



The Proximate Analysis, Phytochemical Screening, Antioxidant Activity and Mineral Composition of *Momordica charantia* and *Ocimum gratissimum* Leaf Powder

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ODO and SOA designed the study, authors ODO, ABF and COO performed the statistical analysis. All authors wrote the protocol and authors ODO, ABF, COO, ISO and SAA wrote the first draft of the manuscript. Authors ODO, ABF and OPAO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aims to evaluate and characterise the *Momordica charantia* and *Ocimum gratissimum* leaf powder.

Methodology: The quantitative analyses for proximate, phytochemicals, minerals, and antioxidant activities of *Momordica charantia* and *Ocimum gratissimum* leaf powder were carried out using standard procedures.

Results: The result of the proximate analysis showed that both plants contain an appreciable amount of moisture, ash, crude fibre, crude fat, crude protein and nitrogen-free extract content,

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with *M. charantia* leaf powder having higher moisture ($5.49 \pm 0.03\%$) content than *O. gratissimum* leaf powder ($5.02 \pm 0.01\%$) ($P < 0.05$). *Ocimum gratissimum* had higher crude fibre content ($25.03 \pm 0.25\%$) than *M. charantia* leaf powder ($20.86 \pm 0.12\%$) ($P < 0.05$). The protein, ash, crude fat and nitrogen-free extract contents were not significantly different ($p > 0.05$) between the two leaf powders. The phytochemical screening revealed the presence of tannin, flavonoid, phenol, alkaloids, saponin and phytate. Except for alkaloid content, *M. charantia* leaf powder had higher tannins (1.20 ± 0.02 mg/g), flavonoids (225.64 ± 14.61 mg/g), phenol (21.04 ± 0.57 mg/g), saponins (57.36 ± 0.08 mg/g) and phytate (7.79 ± 0.25 mg/g) content compared to *O. gratissimum* leaf powder. The antioxidant activity of the plants showed that *M. charantia* leaf powder possessed higher DPPH scavenging free radical activity than that of *O. gratissimum* leaf powder. The mineral constituents revealed that both plants contain a significant amount of zinc, calcium, iron and phosphorus, with *M. charantia* leaf powder having higher zinc and calcium concentration than *O. gratissimum* leaf powder ($p < 0.05$). In conclusion, this study shows that the leaf of *M. charantia* and *O. gratissimum* plants are rich sources of nutrients and phytochemicals and can be used as natural feed additives in animal nutrition.

Keywords: Antioxidant activity; feed additives; nutrients; phytochemicals.

1. INTRODUCTION

The application of plant-derived materials (such as herbs, spices, essential oils, extracts) as natural feed additives is gaining extensive acceptance in livestock nutrition due to their inherent multi-directional activities such as antioxidant, antimicrobial, anti-coccidiosis, anti-stress, anti-helminths, and growth promoters compared to synthetic antibiotic growth promoter [1]. Besides, plant-derived materials are a good source of essential nutrients such as minerals, vitamins, proteins, crude fibre, digestible carbohydrates and lipid [2], deficient in synthetic antibiotic growth promoters. In addition, the dietary utilisation of plant feed additives had shown enormous promising effects on the overall performance and welfare of animals, including cattle, pigs, sheep, goats and poultry, compared to synthetic antibiotic growth promoters.

Precisely, plant feed additives have been reported to increase the production of digestive juices (saliva, gastric juices, pancreatic and intestinal secretion), which in turn enhance feed intake, nutrient digestion, and body weight gain in animals [3,4]. Also, the dietary intake of plant feed additives has been reported to stimulate pancreatic enzyme production, inhibit muscle and serum lipid oxidation and cholesterol level [4], and suppress the pathogenic activity of microflora in the gastrointestinal tract of animals and thus reduce mortality during production [1]. Interestingly, *Momordica charantia* L and *Ocimum gratissimum* L plants with many potentials that can be utilised as feed additives in livestock production [1,2,4].

