

## Determination of the Anti-nutrient Composition of *Ocimum gratissimum*, *Corchorus olitorius*, *Murraya koenigii* Spreng and *Cucurbita maxima*

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**ABSTRACT:** The present study investigates the anti-nutrient composition of *Ocimum gratissimum*, *Corchorus olitorius*, *Murraya koenigii* Spreng and *Cucurbita maxima* following standard methods. Anti-nutritional factors are chemical substances which act to reduce nutrient intake, digestion, absorption and utilization of nutrients which may accumulate in the body to toxic level if the diet is not varied. The qualitative analysis revealed the presence of flavonoids, cardiac glycosides, alkaloids, tannins and phenols in the plant leaves using methanol, ethanol, and petroleum ether as extraction solvents except for saponins in which water was used as the extraction solvent. The differences in mean values were statistically significant at  $p < 0.05$ . The highest level of anti-nutrients were found in the following order: flavonoids ( $7.20 \pm 0.15$  %) in *Cucurbita maxima*; cardiac glycosides ( $13.01 \pm 0.03$  %) in *Ocimum gratissimum*; alkaloids ( $8.82 \pm 0.08$  %) in *Ocimum gratissimum*; phytates ( $0.06 \pm 0.00$  %) in *Corchorus olitorius*; haemagglutinins ( $1.06 \pm 0.00$  mg/l) in *Cucurbita maxima*; saponins ( $9.26 \pm 0.20$  %) in *Cucurbita maxima*; tannins ( $1.45 \pm 0.03$  %) in *Corchorus olitorius*; oxalates ( $1.07 \pm 0.07$  %) in *Murraya koenigii*; and phenols ( $0.68 \pm 0.02$  mg/l) in *Corchorus olitorius*. However, the level of anti-nutrients in these plant leaves falls within safe level but should be consumed with care to prevent over-accumulation.

**KEYWORDS:** Anti-nutrients, *Ocimum gratissimum*, *Corchorus olitorius*, *Murraya koenigii* Spreng, *Cucurbita maxima*.

### 1 INTRODUCTION

Plants are known to be the chief producer of food substances since time immemorial. However, besides the basic nutrients known to be produced by plants which primarily helps in the generation of energy for body metabolism, growth and reproduction, plants produces chemical substances which acts to reduce nutrient intake, digestion, absorption and utilization of nutrients which may accumulate in the body to harmful level if the diet is not varied [1], [2].

In the most general sense, medicinal plants serves as resource for drugs in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [3]. These plants contain bioactive compounds that exhibited physiological activities against bacteria and other microorganisms [4], [5].

It is however pertinent to state that, it is the anti-nutrients in plants that confer to them its medicinal properties. For instance, a 2011 study of girinimbine, a carbazole alkaloid isolated from *Murraya koenigii*, found that it inhibited the growth and induced apoptosis in human hepatocellular carcinoma, HepG2 cells *in vitro* [6]. It is in this light however that the following four plant leaves, *Ocimum gratissimum* (Common name: Scent leaf; family: lamiaceae), *Corchorus olitorius* (family: Malvaceae), *Murraya koenigii* (Common name: Curry; family: Rutaceae) and *Cucurbita maxima* (family: Cucurbitaceae) was analysed to determine the level of the antinutrient they contain.

## **2 MATERIALS AND METHODS**

### **2.1 SAMPLE COLLECTION**

The plant leaves were collected from nearby farmland close to Anambra State University, Uli, Nigeria. The leaves were identified at Biological Science Department of Anambra State University.

### **2.2 SAMPLE PREPARATION**

Plant materials were washed separately under running tap water, followed by distilled water. The washed plant leaves was oven dried, then ground to a fine powder and stored.

### **2.3 METHODS FOR QUALITATIVE ANALYSIS**

#### **2.3.1 DETECTION OF FLAVONOIDS**

Five ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing [4], [7].

#### **2.3.2 DETECTION OF CARDIAC GLYCOSIDES (KELLER-KILLANI TEST)**

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer [8].

