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Original Article

Assessment of the Phytochemical Composition and Antioxidant Properties of Ripe Fruit of *Cucumis metuliferus* E. Meyer Ex. Naudin

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ABSTRACT

Background: Plants' secondary metabolites have great potential as anti-oxidants for slowing or alleviating various diseases. *Cucumis metuliferus* E. Mey. Ex. Naudin Curcubitaceae has been consumed by man and animals as food and used as alternative treatment for some diseases by traditional farmers. However, there is little information on its phytochemical composition and antioxidant properties. This study was carried out to investigate the quantitative phytochemicals and *in vitro* anti-oxidant activity of the ripe fruits of *Cucumis metuliferus* using DPPH radical scavenging assays. **Materials and Methods:** The ripe fruits of *Cucumis metuliferus* were collected on the parent plants in Vom, Jos South Local Government, Plateau State and were dried at room temperature. The ground powder of *Cucumis metuliferus* was made and divided into 3 portions. The first portion was extracted using 80% methanol: distilled water (80:20) to obtain the 80% methanol extract. The second portion of the ground fruits was extracted using distilled water to obtain the Aqueous Extract (AE). The third portion of the ground fruits was sequentially extracted with solvents of different polarities. First, n-hexane was used and the marc from n-hexane was air-dried and then extracted using chloroform, followed by ethyl acetate, methanol and water in that order (each time drying the marc for the subsequent extraction) to obtain the HE, CE, EAE, ME and RE extracts respectively. The extracts were screened for secondary metabolites and evaluated for their *in vitro* anti-oxidant activity using DPPH radical reduction assays. **Results:** The *Cucumis metuliferus* extract contained carbohydrates, cardiac glycosides, flavonoids, steroids, quinones, coumarins, lignins, phenols, alkaloids, phlobatannins, saponins, tannins, terpenoids and volatile oils. The extracts showed anti-oxidant activity, with IC_{50} values ranging between 537.56-2579.91 µg/mL. **Conclusion:** The presence of these phytochemical classes in the ripe *Cucumis metuliferus* fruit and the relatively high anti-oxidant activity reported in this study supports its folkloric use in the treatment of diseases and as an anti-oxidant agent.

Keywords: Phytochemical screening, DPPH, Anti-oxidant activity, *Cucumis metuliferus*.

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INTRODUCTION

Plants have been a source of therapeutic agents in many pharmacological studies. Research is ongoing for the identification, isolation and purification of the bioactive compounds isolated from various plants for leads for multiple medical applications.[1,2] The Cucumis plant family contains some bioactive compounds such as cucurbitacins, sphingolipids, phenols, essential oils, vitamins and minerals that possesses some pharmacological actions in the treatment of hypertension, diabetes mellitus, cancer and oxidative stress.[3-6] Previous phytochemical studies using *Cucumis metuliferus* 70% ethanol extract and methanol extract showed the presence of various bioactive molecules including carbohydrates, cardiac glycosides, flavonoids, steroids, phenols, alkaloids, saponins, tannins and terpenoids.[7,8] These phytoconstituents could improve the well-being of consumers or cause detrimental effects especially when ingested in large amounts. Hence, this study further screened phytochemical constituents from other extracts (100% aqueous, 80% methanol and ethyl acetate) of the ripe fruit of *Cucumis metuliferus* to address the knowledge gap.

Cucumis metuliferus is a climber, or less frequently a trailing herb that grows annually. It is characterized by hairy rough vegetative parts and heart-shaped lobes. The flowers are monoecious or diecious and may be yellow or white in color. The fruits are

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elongated and triangular in shape and usually green in colour when unripe but turns yellow or orange-red when ripe.[9] The seeds are flattened and deeply buried in the jelly-like pulp. *Cucumis metuliferus* grows naturally in tropical and south Saharan Africa, such as Botswana, Namibia, Nigeria, Senegal, South Africa and Swaziland. In Nigeria, it is commonly found in the Northern part of the Country including Jos, Plateau State. The genus *Cucumis* has more than 32 species, of which two species (*Cucumis anguria* L., and *Cucumis metuliferus* have been reported to have significant nutritional and medicinal values.[9,10] Of these 32 species, *Cucumis sativus* L. and *Cucumis melo* L. have been widely studied[11-16] in relation to bioactive compounds. However, there are fewer studies on *Cucumis metuliferus* phytochemical status, anti-oxidant profiling and toxicity.[7,8] A few studies have shown that Cucumis *metuliferus* has antiviral,[17,18] antibacterial,[19] antifungal,[20] antiparasitic,[21,22] antiulcer,[23] antihyperglycaemic[24-26] and antioxidant activities.[7] These activities could be associated with the presence of free radical compounds in the plant.

