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MINERAL AND PHYTOCHEMICAL COMPOSITION OF ADDITIVES PRODUCED BY DIFFERENT EXTRACTION METHODS FROM *Aspilia Africana* LEAVES FOR POULTRY.

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ABSTRACT

This study was conducted to evaluate the mineral, phytochemical and antinutritional composition of additives produced from Aspilia africana leaves extracted by different methods. Fresh leaves were washed in water, shade dried for 48 hours and thereafter, macerated. Extraction of A.africana 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. They were labeled Aspilia africana water decoct (AAWD), Aspilia africana microwave assisted water decoct (AMWD), Aspilia africana ethanol extract (AAEE), Aspilia africana microwave assisted ethanol extract (AMEE), Aspilia africana methanol extract (AAME) and Aspilia africana microwave assisted methanol extract (AMME), then analyzed for mineral, phytochemical and antinutritional composition. A 2x3 factorial in a completely randomized design (CRD) experiment was employed. Results of this study indicated significantly ($p < 0.05$) higher calcium (9.14mg/L) value for AAME than AAWD (2.39mg/l). Potassium values ($p < 0.05$) were highest in AMWD (8.12mg/l) while Iron values were significantly ($p < 0.05$) higher at AMEE (38.49mg/l). Water as an extraction media produced higher values of potassium (7.35mg/l), Iron (32.79mg/l) and manganese (1.46mg/l). Microwave assisted extraction improved mineral values although there was no significant ($p > 0.05$) differences with non-microwave assisted extraction except for magnesium. Phytochemical and antinutritional factor concentration indicated that values obtained fell within tolerable levels especially for monogastric animals. Cyanide was significantly ($p > 0.05$) lower for AMME (0.27mg/100ml) than others while oxalate, phenol and antioxidants were significantly ($P < 0.05$) higher for AAWD. It is concluded microwave assisted extraction in water, ethanol and methanol is more effective than non-microwave assisted. The media of water, ethanol and methanol extraction media are effective in extraction with ethanol producing the higher values in most parameters measured. The values of the minerals, phytochemicals and antinutritional factors obtained makes the additive a candidate for promotion of growth in boiler chicken Aspilia africana as nutritional safe with several mineral attributes.

Introduction

Phytogenic materials are widespread throughout the world (Blumenthal, 2003; Sneader 2005). The beneficial effects of herbs and other plant matter in improved poultry production have been reported severally (Toghyani *et al* 2010; Obuzor and Nutui, 2011; Essiet and Solomon, 2013). This could attributed to the essential components which have antimicrobial, antioxidant, antifungal and anticoccidial activities (Khan *et al.*, 2012). Several herbs offered as leaf meals have been investigated for their ability to enhance feed utilization, growth performance and meat quality of poultry especially broiler chickens (Oko and Agiang, 2011). Most of the leaf meals also contain high amounts of ash and therefore could be a good source of minerals for poultry.

The presence of anti-nutritional factors such as tannin, alkaloids, saponins, flavonoids, cyanide, oxalate, phytate and amino acid imbalance are however, of concern when incorporating leaf meals into poultry diets, due to their effect on nutrient utilization. (Sing

and Panda, 1992; Kanya-Agyemanget *al*, 2007). Factors implicated in poor digestibility and performance of leaf meal- based broiler diets among others is the high levels of crude fibres which boiler chicken, (Santoso and Sartini, 2001; Buragohain, 2016; Ubua *et al.*, 2019).

Extraction of specific components of plants helps to overcome some of the drawbacks to the use of leaf meals in poultry feeding, (Alagbe, 2017; Sebola *et al*, 2019). Extraction of plant secondary metabolites using selective solvents through standard procedures is therefore the basis of phytogenic additive production (Handa and Khanuja, 2008). Studies have shown the impact of different types of solvents, such as methanol, hexane, and ethyl alcohol, on extraction of phytochemicals from various plants parts (Koffi *et al* 2010; Anokwuru, *et al* 2011). Also, Microwave assisted extraction (MVE) method which uses microwave energy to facilitate partition of analytes from the sample matrix into the solvent (Trusheva *et al* 2007) reduces extraction time with comparable solvent volume.

