



Assessment of Nutritional Composition and Antioxidant Properties of *Dysphania ambrosioides* (L.) Mosyakin & Clemants and *Crassocephalum crepidioides* Leaf Meal as Potential Feed Additives[#]

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ABSTRACT

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This study analyzed the proximate, minerals, phytochemical profiles and antioxidant capacity of *Dysphania ambrosioides* (L.) Mosyakin & Clemants and *Crassocephalum crepidioides* (Benth.) S.Moore leaf meals as potential feed additives. The result of the proximate analysis revealed that *C. crepidioides* contained higher moisture (6.66%), ash (21.04%), crude fibre (3.85%), crude fat (5.41%), crude protein (17.11%) contents and lower carbohydrate content (45.60%) than *D. ambrosioides* leaf powder which had 6.31% moisture, 13.69% ash, 3.09% crude fibre, 4.48% crude fat, 15.99% crude protein and 56.12% carbohydrate. The result of the mineral analysis showed that *D. ambrosioides* contained higher concentration of sodium (19.8ppm), potassium (51.05ppm), calcium (29.18ppm), magnesium (24.1ppm), iron (0.42ppm) manganese (0.33ppm) and zinc (0.91ppm) compared to *C. crepidioides* leaf meal at sodium (18.71ppm), potassium (41.87ppm), calcium (25.77ppm), magnesium (20.34ppm), iron (0.28ppm) manganese (0.13ppm) and zinc (0.36ppm). The result of the phytochemical analysis revealed that aqueous extract of *C. crepidioides* possessed higher total phenolic (13.34 mgGAE/g) and flavonoid (2.29 mgrutin/g) contents than that of *D. ambrosioides* at (13.07 mgGAE/g) and (1.62 mgrutin/g) respectively. The Tannin and phytate contents were significantly higher in *D. ambrosioides* leaf meal at 2.39mg/g and 40.28 mg/g respectively, compared to that of *C. crepidioides* at 2.12mg/g and 29.4mg/g, respectively. The aqueous extract of *D. ambrosioides* exhibited higher that antioxidant free radical scavenging activity (24.83%) than that of *C. crepidioides* (16.42%). In conclusion, this study has shown that these two vegetables contained nutrients and antioxidant and could be used as alternative feed additives in animal nutrition.

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Introduction

In recent years, the utilization of phytogenic as a feed additive in the animal diet has been encouraged since the ban of antibiotic growth promoters by the European Union due to the occurrence of antibiotic-resistant pathogenic bacteria and deposition of antimicrobial residues in animal products (Abdelli et al., 2021). The potential of using phytogenic, which are plant-derived materials (such as herbs, spices, fruit, seeds and other plant parts), as a natural alternative to synthetic growth promoters could be attributed to their numerous inherent secondary metabolites (such as carotenoids, vitamins, flavonoids, and other phenolic components) and diverse mechanisms of action such as antioxidant, antimicrobial, anti-stress, anti-inflammatory, anti-toxicogenic, hypocholesterolemic, regulator of the gut flora, etc (Abdelli et al., 2021; Uysal et

al., 2021; Akgül et al., 2022; Falowo et al., 2022; Krupodorova et al., 2022; Mohammed et al., 2022). The supplementation of phytogenics with aromatic characteristics as sensory and flavoring agents helps to enhance feed palatability and taste, which in turn improve feed intake, feed conversion and body weight gain in poultry production (European Commission, 2003; Windisch et al., 2008; Alloui et al., 2014). The extracts of these phytogenics can be incorporated dietary or directly into meat products to improve the oxidative stability, shelf-life and colour during cold storage, because of their inherent bioactive compounds that have anti-oxidative effects on poultry products (Falowo et al., 2014, Falowo et al., 2019, Nduku et al., 2021).

Several phytonutrients and their extracts, especially those with aromatic compounds such as thyme, oregano, cinnamon, rosemary, turmeric, garlic, and ginger, among others have been used as a good replacement for antibiotic growth promoters (Gadde et al., 2017; Pliego et al., 2020; Nduku et al., 2021) in poultry production. Yet, there are still many edible phytonutrients in the tropics that have the potential of being sources of high-quality feed additives that have not been exploited because of the limited information about their nutritional and phytochemical contents. Among such are *Dysphania ambrosioides* (L.) Mosyakin & Clemants and *Crassocephalum crepidioides* plant leaves.

