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Chemicals and Nutritional Composition of Four Botanicals with Fungitoxic Properties

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Abstract: Evaluation of four botanicals *Vernonia amygdalina* L., *Bryophyllum pinnatum* L., *Eucalyptus globules* Labill and *Ocimum gratissimum* Kurz (Clocimum) for chemical and nutritional properties was conducted at Ago Iwoye in South-western Nigeria. The results from the investigation showed that V. *amygdalina* contained large quantity of Thiamine, Pyridoxine, Ascorbic acid, Glycine, Cysteine and Casein hydrolysate significantly more than other botanicals. Similarly, all the botanicals contained carbohydrates, proteins and lipids. However, the acid contents of *B. pinnatum* and *E. globules* were significantly higher than those of *V. amygdalina* and *O. gratissimum*. Similarly, the hydrocyanic acid and oxalic acid contents of *B. pinnatum* and *E. globules* were significantly ($p = 0.05$) higher than those of *O. gratissimum*. Moreover, the four botanicals contained mineral nutrients and sugars; hence, if the botanicals were used to treat cowpea plants, all these would be supplied to cowpea plants and so enhance their performances

Key words: Screening · botanicals · biocidal properties · hydrocyanic acid mineral's composition

INTRODUCTION

The use of botanicals in crop protection has now gained a popular ground in the world of agriculture as an alternative to the use of toxic, persistent and synthetic compounds. Several factors are now responsible for making the use of alternative methods more attractive. The limited external reserves and poor exchange rates of the currency of the developing nations limit the quantity of pesticides that can be imported [1]. The complete removal of subsides on synthetic herbicides, insecticides and fungicides have made them inaccessible to the majority of farmers in many African countries. Since majority of farmers are illiterate, the misuse of these chemicals frequently occur. It is no longer a hidden fact that these chemicals contaminate stored food's commodity, leaving behind harmful residues especially, when an application dosages are not properly followed.

Several researches have been conducted on the use of botanicals [1-3] and several plants with promising biocidal properties have been identified [2]. Most of these plants have also been used *in vitro* and *in vivo* in the control of various plant diseases and pests [2-6]. In Nigeria like any other developing nation, only few of these plants have their bioactive compounds identified [2].

On the other hands, some of the plants with promising bioactive properties also contain useful minerals and food value for human and animal consumption. This however makes such plant to be very scarce due to competition in the usage of such plants.

This investigation, therefore, intends to carry out chemical and nutritional analysis of *Vernonia amygdalina, Bryophyllum pinnatum, Eucalyptus globules* and *Ocimum gratissimum* all botanicals with known fungi toxic properties.

MATERIALS AND METHODS

Preparation of materials for chemical analysis: Leaves of *Vernonia amygdalina, Bryophyllum pinnatum, Eucalyptus globules* and *Ocimum gratissimum* were collected separately, washed with sterile distilled water and kept in the deep freezer to thaw for 24 h [7]. Each set of leaves was later put in brown envelopes and oven-dried at 100°C first for 24 h and later to constant weight after which each set of leaves was blended using an electric miller into powdered form. The powdered samples were sieved through a 0.002 mm wire mesh. Each powdered leaf sample was then packed inside transparent polythene bags in readiness for chemical composition analysis.

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Determination of vitamins

Determination of pyridoxine (Vit. B6): The method described by AOAC [8] was used with some modification. Five hundred milligrams of each leaf sample powder was weighed and put inside separate conical flasks. Forty milliliters of glacial acetic acid was then added to each weighed sample. The solutions were homogenized by shaking gently the conical flasks. Ten milliliters of already prepared 5% (m/v) mercuric acetate solution was then added to the glacial acetic acid solution. The mixture was heated to obtain clear solutions. The solutions were allowed to cool before being filtered using Whatman's No. 1 filter paper. Each filtrate was titrated with 0.1 N perchloric acid ($HClO_4$), while drops of 1% (m/v) methyl rosanidine was used as indicator. The pyridoxine content in each sample was determined using the method of AOAC [8].

Determination of nicotinamide contents: The nicotinamide contents from each sample of the botanicals were determined using the titrimetric method that involved a non-aqueous medium with perchloric acid as described by AOAC [8] with some modifications. One gram each of powdered samples from each botanical was weighed into separate conical flasks using Metler Digital Balance. Twenty milliliters of glacial acetic acid was poured into each conical flask to digest each powder.