Momordica charantia L., also known as bitter gourd or African cucumber, belongs to the family of *Cucurbitaceae* and is commonly found in tropical and subtropical regions of the world, including Africa, Asia and Australia [5]. The plant is widely cultivated as a vegetable for consumption and medicinal purposes [5]. All parts of the plant, especially roots, leaves, fruits, and seeds, are used to treat different diseases such as diabetes, atherosclerosis, and other complications [6]. Some of the medicinal properties of *M. charantia* studied include hypoglycemic, antibacterial, antiviral, anti-tumour, immunomodulation, antioxidant, anthelmintic, antifertility, hepatoprotective and anti-inflammatory activities [6,7].

Ocimum gratissimum L. is a herbaceous perennial plant that belongs to the family of *Lamiaceae* (Labiatae). The plant is popularly known as scent leaf or African basil and is globally distributed [7,8]. The whole plant is used in traditional medicine to treat various diseases like cancer, diarrhoea, headache, fever, ophthalmic, skin disease and pneumonia [8]. Study on the plant's bioactive compounds has shown that it can exhibit numerous biological activities such as anti-diabetic, anti-rheumatic, anti-ulcer, anti-inflammatory, anti-tumour, antioxidants, and antimicrobial properties [9].

Many studies have reported on the use of different plant materials such as moringa, clove, thyme, rosemary, cinnamon, anise, oregano, sage, thyme, garlic, ginger, but to our knowledge; there is a dearth of information on the exploitation of *Momordica charantia* L and *Ocimum gratissimum* L. plant as a feed additive

in animal nutrition. Thus, this study was carried out to determine the proximate, minerals, phytochemical composition and antioxidant activity of *Momordica charantia* and *Ocimum gratissimum* leaf powder as potential feed additives in animal nutrition.

2. MATERIALS AND METHODS

2.1 Plant Part Collection and Processing

In September 2020, leaves of *Momordica charantia* and *Ocimum gratissimum* were collected separately from their mother plant at the Teaching and Research Farm of the Agricultural Technology Department, The Federal Polytechnic, Ado Ekiti (FPA) in Nigeria, and authenticated by a Crop Scientist from the FPA's Crop Production Department. The leaves were dried in the shade for two weeks before being pulverised (with a 0.5mm screen) and frozen in a plastic container until needed for laboratory analysis. The leaf powder samples were analysed in triplicates.

2.2 Proximate Analysis

The AOAC method was used to perform proximate analysis (moisture, crude protein, crude fibre, ether extracts, ash and nitrogen-free extracts) on the leaf samples (AOAC, 1995).

2.3 Phytochemical Analysis

2.3.1 Tannins

Total tannins were determined using the Folin-Ciocalteu technique [11]. 1 ml of the leaf extract was diluted with 49 ml distilled water, 1.7 ml 75 percent ethanol, 0.1 ml metaphosphoric acid, 1.0 mol/ml Na₂CO₃ (10 ml), and 2.5 ml Folin-Ciocalteu in a volumetric flask (100 ml). The mixture was homogenized and allowed to rest for 15 minutes at room temperature. The absorbance of normal solutions and leaf meal combinations was then measured against a blank in a spectrophotometer at 680 nm. The standard curve ($R^2 = 0.9972$) was utilised as a reference, and the sample's total tannin content was represented as tannic acid (TA) mg TA/g DW.

2.3.2 Flavonoids

The protocols used by Surana et al. [12] to determine the flavonoids content of leaf samples were followed. 0.50 mL of leaf powder extracts were carefully quantified in a test tube. After that,

the test tube was filled with 0.1 mL aluminium chloride solution, 1.50 mL methanol, 0.1 mL potassium acetate solution, and 2.8 mL distilled water, all of which were shaken together. Sample blanks for extract and rutin standard dilutions (10-100 g/ml) were generated in the same method but with distilled water rather than aluminium chloride solution. The solution was filtered through Whatman filter paper (No. 1) after that the absorbance ratios were compared to blanks at 510 nm. Thereafter, the total flavonoid content was calculated as 1 mg rutin per gram of extract.