Free radicals are gaining importance in ascertaining many physiological and pathophysiological processes. Most of the oxygen-derived free radicals also known as the Reactive Oxygen Species (ROS) are meant for normal physiological processes such as body homeostasis and defense against microorganisms. However, reduced ingestion, absorption and utilization of antioxidants could lead to oxidative stress causing damage to cellular tissues and change or modification of DNA, lipids and proteins resulting in cell death and subsequent ill health.[27-29] Although the relationship between oxidative stress and the commencement of diseases including diabetes, cardiovascular disease and cancer remains a big challenge, because it could not be ascertained as to which comes first, the disease, or the oxidative stress. Nonetheless, anti-oxidants play a vital role in alleviating the onset of such diseases or delaying their complications.[30] Although the antioxidant activity of 70% ethanol extract of *C. metuliferus* has been reported,[7] this study examined the radical scavenging activity of other extracts of the ripe fruits of *C. metuliferus* in order to address the knowledge gap.

The use of synthetic drugs for the treatment of stress-related diseases is not without unwanted side effects such as nausea, vomiting, diarrhea and hypertension.[31,32] Therefore, the search for a safer natural product is ongoing. This study explores the phytochemistry and anti-oxidant profiles of both polar and non-polar extracts of *Cucumis metuliferus* fruit.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used were of analytical grade. Solvents used (n-hexane, chloroform, ethyl acetate and methanol) and L-ascorbic acid were of Loba Chemie Pvt. Ltd., Mumbai, India. DPPH was of Sigma-Aldrich, USA.

Plant Collection, Identification and Extraction

Ripe fruits of *Cucumis metuliferus* were collected in the environment of the National Veterinary Research Institute (NVRI), Vom, Jos South Local Government Area, Plateau State, Nigeria. Identification and authentication of the plant was done by a plant taxonomist at the Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos, Nigeria and a voucher number juhn19000296 was assigned. The fruits were washed and air-dried completely at room temperature; and then grounded into powdered form using mortar and pestle. The pulverized plant was kept in an air-tight container until required for extraction.

Plant Extraction

Direct and sequential cold maceration method of extraction as described by John *et al.*[33] and Sofowora[34] was adopted in this study.

Preparation of Crude 80% Methanol (80% ME) and Aqueous Extracts (AE)

Seven hundred sixty-three and two-hundredths grams (763.20 g) of the pulverized fruit was extracted with 3000 mL of 80% methanol (2400 mL methanol: 600 mL distilled water) at room temperature for 72 hr with periodic agitation. Four hundred six and six-hundredths grams (406.60 g) of the ground fruits was extracted with 5200 mL of distilled water at 4°C for 72 hr with periodic agitation. Each mixture was filtered using 850 µm and 150 µm pore sizes laboratory sieve, followed by absorbent cotton wool and finally Whatman No. 1 filter paper. The filtrate from the 80% methanol extract was dried in the oven at 50.5°C until completely dried and labelled 80% ME. The filtrate from the distilled water extract was dried at 45°C to obtain crude Aqueous Extract (AE). The yields were calculated and then stored in an air-tight container until needed.[33-34]