#### **2.3.3 DETECTION OF ALKALOIDS (HAGER'S TEST)**

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). The presence of alkaloids is confirmed by the formation of yellow coloured precipitate [9].

#### **2.3.4 DETECTION OF SAPONINS (FOAM TEST)**

About 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins. Few drops of olive oil was added to 0.5g of the extract and vigorously shaken. Formation of soluble emulsion in the extract indicates the presence of saponin [10].

#### **2.3.5 DETECTION OF TANNINS**

First, about 1 ml of the ethanol extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (catechic tannins) or a blue-black (gallic tannins) coloration [11].

#### **2.3.6 DETECTION OF PHENOLS: FERRIC CHLORIDE TEST**

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols [9].

### **2.4 METHODS FOR QUANTITATIVE ANALYSIS**

#### **2.4.1 DETERMINATION OF FLAVONOID**

The method of Boham and Kocipai-Abyazan (1974) was used in which 10g of the plant sample was extracted with 100 ml of 80% aqueous methanol at room temperature and allow to stand for like 5 to 10 minutes. The whole solution was filtered

through whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness and weighed to a constant weight. The percentage flavonoid was calculated by difference.

$$\% \text{ flavonoids} = \frac{W_2 - W_1}{Wt \text{ of sample}} \times 100$$

Where,  $W_1$  = Weight of empty crucible

$W_2$  = Weight of crucible + residue

#### 2.4.2 DETERMINATION OF PHYTATE

The phytate content were determined using the method of Young and Greaves (1940) as adopted by Reference [13]. Two hundred milligram (0.2 g) of the sample was weighed into different 250 ml conical flasks. Each sample was soaked in 100 ml of 2 % conc. HCL for 3hrs. The sample was then filtered. Fifty (50) mls of each filtrate was laced in 250 ml beaker and 100 ml distilled water added to each sample. Ten (10) ml of 0.3 % ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195 g iron per 1 ml.

$$\% \text{ Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19}{2} \times 100$$

#### 2.4.3 CARDIAC GLYCOSIDE

To 1 g of sample in a beaker, add 5 ml of aqueous methanol and allow to stand for 10 minutes. From the mixture take 1 ml of the extract to a 100 ml beaker and add 1 ml of 2 % solution of 3,5-DNS (Dinitro salicylic acid) and 1 ml of 5 % aqueous NaOH. Boil for 2 minutes in a water bath at 95 – 100°C until brick red precipitate is observed (Note: a change in colour from yellow to brick red indicates a positive result). Weigh an empty Whatman filter paper No. 42(125 mm) and use it to filter the boiled sample. Take the filter paper with the absorbed residue and dry in an oven at 50°C till dryness and reweigh the filter paper. The percentage cardiac glycoside was calculated.

$$\% \text{ Cardiac Glycosides} = \frac{(\text{Wt of filter paper} + \text{residue}) - (\text{Wt of filter paper})}{\text{Wt of sample}} \times 100$$

#### 2.4.4 DETERMINATION OF HAEMAGGLUTININ

Two (2g) of sample was placed in a beaker and 20 ml of 0.9 NaCl was added and allowed to stand for 1 hour. Do not filter. Divide the mixture into 4 centrifuge tubes and centrifuge at 2000 rpm (revolution per minute) for 10 minutes. Carefully decant the supernatant from the 4 centrifuge tube into a beaker and filter using Whatman filter paper No. 42(125 mm). The supernatant were collected as crude agglutination extract and absorbance read at 420 nm [14].

$$\text{Conc. of Sample} = \frac{\text{Absorbance of Sample} \times \text{Conc. of standard}}{\text{Absorbance of Standard}}$$

#### 2.4.5 DETERMINATION OF ALKALOIDS

Five (5g) of the plant sample was placed in a 250ml beaker and 200ml of 10% acetic acid ( $\text{CH}_3\text{CO}_2\text{H}$ ) in ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) was added. The mixture was covered and allowed to stand for 4 hours at 25°C i.e. at room temperature. It was then filtered with filter paper No. 42 and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated  $\text{NH}_4\text{OH}$  was added drop wise until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute  $\text{NH}_4\text{OH}$ . The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was calculated by difference [7], [15].