2.3.3 Phenols

The total phenolic content of the leaf sample was determined using the Folin-Ciocalteu method, as described by Otlés and Yalcin [13]. Two hundred fifty μ l of Folin-Ciocalteu reactive were added to 50 μ litres of nettle extract or standard solution. In a dark atmosphere, this mixture was held at ambient temperature for 5 minutes. At the end of this time, a 750 μ L 7 percent Na₂CO₃ solution was added. In this approach, the hydroxyl groups in phenolics could deliver H to water. To dilute the mixture to 5 ml, pure water was utilised. After that, the combination was maintained at room temperature for 120 minutes in a dark setting to react. The absorbance of the samples and standards were measured at 760 nm. An 80 percent methanol solution was added to the blank solution instead of the 50 μ L extract. A calibration curve was used to quantify total phenolic content using gallic acid equivalent standards.

2.3.4 Total saponins

Saponin was quantified using the vanillin and concentrated sulfuric acid colourimetric method previously described by He et al. [14]. The 0.1 ml sample was combined with 0.5 ml ethanol (50%), 4.0 ml sulfuric acid (77%) (w/w), and 0.5 ml freshly made vanillin solution (8% w/v), then allowed to settle to ambient temperature before being heated in a water bath to 60°C for 15 minutes. The absorbance at 545 nm was measured using a UV/Vis spectrophotometer. A tea saponin calibration curve was used to quantify the total saponin content in each sample, which was expressed as mg tea saponin equivalent per g (TSE/g DW).

2.3.5 Alkaloids

The alkaloid content of the leaf sample was determined using the gravimetric technique [15].

In 50 mL of acetic acid solution in ethanol, 5 g of the sample was dispersed (10 percent). After being vibrated, the mixture was left undisturbed for around 240 minutes before being sieved. On a heated plate, the filtrate was reduced to a fraction of its original volume. After that, drops of concentrated ammonium hydroxide were used to precipitate the alkaloids. The precipitate was filtered through filter paper before being rinsed with a 1 percent ammonium hydroxide solution. After that, the precipitate was oven-dried for half an hour at 60°C before being transferred to desiccators and weighed again until it reached a constant weight. The alkaloids' weight was determined as a proportion of the total sample weight.

2.3.5 Phytate

Davies and Reid [16] described anion exchange methods for determining the amount of phytate in leaf samples. 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 were 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 HN_4CNS 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.

2.4 Antioxidant Activity

2.4.1 2, 2-diphenyl-1-picryl-hydrazyl-hydrate

The DPPH radical degradation activity method reported by Otles and Yalcin [12] was used to measure the 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) antioxidant activity of the leaf sample. Firstly, the DPPH radical was prepared daily, using pure methanol at a concentration of 6×10^{-5} M (molar). Then a measure of 2 microlitres of methanolic DPPH solution was added to 100 microlitres of sample extract or reference solution. Thereafter, this mixture was stored in a dark setting for 20 minutes. At the end

of this time, the absorbance was measured at 515 nm. A pure methanol blank solution was used as a control. In the control solution, 100 µL of distilled water were used instead of 100 µL of extract. A calibration curve was created with varying concentrations (10–100 ppm) of the gallic acid solution was used to estimate the antioxidant properties of sample extracts.

2.4.2 Vitamin C

The Benderitter et al. [17] approach was used to determine the vitamin C content of the *Momordica charantia* and *Ocimum gratissimum* leaf samples. 75 µl DNPH solution (i.e. 2 g dinitrophenyl hydrazine, 270 mg copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), and 230 mg thiourea in 100 ml of 5 ml/L H_2SO_4) were added to 500 l extract mixture (300 µl extract dilution with 100µl 13.3% trichloroacetic acid and water). After that, the reaction mixture was incubated at 37°C for 3 hours before adding 0.5 ml of 65 percent H_2SO_4 (v/v) to the medium and using a UV spectrophotometer to measure the absorbance at 520 nm. The vitamin C content of the leaf powder was then determined using ascorbic acid as a reference component.

2.5 Mineral Composition

The amount of phosphorus was determined using the colourimetric method (adapted from AOAC 966.01). To 5 ml aliquot filtrate in 10 ml volume flask, 1 ml NH_4 molybdate solution was added and rotate flask to mix, and thereafter let stand few second. Then, 1 ml hydroquinone solution was added and rotated again before 1 ml Na_2SO_4 solution was added. The solution is diluted to volume (10 ml) with water, stopper flask with thumb and shaken to mix thoroughly. The solution was let stand for 30 minutes and thereafter measured P of the leaf sample with a spectrophotometer set at 650 nm. After wet digestion with a mixture of nitric acid, sulphuric acid, and hydrochloric acid, the Zn, Ca, and Mg of the leaf samples was measured using an Atomic Absorption Spectrophotometer (Bulk scientific, USA, model 210 VGP).

