

COMPARATIVE STUDY ON THE PHYTOCHEMICAL CONTENT OF THE SEEDS AND FRUITS OF THREE CUCURBITS.

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ABSTRACT

A comparative study was carried out on the phytochemical content of the seeds and fruits of *Cucumis melo*(L.), *Lagenaria breviflora*(Benth) and *Citrullus lanatus*(Thunb). Phytochemical analysis of air-dried, powdered epicarp, mesocarp and seeds of the mature fruits were carried out using standard procedures. Data on phytochemical characteristics were collected and subjected to two-way analysis of variance (ANOVA) at $p < 0.05$. Means were compared using LSD. Phytochemicals such as glycosides, tannins, alkaloids, flavonoids, phlobatanin, anthraquinone and saponin were detected in the epicarp, mesocarp and seeds of the Cucurbits under investigation.

INTRODUCTION

Cucurbitaceae comprises both cultivated and wild species. Many of these have been domesticated and grown as vegetables forming basic ingredients for human diet (Thoenissen *et al.*, 2009). They are herbaceous vines (Khan *et al.*, 2013), commonly called the “gourd family” of flowering plants and collectively known as cucurbits (Kocyan *et al.*, 2007), they contain about 125 genera and 980 species (Deyo and O'Malley, 2008) and are well represented in Nigeria by 21 genera, many of which are considered economically important (Ogbonna *et al.*, 2007). They are cultivated in different parts of the world for their medicinal value (Applequist *et al.*, 2006). This value is as a result of certain biological active substance called phytochemicals that are found in them. Phytochemicals are bioactive, non-essential plant nutrient chemical compounds also called phytonutrient. They are rich potential source of drugs as they produce a vast array of novel bioactive molecules many of which probably serve as chemical defenses against infection, making them useful therapeutically

MATERIALS AND METHODS

Fruit and seed collection

Mature fruits of the three members of the Cucurbitaceae family (*Lagenariabreviflora*, *Cucumismelo*, *Citrulluslanatus*) were randomly selected and bought from local farmers at Osiele market in Abeokuta, Ogun State. The seeds were extracted manually from their mature fruits which were left for 10 days to rotten in the case of *Lagenariabreviflora* and *Cucumismelo*. The seeds of *Citrulluslanatus* were also extracted manually after the mature fruit have been cut longitudinally with a knife. Ten grammes (10g) of the seeds was grounded using a sony electric blender into powdery form which was used for proximate and phytochemical analysis.

Each longitudinally dissected mature fresh fruit was peeled to collect their epicarp and mesocarp which were oven dried and ground separately to powdery form for proximate and phytochemical analysis.

Evaluation of Phytochemical constituents of *Lagenariabreviflora*, *Cucumismelo* and *Citrulluslanatus* fruit parts.

This was carried out using the method described by Ciulci (1994). The seeds, mesocarp and epicarp of the fruits were screened for the following phytochemicals including the alkaloids, saponins, tannins, flavonoids, glycosides, phlobatannins, phenol and anthraquinone.

Preparation of extracts

The epicarp, mesocarp and seeds of the three Cucurbitaceae (*Lagenariabreviflora*, *Cucumismelo* and *Citrulluslanatus*) were collected and cut into small pieces and air dried under ambient condition for three weeks. Each was then pulverized into powder with the use of an electric blender. Then 10g of each of powdery samples were separately dissolved in 70ml absolute ethanol and acetone, then each was allowed to stand for 72 hours and was filtered.

Alkaloids

Samples (epicarp, mesocarp and seeds) of 2 g each were weighed into three 100 ml beaker with 200ml of 80 % absolute alcohol added to each beaker. Each mixture was transferred to separate 250ml flask for more alcohol to be added in other to make each one up to 100ml, 1 mg magnesium oxide was then added and digestion in boiling water bath for 1½ hours under a reflux air condenser was carried out occasionally shaking the mixture.

