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Nutritional characterisation and antioxidant capacity of different tissues of *Artemisia annua* L.

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ABSTRACT

Evaluation of different tissues of *Artemisia annua* for their nutritional contents and antioxidant potential demonstrated that the leaves and inflorescences had the highest percentage of protein, crude fat and *in vitro* digestible fractions but the lowest levels of detergent fibres. These tissues also had the highest composition of the major elements as well as manganese and copper. Their relatively high amino acid and vitamin profiles equally reflect a desirable nutritional balance adding to their high antioxidant capacities. Collectively, these high levels of the different nutritional constituents and antioxidant activities coupled with the very low and often negligible levels of inherent anti-nutritive factors, especially in the leaves, which are far below recommended toxic levels, establishes *A. annua* as a good reservoir of nutrients and antioxidants that might favour its use as a potential herbal tonic by humans or an important supplementary feed additive for livestock production systems.

1. Introduction

It is often common that livestock production in many developing countries is hampered by the two major problems of shortage of adequately balanced feed and the increasing cost of feed ingredients, which has inevitably led to poor utilisation of feed. Enzymes and antibiotics in feed, for example, which serve as aids to nutrition and are widely used in intensive livestock production systems in developed societies to improve the digestibility of feeds and utilisation of nutrients, are not available to many resource-poor farmers in developing countries. Aside from this, is the problem associated with the lack of appropriate medication, such that control of many diseases during animal husbandry is mainly periodic. However, even at these levels of medication the cost is huge and often beyond the purchasing capacity of many resource-poor farmers, who have had to resort to the use of natural products such as plant extracts, which are bountiful in their localities and are less harmful to human and animal health, for the treatment of some of these common ailments in livestock. Besides, the fact that some serious disadvantages associated with synthetic drugs such as those used in the treatment of helminthosis and coccidiosis during poultry production have become evident, including resistance and the presence of traces of these drugs at later ends of the food chain due to their indiscriminate use has given farmers further encouragement to seek natural products.

Various types of natural dietary supplements have been explored recently as sustainable alternatives for the control of many diseases afflicting the global livestock industry and they seem to be quite efficacious. Amongst the botanical resources that have been tested and found to be quite effective for possible use as prophylactic or therapeutic feed additives in poultry against coccidiosis, for example, are *Azadirachta indica* (Tipu, Pasha, & Ali, 2002), *Sophora flavescens* (Youn & Noh, 2001) and *Artemisia annua* (Allen & Fetterer, 2002).

Artemisia annua is a vigorous growing annual weedy herb, usually single-stemmed, reaching up to 2–3 m in height. The

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plant produces a beautiful portfolio of bioactive compounds including flavonoids, coumarins, steroids, phenolics, purines, lipids, aliphatic compounds, monoterpenoids, triterpenoids and sesquiterpenoids. Thus far, the most important of the sesquiterpenoids seems to be artemisinin, dihydroartemisinic acid, artemisinic acid and arteannuin B which are stored in the glandular trichomes found in the leaves and inflorescence (Ferreira & Janick, 1995). Artemisinin is a cadinane-type sesquiterpene lactone with an endoperoxide bridge which is presently the most potent and efficacious compound against the late-stage ring parasites and trophozoites of *Plasmodium falciparum* (Brisibe, 2006), the causative agent of malaria. Together with its semi-synthetically prepared derivatives such as dihydroartemisinin, artesunate, artemether, arteether, and artemisinin has also displayed unique pharmacological activities against a wide range of parasitic organisms including *Enterobacter* and *Klebsiella* species, *Streptococcus faecalis*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Escherichia coli* and *Pneumocystis carinii* (Chen et al., 1994), an opportunistic pathogen which causes pneumonia in AIDS and other immune compromised patients. Recent studies have demonstrated that artemisinin derivatives are equally potent and efficacious against several other common infectious parasites of man including *Toxoplasma gondii* (Jones-Brando, D'Angelo, Posner, & Yolken, 2006), which causes toxoplasmosis that is responsible for different forms of behavioural abnormalities in patients, *Trypanosoma* and *Schistosoma* species (Mishina, Krishna, Haynes, & Meade, 2007; Utzinger et al., 2001) that are responsible for human trypanosomiasis or 'sleeping sickness' and schistosomiasis, respectively. Artemisinin derivatives are also effective against some other pathogens including those responsible for cryptosporidiosis, amoebiasis, giardiasis, clonorchiasis and leishmaniasis (Ma, Lu, Lu, Liao, & Hu, 2004; Yang & Liew, 1993). Moreover, artemisinin has been recently indicated as a potential and effective compound against a number of viruses including hepatitis B, C and others (Efferth et al., 2008).