Sequential Plant Extraction with Solvents of Different Polarities

The sequential extraction method was used to extract the dried ground fruit with solvents of different polarities (n-hexane, chloroform, ethyl acetate, methanol and water) at room or refrigeration temperature for 72 hr with periodic agitation. Briefly, two thousand six hundred ninety-two and one-hundredths grams (2,692.10 g) of the ground fruit was soaked in 5250 mL of n-hexane and then filtered as described above (in direct extraction). The filtrate was placed under a constant flow of air using a laboratory fan to obtain the n-Hexane Extract (HE). The marc from n-hexane (2,445.95 g,) above was air-dried and used to extract with 5200 mL of chloroform to obtain the Chloroform Extract (CE). The marc from chloroform (1,782.80 g) above was air-dried and then extracted with 3700 mL of ethyl acetate to obtain the Ethyl Acetate Extract (EAE). The marc from ethyl acetate (1345.10 g) above was air-dried and then extracted with

3000mL of methanol to obtain the Methanol Extract (ME). The marc from methanol (753.21 g) above was air-dried and then extracted with 3000 mL of distilled water at 4°C to obtain the Residual Extract (RE). The yields were calculated and each extract was stored in an air-tight bottle until used.[8,33]

Test for Antioxidant Activity

The method described by Elisha *et al.*[35] was used to test for Antioxidant activity. A DPPH (2,2-diphenyl-1-picrylhydrazyl) working solution was prepared by making a 0.1 mM solution dissolved in methanol. A varying concentration of the extract (40-400 µg/mL) was prepared. Four parts DPPH solution (800 µL) to one part of extract sample (200 µL) was mixed in a test tube and the absorbance was taken at 517 nm. This was carried out in triplicate and the average was taken. DPPH without antioxidants was the control, while ascorbic acid was used as positive control. The concentration of the extract required to reduce the DPPH radical by 50% (percent radical scavenging activity) was calculated and the IC_{50} was also determined as follows:

$$\% \text{ RSA}=[(\text{AC} - \text{AS})/\text{AC})] \times 100$$

Where,

RSA=Percent Radical Scavenging Activity, AC=absorbance of the control, AS=absorbance of the sample.

The IC_{50} values were determined from a graph plotted from the percent inhibition against the extract concentration using the linear regression formula, y=mx+b,

$$IC_{50}=(50-b)/m$$

Where,

m=slope, b=intercept, y=concentration (50% inhibition), x=IC_{50}

Data Analysis

Data analysis was carried out using SPSS (Statistical Package for Social Sciences) IBM® version 25 for the one-way analysis of variance (ANOVA. Results were presented as mean±SEM, where $p \leq 0.05$ was considered significant.

RESULTS

Percentage Yield of Direct and Sequential Extractions

The result of percentage yield of extracts is presented in Table 1. The percentage yields obtained from the direct extraction of the ripe fruits of *Cucumis metuliferus* with distilled water and 80% methanol, showed Aqueous Extract (AE) to have a higher percentage yield of 22%, while 80% methanol (80% ME) yielded 12.31%. The value percent yields of sequential extraction showed residual extract (10.18%) to have the highest percent yield, followed by methanol extract (7.38%), hexane extract (4.22%), chloroform extract (1.87%) and the least percent yield was ethyl acetate extract (1.03%).

Phytochemical Screening

The result of phytochemical screening is shown in Table 2. The phytochemical study revealed that the Ground Powder (GP) and all extracts [n-hexane (HE), Chloroform (CE), Ethyl Acetate (EAE), Methanol (ME), Residual (RE), 80% Methanol (80% ME) and Aqueous (AE)] contained carbohydrates, cardiac glycosides, flavonoids, steroids, terpenoids and quinones. Alkaloids were identified in all except CE and ME. Coumarin was detected in all extracts except HE. Lignins and phenols were detected in all extracts except CE. Phlobatannins were only detected in GP, AE and EAE. Saponins were detected in GP and RE only. Tannins were detected in all except HE, CE and EAE. Volatile oils were only detected in AE and HE. Anthocyanins and anthraquinones were not detected in the GP or the extracts.

Antioxidant Activity

Table 3 shows the antioxidant activity of *C. metuliferus* fruit extracts. The 80% ME had the highest percent Radical Scavenging Activity (RSA) with an IC_{50} of 537.56 µg/mL, followed by ME that had IC_{50} of 594.78 µg/mL. The RE showed the least antioxidant activity with an IC_{50} of 2579.91 µg/mL. The antioxidant activity of the crude fruit extract were lower than that of ascorbic acid which had an IC_{50} of 1.45 µg/mL.