D. ambrosioides which is commonly known as epazote, Mexican tea or Ewe asin, is an aromatic herbaceous medicinal plant that belongs to the family of *Amaranthaceae* and the genus *Dysphania*. The plant originates from America but is now widely found in West Africa (including Nigeria, Senegal, Ghana and Cameroon) and other regions of the world (Reyes-Becerril et al., 2019). The leaves of the plant are used as a vegetable and for the treatment of viral, bacterial, parasitic and fungal infections in biological system (Kumar et al., 2007). The utilization of tea made from dried leaves of *D. ambrosioides* has been reported for the treatment of uterine fibroids (Diroff, 2008).

C. crepidioides which is commonly known as fireweed plant is a succulent leafy vegetable and herb that belongs to the family *Asteraceae* (Compositae). It is widely found in African, Asia, and Australia (Can and Thao, 2020). The leaves and stem of this plant are used in the preparation of soups and treatment of ailments such as indigestion, stomach pain, epilepsy, headache and wounds, especially in West and Central Africa (Sakpere et al., 2013). According to Nupo et al (2013), every part of plants is known to contain rich phytochemicals and bioactive compounds with the capacity to elicit antibacterial, hypoglycemic, antioxidant, anti-inflammatory, antitumor, and antidiabetic activities (Can and Thao, 2020). The dietary intake of this plant has been reported to boost red blood cells and reduce oxidative stress in mice (Can and Thao, 2020). Based on these potentials, this study aimed to examine the nutritional composition and antioxidant capacity of *D.ambrosioides* and *C. crepidioides* leaf meal as potential additives in animal nutrition.

Materials and Methods

Sample collection and preparation

Freshly harvested leaf of *D. ambrosioides* and *C. crepidioides* were purchased from Ondo and Ajowa markets, respectively, in Ondo state, Nigeria. The leaves were cleaned and air-dried in an open shade. The dried leaves were ground using electric blending machine and the powdered samples were packed into a black polyethylene bag before further analysis and extraction.

Determination of Nutritional Composition

The proximate composition (moisture, fat, protein, ash, crude and fibre) of dried plant samples were determined according to the procedures described by the Association of Official Analytical Chemist (1990). The carbohydrate content of the plant samples was calculated by the difference method (A.O.A.C., 1990) by subtracting the

sum (g/100g dry matter) of crude protein, crude fat, ash and fibre from 100g.

Determination of mineral Composition

Each dried plant sample was ashed and digested with 2M HNO₃. The mixture was filtered and the filtrate was made up to 100mL with de-ionized water in a 100mL volumetric flask. The concentration of calcium, sodium and potassium in the dried leaf samples were measured using the flame photometer of Jenway Digital Flame Photometer (PFP7 Model) while the concentration of magnesium, iron, zinc and manganese were determined using Scientific Buck Atomic Absorption Spectrophotometer (BUCK 210 VGP Model) at their respective wavelengths.

Determination of Tannin content

About 0.2g each of finely ground samples were weighed into a 50mL sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hours at 30°C. Each solution was then centrifuged and the supernatant was stored in ice. 0.2mL of each solution was pipetted into the test tube and 0.8mL of distilled water was added. Standard tannin acid solutions were prepared from 0.5mg/mL of the stock and the solution was made up to 1ml with distilled water. 0.5mL of Folin ciocateau reagent was added to both sample and standard followed by 2.5mL of 20% Na₂CO₃ the solution was then vortexed and allowed to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared (Makkar and Goodchild. 1996).

Determination of phytate content

The phytate contents of the plant samples were determined by anion exchange methods as described by Davies and Reid (1979). The filter (0.2-1.0 mL) was diluted to a final volume of 1.4 mL with distilled water, then 1.0 mL ferric ammonium sulphate solution containing 50µg Fe was added and thoroughly mixed. After that, the test tubes were sealed and placed in a 20-minute boiling water bath. After the test tube had cooled to room temperature, 5 mL amyl alcohol was added, followed by 0-1 mL of a 100 g/L ammonium thiocyanate solution. Inversion and shaking were immediately used to mix the contents of the test tubes. Following brief centrifugation for 10 minutes at low speed, the colour intensity in the amyl layer was measured using a spectrophotometer at 465 nm against an amyl alcohol "blank" 15 minutes after the HN4CNS was applied. The extinction at 465 nm in the amyl layer is inversely related to the phytate anion concentration because ferric ions complexed with phytate at pH 1-2 cannot interact with thiocyanate ion to create the characteristic pink complex.

