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Mineral and phytochemical composition of additives produced from *Chromolaena odorata* leaf extracts by different extraction methods

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Abstract

This study was conducted to determine the mineral, phytochemical and antinutritional composition of additive produced from Chromlaena odorata leaves extract by different extraction methods. Fresh leaves werewashed in water, shade dried for 48 hours and thereafter, macerated. Extraction of Chromolaena odorata was performed using six methods; water decoction, methanol, and ethanol extraction, followed by microwave assisted repeats of the three extraction media. The additive production was completed by the addition of sodium benzoate as preservative and Aspartame® as sweetener to each extract. The additives werelabeled Chromolaena odorata water decoct (COWD), Chromolaena odorata microwave assisted water decoct (CMWD) Chromolaena odorata ethanol extract (COEE), Chromolaena odorata microwave assisted ethanol extract (CMEE), Chromolaena odorata methanol extract (COME) and Chromolaena odorata microwave assisted methanol extract (CMME)then analysed for mineral,phytochemical and antinutritional composition. A 2x3 factorial in a completely randomized design (CRD) was employed.Results of this study indicated significantly ($p<0.05$) highest calcium (8.24mg/L) value for COME. Potassium value ($p<0.05$) was highest in CMWD (5.24mg/l) while Ironvalue was highest ($p<0.05$) at COWD (49.69mg/l). Methanol as an extraction media produced the highest value of calcium (7.72mg/l), magnesium (0.88mg/l), potassium (4.97mg/l) and Iron (40.20mg/l). There was no significant ($p>0.05$) differencein parameters analysedbetween microwaveand non-microwave assisted extraction except for manganese. Phytochemical and antinutritional factor concentration indicated that values obtained fellwithin tolerable levels especially for monogastric animals. Cyanide was significantly ($p<0.05$) lower for CMEE (0.99mg/100ml) than others while oxalate, saponin, alkaloid and antioxidants were significantly ($p<0.05$) higher for COME. The values of minerals, phytochemicals and antinutritional factors obtained makes the additive a potential candidate for promotion of growth in broiler chicken.

Key words: Mineral; Phytochemical; *Chromolaena odorata*; Extraction; Microwave

Description of Problem

Additives are added in poultry feeds to improve nutritive value of ingredients and enhance poultry performance by improving feed utilization and promoting growth (1). Additives therefore are useful since growth is certainly the most important element and the objective in most poultry production operation (2).Phytogenic additives (herbs, spices, plant extracts and essential oils) have been used for centuries to preserve food for man and to make it more appetizing. They are well recognized for their health benefitsin many countries around the world. Consequently, medicinal plants have been developed and proposed for use in feed as natural antimicrobials (3; 4). Compared with synthetic inorganic chemicals, these plant-derived products have proven to be natural, less toxic, residue free and are thought to be ideal feed additives in food animal production (5). Although a range of phytogenic materials have been studied in livestock production with the aim of enhancing feed utilization, growth performance and meat quality,attempts to use their leaves as feed in monogastric animal feeding is usually limited by their poor intake, high fibre content and, in some cases the presence of anti-nutritional

substances or metabolic inhibitors suchas cyanogens, alkaloids, saponins and tannins as well as low digestibility, low nutrient content and subsequently poor animal performance (6; 7). The negative effects of high dietary inclusion levels of most leaf meals have been linked to their bulkiness, reduced energy supply and essential amino acids deficiency, with bulkiness due to high fibre content being probably the major limiting factor at high level of inclusion (7; 8; 9). Therefore, elimination of this fibre effect by extraction of the phytogenic constituents of the leaf and applying them appropriately to poultry production may solve these problems (10; 11).