2.6 Statistical Analysis

The results of the study were computed using the averages of triplicate values. The data were analyzed using the statistical software tool SPSS version 20. A one-way ANOVA was used to analyze the significant differences in mean values.

3. RESULTS AND DISCUSSION

Evaluation of nutritional content of feed ingredients is critical in animal nutrition to ensure nutrient accuracy during feed formulation. The result of the proximate composition of *M. charantia* and *O. gratissimum* leaf powder is presented in Table 1. The results revealed that both *M. charantia* and *O. gratissimum* leaf powder are rich sources of moisture, ash, crude fibre, crude fat, crude protein and nitrogen-free extract content. This indicates that the inclusion of *M. charantia* and *O. gratissimum* leaf powder as feed additives could increase the nutritional content of the diets and improve the metabolic activities of the animal. However, the amount of moisture content in *M. charantia* leaf powder ($5.49 \pm 0.03\%$) was significantly higher ($P < 0.05$) than that of *O. gratissimum* leaf powder ($5.02 \pm 0.01\%$). The variation in the moisture contents of *M. charantia* and *O. gratissimum* could be due to the differences in the nutrient requirements, environmental conditions and soil types that support their individual optimal growth [18] As earlier reported by Lamidi et al. [19] soil type having more organic matter could add more sap (moisture) to the crop. The crude fibre content was significantly higher in *O. gratissimum* ($25.03 \pm 0.25\%$) than *M. charantia* leaf powder ($20.86 \pm 0.12\%$) ($P < 0.05$). Plant species differences could explain the observed disparities in crude fibre content. Between-species and within-species variance in the neutral detergent fibre value, for example, has been discovered [18]. The protein, ash, crude fat and nitrogen-free extract contents were not significantly different ($p > 0.05$) between the two leaf powders. The result of the proximate composition of the *M. charantia* and *O. gratissimum* leaf powder recorded in this study was relatively lower compared to those reported by Bakare et al. [20] for *M. charantia* leaf and Adewole [21] for *O. gratissimum* leaf in their studies. This difference could be attributed to the processing technique, geographical location, maturity at harvest, season and time of harvest, soil factor, climate change, and plant genotype [22].

The phytochemical composition of the *M. charantia* and *O. gratissimum* leaf powder is presented in Table 2. The results showed significant differences in tannins, flavonoids, phenol, saponins, alkaloids, phytate content between the two leaf powders ($p < 0.05$). Except for alkaloid content, *M. charantia* leaf powder had higher tannins (1.20 ± 0.02 mg/g), flavonoids (225.64 ± 14.61 mg/g), phenol (21.04 ± 0.57 mg/g),

saponins (57.36 ± 0.08 mg/g) and phytate (7.79 ± 0.25 mg/g) content compared to *O. gratissimum* leaf powder. This shows that *M. charantia* leaf powder possessed more secondary metabolites (phytochemicals) than *O. gratissimum* leaf powder. The presence of secondary metabolites has been documented to exhibit antioxidant activity that helps to prevent oxidative stress and boost the immune system of animals [23,24]. Notably, phenols have been reported as antioxidant and scavenging agents against free radicals associated with oxidative damage [25]. Flavonoids have been reported as an active agent that can modulate lipid peroxidation [26]. At the same time, saponins are used to lower serum cholesterol levels, improve feed efficiency and body weight gain of animals, increase the permeability of intestinal mucosal cells *in-vitro*, inhibit active mucosal transport and facilitate uptake of substances that are usually not absorbed [27]. Plant tannins have been recorded to control bloat, intestinal parasite and pathogenic bacteria load in ruminant animals raised on pasture [28]. The total phenol and flavonoid contents recorded in this study were relatively higher than those reported by Horax et al. [29] and Igbiosa et al. [8] for *M. charantia* and *O. gratissimum* leaf, respectively.