DISCUSSION

The result of direct extraction revealed that the aqueous extract had a higher percent yield than the 80% methanol extract, this result shows that water is a better solvent for the extraction of *C. metuliferus* which is in agreement with the findings of Gonfa *et al.*,[36] who documented that the addition of water increased percent yield of plant material. In the sequential extraction, the result showed residual extract to have a higher percent yield (10.18%), this finding seems to be consistent and similar to our previous sequential extraction where Crude Aqueous Extract

Table 1: The percentage yield of crude extracts of the ripe fruits of *Cucumis metuliferus*.

Extraction	Extract	Yield (%)
Direct	AE	22
	80% ME	12.31
Sequential	HE	4.22
	CE	1.87
	EAE	1.03
	ME	7.38
	RE	10.18

Key: 80% ME=80% Methanol extract, AE=Aqueous extract, HE=n-Hexane extract; CE=Chloroform extract; EAE=Ethyl acetate extract; ME=Methanol extract; RE=Residual extract.

Table 2: Phytochemical screening of the powdered and the crude extracts of *Cucumis metuliferus* Fruit.

		Inference							
Phytochemical constituents	**Type of test**	**GP**	**HE**	**CE**	**EAE**	**ME**	**RE**	**80% ME**	**AE**
Anthocyanins		−	−	−	−	−	−	−	−
Anthraquinones	Free anthraquinone	−	−	−	−	−	−	−	−
	Combined anthraquinone	−	−	−	−	−	−	−	−
Alkaloids	Dragendorff's	−	−	−	+	−	−	−	−
	Mayer's	+	+	−	+	−	+	+	+
	Wagners	+	+	−	−	−	−	−	−
	Hager's	−	+	−	+	−	+	+	+
	Tannic acid	+	+	−	+	−	−	+	−
	Scheibler's	−	−	−	+	−	+	+	+
Coumarins	Sodium hydroxide	+	−	+	+	+	+	+	+
Carbohydrates	Molisch's (general test)	+	−	+	−	−	−	−	−
	Fehling's (free reducing sugars)	+	+	−	−	+	+	−	−
	Fehling's (combined reducing sugars)	−	+	−	−	+	−	+	−
	Selivanoff's (ketoses and aldoses)	+	−	−	−	+	+	+	+
	Keller killiani for deoxysugars	+	+	+	+	+	+	+	+
Cardiac glycosides	Keller killiani	+	+	+	+	+	+	+	+
	Liebarmans	+	+	+	−	−	−	−	+
Flavonoids	Shinoda's	−	−	−	−	+	−	+	+
	Ferric chloride	−	−	−	−	−	−	+	−
	Lead acetate		−	−	−	+	−	+	−
	Sodium hydroxide	−	+	−	+	−	−	−	−
	Pew test	+	+	+	+	+	+	+	+
Lignin	Aniline sulphate	+	+	−	+	+	+	+	+
Phenols	Ferric chloride	−	−	−	−	−	−	−	−
	Gelatin	−	−	−	−	−	−	−	+
	Litmus paper	+	+	−	+	+	+	+	+
	Phosphomolybdic acid	+	−	−	+	+	+	−	+
Phlobatannins	Hydrochloric acid	−	−	−	−	−	−	−	−
	Lime water	+	−	−	+	−	−	−	+
Saponins	Frothing	+	−	−	−	−	+	−	−
Steroids/Terpenoids	Liebermann's	+	+	+	+	+	+	+	+
	Salkowski's	+	+	+	+	+	+	+	+
Tannins	Ferric chloride	−	−	−	−	−	−	−	−
	Formaldehyde	−	−	−	−	−	−	−	−
	Chlorogenic acid	−	−	−	−	−	−	−	−
	Lead acetate	+	−	−	−	+	+	+	+
Quinones	Sulphuric acid	+	+	+	+	+	+	+	+

		Inference							
Phytochemical constituents	**Type of test**	**GP**	**HE**	**CE**	**EAE**	**ME**	**RE**	**80% ME**	**AE**
	Hydrochloric acid	–	–	–	–	–	–	–	–
Volatile oils	Sodium hydroxide	–	+	–	–	–	–	–	+

+=Detected; -=Not detected; GP=Ground powder; HE=n-Hexane extract; CE=Chloroform extract; EAE=Ethyl acetate extract; ME=Methanol extract; RE=Residual extract; 80% ME=80% Methanol extract; AE=Aqueous extract.