The mixture was then warmed slightly to obtain a solution. A hundred milliliters of benzene was added to each solution. Each solution was titrated with 0.1 M perchloric acid solution. The values of nicotinamide were then determined using method of AOAC [8].

Determination of glycine, cystein and casein hydrolysate contents: Each amino acid content as listed above per sample was determined using the modified Ninhydrin Colorimetric Analysis. The processes used involved include centrifugation, distillation, quantitative determination of amino acid profiles as described by Rosen [9]. The amino acid profiles were estimated by determining the optical density at 570 nm (w/v) using visible spetrophometer model (Spectronic 20D). The amounts of each amino acid type present were then calculated from the standard curve of known concentration of leucine at (10 mg g^{-1}) prepared [8].

Determination of sugar contents: The sugar contents were determined as described by AOAC [8] with some modifications. The sugar contents were determined using the principle of ethanolic extraction of sugar from samples by the organic solvent - petroleum ether using absorbance method. One gram of each sample leaf powder was weighed into 200 mL volumetric flask. Cotton wool was placed over the sample in the volumetric flask to prevent splashing. Fifteen milliliters of 85% ethanol was added to each flask. The solutions were each homogenized by shaking gently. The solutions were later filtered each through Whatman's No. 1 filter paper. Four separating funnels were set up. Ten milliliter chloroform solution was added to each filtrate. Further extraction was carried out in the separating funnels. Each filtrate formed a layer on the chloroform. The chloroform layer was discarded. Ten milliliters of petroleum either was added to each filtrate to remove the lipid content present. The petroleum ether layer below was used to determine the total sugar contents. Five milliliters of petroleum ether was weighed out inside a boiling flask. 0.5 mL of distilled water was added to obtain a diluted extract. 0.5 mL of 5% (w/v) phenol was added to each content in each flask along with 2.5 mL of concentrated sulphuric acid (Conc. H_2S0_4) for colour development [8]. Each flask content was then heated and allowed to cool after which the absorbance was read off at 490 nm wavelength using Spectronic 20D - Spectrophotometer.

Preparation of standard glucose curve: One gram of glucose was dissolved in 1 L of sterile distilled water. Different concentrations of stock solution were from 2 mL/100 mL. And 20 mL/100 mL of sterile distilled water. One milliliter of each stock was withdrawn from each dilution into test tubes. Colour development was carried out by adding 2.5 mL Conc. H_2S0_4 to each test tube. Colour development was carried out by adding 2.5 mL Conc. H_2S0_4 to each test tube. The blank was done same way using one milliliter water instead of the solutions of botanicals. The sugar contents of each sample were estimated as described by AOAC [8].

Determination of lipid contents: The lipid contents were extracted using the principle gravimetric extraction of fats from samples by organic solvent - petroleum either - using Soxhlet extraction method [8, 10, 11].

Determination of acid value: Five hundred milligrams of oil from each leaf sample was weighed into separate beakers and mixed with a neutral solvent (ethanol) few drops of phenolphthalein solution was added as an indicator. The solutions were well homogenized before being titrated with aqueous 0.1 M of NaOH, till pink-like colour persisted for 15 sec. The amount of acid contents was determined using AOAC [8] method.

Determination of iodine contents: Five hundred milligrams of each sample from the botanicals were measured into 100 mL. Volumetric flasks and made up to mark with chloroform solution. Five milliliters of the solution from each sample placed in different flasks. Ten milliliters of 10% Potassium Iodine and 2 mL of sterile distilled water were added to each flask content. Two drops of starch solution were added as indicator. Each sample solution was titrated with N/40 Sodium thiosulphate solution.

Determination of hydro cyanic acid and oxalic acid contents: These were determined for each botanical sample using the method described by Oke [12]. The qualitative analysis of different sugar was carried out as described by Alabi [13].

Determination of mineral contents: The determination of the mineral contents of each botanical sample, was carried out using method described by Alabi [13] and Hack [14].