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{Wt \text{ of sample}} \times 100$$

Where,  $W_1$  = Weight of empty filter paper  
 $W_2$  = Weight of filter paper + Alkaloid

#### 2.4.6 DETERMINATION OF TOTAL SAPONINS

Five (5g) of the sample was put into 20 % acetic acid in ethanol and allowed to stand in a water bath at 50<sup>0</sup>C for 24 hours. This was filtered and the extract was concentrated using a waterbath to one-quarter of the original volume. Concentrated NH<sub>4</sub>OH was then added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage [15].

$$\% \text{ Saponins} = \frac{W_2 - W_1}{Wt \text{ of sample}} \times 100$$

Where,  $W_1$  = Weight of filter paper  
 $W_2$  = Weight of filter paper + residue

#### 2.4.7 DETERMINATION OF TOTAL TANNIN BY TITRATION

The Follin Denis titrating method as described by Reference [16] was used. To 20 g of the crushed sample in a sample conical flask was added 100 ml of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100 ml of 10 % acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected. 25 ml of NH<sub>4</sub>OH were added to the filtrate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH<sub>4</sub>OH still in solution. The remaining volume was measured to be 33 ml. 5 ml of this was taken and 20 ml of ethanol was added to it. It was titrated with 0.1 M NaOH using phenolphthalein as indicator until a pink end point is reached. Tannin content was then calculated in % ( $C_1V_1 = C_2V_2$ ) molarity. **Where**,  $C_1$  = Conc.of tannic acid ,  $C_2$  = Conc. of base,  $V_1$  = volume of tannic acid and  $V_2$  = Volume of base.

#### 2.4.8 DETERMINATION OF OXALATE BY TITRATION METHOD

This determination involves three major steps viz digestion, oxalate precipitation, and permanganate titration [17].

##### 1. Digestion

- 2 g of sample was suspended in 190 ml of distilled water in a 250 ml volumetric flask.
- 10 ml of 6M HCL was added and the suspension digested at 100<sup>0</sup>C for 1 hour.
- Cool and then make up to 250 ml mark before filtration.

##### 2. Oxalate precipitation

Duplicate portions of 125 ml of the filtrate were measured into beakers and 4 drops of methyl red indicator added. This was followed by the addition of NH<sub>4</sub>OH solution (dropwise) until the test solution changes from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion was then heated to 90<sup>0</sup>C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90<sup>0</sup>C and 10 ml of 5 % CaCl<sub>2</sub> solution was added while being stirred constantly. After heating, it is cooled and left overnight at 25<sup>0</sup>C. The solution is then centrifuged at 2500 rpm for 5 minutes. The supernatant is decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H<sub>2</sub>SO<sub>4</sub> solution.

##### 3. Permanganate titration

At this point, the total filtration resulting from digestion of 2 g of flour was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized KMNO<sub>4</sub> solution to a faint pink colour which persists for 30 sec. The calcium oxalate content was then calculated using the formula.

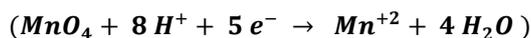
**T** = Titre value

**Vme** = Volume of mass equivalent (i.e. 1 ml of 0.05 M KMNO<sub>4</sub> solution is equivalent to 0.00225 g anhydrous oxalic acid).

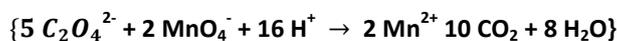
**Df** = Dilution factor  $Vt/A$  (2.4 where  $Vt$  is the total volume of titrate (300 ml) and  $A$  is the aliquot used (125 ml).