Solvent Extraction

Ten gram of each powdered sample was weighed into five cleaned and dried reagent bottles and 100mL of water was added to each bottle and left for 72hours during which it was intermittently shaken on a shaking orbit machine. The mixture was thereafter filtered through a 0.45µm Nylon membrane filter. The extracts were evaporated to

dryness under reduced pressure at 40°C by a rotary evaporator. The collected aqueous extracts were used for the determination of antioxidant content and activity.

Determination of antioxidant content

Total Phenol content

The total phenol content of each aqueous extract was determined by the method described by Singleton et al. (1999). In brief, 0.2mL of each extract was mixed with 2.5mL of 10% Folin-ciocalteu's reagent and 2mL of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45°C for 40mins, and the absorbance was measured at 700nm in the spectrophotometer. The phenol content was expressed as mg gallic acid equivalent.

Total flavonoid content

The total flavonoid content of each aqueous extract was determined using a colorimeter assay described by Bao (2005). In brief, 0.2mL of each extract was added to 0.3mL of 5% NaNO₃ at zero time. After 5min, 0.6mL of 10% AlCl₃ was added and after 6min, 2ml of 1M NaOH was added to the mixture followed by the addition of 2.1mL of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

Determination of free radical scavenging ability

The free radical scavenging ability of the aqueous extracts against DPPH (1, 1- diphenyl-2-picrylhydrazyl) was determined using Gyamfi et al. (1999) method. 1mL of the extract was mixed with 1ml of the 0.4mM methanolic solution of the DPPH the mixture was left in the dark for 30min before measuring the absorbance at 516nm.

Statistical analysis

Data obtained on proximate, mineral, phytochemical and antioxidant contents of the plant extracts were analyzed using Student's t-test and PROC ANOVA procedures of the Statistical Analysis System (SAS, version 1.9.3 of 2007).

Results and Discussion

Proximate composition

Evaluation of nutritional content of phytochemicals as feed ingredients in animal nutrition is essential in ensuring nutrient balance and accuracy during feed formulation. Since some phytochemicals (such as *Moringa oleifera*, *Biden pilosa* etc) are being utilized in animal ration as source of protein to complement the conventional protein sources which are mostly expensive. The result of the proximate composition of *C. crepidioides* and *D. ambrosioides* leaf meals is shown in Table 1. The results revealed that both plant samples are rich sources of moisture, ash, crude fibre, crude fat, crude protein and carbohydrate. This indicates that the inclusion of *C. crepidioides* and *D. ambrosioides* leaf meals as a feed ingredients in animal diets could enhance animal growth and performance. However, the leaf meal of *C. crepidioides* produced a higher amount of moisture (6.66%), ash (21.04%), crude fibre (3.85%), crude fat (5.41%), crude protein (17.11%), and lower carbohydrate content (45.60%) compared to *D. ambrosioides* leaf meal ($p < 0.05$) which had (6.31%), ash

(13.69%), crude fibre (3.09%), crude fat (4.48%), crude protein (15.99%) and carbohydrate (56.12%). This means that *C. crepidioides* leaf meal possessed more organic content and is, therefore, more nutritious than *D. ambrosioides* leaf meal. Moreover, the variation in the content of their proximate analysis could be due to difference in species and geographical location. Ng et al. (2021) had earlier reported that variation in nutritional contents of vegetable leaf powders could be attributed to the difference in species of the plant. However, the concentration of crude fibre, crude fat, crude protein, and ash of *C. crepidioides* leaf meal in this study were higher than those reported by Nupo et al. (2013) but similar to values reported by Adjatin et al. (2013). Similarly the concentration of ash, crude fiber and crude fat content of *D. ambrosioides* leaf meal were lower than the values reported by Lohdip et al. (2017) in their study. These discrepancies in proximate values of these plants between studies could be a result of differences in factors such as time of harvest, climatic condition, soil type, type of cultivars used, physiological states and maturity, post-harvest treatment, and experimental method of analysis (Adjatin, 2013; Falowo et al., 2021).