We observed from a series of preliminary studies, which have not been reported up until now, that in addition to the very unique pharmacological properties of many of the biologically active compounds found in *A. annua*, different tissues of the plant, especially the leaves and inflorescence, also contain comparable levels of nutrients and mineral elements (dry matter basis) to many other forage plants that have been investigated previously, leading us to suspect that this crop could also serve as an alternative source of nutrients for humans or livestock, besides other bioactive compounds such as antioxidants.

Undoubtedly, there is growing interest in natural sources of nutrients and health-promoting compounds. Within these compounds, polyphenols and antioxidants have aroused special attention, which is understandable because of their role as potential protective and preventive molecules against chronic ailments, such as atherosclerosis and cardiovascular diseases, ischemic heart disease, Alzheimer's disease, Parkinson's disease, cancer, osteoporosis and in the entire aging process (Aruoma, 2003; Coruh, Celep, & Ozgokce, 2007; Dasgupta & De, 2004). It is indeed a paradox that though green plants generally are known as sources of bioactive compounds with potential use as antioxidants and immune system modulators, information on the antioxidant capacity of non-traditional forages and medicinal plants such as *A. annua* have not been reported. Consequently, encouraged by our preliminary results and the need to reveal the antioxidant profile of *A. annua*, the primary objective of the current study was to determine the proximal composition, nutritional value and antioxidant potential of *A. annua* as a possible source of a very useful supplementary feed additive for the production of monogastric and polygastric livestock or as an herbal tonic for humans.

2. Materials and methods

2.1. Plant material and tissue culture conditions

Seeds of an *A. annua* hybrid line (3 M) identified from an earlier study with enhanced agronomic performance and high artemisinin content under humid lowland conditions in Nigeria were used in the current study. The seeds were surface sterilised by soaking in 70% ethanol for 5 min. Thereafter, they were soaked in 0.1% mercuric chloride solution for 10 min. The seeds were thoroughly rinsed with sterile distilled water and then germinated under aseptic conditions in 500 ml vessels containing 100 ml of half-strength Murashige and Skoog (1962) basal medium supplemented with 1% sucrose and 7% agar that was autoclaved after the adjustment of the medium pH to 6.0. The cultures with the seeds were then incubated at 26 ± 2 °C in a growth room, where they germinated within 3–4 days after transfer to this medium. After four weeks the seedlings were sectioned into small portions and transferred to Murashige & Skoog (MS) basal medium supplemented with 3% sucrose, 0.05 mg/l naphthalene acetic acid and 2.0 mg/l benzyl amino purine to induce the formation of multiple shoots mainly from the leaves. These were later transferred after eight weeks to another medium containing half-strength MS basal salts, which was supplemented with 1% sucrose, 0.01 mg/l naphthalene acetic acid, 0.2 mg/l indole butyric acid and solidified with 7% agar for the induction of roots. Cultures were maintained at 28 ± 2 °C under a photoperiod of 16 h (light)/8 h (dark) at 45 μmol photons m^{-2} s^{-1} and a relative humidity of 60% throughout this study. Fully developed plants grown in 20 l plastic containers of 30 cm diameter, filled with sandy loam top soil that was mixed properly with poultry droppings, were maintained in an air-conditioned greenhouse using fluorescent lamps (with a light intensity of 3000 lux) at a temperature of 30 ± 2 °C and a relative humidity of 50%.

Unless specifically indicated as in the case of antioxidants, different tissues including leaves, inflorescence, stems and roots were harvested separately from six-month old, fully mature tissue culture derived-plants, sun dried and pulverised to a fine powder in a blender and stored in airtight glass jars until required for analysis.

2.2. Reagents and samples

Distilled, deionised water of 18 M ohm cm resistivity, obtained from a Labconco WaterPro PS (Kansas City, MO) was used to prepare all solutions for ICP mineral analysis. Fisher Scientific Trace Metal Grade HNO_3 (Pittsburgh, Pa) was used for the microwave acid digestion of samples. PlasmaCal Multi-Element Standard 600-144-604 in 5% HNO_3 (Champlain, NY) was used to prepare Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn reference solutions which ranged from 9.98 to 5003 μg/ml. All glass and plastic wares were washed with diluted neutral cleaning solution, soaked overnight in 300 ml HCl + 35 l deionised water and rinsed three times with deionised water before use.