**ME** = The molar equivalent of  $\text{KMnO}_4$  in oxalate ( $\text{KMnO}_4$  redox +5 reaction).

**Mf** = The mass of sample used.



The equation for the reaction between oxalate and permanganate is



$$\text{Conc. of Oxalate} = \frac{T \times V_{me} \times D_f \times 10^5}{ME \times M_f} \text{ (mg/100g)}$$

#### 2.4.9 DETERMINATION OF TOTAL PHENOLS BY SPECTROPHOTOMETRIC METHOD

The fat free sample was boiled with 50 ml of diethylether ( $\text{CH}_3\text{CH}_2)_2\text{O}$ . 5 ml of the boiled extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. After the addition of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol ( $\text{CH}_3(\text{CH}_2)_3\text{CH}_2\text{OH}$ ), were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm [8].

$$\text{Conc. of Sample (mg/l)} = \frac{\text{Absorbance of Sample} \times \text{Conc. of Sample}}{\text{Absorbance of Standard}}$$

### 3 STATISTICAL ANALYSIS AND DATA PROCESSING

One-way analysis of variance (ANOVA) was conducted on each of processing methods and Least Significant Difference (LSD) test at significant level of  $p < 0.05$  was performed using SPSS version 17 software for windows to compare the difference between treatment means. The results were expressed as means  $\pm$  standard deviation of three separate determinations.

### 4 RESULTS AND DISCUSSION

The qualitative analysis for the four leaf extracts is shown in Table 1 which was carried out following standard procedures. Flavonoids were found to be present in all the ethanolic extracts. *Corchorus olitorius*, *Murraya koenigii*, and *Cucurbita maxima* contained cardiac glycosides with *Ocimum gratissimum* containing the highest cardiac glycoside which is in conformity with the results of the quantitative analysis presented in Table 2 as well as those reported by [2]. Alkaloids, tannins, saponins and phenols were also present in the leaf extracts.

The amount of phytochemicals found in the four leaf extracts was quantitatively determined by standard procedures and expressed as mean  $\pm$  standard deviation of three determination. However, analysis revealed that *Cucurbita maxima*, *Murraya koenigii* contained  $7.20 \pm 0.15 \%$ ,  $7.20 \pm 0.02 \%$  flavonoids whilst *Corchorus olitorius* and *Ocimum gratissimum* has  $7.02 \pm 0.03 \%$  and  $5.40 \pm 0.02 \%$  respectively. The relatively low toxicity of flavonoids compared to other active plant compounds like alkaloids means that many animals, including humans could ingest significant quantities in their diet. In vitro studies showed that flavonoids have anti-allergic, anti-inflammatory, anti-microbial [18] and anti-diarrheal activities [19].

*Ocimum gratissimum* contained the highest concentration of cardiac glycosides in the tune of  $13.00 \pm 0.03 \%$  which justifies its use in the treatment of congestive heart failure and cardiac arrhythmia though the dosage must be controlled carefully, since the therapeutic dose is close to its toxic dose [20]. The alkaloids content of the plants leaf was found in the following order *Ocimum gratissimum* ( $8.82 \pm 0.08 \%$ ), *Cucurbita maxima* ( $8.40 \pm 0.10 \%$ ), *Murraya koenigii* ( $7.60 \pm 0.02 \%$ ) and *Corchorus olitorius* as ( $7.21 \pm 0.04 \%$ ). The result justified the medicinal value of *Ocimum gratissimum*. In higher concentration, alkaloids could be toxic especially when it exceeds the lethal dose of 20 mg/100g [14]. The highest level of phytate was found in *Corchorus olitorius* ( $0.06 \pm 0.00 \%$ ) with *Ocimum gratissimum* ( $0.01 \pm 0.00 \%$ ) as the least. For best health, phytate should be lowered as much as possible, ideally to 25 mg or less per 100 grams or to about 0.03 % of the phytate containing food eaten. At this level, micronutrient losses are minimized. Reference [14] stated that the lethal dose of phytate is 50-60 mg/kg.

Relatively low concentration of haemagglutinin was found in the extracts. The highest haemagglutinin content was found in *Cucurbita maxima* as  $1.06 \pm 0.00$  mg/l which is far below the lethal dose of 50 mg/kg [14]. The highest saponins content was recorded in *Cucurbita maxima*. Generally saponins are toxic, but Reference [21] showed that consumption of saponins by human beings may be beneficial in reducing heart disease (by binding of saponins with plasma membrane and cholesterol).