Several cheaper alternative technologies have however, recently been used in the production and extraction of phytogenic products from leaf meals (12; 13; 10; 14). These technologies have recorded varying levels of success and are adaptable to local conditions and practices especially in Nigeria. These alternative methods also employ the time tasted water decoction and alcohol-based solvent extraction methods to achieve their phyto-extraction process. *Chromolaena odorata* has been reported to have multipurpose properties (15). The leaves are rich sources of micro and macro minerals (16), with proximate composition of 18.7% crude protein, 11.7% crude fibre, 3.63% Ash, 1.01% ether extract and 65% nitrogen free extract (17), indicating high levels of essential primary nutrients (18). (19) Noted that *Chromolaena odorata* leaf extract have immune-stimulating and prophylactic properties as well as anti-anaemic property, which has been attributed to the high iron content of its chlorophyll. Therefore, directing research efforts at the exploitation of neglected phytogenic plants such as *Chromolaena odorata* which is indigenous, readily available, of high quality and cheaper could yield products that are cost effective in poultry production (20). There is therefore the need toextract optimally and enhance continuous use of such additivesfrom *Chromolaena odorata* leaves using appropriate methods of extraction, preservation and to offer same in a palatable form.

Materials and Methods

Production of the additives involved harvesting and processing of *Chromolaena odorata* leaves, extraction using different methods, addition of preservative and sweetener.

Harvesting and processing of *Chromolaena odorata* leaves

The leaves of *Chromolaena odorata* (CO) for this experiment were harvested from Umudike in Ikwuano Local Government Area,Abia State.The harvested fresh leaves were weighed then washed in water to remove sand and debris, shade dried for 48 hours to reduce the moisture content after which the shade-dried leaves were maceratedusingMastercheff® blender (Model Mc-211) to increase surface tension.

Experimental design and Extraction Procedures

The macerated *Chromolaena odorata* leaves were extracted ofin three media; water, methanol and ethanol, eachin non-microwave assisted and microwave assisted. Each extraction process was replicated three times processes were replicated repeats of the three extraction methods in a 2x3 factorial of a completely randomized design (CRD) experiment.

Non-microwave assisted water decoction (WD)

Extraction of the macerated *Chromolaena odorata* (CO) leaves was by soaking in water in an earthenware

container. Five hundred (500) mls of water was used for every 300gramsof CO. The containerwas covered and boiled for 10 minutes, until water volume was halved; the content wasthen lablelled, cooled, and refrigerated. Water decoctions waskept for 2-3 days (21).

Microwave assisted water decoction (MW)

Three hundred (300) grams of the macerated CO were transferred to a 500 mL conical flask and 400mLof boiled water was added. The mixture was shaken thoroughly and left for 45 minutes so that the absorption of water by CO was thorough. The flask was the kept in the microwave oven for 5 minutes with irradiation power set at 480 W for extraction to take place. After the extraction, the conical flask was taken out from the microwave oven and the content filtered with Whatman paper No. 4. Concentration of extract was carried out in a water bath and calculated percentage yield of extract (%w/w) was determined (22)

Non-microwave assisted methanol Extraction (ME)

Three hundred grams (300g) of the macerated plant materials were measured separately into different containers and soaked with 400Ml of methanol. The mixtures were allowed to stand for 72 hours with intermittent shaking and then filtered with muslin cloths.

Microwave assisted methanol extraction (MM)

Three hundred (300) grams of the macerated plant materialswere transferred to a 500 mL conical flask and 400mL of methanol added. The setup was thoroughly mixed and left for 45 minutes so that the absorption can properly take place. The flask was transferred to the microwave oven. The extraction time in the microwave oven was set at 5 minutes and irradiation power set at 480 W. After the extraction, the conical flask was taken out from the microwave oven and filtered with Whatman paper No. 4 (23).

Non-microwave assisted Ethanol Extraction (EE)

The ethanol extraction of macerated CO was prepared as outlined by (24). Three hundred grams of CO was soaked in 400mL of 80% ethanol in a container, thoroughly mixed and left to stand for 72 hours. Thereafter, it was filtered with Whatman paper No. 4. (24)

Microwave Assisted Ethanol Extraction (ME)

Three hundred grams (300g) of each of the macerated test materials will be placed in a 500mL conical flask, 400mL of 80% ethanol poured into each of the flask and left for 45 minutes for proper absorption. Thereafter, they will be transferred to the microwave oven and treated for microwave process. The extraction time will be set at 5 minutes and irradiation power set at 480W. After the extraction process, the conical flask will be taken out from the oven, and each filtered with Whatman paper No. 4.