2.3. Proximal and chemical analyses

All assays of the proximal composition of the experimental materials were undertaken at the main soil analysis laboratory of the Soil Science Department, Faculty of Agriculture, University of Calabar, Calabar, Nigeria. The ash and moisture contents were determined as described by AOAC (1990) whilst the crude fat was extracted from about 4 g of each sample using the Soxhlet method with petroleum ether (40–60 °C) for 8 h. Whilst the protein content of each tissue was determined using a Foss automatic protein analyser after digesting the samples for 1 h, the fibre and total non-structural carbohydrate contents were analysed according to Denison, Fedders, and Tong (1992). Phytate was determined by the technique

of Igbedion, Olugbemi, and Akpapunam (1994), tannin was determined according to Makker and Goodchild (1996) and oxalate by the method of Day and Underwood (1986). All determinations were done in triplicates and results expressed as averages of percent values on a dry weight basis.

2.4. Determination of mineral elements

A microwave accelerated reaction system, MARSXPress model 907501 (CEM, Matthews, NC, USA) was used for sample digestion and set at 1600 W (80% power) with an extraction time of 15 min, at 200 °C. A Genesis OES-ICP spectrometer (Spectro, Germany) with axial plasma configuration was used for elemental determination with a plasma power of 1400 W, coolant flow at 13.5 l/min, auxiliary flow of 1.2 l/min and a nebuliser flow of 0.8 l/min. The plasma was operated with a cross-flow nebulizer attached to a Scott spray chamber. Microwave assisted acid decomposition of the samples before elemental analysis was performed. Samples of *A. annua* tissues weighing 0.1 g were transferred to teflon vessels. Two millilitres of concentrated HNO_3 was then added to each of these vessels. The samples were allowed to predigest for a minimum of 15 min in the vessels. Predigested sample vessels were capped, placed in the CEM, and subjected to the microwave operational parameters. Upon completion of the digestion programme, the vessels were cooled and the digests were transferred to 10 ml volumetric flasks and brought to volume with distilled deionised water. Elemental concentrations in the samples were then determined by ICP with Analytes' spectral lines based on their sensitivity and spectral interference.

2.5. Evaluation of amino acid profile

The amino acid content of the protein in the different tissues was determined according to Simpson, Neuberger, and Liu (1976), using an amino acid auto analyser (JLC-500 V, JEOL; Nippon electric, Tokyo, Japan) courtesy of Mr. Gabriel Gana, Laboratory of Fish Aquaculture, Faculty of Marine Science, Tokyo University of Marine Science & Technology, Minato-ku, Tokyo, Japan.

2.6. Determination of vitamin composition

Sun-dried samples of *A. annua* grown and harvested in Nigeria were sent to and analysed by CBO Assesoria and Análise Limited, Campinas, Brazil, using high performance liquid chromatography at a wavelength of 270 nm.

2.7. Antioxidant analysis

Leaves of *A. annua* plants that were field-grown in West Virginia, USA, were harvested and oven dried at 45 °C. Thereafter, they were ground in an electric blender and kept in a freezer until analysis. Together with these pulverised leaves, inflorescence, stems and roots of plants which were field-grown in 2007 that were harvested, oven dried and ground into a fine powder were analysed for antioxidant capacity. Both hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC) assays were carried out on a FLUOstar Galaxy plate reader. Procedures were based on a modified $ORAC_{FL}$ method previously reported by Wu et al. (2004). Tests were run in triplicates for leaves and in duplicates for inflorescence, stems and roots. Data are expressed as micromoles of trilox equivalent (TE) per gram of sample (μmol TE/g).

2.8. Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) to determine the level of significance among means. Significant differences among group means were determined using the least significant difference (LSD) at the level of $p < 0.05$.