The mixture was filtered while hot using a small bucher funnel and residue was returned to the flask for re-digestion for 30minutes by adding 50ml alcohol. The alcohol was evaporated and hot water was added for replacement, 3 drops of 10% hydrochloric acid was also added to the solution. Each solution was again transferred into a 250ml volumetric flask, to which 50ml zinc acetate solution and 5ml potassium ferrocyanide solution was added to each and thoroughly mixed. Each flask was left to stand for a few minutes, after which they were filtered through a dry filter paper and 10ml of filtrate was transferred into a separating funnel for the extraction of alkaloids present by shaking vigorously in five successive portions of chloroform.

Residue obtained was then dissolved in 10ml hot distilled water and was transferred into a Kjeldahl tube and 0.20 g of sucrose, 10ml concsulphuric acid and 0.02 g selenium solutions were added to determine % N using Kjeldahl distillation method. % N was converted to % total alkaloid by multiplying by a factor 3.26

i.e % Total Alkaloid = % N x 3.26

Flavonoids

Samples (epicarp, mesocarp and seeds) of 0.5 g were weighed into separate 100ml beaker with 80ml of 95 % ethanol added and stirred with a glass rod to prevent lumping. The mixture were then filtered separately through a whatman no 1 filter into three 100 ml volumetric flasks, each made up to the mark by adding ethanol. 1ml of each extract were pipetted into

separate 50ml volumetric flask and 4 drops of conc HCl was added to each dropwisely, 0.5 g magnesium turnings was added for the development of a magenta red colouration.

Standard flavonoid solution of range (0 – 5) ppm was prepared from 100ppm stock solution and was treated similarly, i.e adding HCl and magnesium turnings. The absorbance of magenta red colouration of sample and standard solution was read on a digital Jenway V6300 spectrophotometer at a wavelength at 520nm.

% Flavonoid was calculated thus;

$$\% \text{ Flavonoid} = \frac{\text{Absorbance} \times \text{Average Gradient Factor} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 10,000}$$

Tannin

Samples (epicarp, mesocarp and seeds) of 0.20 g each were measured into separate 50ml beaker with 20ml of 50 % methanol added to each. Each beaker was then covered with parafilm and placed in a water bath at (77 – 80)^oc for an hour shaking thoroughly to ensure uniformity. The extract was quantitatively filtered using double layered whatman no 41 filter paper into three 100ml volumetric flasks, with 20ml distilled water, 2.5ml Folin – Denis reagent and 10 ml of 17% Na₂CO₄ added and the mixture properly mixed together. Each mixture was made up to the mark by adding distilled water and allowed to stand for 20 minutes.

The bluish – green colour development at the end of range (0 – 10) ppm was treated similarly as sample above. Absorbance of Tannic acid standard solutions as well as that of the samples were read after colour development on a spectronic 2ID spectrophotometer at a wavelength of 760nm.

% Tannin was calculated thus;

$$\% \text{ Tannin} = \frac{\text{Absorbance of sample} \times \text{Average Gradient Factor} \times \text{Dilution Factor}}{\text{Weight of sample} \times 10,000}$$

Saponin

Samples (epicarp, mesocarp and seeds) of 1g each were weighed into separate 250ml beakers and 100 ml Isobutyl alcohol was added to each. Mixture was shaken on a UDY shaker for 5 hours for uniformity after which each was filtered through a whatman number 1 filter paper into separate 100ml beaker with 20ml of 40% saturated solution of Magnesium carbonate added, the mixture was filtered again using whatman number 1 filter paper to obtain a clear colourless solution. Colourless solution of 1 ml each were pipetted into three 50ml volumetric flasks and 2ml of 50% FeCl₃ solution was added to each flask. Each was then made up to mark with distilled

water and allowed to stand for 30 minutes for a blood red colour to develop. 0 -10 ppm standard Saponin solution was prepared from Saponin stock solution and was treated in a similar way that is, with the addition of 2 ml of 5% FeCl₃ solution.

The absorbance of the sample as well as that of the standard was read after the colour development in a Jenway V6300 Spectrophotometer at wavelength 380 nm.