The DPPH scavenging activity assay is used to determine the antioxidant potentials of plant materials. In this study, the level of inhibitions of DPPH scavenging activity in *M. charantia* leaf powder was significantly higher ($P < 0.05$) than that of *O. gratissimum* leaf powder. The observed higher antioxidant activity of *M. charantia* leaf powder compared to *O. gratissimum* leaf powder, as shown in the present study, could be attributed to high contents of inherent phenolic compounds (phenol and flavonoid, Table 2). The study has shown that plant material's antioxidant potential is mainly due to the concentration of phenolic compounds (Phenol and flavonoid contents) [8]. This study, therefore, shows that both plants possessed significant antioxidant activities. The result of the present study is in agreement with the reports of Kubota and Siriamompun [30] and Igbiosa et al. [8]. They found that *O. gratissimum* and *M. charantia* leaf possessed high DPPH scavenging capacities. Moreover, the leaf powder of *O. gratissimum* and *M. charantia* plant contains a relatively lower vitamin C level (Fig. 1). This result is also in agreement with the report of Bakare et al. [20] and Moon et al., [31], who found that *M. charantia* and *O. gratissimum*

plant possessed a low amount of vitamin C. Vitamin C is a natural antioxidant that helps to scavenge free radicals, strengthen the body

immunity against infections and helps in collagen and thyroxin synthesis and enhance iron absorption [32].

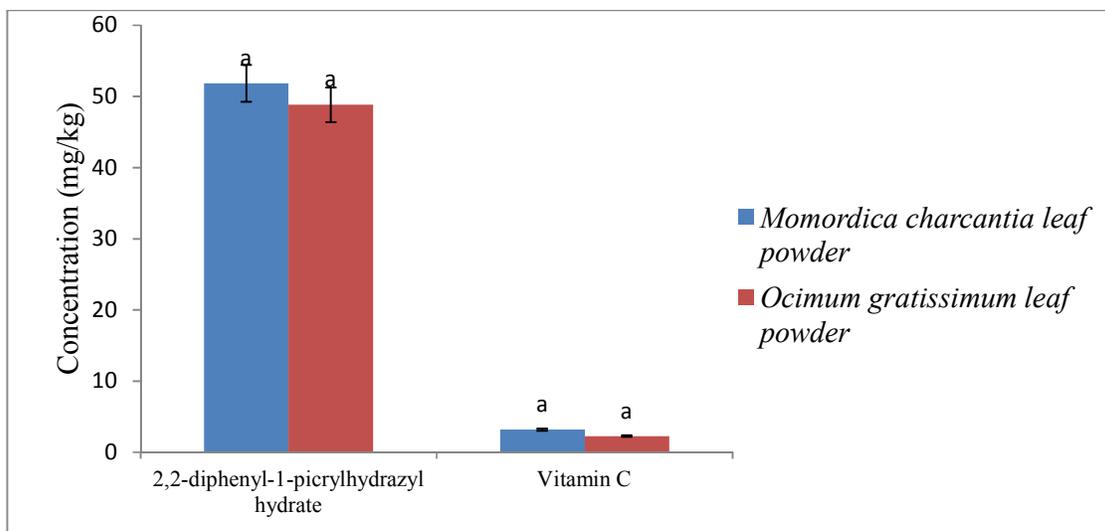


Fig. 1. The antioxidant properties of *M. charcantia* and *O. gratissimum* leaf powder