3. Results

3.1. Proximal composition and nutritive potential of different tissues of *Artemisia annua*

The proximal composition and nutritive value of the different tissues of *A. annua* are presented in Tables 1 and 2. As expected, the levels of different nutritional factors such as protein, digestible fibre, total non-structural carbohydrates, etc. were significantly different ($P < 0.05$) between the different tissues of the plant. As shown in the tables, the inflorescence and the leaves of *A. annua* were richer in protein, crude fat and digestible carbohydrates when compared to the stems and roots, respectively. It was observed that the leaves were the richest in protein content; having the highest concentration of 27.1%, which is slightly higher than the 25.38% that has been reported in *Tridax procumbens*, a forage crop, by Asuquo (1993). On the other hand, the roots had the lowest protein level of 8.23% whilst the stems had a value that was slightly higher. Not surprisingly, the roots equally contained the highest amount of acid and neutral detergent fibres whilst those for the inflorescence and leaves were lower. For example, the values of the acid detergent fibre for the inflorescence and leaves were 15.2% and 13.1%, respectively.

In addition to percentage protein, Table 1 equally shows the composition of crude fat, ash and moisture contents of the different tissues of *A. annua.* Whilst the inflorescence and leaves again had the greatest percentages of fat content, the lowest amount was found in the roots (2.13%). Interestingly, the contents of crude fat seen here in the *A. annua* leaves and inflorescence were significantly higher than what has been shown in *Ipomoea batatas* (4.20%), *Tridax procumbens* (4.6%) and *Centrosema pubescens* (4.8%), respectively; even though the values in the stems and roots were lower than what was recorded in each of these three forage crops. With regards to the percentage ash content, the leaves had the highest level of 10.6% whilst the stem recorded the lowest (3.43%). The inflorescence had 6.99% whilst the roots had 5.46%, respectively. As shown in Table 2, the *in vitro* digestible fraction in the different tissues of the plant was also relatively high though values varied between 37.7% in the roots to a high level of 77.5% in the inflorescence.

Table 3 is a summary of the contents of various anti-nutritive factors (phytate, tannin and total oxalate) found in the different tissues of the plant. It is striking that these components were higher in the leaves than in all the other parts examined in this study. Whilst the phytate content of the leaves was significantly ($P < 0.05$) higher than those of all other parts put together, on the other hand the tannin and total oxalate contents of the leaves were significantly higher than those of the inflorescence, stems and roots. However, it is of great interest and importance that the levels of these anti-nutrients are rather low and below the established toxic levels to significantly interfere with the utilisation of nutrients.

Table 1
Proximal composition in different tissues of *Artemisia annua* (% dry basis).

Plant tissue	Protein content	Crude fat	Ash content	Moisture content
Inflorescence	18.4	10.5	6.99	9.95
Leaves	27.1	8.34	10.6	10.5
Steams	10.7	2.60	3.43	7.72
Roots	8.23	2.13	5.46	4.57

Table 2
Nutritional analysis of different tissues of *Artemisia annua*.

Plant tissue	ADF (g/100 g)	NDF (g/100 g)	IVDF (g/100 g)	TNC (g/100 g)	Starch (as % of TNC)	Dry matter (g/100 g)
Inflorescence	15.2	27.0	77.5	11.6	6.15	93.6
Leaves	13.1	39.5	64.7	7.6	0.27	91.5
Stems	47.4	76.4	45.0	9.7	5.77	96.1
Roots	49.1	82.4	37.7	6.2	8.51	97.0

ADF = Acid detergent fibre.
NDF = Neutral detergent fibre.
IVDF = In vitro digestible fraction.
TNC = Total non-structural carbohydrates.

Table 3
Proximal composition of anti-nutritive substances in different tissues of *Artemisia annua* (mg/100 g dry matter).

Plant tissue	Phytate	Tannin	Total oxalate
Inflorescence	25.1	0.115	0.004
Leaves	120	0.524	30.94
Steams	10.6	0.200	0.003
Roots	16.5	0.110	0.004
Mean	43.1	0.237	7.74

3.2. Mineral analysis of different parts of Artemisia annua

The mineral constituents from the different tissues of *A. annua* are listed in Table 4. There were considerable differences in the contents of all the minerals analysed, except for boron. As expected, all the five major elements evaluated in this study were found in all the plant tissues, with potassium being the highest in all the tissues, especially in the leaves and inflorescence. The stems contained four of these major elements (calcium, magnesium, phosphorus and sulphur), albeit at significantly low concentrations, compared to the leaves and inflorescence. The concentrations of phosphorus and sulphur in the different plant parts were similar, being fairly high in the inflorescence and leaves but low in the stems and roots.