RESULTS

Vernonia amygdalina has the highest amount of amino acids such as Thiamine, Pyodoxine, Ascorbic acid, glycine, Cysteine and Casein hydrolysate which are significantly higher (p = 0.05) than those of *B. pinnatum, E. globules* and significantly higher (p = 0.05) in both *V. amygdalina* and *B. pinnatum* than in *E. globules* and *O. gratissimum* (Table 1). The amount of glucose obtained from *V. amygdalina* and *B. pinnatus* are almost the same but the two values are significantly higher than the values obtained for *E. globules* and *O. gratissimum*. The crude protein value of *V. amygdalina* is significantly higher (p = 0.05) than values obtained for remaining botanicals (Table 2). *Bryophyllum pinnatum* was significantly (p = 0.05) higher in Lipid content than the remaining three botanicals. The acid values of *E. globulus* were the highest followed by that of *B. pinnatum* and by *O. gratissiumum*, the three values of the botanicals were significantly (p = 0.05) higher than the value obtained for *V. amygdalina* (Table 2). The iodine values of O. *gratissimum* and that of *V. amygdalina* was not significantly different but the two values were significantly higher than those of *B. pinnatum* and *E. globulus*. Extract from *O. gratissimum* has the highest value of Hydrocyanic acid, which is significantly greater than those of other botanicals while *E. globulus* has the highest amount of total oxalate content (Table 2). The extracts of *V. amygdalina* contained the highest amount of Na, K, Mg, Mn, Fe, Cu, Zn and ash contents, which were significantly higher in values than the other botanicals. *B. pinnatum* and *O. gratissimum* contained the same amount of ash contents as *V. amygdalina* but the contents were not significantly (p = 0.05) different (Table 3). *Vernonia amygdalina* contains the highest

Table 1: Analysis of the amino Acid contents of, *Vernonia amygdalina Bryophyllum pinnatum, Eucalyptus globules* and *Ocimum gratissimum*

	Types of amino acids analysis (mg^{-1}/100 g)						
Plant materials used	Ihiamine	Pyrdoxine	Ascorbic acid	Glycine	Cysteine	Casein hydrolysate	Nicotinamide
V amygdalina	170.00a	2.06a	20.49a	4.63a	1.84a	96.99a	1.65a
B. pinnatum	0.30b	0.74b	13.28c	2.71c	0.64d	53.68d	1.60a
E. globules	0.16c	0.52	10.60d	2.52c	0.80c	80.45c	0.52c
O. gratissimum	0.20bc	0.41d	15.35b	3.82b	1.60b	89.99b	0.86b

Table 2: Analysis of chemical and food contents of *Vernonia amygdalina*, *bryophyllum pinnatum*, E*ucalyptus globulus* and *Ocimum gratissimum*

	Chemical and food contents determined (mg^{-1}/100 g)						
Plant materials used	Carbohydrate	Protein	Lipids	Acids	Iodine	Hydrocyanic acid	Total oxylate
V. amygdalina	4.31a	20.2a	15.0b	10.26d	35.82a	0.46c	0.62d
B. pinnatum	4.29a	14.5b	24.3a	36.60b	10.20b	3.85a	1.96b
E. globules	0.65c	12.3c	10.6d	42.20a	10.30b	3.96a	2.13c
O. gratissimum	2.38b	11.4d	13.7b	12.25c	34.25a	0.51b	0.86b

Table 3: Analysis of the mineral Ash and fibre content of *Vernonia amygdalina*, *Bryophyllum pinnatum*, *Eucalyptus globulus* and *Ocimum gratissimum*

	(mg^{-1}/100 g)										
Botanicals used	Na	Ca	K	P	Mg	Mn	Fe	Cu	Zn	Ash	Fibre
V. amygdalina	8.48a	67.39b	60.90a	60.90a	88.10	5.56a	14.20a	6.01a	8.05a	10.22a	9.75b
B. pinnatum	7.65b	65.40b	90.25a	60.46a	87.62b	5.10a	14.13a	5.94a	7.92a	10.22a	10.25b
E. globules	8.05a	70.22a	84.71b	20.24b	83.95c	4.56b	12.04c	6.00a	6.86a	7.33b	11.36a
O. gratissimum	8.25a	64.80c	86.24b	61.25a	84.10c	4.65b	13.36b	5.69b	6.85b	10.25a	9.26c

Each of the data is mean of four replicates. Figures followed by the same alphabet along the columns are not significantly different at p = 0.05. Using (DMRT) Duncan Multiple Range Test