Aspilia africana an indigenous weed found in abundance in compound bushes and farms in southeastern Nigeria (Okoli *et al*, 2003) but has received limited study as phytogetic additive candidate. (Adedeji *et al* 2014; Uchewa *et al* 2018). *Aspilia africana* leaf contains, 14-26% crude protein, reasonable amount of carbohydrates, amino acids (Adeniyi and Odufowora, 2000; Taiwo *et al* 2008; Abi and Onuoha, 2010; Oko and Agiang, 2011; Maduka *et al*, 2018), flavonoids, alkaloids, saponin, phenols, vitamins, A, C and E.

This study therefore determined the mineral and phytochemical composition of additives produced by different extraction methods from *Aspilia Africana* leaves for poultry and pigs.

Materials and Methods

Harvesting and processing of *Aspilia africana* Leaves

The leaves of *Aspilia africana* for this experiment were harvested from Umudike in Ikwuano Local Government Area, Abia State. The harvested fresh leaves was 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 macerated using Mastercheff® blender (Model Mc-211) to increase surface tension.

Experimental design, Extraction Procedures and production of additives

The macerated leaves were extracted of *A. africana* in three media; water, methanol and ethanol, each in 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 *Aspilia africana* leaves (AA) was by soaking in water in an earthenware container. Five hundred (500) mls of water was used for every 300 grams of AA. The container was covered and boiled for 10-20 minutes, until water volume was halved; the content was then strained, cooled, and refrigerated. Water decoctions usually keep for 2-3 days (Santaigo-flores, 1998).

Microwave assisted water decoction (MW)

Three hundred (300) grams of the macerated AA were transferred to a 500 mL conical flask and 400 mL of boiled water was added. The mixture was shaken thoroughly and kept for 45 minutes so that the absorption of water by AA was thorough. The flask was 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 (Saurabh, *et al* 2015)

Non-microwave assisted methanol Extraction (ME)

Three hundred grams (300g) of the macerated plant materials were measured separately into different containers and soaked with 400 mL 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 materials were transferred to a 500 mL conical flask and 400 mL of methanol added. The setup will be thoroughly mixed and kept 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 (Verma *et al.*, 2011).

Ethanol Extraction (EE)

The ethanol extraction of macerated AA was prepared as outlined by Smith (2001). Three hundred grams of each of the test material was soaked in 400 mL of 80% ethanol in a container, thoroughly mixed and left to stand for 72 hours. Thereafter, it was filtered with Whatman paper No. 4. (Smith, 2001)

Preservation and sweetening of leave extract

To preserve the extract, 0.05g each of sodium benzoate was dissolved in 3 mL 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, in order to sweeten and mask the bitterness usually associated with phytochemicals. The additives produced from the extracts were stored at room temperature in properly labeled sample bottles for easy identification as follows.

Aspilia africana water decoct (AAWD)

Aspilia africana microwave assisted water decoct (AMWD)

Aspilia africana ethanol extract (AAEE)

Aspilia africana microwave assisted ethanol extract (AMEE)

Aspilia africana methanol extract (AAME)

Aspilia africana microwave assisted methanol extract (AMME)

Mineral analysis

The concentration of macro minerals such as calcium, phosphorus, magnesium and potassium and micro mineral such as iron, zinc, manganese and copper in the test samples were determined using Dry Ashing method using the Atomic Absorption Spectrophotometer (AAS), Bulk Scientific, Model 210 VGB)

Phytochemical analysis

Phytochemical evaluation of the additives was carried out on the additives produced from the extract to determine the concentration of essential bioactive substances using the procedure described by Harbone (1973) and Sofowora (1980). The phytochemicals assayed include alkaloids, polyphenols, tannins, flavonoids, saponins, antioxidants, trypsin inhibitor, terpenoids, oxalates, carotenoids, phytate and cyanide all in mg/L.

Statistical Analysis

Means of data collected were subjected to analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1978). Duncan New multiple Range Test (DNMRT) as outlined by Obi (1990) was used to separate the means where significant treatment effect existed. These statistical analyses were carried out using R – Core Team (2012) application.