Regarding the minor elements, the inflorescence had high concentrations of iron, manganese and zinc, whilst the leaves followed the same trend for these elements except for the fairly high levels of aluminum. Boron was also found in all the tissues of the plant even though its levels were the lowest compared to the other microelements (Table 4). The very negligible concentration of sodium in some of the tissues was particularly fascinating. Except for the roots that indicated a low presence, its level was almost insignificant ($P > 0.01$) in all the other tissues examined. The roots also contained fairly high levels of iron and aluminum, which were significantly higher than those of all the other tissues including the leaves.

Overall, it is interesting to note that whilst there were statistically significant differences ($P < 0.05$) in the concentrations of calcium, manganese, iron and aluminum between the different plant parts, the differences in the concentrations of magnesium, phosphorus, potassium, copper and sodium were not highly significant.

Table 4
Mineral composition in different tissues of *Artemisia annua* L determined by ICP spectrometry.*

Mineral	Inflorescence	Leaves	Stems	Roots
Potassium	24,568 (3.4)	26,332 (0.4)	13,488 (0.9)	11,126 (4.2)
Calcium	4,405 (0.1)	11,470 (0.4)	2,037 (0.2)	946 (0.9)
Magnesium	2,291 (2.2)	7,138 (0.4)	1,704 (2.3)	850 (14.6)
Phosphorus	3,354 (1.8)	3,665 (0.4)	871 (0.7)	664 (15.8)
Sulphur	4,585 (3.8)	3,908 (1.4)	742 (1.9)	471 (16.2)
Iron	224 (0.6)	196 (0.7)	22.2 (13.0)	682 (3.0)
Manganese	296 (3.6)	219 (0.3)	21.0 (0.7)	19.8 (3.9)
Aluminum	64.1 (2.4)	134 (0.0)	21.9 (2.6)	848 (0.7)
Zinc	60.4 (0.8)	64.5 (43.2)	46.2 (87.0)	75.9 (34.5)
Copper	31.6 (1.6)	14.3 (3.0)	6.7 (13.8)	22.5 (0.0)
Boron	17.5 (1.2)	18.0 (8.0)	4.5 (32.4)	5.0 (16.0)
Sodium	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	106 (5.3)

*The concentrations of all elements given here are in parts per million (ppm) whilst the values in parentheses represent relative standard deviation (RSD). All analyses were run in duplicates.

3.3. Amino acid and vitamin composition of the different plant parts

In order to assess the nutritional quality of the protein found in *A. annua*, the amino acid composition of the different tissues was compared to that of a World Health Organisation standard protein (WHO, 1973). As shown in Table 5 and in reference to the WHO standard, it is obvious that there was a favourable nutritional balance of both essential and non-essential amino acids present in the plant. Reflective of the protein content in the various tissues, the highest concentrations of amino acids were found in the leaves and the inflorescence. Either of these tissues had appreciable levels of most of the eight essential amino acids or amino acid pairs. Equally important was the fact that when put together as a whole the plant had an essential amino acid profile, expressed as a percentage of total amino acids, that were slightly better than the WHO standard for most of the amino acids, with the leaves (the most used tissue for Artemisia herbal tea preparations or browsed

Table 5
Constitutional amino acid profile (g/100 g) in different tissues of *Artemisia annua*.

Plant tissue	Inflorescence	Leaves	Stems	Roots	WHO Standard*
Essential amino acid					
Arginine	1.14	2.23	0.90	0.79	
Histidine	0.54	0.56	0.30	0.21	
Isoleucine	1.21	1.49	1.04	0.55	4.0
Leucine	1.82	2.87	1.08	0.91	7.0
Lysine	1.70	2.17	0.76	0.52	5.0
Methionine	0.44	0.96	0.00	0.00	3.5[a]
Phenylalanine	1.02	2.25	0.45	0.21	6.0[b]
Valine	1.12	1.91	0.56	0.48	5.0
Threonine	1.15	1.66	0.82	0.67	4.0
Tryptophan	–	–	–	–	4.0[c]
Non-essential amino acid					
Taurine	–	–	–	–	
Alanine	1.21	1.98	0.57	0.44	
Glycine	1.11	2.23	0.92	0.89	
Glutamic acid	2.98	3.78	1.52	1.12	
Serine	1.60	1.40	0.51	0.49	
Aspartic acid	1.21	2.10	0.86	0.72	
Total	18.3	27.6	10.3	8.01	38.5

*Based on the essential amino acid needs of a preschool child according to the WHO/FAO Report: Energy and Protein Requirements. WHO Technical Report Series No. 522 (World Health Organisation, 1973).
[a] The values indicated here of the WHO standard are representative of methionine and cystine in combination.
[b] The values indicated here of the WHO standard are representative of phenylalanine and tyrosine in combination.
[c] Tryptophan was not determined in the present study.