Mineral composition

The mineral composition of the *C. crepidioides* and *D. ambrosioides* leaf meal is presented in Table 2. The results showed that both *C. crepidioides* and *D. ambrosioides* leaf meals are very rich in sodium, potassium, calcium, magnesium, iron manganese and zinc. The presence of some of these minerals in the plants justifies their usage as important vegetables in the diet. Minerals are very essential in the animal diet because they are needed for several biological processes such as bone mineralization, energy production, metabolism, cell growth, muscle function, hormonal secretion, regulation of electrolytes, cofactor to enzymatic reactions etc (Falowo, 2021). Specifically, potassium and sodium are required to maintain the osmotic balance of the body fluid, regulate muscle and nerve irritability, control glucose absorption and enhance the normal retention of protein during growth (Eke et al., 2013, Falowo, 2021). The leaf meal of *D. ambrosioides* exhibited higher concentration of sodium (19.8), potassium (51.05ppm), calcium (29.18ppm), magnesium (24.1ppm), iron (0.42ppm) manganese (0.33ppm) and zinc (0.91ppm) compared to *C. crepidioides* leaf meal which had at 18.71ppm sodium, 41.87ppm potassium, 25.77ppm calcium, 20.34ppm magnesium, 0.28ppm iron, 0.13ppm manganese and 0.36ppm zinc. The amount of sodium, potassium, calcium, and magnesium concentration recorded in leaf meal of *C. crepidioides* and *D. ambrosioides* were higher than those reported by Dairo and Adanlawo (2007) and Ng et al. (2012) in their studies, respectively. On the other hand, the amount of iron, manganese and zinc recorded in leaf meal of *C. crepidioides* and *D. ambrosioides* were lower than those reported by Dairo and Adanlawo (2007) and Lohdip et al (2015), respectively. These differences could be a result of the difference in soil mineral composition, the content of soil organic matter, geographical location, and rate of nutrient uptake by the plant during growth (Dairo and Adanlawo, 2007, Oladeji et al., 2017).

Table 1. Proximate composition of *C. crepidioides* and *D. ambrosioides* leaf meal

Parameter (%)	Plant Species	Moisture	Ash	Crude Fat	Crude protein	Crude fibre	Carbohydrate
	<i>C. crepidioides</i>	6.66±0.01 ^a	21.04±0.14 ^a	5.41±0.12 ^a	17.11±0.06 ^a	3.85±0.07 ^a	45.60±0.41 ^b
	<i>D. ambrosioides</i>	6.31±0.02 ^b	13.69±0.01 ^b	4.48±0.07 ^b	15.99±0.11 ^b	3.09±0.04 ^b	56.12±0.09 ^a
<i>Pvalue</i>		<0.01	<0.01	0.02	0.01	0.01	<0.01

Means within a row with different letters and significantly different (P<0.05). SEM Standard error

Table 2. Mineral Composition of *C. crepidioides* and *D. ambrosioides* leaf meal

Parameter (ppm)	Na	K	Ca	Mg	Fe	Mn	Zn
<i>C. crepidioides</i>	18.71±0.12 ^b	41.87±0.08 ^a	25.77±0.14 ^b	20.34±0.09 ^a	0.28±0.01 ^a	0.13±0.00 ^a	0.36±0.00 ^a
<i>D. ambrosioides</i>	19.8±0.11 ^a	51.05±0.02 ^b	29.18±0.17 ^a	24.1±0.06 ^b	0.42±0.01 ^b	0.33±0.00 ^b	0.91±0.01 ^b
<i>Pvalue</i>	0.003	0.001	0.0001	0.001	0.001	0.001	0.001

Means within a row with different letters and significantly different (P<0.05). SEM Standard error

Table 3. Phytochemical, antioxidant content and activity of *C. crepidioides* and *D. ambrosioides* leaf meal

Plant	Phenol (mgGAE/g)	Flavonoid (mg rutin/g)	Tannin (mg/g)	Phytate (mg/g)	DPPH (%)
<i>C. crepidioides</i>	15.34±0.01 ^a	2.29±0.03 ^a	2.39±0.01 ^a	40.28±0.48 ^a	16.42±0.09 ^b
<i>D. ambrosioides</i>	13.07±0.11 ^b	1.62±0.02 ^b	2.12±0.01 ^b	29.57±0.48 ^b	24.83±0.08 ^a
<i>Pvalue</i>	0.014	0.001	0.001	0.001	0.001

Means within a row with different letters and significantly different (P<0.05). SEM Standard error