Preservation and sweetening of leaf extract

To preserve the additive produced from the extract, 0.05g each of sodium benzoate was dissolved in 3mL of distilled water and shaken thoroughly inside a beaker. One and half (1.5) mL of the solution was then added to a litre of extractants according to manufacturer's prescription to preserve the extracts. Aspartame® was added to the extracts at 20mg/liter, inorder to sweeten and mask the bitterness usually associated with phytogenics.The additives produced from the extracts were stored at room temperature in properly labeled sample bottles for easy

identification as follows;

Chromolaena odorata water decoct (COWD)
Chromolaena odorata microwave assisted water decoct (CMWD)
Chromolaena odorata ethanol extract (COEE)
Chromolaena odorata microwave assisted ethanol extract (CMEE)
Chromolaena odorata methanol extract (COME)
Chromolaena odorata microwave assisted methanol extract (CMME).

Mineral Analysis

The concentration of macro minerals such as calcium, phosphorus, magnesiumand potassium and micro mineral such as iron, zinc, manganese and copper in the additive produced from*C.odorata* leaf extracts was determined using Dry Ashing method as described by (25) using theAtomic AbsorptionSpectrophotometer (AAS), Bulk Scientific, Model 210 VGB.

Phytochemical Analysis

Phytochemical tests were carried out on the additives produced from the *C. odorata*leave extracts to determine the concentration of essential chemicals using the procedure described by (26) and (27). The phytochemical assayed include Alkaloids, Phenols, Saponins, Antioxidants, Trypsin inhibitor, Oxalates, Phytate Cyanide and Total carotenoid.

Statistical Analysis

Means of all the data collected were subjected to analysis of variance (ANOVA) as outlined by (28). Duncan New multiple Range Test (DNMRT) as outlined (29) was used to separate the means where significant treatment effect exists. These statistical analyses were carried out using (30).

Results and Discussion

Mineral composition of additives produced from *Chromolaena odorata* leafextrctis presented in Table 1. Calcium levels of the extracts ranged from 2.89 to 8.24mg/l with COME containing higher ($p<0.05$) values than other treatment groups, this Results are in disagreementwith (31) who obtained higher values for green and black tisane of *Chromolaena odorata* leave, disparity observed maybe due to the activities of extraction media used (32).Calcium is associated reduced risk of osteoporosis, it also plays important role in blood clothing and muscle (33). This could be the reason for which *Chromolaena odorata* leaves are excllent in stopping bleeding topically. Magnesium values ranged from 0.35 to 0.96mg/l, result obtained from CMME was higher ($p<0.05$) when compared to other groups, similar ($p>0.05$) results were obtained in CMEE and COME followed by similar ($p>0.05$) results from COWD, CMWD and COEE. Thelevel of magnesium obtained in this studywould help contribute to the dietary intake. Magnesium is required for the muscular contractions (33). Potassium values ranged from 3.57mg/l in COEE to 5.2.4mg/l in CMWD. Iron values ranged from 29.82mg/l in COEE to 49.69mg/l in COWD while Zinc levels ranged from 0.41mg/l in COME to 0.73mg/l in COEE with COEE having significantly ($p<0.05$) higher level of zinc than other than others. The results were slightly higher than results obtained by (31) this could be because of higher quantity of extraction medium used by this authorIron and Zinc are essential trace elements required for optimal growth, development and maintenance of immune function (33). Phosphorus value is similar ($p>0.05$) among the group. The presence of phosphorus in *Chromolaenaodorata*makes it essential because phosphorus is neededto repair tissue

cells and to build stron bones (34). Manganese levels ranged from 1.10mg/l in COWD to 1.93mg/l in CMWD. Copper values were significantly ($p<0.05$) higher in COEE, least Copper values ($p>0.05$) was reported in COME while similar results were obtained in other groups.