Table 6
Concentration of vitamins A and E in different tissues of *Artemisia annua*.*

Plant tissue	Vitamin A	Vitamin E
Inflorescence	<0.3 μg/100 g	19.38 mg/kg
Leaves	<0.3 μg/100 g	22.63 mg/kg
Stems	<0.3 μg/100 g	1.19 mg/kg
Roots	<0.3 μg/100 g	1.36 mg/kg

*Vitamin values from fresh or freeze-dried plant materials will provide much higher values than the sun-dried tissues evaluated in the current study.

by animals) containing approximately 42% (16.1 g/100 g) of the total (38.5 g/100 g) recommended WHO standard.

The concentrations of vitamins A and E in the different tissues of the sun dried plant materials were equally investigated. It was observed that whilst vitamin A was found only in marginal levels (usually below 0.3 μg/100 g) in all the different tissues, vitamin E was detected in varying concentrations with the highest levels found in the leaves and inflorescence, respectively (Table 6).

3.4. Antioxidant capacity

Total antioxidant capacity (TAC), expressed as the sum of the antioxidant capacity of the hydrophilic and lipophilic fractions, averaged 1,125 μmol TE/g dry matter for the leaves and 1,234 μmol TE/g dry matter for the inflorescences. The values for the stems and roots were similar with 292 and 287 μmol TE/g dry matter, respectively. Both hydrophilic and lipophilic fractions for the inflorescences and leaves had higher ORAC values than those for the stems or roots (Table 7).

4. Discussion

Recently, a high degree of renewed interest has been placed on the pharmacological properties of *A. annua*, a seemingly innocuous, yet versatile herbaceous plant with a long history of use as an herbal tea preparation in China, where it is renowned for its treatment of haemorrhoids and fevers associated with malaria. Aside from this, some of the biologically active compounds isolated from the plant have also proven to have very effective anti-microbial, anti-inflammatory and anti-tumour properties. Results of the present study clearly indicate that the plant is equally highly nutritive and contains high antioxidant capacities; a tacit indication that it can be used as a healthy, supplementary feed additive in the production of monogastric and polygastric animals.

There is no better proof that *A. annua* leaves could serve as a good supplement in animal feed, particularly when used as an additional protein, mineral and antioxidant resource to improve the health of livestock, than as exemplified by the proximal composition, mineral constituents, amino acid, vitamin (vitamin contents would be higher if analysed from fresh or freeze-dried tissues rather than the sun-dried tissues used here) and antioxidant profiles of the different plant tissues evaluated in this study. It is very interesting that the protein content and amino acid profiles of the different parts of the plant compared favourably, and in some cases even surpassed those reported previously for many common forage plants and some conventional protein sources used in the diets of monogastric and polygastric animals. For example, data on the protein content found in the leaves and inflorescence of *A. annua* in the present study were either only slightly lower than the levels that have been reported in *Tridax procumbens, Centrosema pubescens* and *Ipomoea batatas* (Asuquo, 1993; Cheeke, 1984) or similar to those found in the fresh whole leaves of *Acacia albida* or even greatly superior to most tropical grasses as well as those observed at various developmental stages of some legumes including *Cajanus cajan, Glycine javanica, Phaseolus lunatus, Pueraria phaseoloides* and *Desmodium heterophyllum*. This implies that *A. annua*, especially if the leaves are used in combination with the inflorescence, could serve as a supplementary source of protein in animal diets, provided that there are no palatability issues with the target animals. This high protein content of the leaves and inflorescence is of particular nutritional significance since it has been suggested that amino acid supplementation derived from leafy vegetable meals is important in meeting a substantial proportion of an animal's protein and energy requirements (Aletor & Adeogun, 1995), and could be beneficial in boosting the immune system of animals which has been reported to help them fight gastrointestinal parasite infestation (Koski & Scott, 2001; Kyriazakis & Houdijk, 2006). In goat feeding studies, for example, the animals consumed approximately 450 g of leaves of *A. annua* and flowering tops of *A. ludoviciana* in their daily diet for 4–5 days without rejecting the material and with a stagnation or reduction in faecal egg counts of gastrointestinal nematodes provided by the plant materials compared to a control group (Hart, Ferreira, & Wang, 2007).