The percentage concentration of tannin was recorded at the highest level in *Corchorus olitorius* as leaf extracts contained tannin in appreciable amount. The lethal dose of tannin was reported to be 30mg/kg [14]. The highest concentration of oxalate was found in the leaf of *Murraya koenigii* which serves as a caution in its consumption as oxalates chelates metals. The toxic dose was reported to be 2.5 g/kg [14]. The highest phenol content was found in *Corchorus olitorius* ( $0.68 \pm 0.02$  mg/l).

Table 1. Qualitative Analysis For Four Plant Leaves

	<i>Ocimum gratissimum</i>			<i>Corchorus olitorius</i>			<i>Murraya koenigii</i>			<i>Cucurbita maxima</i>		
	Met.	Eth.	Pet.	Met.	Eth.	Pet.	Met.	Eth.	Pet.	Met.	Eth.	Pet.
Flavonoids	-	+	-	+	+	-	-	+	-	-	+	-
Cardiac Glycosides	+++	++	+	-	++	++	+	++	+	+	++	-
Alkaloids	+	+	-	+	-	-	+	-	+	+	-	-
Tannins	+	++	+	++	+	-	-	++	+	+	++	-
Phenols	+	++	+	-	+	-	-	+	-	+	++	-
<i>For saponin screening, leaf extracts was gotten using distilled water only</i>												
Saponins	+++			++			+			++		

Key: -, Not present; +, present at low concentration; ++, present at moderate concentration; +++, present at high concentration. Met. =Methanol, Eth. = Ethanol, Pet. = Petroleum ether.

Table 2. Quantitative Analysis For Four Plant Leaves

Phytochemicals	<i>Ocimum gratissimum</i>	<i>Corchorus olitorius</i>	<i>Murraya koenigii</i>	<i>Cucurbita maxima</i>
Flavonoids (%)	$5.40 \pm 0.02^d$	$7.02 \pm 0.03^c$	$7.20 \pm 0.02^a$	$7.20 \pm 0.15^b$
Cardiac Glycosides (%)	$13.01 \pm 0.03^a$	$8.03 \pm 0.06^d$	$9.04 \pm 0.05^c$	$11.05 \pm 0.05^b$
Alkaloids (%)	$8.82 \pm 0.08^a$	$7.20 \pm 0.04^d$	$7.60 \pm 0.02^c$	$8.40 \pm 0.10^b$
Phytates (%)	$0.01 \pm 0.00^d$	$0.06 \pm 0.00^a$	$0.04 \pm 0.00^b$	$0.03 \pm 0.00^c$
Haemagglutinins (mg/l)	$0.51 \pm 0.00^b$	$0.43 \pm 0.00^c$	$0.29 \pm 0.00^d$	$1.06 \pm 0.00^a$
Saponins (%)	$5.24 \pm 0.06^d$	$6.00 \pm 0.00^c$	$6.40 \pm 0.01^b$	$9.26 \pm 0.20^a$
Tannins (%)	$0.86 \pm 0.00^d$	$1.45 \pm 0.03^a$	$0.97 \pm 0.02^b$	$0.93 \pm 0.01^c$
Oxalates (%)	$0.92 \pm 0.12^b$	$0.17 \pm 0.00^d$	$1.07 \pm 0.07^a$	$0.19 \pm 0.00^c$
Phenols (mg/l)	$0.42 \pm 0.02^c$	$0.68 \pm 0.02^a$	$0.48 \pm 0.01^b$	$0.30 \pm 0.00^d$

Values were expressed as Mean  $\pm$  Standard Deviation of three Determinations. Means within a row followed by different superscript letters are statistically significant by LSD (Least Significance Difference) test at  $p < 0.05$ .

## 5 CONCLUSION AND RECOMMENDATION

The level of antinutrients found in the four leaf extracts are within safe level and the moderate consumption would be very vital in improving health conditions and could serve as a source for useful drugs. However, since no one method is completely efficient in determining the chemical composition for a particular phytochemical and the fact that environmental conditions could influence the level of the phytochemical in the plant, we recommend that research into this field should be carried out randomly as often as possible with adaptable methods within the reach of the investigator.

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