Table 1: Mineral composition of additives produced from *Chromolaena odorata* leaf extracts

Parameters (mg/l)	COWD	CMWD	COEE	CMEE	COME	CMME	SEM
Calcium	2.89^{e}	2.20^{f}	7.79^{b}	4.56^{d}	8.24^{a}	7.19^{c}	0.58
Magnesium	0.35^{c}	0.41^{c}	0.41^{c}	0.80^{b}	0.81^{b}	0.96^{a}	0.05
Potassium	3.86^{e}	5.24^{a}	3.57^{f}	4.14^{d}	5.06^{b}	4.88^{c}	0.15
Iron	49.69^{a}	38.05^{d}	29.82^{f}	33.38^{e}	41.91^{b}	38.50^{c}	1.53
Zinc	0.54^{c}	0.60^{b}	0.73^{a}	0.44^{d}	0.41^{d}	0.68^{a}	0.03
Phosphorus	0.53^{a}	0.62^{a}	0.54^{a}	0.34^{a}	1.28^{a}	0.76^{a}	0.15
Manganese	1.10^{e}	1.93^{a}	1.30^{d}	1.73^{b}	1.63^{c}	1.59^{c}	0.06
Copper	0.04^{bc}	0.05^{b}	0.07^{a}	0.05^{bc}	0.02^{d}	0.03^{cd}	0.04

^{abcdef}Means within a row with different superscripts are significantly different ($p<0.05$)
COWD=*Chromolaena odorata* water decoct, CMWD=*Chromolaena odorata* microwave assisted water decoct COEE=*Chromolaena odorata* ethanol extract, CMEE=*Chromolaena odorata* microwave assisted ethanol extract
COME=*Chromolaena odorata* methanol extract, CMME=*Chromolaena odorata* microwave assisted methanol extract.

Effect of extraction media on mineral composition of additives produced from *Chromolaenaodorata* leaf extractis presented in Table 2. Calcium and Magnesium values for methanol medium were significantly ($P<0.05$) highest compared to water and ethanol media, this maybe due to calcium ion elevation properties of methanol (35). Potasium and Iron concentration in this additive showed similar ($p>0.05$) results for water and methanol. Similar ($p>0.05$) values was observed for zinc, phosphorus and manganese with all the extraction media. Copper concentration showed similar ($p>0.05$) results in water and ethanol extraction media while lowest ($p<0.05$) copper concentration was oberseved in methanol.

Table 2: Effect of extraction media on the mineral composition of additives produced from *Chromolaena odorata*leaf extract

Parameters (mg/l)	Water	Ethanol	Methanol	SEM
alcium	2.54^{c}	6.17^{b}	7.72^{a}	0.58
agnesium	0.38^{c}	0.61^{b}	0.88^{a}	0.06
Potassium	4.54^{a}	3.85^{b}	4.97^{a}	0.15
Iron	43.87^{a}	31.60^{b}	40.20^{a}	1.53
Zinc	0.57	0.59	0.54	0.03
Phosphorus	0.57	0.44	1.02	0.15
Manganese	1.51	1.52	1.61	0.07
Copper	0.05^{a}	0.06^{a}	0.03^{b}	0.00

^{abc}Means within a row with different superscripts are significantly different ($p<0.05$)

Effect of extraction method on the mineral composition of additives produced from *Chromolaena odorata* leaves extract is presented in Table 3. Microwave assisted method of extraction produced similar ($p>0.05$) but numerically higher potassium, iron, zinc, phosphorus and manganese values in the additives with magnesium value significantly higher in microwave method than non microwave method while calcium and copper numerically higher in non-microwave assisted method. These results agree substantially with (36) who reported that microwave assisted extraction was better and more efficient than conventional extraction methods.

Table 3: Effect of extraction method on the mineral composition of additives produced from *Chromolaena odorata* leaf extract

Parameters (mg/l)	Microwave Method	Non Microwave Method	SEM
Calcium	4.65	6.31	0.58
Magnesium	0.67	0.57	0.06
Potassium	4.75	4.16	0.15
Iron	36.64	40.47	1.53
Zinc	0.57	0.56	0.03
Phosphorus	0.58	0.78	0.15
Manganese	1.75[a]	1.35[b]	0.07
Copper	0.04	0.05	0.00

[ab]Means within a row with different superscripts are significantly different ($p<0.05$)