It is also of remarkable interest that *A. annua* contains a high percentage of *in vitro* digestible fractions, but low acid and neutral detergent fibres compared with most forage plants. It is obvious that the high ash content in *A. annua* is a reflection of the high deposit of mineral elements, especially the major minerals such as potassium, calcium, phosphorus, and magnesium when compared with other common vegetable sources. Interestingly, even iron, which is commonly deficient in many plant-based diets, is fairly abundant in the plant, especially in the inflorescence, leaves and roots. Iron plays a critical role in the transport of oxygen throughout the body and in cellular processes of growth and division. Its deficiency results in a decrease in the haemoglobin concentration in the red blood cells, which leads to anaemia. Quite often the poor health outcomes associated with iron deficiency and anaemia in animals include direct insidious losses as a result of a reduction in physical working capacity, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity. As with

Table 7
Antioxidant activity of hydrophilic ($ORAC_{Hydro}$) and lipophilic ($ORAC_{Lipo}$) fractions of different tissues of *Artemisia annua* (μmol TE/g of dry matter) and total phenolics.

A. annua plant part	$ORAC_{Hydro}$	$ORAC_{Lipo}$	TAC	SD (of TAC)	Total phenolics
Inflorescence	1,196	38.0	1,234	1.26	0.49
Leaves	1,090	34.6	1,125	1.27	0.38
Stems	288	4.0	292	7.64	0.14
Roots	284	2.6	287	12.2	0.10
Oregano*	2,820	18.3	2,839	1.43	0.66
Alfalfa hay*	166	6.0	172	0.87	0.09

$ORAC_{Hydro}$ = ORAC for hydrophilic fraction.
$ORAC_{Lipo}$ = ORAC for lipophilic fraction.
TAC = Total antioxidant activity ($ORAC_{Hydro}$ + $ORAC_{Lipo}$).
SD = Standard deviation.
*The values for the aerial parts of oregano and alfalfa hay (leaves and stems) indicated here are only for the purposes of comparison of antioxidant and total phenolic contents with those of *A. annua*.

iron, the fairly high levels of zinc found in the different tissues of the plant, especially the aerial parts, may perhaps be of special interest in view of the importance of adequate zinc in the diets of animals and humans, where it is known to be essential for the function and/or structure of several enzymes including peptidases, transphosphorylase, aspartate transcarbamylase, etc. It has also been found to be an essential component of both DNA and RNA polymerases (Bryce-Smith, 1989; Riordan, 1976). In addition, zinc is also known for its anti-viral, anti-bacterial, anti-fungal and anti-cancer properties, and has been found to protect animals against, otherwise, lethal irradiation by neutrons (Bryce-Smith, 1989). The effects of zinc deficiency in animals are particularly worrisome. These include the retardation of growth in young animals, low efficiency of feed conversion (Williamson & Payne, 1978) and the lack of ability to maintain a good immune system (Nauss & Newberne, 1982; Oliver, 1997). Usually zinc-deficient humans also have weak immune systems and are extremely vulnerable to a wide range of infections (Nauss & Newberne, 1982). This is especially a very serious case amongst human immunodeficiency virus (HIV)-infected individuals, who are particularly susceptible to zinc deficiency (Wellinghansen, Kern, Jochie, & Kern, 2000).

Now apart from iron and zinc, all the major minerals, especially potassium, function among other areas, in the maintenance of cell osmotic pressure and water distribution in the various body compartments and stimulation of normal movement in the intestinal tract. Taken together, this probably explains the traditional use of the plant as an herbal tonic in China, because of its high levels of readily available essential nutrients and mineral resources, which may be required for the maintenance of electrical potential of nervous tissues and cell membranes as well as treatment of blood related disorders that is necessary for the improvement of the overall well-being of the body.

Another interesting aspect of the results reported here is the low percentages of anti-nutritional factors in the plant material, which though present, are negligible. For instance, oxalates which are known to cause irreversible nephrosis in animals when ingested in doses in excess of 5.0 g/kg of body weight, may serve as a cautionary parameter not to use a plant material as a feed supplement. However, this is not the case with *A. annua* as the levels seen here in the various tissues are far below established toxic levels.

