing the body from absorbing beneficial bioavailable substances [33]. Tannins can also bind and form complexes with proteins. Theses complexes may cause inactivation of digestive enzymes and reduction in protein digestibility caused by protein substrate and ionisable iron interaction [34]. The high composition of Tannin in Cucumis metuliferus juice shows that the juice could be useful therapeutically for wound treatment. The second most abundant phytochemical in the *Cucumis metuliferus* juice was oxalate (3.24 ± 0.38 mg/ml). Oxalate is also an antinutrient. Oxalic acids form soluble (with potassium and sodium) or insoluble (with calcium, magnesium, iron) salts or esters called oxalates that are commonly found in plants or synthesized in the body [35]. These insoluble salts cannot be processed out of the urinary tract once processed through the digestive system. Calcium oxalate can have a deleterious effect on human nutrition and health by accumulating as kidney stones [36]. Other phytochemicals present in the juice were phytate, alkaloids, saponin, cardiac glycoside and phenol. Phytate is said to be the major storage form of phosphorus especially in leafy vegetables [37]. However, phytase; the digestive enzyme can unlock the phosphorus stored as phytic acid. But in the absence of phytase, phytic acid can hinder the absorption of other minerals such as iron, magnesium, zinc and calcium by binding and forming complexes with them [38]. This results in highly insoluble salts that are poorly absorbed by the gastrointestinal tract leading to lower bioavailability of minerals. However, it is an antioxidant by inhibiting iron-mediated free radical generation [39]. Alkaloids possess various pharmacological activities such antihypertensive, antiarrhythmic, antimalarial and anticancer activity [40]. Alkaloids isolated in its pure form and their synthetic compounds have been used as an analgesic, antispasmodic and bactericidal agents [41]. Plants having alkaloids are used in medicines for reducing headache and fever [42]. These are attributed to their antibacterial and analgesic properties [43]. Alkaloids have been reported to act as central nervous system stimulant [44]. Saponins are also anitinutrients with a bitter taste and are toxic in high concentrations. They can affect nutrient absorption by inhibiting enzymes as well as by bind with nutrients such as zinc. Saponins are naturally occurring substances with various biological effects. They have hypocholesterolemic and hypoglycemic effects [45, 46]. They also impair digestion of protein, vitamins and minerals uptake in the gut, as well as lead to the development of a leaky gut [47]. Cardiac glycosides have been reported to inhibit the membrane-bound Na^+- K^+-ATPase pump responsible for Na^+-K^+ exchange, and are used as heart drugs [48]. The phenol content was very low in the concentration of 0.06 ± 0.01 mg/ml. Phenolic compounds are metabolites with antioxidant activity and act as free radical scavengers [49].

From the result of vitamin analysis in Table 2, it was shown that the juice contains very high amount of vitamins B6, C and A. Others were present in moderate amounts. These vitamins are important in human health playing protective roles and participate in energy production. The B vitamins are coenzymes needed in the metabolism of carbohydrates, amino acids and fatty acids. Vitamins C and E are antioxidants which protect the cell membranes from free radicals oxidative damages [50]. Vitamin C in addition to its antioxidant role, possesses several health benefits [51].Vitamin C is also required for maintenance of normal connective tissues, wound healing and as well as facilitate the absorption of dietary iron from the intestine [52]. Vitamin E is known as anti-sterility vitamin and important in reproduction, in the development and normal functioning of the red blood cell and muscles [53, 54]. Vitamin D is needed for increase intestinal uptake of calcium, phosphate and magnesium [55].

CONCLUSION

From this study, it has been shown that *Cucumis metuliferus* juice contains important secondary metabolites which are of great health benefits. This finding suggest that the juice could be utilized as nutraceutics and it is a good source of vitamins being high in a number of them.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

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