Phytochemical and antinutritional composition of additives produced from *Chromolaenaodorata leaves* extract is presented in Table 4. Oxalate concentration ranged from 5.47mg/100ml in CMWD to 8.00mg/100ml in COME. Phytate concentration was significantly ($p<0.05$) higher in CMEE, similar results were recorded in COME and CMME followed by CMWD and COEE. This result differs with (37) who reported 0.22 and 0.58% phytate concentration for wet and dry Chromolaena odorata leaves respectively, this variation maybe due to the processing and extraction procedure which led to an increase in phytate concentration. Phytate has been suggested to serve a store of cationsof high energy phosphoryl group and at the same time chelating free iron as a potent natural antioxidant (38). Phenol levels ranged from 35.00mg/100ml in CMME to 55.49mg/100ml in COWD. Phenolics are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts (39). Trypsin inhibitor level ranged from 5.11mg/100ml in CMWD to 55.49mg/100ml in COWD. Cyanide concentration in COME was significantly ($P<0.05$) higher than others and the low cyanide concentration reported in COEE and CMEE agrees with (13) who reported a low cyanide level for ethanolic extracts of phytogenic plantwhich wastolerable levels especiallyfor monogastric animals.Alkaloid concentration was hghest in COEE. Saponin concentration ranged ($p<0.05$) from 0.97g/100ml in CMEE to 1.54g/100ml in COEEThe presence of saponins in this study justifies the cholesterol lowering properties of *Chromolaena odorata* as reported in a study by (40). Total carotenoid values ranged from 0.40g/100ml in CMWD to 1.45g/100ml in COEE while antioxidants ranged from 23.41% in CMEE to 36.24% in COME.

Table 4: Phytochemical and Antinutritional composition of additives roduced from *Chromolaena odorata* leaf extracts

Parameters	COWD	CMWD	COEE	CMEE	COME	CMME	SEM
Oxalate (mg/100ml)	7.01^{c}	5.47^{e}	6.83^{d}	7.50^{b}	8.00^{a}	7.00^{c}	0.18
Phytate (mg/100ml)	0.82^{d}	1.11^{c}	1.13^{c}	1.38^{a}	1.28^{b}	1.26^{b}	0.04
Phenol (mg/100ml)	55.49^{a}	39.32^{d}	39.05^{d}	35.00^{e}	53.35^{b}	40.91^{c}	1.86
Trypsin inhibitor (mg/100ml)	12.72^{e}	5.11^{f}	39.10^{c}	37.27^{d}	49.21^{a}	44.32^{b}	3.98
Cynide (mg/100ml)	1.51^{b}	1.50^{b}	1.21^{c}	0.99^{d}	1.57^{a}	1.18^{c}	0.05
Alkaloid (g/100ml)	0.30^{bc}	0.37^{b}	0.54^{a}	0.20^{c}	0.49^{a}	0.52^{a}	0.03
Saponin (g/100ml)	1.16^{b}	1.21^{b}	1.54^{a}	0.97^{c}	1.48^{a}	1.51^{a}	0.05
Total carotinoid (g/100ml)	0.66^{e}	0.40^{f}	1.45^{a}	0.77^{d}	1.18^{c}	1.32^{b}	0.09
Antioxidant (%)	30.73^{b}	27.23^{c}	34.17^{a}	23.41^{d}	36.24^{a}	31.33^{b}	1.07

^{abcdef}Means within a row with different superscripts are significantly different ($p<0.05$)

COWD=*Chromolaena odorata* water decoct, CMWD=*Chromolaena odorata* microwave assisted water decoct COEE=*Chromolaena odorata* ethanol extract, CMEE=*Chromolaena odorata* microwave assisted ethanol extract

COME=*Chromolaena odorata* methanol extract, CMME=*Chromolaena odorata* microwave assisted methanol extract