Now aside from its potential as a source of feed additive, *A. annua* has two other great advantages associated with enhanced feeding efficiency in animals. First and foremost, the plant has fairly high contents of essential amino acids. Many of these including isoleucine, lysine and tryptophan, when taken together from the different tissues, were more than the WHO standard recommended as the needs of a preschool child (WHO, 1973). Moreover, the plant also has a high content of vitamin E and antioxidants, similar to that reported for *Brassica chinensis, Allium cepa*, and *Artemisia vulgaris* (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004), which are known to contain high levels of vitamins and flavonoids such as quercetin. This is not surprising since Artemisia species are generally known as rich sources of antioxidants including flavonoids, coumarins (El-Massry, El-Ghorab, & Farouk, 2002; Liu, Murch, El-Demerdash, & Saxena, 2004; Toda, 2005) and estrogenic flavonoids (Lee et al., 1998). Generally, antioxidants are very important as they help to reduce free radical damage caused by normal cellular activity and several stresses including gastrointestinal mucosal injuries (Bagchi et al., 1999). They are also known to be very good modulators of the immune system in animals and in humans (Bendich, 1993).

In addition to antioxidants, Artemisia species also have high concentrations of essential oil compared to traditional forages that are useful in the maintenance of a favourable micro-floral balance and suppression of protozoa in the rumen, increases nitrogen uptake and reduces methane production, which collectively increases feed efficiency (Greathead, 2003).

Second and equally important, is the ability of *A. annua* leaves to control protozoan parasites when used as feed additives in the poultry industry (Allen & Fetterer, 2002; Brisibe, Owai, Umoren, & Brisibe, 2008) as well as having the potential to be an effective anthelmintic in small ruminants destined for the meat market (Ferreira, Ritchey, Cassida, & Turner, 2006; Hart et al., 2007; Turner & Ferreira, 2005). Part of the studies by Turner and Ferreira (2005) demonstrated that the *in vitro* organic matter disappearance of *A. annua* leaves is comparable to that for whole alfalfa plants, indicating that the contents of biologically active secondary metabolites in the leaves did not have a negative impact on the micro-flora and fermentation in the rumen.

Though it is very difficult to say with utmost certainty the exact compounds that are responsible for these anti-protozoan and anthelmintic effects seen in poultry and small ruminants fed with *A. annua* leaves, it is reasonable to assume that this anti-parasitic effect is due, in part, to the presence of fairly high levels of artemisinin, usually between 0.6% and 1.1%, in most commercial cultivars of the plant. Artemisinin is known to be very effective against a wide spectrum of microorganisms including blood flukes, protozoa, bacteria, fungi and viruses (Allen & Fetterer, 2002; Turner & Ferreira, 2005; Utzinger et al., 2001; Brisibe et al., 2008; Efferth et al., 2008). It destroys parasitic organisms through the generation of highly reactive oxygen-based free radicals or electrophilic intermediates, by alkylating and oxidising proteins and lipids of parasite membranes as well as inactivation of channel proteins. It has been demonstrated that the effect of artemisinin is equally mediated through disruption of membrane potential by interacting with the electron transport chain in the mitochondrial membrane, resulting in free radical damage and impairment of mitochondrial function (Li et al., 2005). However, an alternative mechanism of action based on inhibition of sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA) of *Plasmodium,* the protozoan causative agent of malaria, has been suggested which has reconciled some intriguing observations on the actions of artemisinin and its derivatives against the malarial parasite (Woodrow, Haynes, & Krishna, 2005). Just like has been demonstrated above, it is possible that artemisinin, either acting alone or in combination with a few other constituents of Artemisia leaves, possibly polymethoxyflavones such as casticin, artemetin, chrysosplenetin and chrysosplenol-D, which are known to have a synergistic effect on artemisinin, might be effective as an anti-coccidial and anthelmintic agent in a manner similar to what has been reported for *Plasmodium* (Brisibe et al., 2008).

In conclusion, the data derived from the proximal, nutrient and antioxidant analyses of the different tissues of *A. annua* are clear indications of the nutritional potential of the plant as a possible feed additive with multiple purposes. These include serving as a protein, mineral and antioxidant resource for animal feed formulations besides being a cheap and natural source of anti-microbial compounds that could be supplemented as part of the daily ration. Another interest is also the desirability of the high contents of some of the functional properties of the leaves including minerals such as iron, sodium and zinc, which are important nutritional indicators of the usefulness of the plant as a likely feed resource. Thus taken together, the results reported in the current study therefore show that *A. annua*, when used effectively, can assist in meeting some of the important nutrient and mineral requirements of monogastric and polygastric animals. Thus the leaves and inflorescences of *A. annua* should be further explored and utilised fully as a feed supplement in the production of livestock.

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