Phytochemical content and antioxidant activity

The phenol, flavonoid, tannin and phytate content of the plant samples are reported in Table 3. The results showed that both aqueous extracts of leaf meals contained a relative amount of phenol and flavonoid content. Phenols are important plant constituents that contain hydroxyl groups and possesses the ability to inhibit or scavenge free radicals, chelate metal catalysts, activate antioxidant enzymes, and inhibit oxidases to protect the body against peroxidations (Leye and Oboh, 2017). Flavonoids are powerful antioxidant agents that help to regulate cellular activity, fight off free radicals, and prevent diseases that are related to oxidative stress in the body (Kumar and Pandey, 2013; Jovana et al., 2018). In this study, the total phenolic (13.34 mgGAE/g) and flavonoid (2.29 mg rutin/g) contents in the aqueous extract of *C. crepidioides* were significantly higher than that of *D. ambrosioides* at (13.07 mgGAE/g) and (1.62 mg rutin/g) respectively, (P < 0.05). The results of phenolic and flavonoid content in this study were slightly higher than those reported by Ng et al (2012) for of *C. crepidioides* and lower than the amount reported by Leye and Oboh (2017) for *D. ambrosioides*.

Tannin and phytate are phytochemicals that play an antioxidant roles in biological system. The tannin content in *C. crepidioides* and *D. ambrosioides* leaf meals were 2.39 and 2.12 mg/g, respectively. The values of tannin recorded in this study is relatively lower compared to those reported for other leafy vegetables such as *Moringa oleifera* (3.74mg/g) and rosemary (*Rosmarinus officinalis*) (9.44mg/C Eg) (Kilic et al., 2017). High tannin intake has been reported to form a high polyphenol complex with protein thereby making it unavailable in the diet (Nupo et al., 2013). However, the trace amount of tannin in recorded in the leaf meal of *C. crepidioides* and *D. ambrosioides* in this study is suggesting that these plants are less harmful and can be successively utilized as feed additives or supplements in animal diets.

The amount of phytate recorded *C. crepidioides* (40.28) was higher than that *D. ambrosioides* (29.54). Phytates are known to be beneficial to health because of their ability to prevent colorectal carcinoma and hypercholesterolaemia and also lower blood glucose in humans (Abulude, 2007;

Gemedet et al., 2014). However, a high intake of phytate has been reported to form complexes with mineral ions thereby rendering them unavailable for intestinal absorption. Consequently, this can lead to mineral deficiency depending on its effect on the bioavailability of the minerals such as Ca, Fe, Mg, Mn and Zn (Oladeji et al., 2017). The amount of phytate recorded in this study is were relatively higher than those reported by Agabire (2011); Okezie et al. (2017) and Ukom and Obi (2018) for leafy vegetables. This result is suggesting that the *C. crepidioides* and *D. ambrosioides* leaf meals should not be used at high concentrations in animal diets to prevent interference with the bioavailability of other minerals in animal diets. However, variation in phytate contents of plants have been associated with changes in environmental conditions, processing procedures and level of plant maturation and (Abulude, 2007).

The antioxidant activity potential of aqueous extract of *C. crepidioides* and *D. ambrosioides* leaf meals to scavenge 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radicals is presented in Table 3. The result showed that both leaf possessed antioxidants activity potentials. DPPH was significantly higher in *D. ambrosioides* (24.83%) than *C. crepidioides* (16.42%) leaf extracts. This is implying that *D. ambrosioides* leaf extract has higher antioxidant potential to scavenge free radicals and other reactive species compared to *C. crepidioides* leaf extract. The observed antioxidant potential of the aqueous extracts in this study is lower compared to the values reported for other leafy vegetables such as *Limnophila aromaticoides*, *Ceratopetris thalictroides*, *Etilingera elatior* and *Monochoria vaginalis* (Ng et al., 2012). These differences could be as a result of the method and solvent of extraction, geographical location and method of analysis (Sevindik et al., 2017).

Conclusion

This study revealed that the leaf meal of *C. crepidioides* and *D. ambrosioides* plant contained both macro and micronutrients especially protein, calcium, magnesium, potassium and iron. Specifically, the leaf meal *C.*

crepidioides exhibited higher proximate but lower mineral content compared to *D. ambrosioides* leaf meal. The result of the aqueous extract also shows that both leaf meals possessed moderate phenol and flavonoid contents that can exhibit antioxidant capacity. Therefore, based on the result of the nutritional and antioxidant contents, both plant leaves have the potential to be used as feed additives to animal diets.

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