% saponin was calculated thus;

$$\% \text{ Saponin} = \frac{\text{Absorbance of sample} \times \text{Average Gradient Factor} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 10000}$$

Glycosides

Extract (epicarp, mesocarp and seeds) were pipette (10 ml) into three separate 250ml conical flask, 50ml chloroform was added to each, after which the mixture was shaken on a Vortex mixer for 1 hour. Each mixture were filtered into three 100 ml conical flask with 10ml pyridine, 2ml of 2 % sodium Nitroprusside added respectively and then mixed thoroughly for 10 minutes.

Then 3ml of 20 % NaOH were added to each for a brownish yellow colour development. Glycoside standard of concentration ranges from 0 – 5 mg/ml was prepared from 100mg/ml stock glycoside standard. The series of standard was treated similarly like the sample and absorbance of both sample and standards was read on a spectronic 2ID digital spectrophotometer at wavelength 510nm.

% Glycoside was calculated thus;

$$\% \text{ Glycoside} = \frac{\text{Absorbance of Sample} \times \text{Gradient Factor} \times \text{Dilution factor}}{\text{Weight of Sample} \times 10000}$$

Phenol

Sample extracts (epicarp, mesocarp and seeds) of 0.5 g were weighed into three separate 100ml beaker with 20ml chloroform – Methanol added at ratio 2:1, this was to dissolve the extract upon shaking for 30 minutes on a shaker.

Each mixture was then filtered through a whatman number 1 filter paper into a dry clean 100ml conical flask or beaker. The resultant residue were repeatedly treated with chloroform – methanol mixture until it was phenol free. 1 ml of each filtrate were pipetted into a 30ml test tube with 5 ml alcoholic KOH added and shaken thoroughly. Each mixture was placed in a water bath set at 37^oc – 40^oc for 90 minutes, after which cooled to room temperature with 10 ml of petroleum ether then added followed by the addition of 5ml distilled water.

Each mixture was evaporated to dryness on a water bath and 6ml of Liebermann Burchard reagent

was added to the residue in a dry bottle for absorbance to be taken. The absorbance was taken at wavelength 620nm on a 2ID digital spectrophotometer. Standard phenol of concentration 0 – 4 mg/ml was prepared from 100 mg/ml stock phenol solution and treated similarly.

% Phenol was calculated thus;

$$\frac{\text{Absorbance of Sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of Sample} \times 10000}$$

Phlobatannin

Sample (epicarp, mesocarp and seeds) of 0.5 g were weighed into three separate 50ml beaker and 20ml of 50% Methanol was added to each beaker. Each mixture was covered with parafilm and placed in a water bath set at 77 – 88°c for 1 hour. The mixture was properly shaken to ensure it was well mixed and then it was be filtered through using whatman number 1 filter paper into three 50ml volumetric flask, using aqueous methanol to rinse.

Each was made up to mark with distilled water and 1 ml of the sample extract was pipetted into three 50ml volumetric flask and 20 ml distilled water, 2.5ml Folin – Dennis reagent and 10ml of 17% Sodium Carbonate solution were added to the extracts respectively. The mixtutre were then shaken thoroughly for 20 minutes with 0 – 5 mg/ml of Phlobatanin standard concentrations was prepared from 100mg/ml.

Phlobatanin stock solution and it was treated the same way as sample. The absorbance of standard solutions as well as that of Sample was read on the spectronic 2ID spectrophotometer at wavelength 550 nm.

% Phlobatannin was calculated thus;

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10000}$$

Statistical Analysis

All data collected from the above experiments were analyzed using two way analysis of variance (ANOVA) and means were compared using LSD. P value was set at ≤ 0.05.

RESULT

Phytochemical concentration in the epicarp, mesocarp and seed of *C. melo*, *L. breviflora* and *C. lanatus* The percentage concentration of some phytochemicals detected in the epicarp, mesocarp and seed of *C. melo*, *L. breviflora* and *C. lanatus* is shown in Table 1.

The concentrations of the phytochemicals were significantly (p < 0.05) different among the epicarp, mesocarp and seeds of *C. melo*, *L. breviflora* and *C. lanatus* except for alkaloid, phenol and anthraquinone present in *L. breviflora* (epicarp, mesocarp and seed) which were not significantly (p > 0.05) different.