Results and Discussion

The mineral composition of additives produced from *Aspilia africana* leaves extract is presented in table 1. Calcium level in the additives ranged from 2.39 - 9.14mg/l with AAME containing significantly ($P<0.05$) higher values than other treatment groups. This result agrees with Okwu and Josiah (2006) who reported the presence of macro elements in *Aspilia africana* leaves with calcium as the most abundant. The differences in concentration maybe as result of differences in extraction methods. the high calcium concentration of *Aspilia africana* may be the reason

behind its effectiveness in wound healing and blood coagulation. These elements also act as inorganic cofactors in metabolic processes hence their absence can lead to impaired metabolism (Iheanacho and Udebuani, 2009). Magnesium values ranged from 0.29 – 0.53mg/l and was significantly ($p<0.05$) higher in AAME and AAME but similar ($P> 0.05$) in AMWD, AAME and AMME. Also, magnesium values was in line with those reported by Okwuonu *et al* (2017) who observed lower concentration of magnesium in Aqueous *Aspilia Africana* compared with crude *Aspilia africana*. Potassium values ranged from 3.24mg/l in AAEE to 8.12mg/l in AMWD, with AMWD recording significantly ($P<0.05$) higher values than others. Iron and zinc values were significantly higher ($p<0.05$) in AMEE than others, these results were higher than values reported by Okwuonu *et al* (2017). This disparity maybe due the different methods and media of extraction (Tomaniova *et al.*, 1998). In almost all the metabolic process in living organisms, iron is implicated as a key which forms an integral part of most enzymes and protein, acting as a catalytic centre in most enzymatic activities and playing roles in the process of oxygen binding in haemoglobin (Okaka and Okaka, 2001) Phosphorus value was significantly ($p>0.05$) lower in AMEE than others and manganese values ranged from 1.03mg/l in AAEE to 1.70mg/l in AAWD. Copper values were significantly ($P<0.05$) higher in AAME while similar results were obtained results in AMWD and AAEE. The presence of copper in *Aspilia africana* provides intrinsic ability to destroy a wide range of disease-causing pathogens (Feder, 2008).

Table 1: Mineral composition of additives produced from *Aspilia africana* leaves extract

Parameters (mg/l)	AAWD	AMWD	AAEE	AMEE	AAME	AMME	SEM
Calcium	2.39 ^e	5.18 ^c	2.06 ^f	4.96 ^d	9.14 ^a	5.83 ^b	0.57
Magnesium	0.35 ^c	0.51 ^{ab}	0.29 ^d	0.53 ^a	0.50 ^{ab}	0.49 ^b	0.02
Potassium	6.58 ^c	8.12 ^a	3.42 ^e	4.41 ^d	7.11 ^b	4.65 ^d	0.40
Iron	37.14 ^b	28.46 ^c	22.50 ^d	38.49 ^a	20.58 ^e	19.47 ^f	1.85
Zinc	0.55 ^b	0.85 ^a	0.49 ^c	0.86 ^a	0.49 ^c	0.54 ^b	0.04
Phosphorus	0.24 ^d	0.14 ^e	0.32 ^c	0.12 ^e	0.40 ^b	0.71 ^a	0.05
Manganese	1.70 ^a	1.22 ^c	1.03 ^d	1.48 ^b	1.23 ^c	1.50 ^b	0.05
Copper	0.04 ^b	0.02 ^{cd}	0.02 ^d	0.03 ^c	0.05 ^a	0.03 ^c	0.03

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

Effect of extraction media on mineral composition of additives produced from *Aspilia africana* leaves extracts presented in table 2. Calcium values for methanol medium were significantly ($P<0.05$) higher compared to water and ethanol media, this higher value maybe due to calcium ion elevation properties of methanol (Wayen *et al* 1999). Magnesium values were similar ($P>0.05$) among the different media of extraction though methanol media recorded the highest numerical value. Potassium values on the other hand, was highest in water medium and significantly ($p<0.05$) different from others. Iron

values of additives produced from *Aspilia africana* was higher ($p<0.05$) for water media. , Substantial amount of iron in water may have contributed to this result (Dutra de Oliveira *et al.*, 2013). Zinc and manganese concentration were similar ($p>0.05$) among all extraction media. Phosphorus levels were similar ($p>0.05$) in water and ethanol media while methanol produced significantly ($P<0.05$) higher values than other media. Copper concentrations were similar ($p>0.05$) in water and ethanol media while methanol produced significantly ($p<0.05$) higher value than other media.