Effect of extraction media on phytochemical and antinutritional composition of additives produced from *Chromolaena odorata*leaf extract is presented in Table 5. Oxalate, Phytate, Trypsin inhibitor and total carotinoid concentration were significantly ($p<0.05$) higher in ethanol and methanol media when compared to water. Phenol concentration was significantly ($p>0.05$) lower in ethanol medium compared to others which had similar ($p>0.05$) values.Cyanide and alkaloid concentration were significantly ($p<0.05$) higher for water and methanol media respectively than ethanol. Saponin concentration for methanol medium was significantly ($p<0.05$) higher than other media. Antioxidants value were similar ($p>0.05$) in water and methanol mediawhile similar ($p>0.05$) results were obtained in ethanol and water media.These results have placed the additive produced from *Chromolaena odorata* leave exract in a good position as potential feedstuffs and additives, especially in poultry birds(41)

Table 5: Effect of extraction media on the Phytochemical and Antinutritional Composition of additives produced from *Chromolaena odorata*leaf extract

Parameter	Water	Ethanol	Methanol	SEM
Oxalate (mg/100ml)	6.24^{b}	7.16^{a}	7.50^{a}	0.19
Phytate (mg/100ml)	0.96^{b}	1.25^{a}	1.26^{a}	0.04
Phenol (mg/100ml)	47.40 ^{a}	37.02^{b}	47.13^{a}	1.87
Trypsin inhibitor (mg/100ml)	8.91^{b}	38.18^{a}	39.26^{a}	4.11
Cyanide (mg/100ml)	1.50^{a}	1.10^{b}	1.37^{a}	0.05
Alkaloid (g/100ml)	0.34^{b}	0.37^{ab}	0.51^{a}	0.03
Saponin(g/100ml)	1.18^{b}	1.25^{b}	1.49^{a}	0.05
Total carotinoid(g/100ml)	0.94^{b}	1.12^{a}	1.25^{a}	0.09
Antioxidant (%)	28.98^{ab}	24.62^{b}	33.78^{a}	1.88

^{ab}Means within a row with different superscripts are significantly different ($p<0.05$)

Effect of extraction method on the phytochemical and antinutritional composition of additives produced from *Chromlaena odorata* leaves extracts is presented in table 6. Non microwave method of extraction reduced ($p<0.05$) phytate concentration while microwave extraction method reduced ($p<0.05$) phenol, cyanide, and antioxidants. Values of oxalate, Trypsin inhibitor, alkaloid, saponin and total caotinoid were similar ($p>0.05$) among the extraction methods. (42) Reported similar oxalete, alkaloid and saponins values in microwave and non microwave methods of aspilia africana extracts and eqaully observed significant increase in cynide and antioxidant concentration for the same extract.

Table 6: Effect of extraction method on the Phytochemical and Antinutritional Composition of additives produced from *Chromolaena odorata* leaf extract

Parameters	Microwave Method	Non Microwave Method	SEM
Oxalate (mg/100ml)	6.65	7.28	0.19
Phytate (mg/100ml)	1.25[a]	1.07[b]	0.04
Phenol (mg/100ml)	38.41[b]	49.29[a]	1.87
Trypsin inhibitor (mg/100ml)	28.90	28.67	4.11
Cynide (mg/100ml)	1.22[b]	1.43a	0.05
Alkaloid(g/100ml)	0.36	0.44	0.03
Saponin(g/100ml)	1.23	1.39	0.05
Total carotinoid(g/100ml)	0.83	1.10	0.09
Antioxidant (%)	24.54[b]	33.71[a]	1.88

[ab]Means within a row with different superscripts are significantly different ($p<0.05$)

Conclusion and Application

1. Additives produced from *Chromolaena odorata* leaf extracts using different extraction methods varied in in some mineral and phytochemical and anti-nutritional composition.
2. Additives produced form *Chromolaena odorata* extract using non microwave assisted and methanol showed higher values of minerals except zinc, iron and copper. Similarly, methanol as an extraction media appears to have produced better phytochemical and antinutritional values.
3. Microwave assisted extraction method however, produced better values of most phytochemical and antinutritional parameters of the additives.
4. Based on the values obtained, the additives could be candidates for enhancing the performance of poultry and indeed other livestock.
5. From this study it could be concluded that *Chromolaena odotrata* extraction using methanol improved mineral and antinutritional constituents even though extraction methods seem not have any effect significant on them making this extract a potential candidate as an additive in poultry and livestock production

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