Phenol was not detected in the epicarp and mesocarp of *C. lanatus* and seeds of *L. breviflora*. However, concentrations of phenol detected in the different parts of *C. melo* and *L. breviflora* (epicarp, mesocarp and seed) were not significantly different (p > 0.05).

Highest concentrations of saponin, alkaloid, glycoside, anthraquinone and phlobatannin were recorded in the epicarp of *C. melo*, *L. breviflora* and *C. lanatus*. These were lowest in their seeds (Table 1).

Tannin was not detected in the epicarp, mesocarp and seeds of *C. lanatus* and in the seeds of *C. melo* and *L. breviflora*.

Flavonoid was also significantly higher in the epicarp of *C. melo* and *L. breviflora* than in their mesocarp and seeds. On the other hand, flavonoid was higher in the seeds of *C. lanatus* than in its epicarp and mesocarp (Table 1).

Table 1: Concentrations of some phytochemicals in seeds of *C. melo*, *L. the* epicarp, mesocarp and *breviflora* and *C. lanatus*

Phytochemicals %	Epicarp			Mesocarp			Seed		
	<i>Cucumismelo</i>	<i>Lagenariabreviflora</i>	<i>Citrulluslanatus</i>	<i>Cucumismelo</i>	<i>Lagenariabreviflora</i>	<i>Citrulluslanatus</i>	<i>Cucumismelo</i>	<i>Lagenariabreviflora</i>	<i>Citrullus</i>
<i>lanatus</i>									
Tannin	0.16±0.00 ^c	0.23±0.01 ^c	ND	0.95±0.07 ^b	0.17±0.01 ^b	ND	ND	ND	ND
Saponin	2.47±0.05 ^c	2.64±0.03 ^c	2.07±0.03 ^c	2.19±0.08 ^b	2.15±0.04 ^b	1.49±0.04 ^b	0.21±0.00 ^a	1.23±0.01 ^a	0.27±0.02 ^a
Alkaloid	2.04±0.06 ^c	12.43±10.02 ^a	1.87±0.01 ^c	0.95±0.01 ^b	1.25±0.02 ^a	1.13±0.01 ^b	0.42±0.11 ^a	0.54±0.03 ^a	0.45±0.01 ^a
Flavonoid	1.84±0.02 ^c	2.19±0.08 ^c	1.74±0.07 ^c	1.56±0.01 ^b	1.74±0.02 ^b	1.43±0.01 ^b	0.82±0.04 ^a	0.93±0.01 ^a	0.82±0.01 ^a
Glycoside	0.82±0.01 ^c	0.92±0.01 ^c	0.63±0.01 ^c	0.52±0.01 ^b	0.69±0.03 ^b	0.31±0.01	0.09±0.00 ^a	0.13±0.00 ^a	0.13±0.02 ^a
Phenol	0.11±0.02 ^a	0.06±0.06 ^a	ND	0.05±0.05 ^a	0.09±0.01 ^a	ND	0.06±0.06 ^a	ND	0.07±0.07 ^a
Anthraquinone	1.22±0.02 ^c	0.89±0.53 ^a	1.11±0.01 ^c	1.09±0.03 ^b	0.87±0.01 ^a	0.70±0.02 ^b	0.63±0.010 ^a	0.58±0.01 ^a	0.23±0.02 ^a
Phlobatanin	0.41±0.01 ^c	0.55±0.01 ^c	0.23±0.02 ^c	0.21±0.01 ^b	0.43±0.01 ^b	0.17±0.01 ^b	0.10±0.010 ^a	0.21±0.01 ^a	0.05±0.01 ^a

abcMean values (± Standard Error) with the same superscript in the same row for each species are not significantly different (p > 0.05) (ND = Not Detected)

DISCUSSION

Phytochemicals of medicinal importance such as glycosides, tannin, alkaloids, flavonoids, anthraquinone, phenol, saponins and phlobatanins were detected in the epicarp, mesocarp and seed of *C. melo*, *L. breviflora* and *C. lanatus*. Varadarajan *et al.* (2008) reported that phytochemicals and other chemical constituents are responsible for the medicinal value of plants. The presence of these phytochemical constituents in fruits has also been reported to account for the counter reaction observed against metallic taste due to chemotherapy noticed in the mouth of cancer patients (Slater, 2007). The occurrence of these phytochemical in the fruit parts utilized in this study conforms to the report of Yuan *et al.* (2006) he observed that cucurbits are known to contain these bioactive compounds which aids colour, flavour and generally play protective roles in these fruits.