Table 2: Effect of extraction media on the mineral composition of additives produced from *Aspilia africana* leaves extract

Parameters(mg/l)	Water	Ethanol	Methanol	SEM
Calcium	3.78 ^b	3.51 ^b	7.48 ^a	0.57
Magnesium	0.43	0.41	0.50	0.02
Potassium	7.35 ^a	3.91 ^c	5.88 ^b	0.41
Iron	32.79 ^a	30.49 ^a	20.02 ^b	1.85
Zinc	0.70	0.68	0.52	2.63
Phosphorus	0.18 ^b	0.22 ^b	0.55 ^a	0.05
Manganese	1.46	1.25	1.37	6.70
Copper	0.02 ^{ab}	0.02 ^b	0.04 ^a	0.03

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

Effect of extraction method on the mineral composition of additives produced from *Aspilia africana* leaves extract is presented in table 3. Microwave assisted method of extraction produced similar (p>0.05) but numerically higher calcium, potassium, iron, zinc, and manganese values in the

additives with magnesium value significantly higher and copper numerically lower than non-microwave assisted method. These results agree substantially with Tomaniova *et al.* (1998) who reported that microwave assisted extraction was more efficient than conventional extraction methods.

Table 3: Effect of extraction methods on the mineral composition of additives produced from *Aspilia africana* leaves extract

Parameters(mg/l)	Microwave assisted method	Non microwave assisted method	SEM
Calcium	5.32	4.53	0.57
Magnesium	0.51 ^a	0.38 ^b	0.02
Potassium	5.73	5.70	0.41
Iron	28.80	26.74	1.86
Zinc	0.75	0.51	2.63
Phosphorus	0.32	0.32	0.05
Manganese	1.39	1.32	6.70
Copper	0.02	0.03	0.00

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

Phytochemical and antinutritional composition of additives produced from *Aspilia africana* leaves extract is presented in table 4. Oxalate concentration ranged from 3.80mg/100ml in AAME to 4.46mg/100ml in AAWD, Oxalate values obtained in this study were higher than those reported by Oko and Agiang (2011). This disparity could be as a result of differences in reagents used and/or analytical methods environmental of the plant materials could also affect the values obtained as well as geographic location (Okwuonu *et al* 2017). Phytate concentration was significantly (p<0.05) higher in AMWD, AAEE than AAWD, AMEE and AMME which recorded the least value. Phenol levels ranged from 5.49mg/100ml in AAME to 23.49mg/100ml in AAWD. Their significant (p>0.05) reductions in ethanol (AAEE and AAME) extractions medium might explain its use, in traditional medicine, as anti-inflammatory, bactericidal, analgesic, and astringent agents (Okwu and Okwu, 2004). Trypsin inhibitor level ranged from 3.48mg/100ml in AMME to 7.39mg/100ml in AAEE.

Cyanide concentration in AAWD was significantly (P<0.05) higher than others and the low cyanide concentration reported in AAEE and AMEE agrees with Oko and Agiang (2011) who reported a low cyanide level for ethanolic extracts of *Aspilia africana* which was tolerable levels especially for monogastric animals. Alkaloid concentration in AMME was significantly (P<0.05) higher than others. The result is at variance with the report of Uduak and Emana (2013) who noticed complete absence of alkaloid in *Aspilia africana* leaf extract, this may be due to the processing procedure used which have been reported to affect phytochemical concentration (Okwuonu *et al*, 2017). Saponin concentration ranged from 1.51g/100ml in AMWD to 11.1g/100ml in AAME, the saponin values were significantly (p<0.05) different among the groups. Total carotenoid values ranged from 0.37g/100ml in AMME to 0.89g/100ml while Antioxidants ranged from 19.03% in AMEE to 28.51% in AAWD.

Table 4: Phytochemical and antinutritional composition of additives produced from *Aspilia africana* leaves extract.