Table 4: Qualitative analysis of sugar contents of *Vernomia amygdalina Brypophyllum pinnatum*, *Eucalyptus globulus* and *Ocimum gratissimum*

	Sugars qualitatively analyzed (mg^{-1}/100 g)							
Plant material used	Raffinose	Lactose	Sucrose	Glucose	Galactose	Fructose	Maltose	Arabinose
V. amygdalina	5.1a	2.61a	13.20a	7.20a	6.56a	6.00a	7.24a	9.25a
B. pinnatum	2.8b	0.20c	4.92c	2.62c	5.82b	2.30c	5.64b	8.66b
E. globules	2.3b	0.10c	2.65b	1.45d	2.46c	1.20d	5.88b	8.90ab
O. gratissimum	5.0a	2.15b	10.85b	6.05	5.24b	4.80b	6.92a	9.10a

Each of the data is a mean of four replicates. Figure followed by the same alphabet along the columns are not significantly different at p = 0.05. Using (DMRT) Duncan Multiple Range Test

amount of raffinose, lactose, sucrose, glucose, glatactose and fructose, which were significantly (p = 0.05) higher than the values obtained from other three botanicals, While the Maltose and Arabinose sugar contents of *V. amygdalina* and *O. gratissimum* were not significantly different (Table 4).

DISCUSSION

Result from this work showed that extracts from both fresh and dry leaves of *bryophyllum pinnatum*, *Eucalyptus globulus* and *Ocimum gratissimum* have high amount of acid, hydrocyanic acid and oxalic acid contents, which would make the extracts toxic and poisonous. This is in agreement with report of Oke [12] on mushrooms. Those contents suggest that the extracts of the three botanicals probably contain chemicals that can prevent attack of plants especially cowpea, pathogens such as *Pythium alphanidermatum* and *Sclerotium rolfussiiIi* Sacc. Which normally cause disease on cowpea and lower the yield especially during raining seasons. This observation is similar to that of *Kurucheve et al*. [15], who reported that extracts of *E. globulus* and fungitoxic against *Rhizoctinia solani*, the result also agrees with that of Oluma and Garba [16], who reported that extracts of *E. globulus* and *O. gratissimum* were fungi toxic against *Pythium aphanidermatum*. Tripathri *et al*. [17] also reported that the principal to exert a pronounced inhibitory effect on *Altennaria alternata* (Fr) Kessl; *Collectorihum capsici* (Syd) Bull and Bisby and *Sclerotium rofusii* Sacc. Awua [18] reported that engenol may be responsible for the fungitoxic effects of this plant as noticed on *Ustilago maydis*, *Curvularia lunata* and *Rhizopus* species. This gratissimum since the analysis of the contents showed that the three botanicals contained high amount of acid, contents Sofowora [19] established the *antimicrobial* activities of *thymol*, which forms 75% of the volatile oil of *O gritissimum*. Similarly Sofowora [19] reported *O. gratissimum* as an insect repellant. Furthermore, the higher values of hydrocynamic acid and oxalic acid obtained in their extracts may make them toxic and poisonous. Probably, this may be the reason why plants are popular for the treatment of diarrhea [20]. Kay *et al*. [21] reported that thymol from the oil of *O. gratissimum* is used in the treatment of throat lozenges and gargles as trihalogenophenols. *Vernonia amygdalina* contained a lot of thiamine and other amino acids. The extract's oil is less acidic. Hence, V. *amygdalina* and *O. gratissiumum* plants are popular condiments in sauce and flavoring agents, moreso in Africa.

The earlier reports on *O. gratissimum* [16, 17, 19, 20] confirm this our findings that extracts from *B. pinnatum*, O. *gratissimum* and *E. globulus* have possible board spectrum efficiency level to combat of pathogens of cowpea seedlings. The extracts from *V. amygdlina* contained a high amount of Thiamine and other amino acids while the oil contained less acid value and high iodine value hence could be used in treating goiter.

The resistance of the extracts against the autoclave treatment, which made the four extracts to retain their active ingredients, is an indication that the active ingredients (i.e.) are a thermos table. This agrees with the findings of Oluma and Garba [16]. In spite of those interesting findings, efforts are being made to test the effects of these extracts on plants in the field to actually determine their agricultural usefulness.

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