This study recorded high saponin concentrations in the epicarp of the fruits of *C. melo*, *L. breviflora* and *C. lanatus*. Saponin is said to have hypotensive and cardiodepressant properties (Olaleye, 2007). Recent studies have shown that saponins possesses haemolytic and induced cytotoxicity effect (Ayoola and Adeyeye, 2010), anti-tumor and anti-mutagenic properties and can reduce the risk of cancer by preventing the growth of the cancer cells (Nafiu *et al.*, 2011). Cucurbits plants as *L. breviflora* are used in the treatment of wounds and also to stops bleeding (Okwu and Josiah, 2006). Saponin exhibit foaming or soapy properties and cell membrane permeabilizing properties, this soapy character is due to their surfactant properties (Noudeh *et al.*, 2010). This study revealed levels of glycosides in the epicarp, mesocarp and seed of *C. melo*, *L. breviflora* and *C. lanatus*. Glycosides have anti-inflammatory effects, protecting against lethal endotoxemia (Shah *et al.*, 2011) and are useful in cardiac treatment of congestive heart failure and cardiac arrhythmia (Ayoola and Adeyeye, 2010).

C. melo and *L. breviflora* contained some percentage of tannin. Ayoola *et al.* (2008) reported that tannin has astringent properties, (causes the dry and puckery feeling felt in the mouth following consumption of unripe fruit) and hasten wound healing (Njoku and Akumefula, 2007). Tannins are potential metal chelators, precipitators and biological

antioxidants, they usually form insoluble complexes with protein, interfering with their bioavailability (Okonkwo, 2009). Tannins have been reported in the fruits of other Cucurbits as *Cucumis sativa* and *Praecitrullus fistulosus* (Lai *et al.*, 2010). Their high content in diets leads to poor palatability.

Tannin was not detected in *C. lanatus* probably due to loss of its astringency to fruit ripening since the destruction and modification of tannins overtime plays an important role in ripening of fruits (McGee, 2004).

Flavonoids are known to aid colour and flavour in plant, its content in the epicarp of the fruits parts in this study was high probably accounting for the attractive colouration of the epicarp of these fruits. The content of Flavonoid in the epicarp of *C. melo* and *L. breviflora* was significantly high, the same was also recorded in the seeds of *C. lanatus*. This conforms with the report of Ekoh (2009) for African elemi however, George *et al.* (2012) reported that high content of flavonoids help protect blood vessels from rupture or leakage, also enhances the power of vitamin c protecting cells from oxygen damage.

Flavonoids are anti-microbial and inhibitory in nature, this attribute is ernested in preventing the initiation and promotion of tumours (Okwu, 2005). Flavonoids help in stress reduction by scavenging hydroxyl and lipid peroxy radicals, superoxide and anions and has been recognized as an anticoagulant and an aphrodisiac (Herve *et al.*, 2008).

In contrast, phenol is known to promote the production of undesirable colour, flavour and also loss of nutrient, their content in the fruit parts studied here were very low this probably giving these fruit an edge nutritionally

Alkaloids was present in the fruit parts of *C. melo*, *L. breviflora* and *C. lanatus* examined in this study. Alkaloids are generally known to be highly toxic made up of ammonium compounds, they defend plants against herbivores and pathogens (Madziga *et al.*, 2010). This property is ernested and used in the reduction and elimination of cancer cells, also applied as anesthetics and CNS stimulants (Kam and Liew, 2002). Due to its toxicity, it is normally applied at a strictly controlled dose in herbal medicine (Jimam, 2008). Cooking lowers the toxicity of alkaloids (Hotpkins, 1995), in this study the alkaloid content was within the dietary recommended allowance.

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