Parameters(mg/100ml)	AAWD	AMWD	AAEE	AMEE	AAME	AMME	SEM
Oxalate	4.46 ^a	4.31 ^b	4.00 ^c	4.41 ^a	3.80 ^d	3.81 ^d	0.06
Phytate	1.02 ^b	1.23 ^a	1.21 ^a	1.06 ^b	1.10 ^{ab}	0.87 ^c	0.03
Phenols	23.48 ^a	16.10 ^c	6.47 ^e	5.49 ^f	16.98 ^b	17.96 ^d	1.59
Trypsin inhibitor	3.50 ^e	4.03 ^d	7.39 ^a	5.17 ^c	7.27 ^b	3.48 ^e	0.39
Cyanide	0.54 ^a	0.37 ^c	0.33 ^d	0.27 ^e	0.46 ^b	0.37 ^c	0.02
Alkaloid (g/100ml)	0.31 ^{bc}	0.19 ^d	0.27 ^c	0.20 ^d	0.32 ^b	0.37 ^a	0.01
	1.40 ^b	1.51 ^a	1.02 ^e	1.22 ^c	1.11 ^d	1.24 ^c	
Saponin (g/100ml)							0.04
	0.57 ^c	0.72 ^b	0.72 ^b	0.59 ^c	0.89 ^a	0.37 ^d	
Total carotenoid (g/100ml)							0.03
Antioxidant (%)	28.51 ^a	22.69 ^c	26.52 ^b	19.03 ^e	20.13 ^d	20.80 ^d	0.84

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

Effect of extraction media on phytochemical and antinutritional composition of additives produced from *Aspilia africana* leaves is presented in table 5. Oxalate concentration was significantly (p<0.05) different among alle extraction media. There was no significant (p>0.05) difference in the extraction media for phytate and phenol values. Trypsin inhibitor values showed that water medium produced significantly (p>0.05) lower concentration than other extraction media while values for ethanol and methanol were similar (p>0.05). Cyanide concentration was significantly (p<0.05) higher for water and methanol media than ethanol. This result agrees with Oko and Agiang (2011) who reported that

ethanol could lower cyanide concentration in extracts of *Aspilia africana*. Alkaloids concentration was significantly (p<0.05) higher for methanol medium compared to others which had similar (p>0.05) values. Saponin concentration for water medium was significantly higher than others while total carotenoid values were similar for all the media. Antioxidant value was significantly higher in water medium than methanol but similar (p>0.05) to value for ethanol medium This result is indicative of the disease risk reducing ability of *Aspilia africana* and therefore, a growth promoting candidate additive for poultry (Uchewaet *al*, 2018)

Table 5: Effect of extraction media on the phytochemical and antinutritional composition of additives produced from *Aspilia afriacana* leaves extract

Parameters (mg/100ml)	Water	Ethanol	Methanol	SEM
Oxalate	4.38 ^a	4.20 ^b	3.80 ^c	0.06
Phytate	1.13	1.13	0.99	0.03
Phenol	19.79	5.98	25.60	4.43
Trypsin inhibitor	3.76 ^b	6.27 ^a	5.37 ^{ab}	0.39
Cyanide	0.45 ^a	0.29 ^b	0.41 ^a	0.02
Alkaloid(g/100ml)	0.25 ^b	0.24 ^b	0.35 ^a	0.01
Saponin(g/100ml)	1.45 ^a	1.12 ^b	1.17 ^b	0.04
Total carotenoid(g/100ml)	0.64	0.66	0.64	0.03
Antioxidant (%)	25.59 ^a	22.77 ^{ab}	20.46 ^b	0.84

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

Effect of extraction method on the phytochemical and antinutritional composition of additives produced from *Aspilia Africana* leaves extracts presented in table 6. Values for oxalate, phytate, alkaloid and saponin similar (P>0.05) among the extraction

methods. While non-microwave method of extraction produced significantly (p<0.05) higher than microwave extraction method for trypsin inhibitor, cyanide, total carotenoids and antioxidants.

Table 6: Effect of extraction method of Phytochemical and Antinutritional composition of additives produced from *Aspilia afriacan* aleaves extract

Parameters (mg/100ml)	Microwave method	Non microwave method	SEM
Oxalate	4.17	4.08	0.07
Phytate	1.05	1.11	0.03
Phenol	18.60	15.64	4.43
Trypsininhibitor	4.23 ^b	6.05 ^a	0.40
Cynide	0.32 ^b	0.45 ^a	0.02

Alkaloid (g/100ml)	0.25	0.31	0.02
Saponin (g/100ml)	1.32	1.17	0.04
Total carotenoids (g/100ml)	0.55 ^b	0.73 ^a	0.04
Antioxidant (%)	20.83 ^b	25.05 ^a	0.84

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

Conclusion

This study have shown that the use of microwave and non-microwave extraction method could reveal the presence and abundance of active phyto minerals and phytochemicals in *Aspilia africana* thereby validating it as nutritionally safe for consumption especially in monogastric animals

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Conflict of Interest

There is no conflict of interest.

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