Table 3: DPPH percent Radical Scavenging Activity (% RSA) of the various extract of *Cucumis metuliferus* fruit and ascorbic acid.

Extracts	IC_{50} (µg/mL)
80% ME	537.56
AE	932.88
HE	1882.90
CE	2180.94
EAE	2464.16
ME	594.78
RE	2579.91
AA	1.45

Key: 80% ME-80% Methanol extract, AE-Aqueous extract, HE-n-Hexane extract, CE-Chloroform extract, EAE-Ethyl acetate extract, ME-Methanol extract, RE-Residual extract, AA-Ascorbic acid, IC_{50}- half-maximal inhibitory concentration.

(CAE), which is same as Residual Extract (RE) in this study, had a higher percent yield.[8] The least percent yield in the sequential extraction obtained in this study was recorded by ethyl acetate (1.03%), followed by chloroform extract (1.87%), which seems to differ from[8] who recorded the least percent yield by chloroform extract (3.16%). This variation observed could be attributed to the fact that they hadn't extracted with ethyl acetate.

Most of the phytochemicals screened in this work correspond to our earlier findings,[8] albeit, some phytochemicals in this study were not screened in the previous work, such as anthocyanins, coumarins, lignin, phenols, quinones and volatile oils. In contrast to our previous study, some phytochemicals previously detected were not detected in this study. For example, alkaloids were detected in the crude methanol extract of our previous work but weren't detected in the present study. This difference could be attributed to the fact that ethyl acetate was used in the extraction before methanol extraction, thereby extracting most of the alkaloids into the ethyl acetate (Table 2). In addition, pew test for the confirmation of flavonoids was not carried out in the former work.[8] However, performing this test in this present study, confirmed the presence of flavonoids in the n-Hexane (HE) and the Chloroform (CE) extract, which was earlier reported to be absent in both extracts.

From this study, the IC_{50} of the plant extracts ranged from 537.56 to 2579.91 µg/mL, with 80% ME showing the highest anti-oxidant activity while RE showed the least anti-oxidant effect. In all the extracts' anti-oxidant activity, their results were lower than that of the standard drug (ascorbic acid) which had IC_{50} of 1.45 µg/mL. Therefore, this study has established the anti-oxidant activity of *C. metuliferus* which may be attributed to the presence of flavonoids, quinones and phenols that were detected in the fruit extracts. These classes of phytochemicals were documented to possess anti-oxidant effects.[37-42] Furthermore, Busuioc *et. al.*[7] reported the anti-oxidant activity of ursolic acid isolated from *C. metuliferus* with IC_{50} of 4.27 µg/mL, to be better than that of ascorbic acid and Trolox, which had IC_{50} of 20.34 µg/mL and 10.57 µg/mL respectively. In addition, the fruit of *C. metuliferus* was reported to contain quercetin, rutin, epicatechin, catechin, ursolic acid, chlorogenic acid, gallic acid and oleanolic acid.[7] These chemicals have been shown to possess anti-oxidant activities by their effects on ROS, signal transduction pathways and enzymes of oxidative stress such as catalase, glutathione peroxidase and superoxide dismutase.[7,43,44] Although, this study did not utilized specific class of phytochemicals for antioxidant assay, it would be considered in future research.

CONCLUSION

In conclusion this study has revealed the presence of carbohydrates, cardiac glycosides, flavonoids, steroids, terpenoids and quinones in *Cucumis metuliferus*. Other bioactive compound also present are coumarin, lignins, phenols, alkaloids, phlobataninis, tannins and volatile oils. The fruit extracts of *C. metuliferus* showed anti-oxidant activity with IC_{50} ranged between 537.56 to 2579.91 µg/mL compared to ascorbic acid value of 1.45 µg/mL.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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