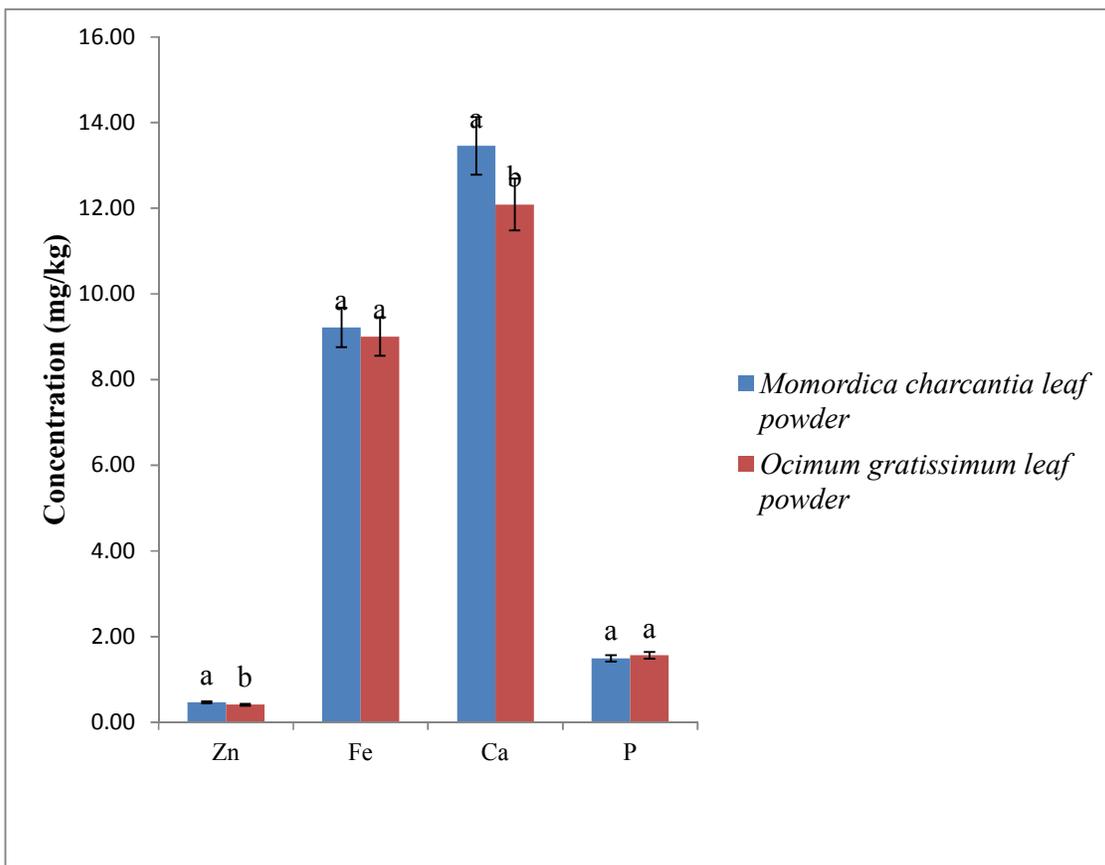


Fig. 2. The mineral composition of *M. charcantia* and *O. gratissimum* leaf powder

Table 1. The proximate composition (%) of *Momordica charantia* and *Ocimum gratissimum* leaf powder

Proximate composition (%)						
Plant species	Moisture	Ash	Crude fibre	Crude fat	Crude protein	Nitrogen free extract
<i>Momordicacharantia</i>	5.49±0.03 ^a	22.67±0.08	20.86±0.12 ^b	5.37±0.28	9.89±0.31	35.69±0.03
<i>Ocimumgratissimum</i>	5.02±0.01 ^b	22.33±0.19	25.03±0.25 ^a	5.21±1.54	9.80±0.03	32.59±1.97
P value	0.00	0.18	0.00	0.92	0.79	0.19

Means on the same column with different superscripts are significant ($P<0.05$)

Table 2. The phytochemical composition (mg/g) of *Momordica charantia* and *Ocimum gratissimum* leaf powder

Phytochemical composition (mg/g)						
Plant species	Tannins	Flavonoids	Phenols	Saponins	Alkaloids	Phytate
<i>Momordicacharantia</i>	1.20±0.02 ^a	225.64±14.61 ^a	21.04±0.57 ^a	57.36±0.08 ^a	191.49±0.86 ^a	7.79±0.25 ^a
<i>Ocimumgratissimum</i>	0.61±0.03 ^b	182.18±1.06 ^b	14.72±0.41 ^b	40.96±0.23 ^b	123.00±0.57 ^b	5.66±0.06 ^b
P value	0.00	0.04	0.00	0.00	0.00	0.00

Means on the same column with different superscripts are significant ($P<0.05$)

The result of the macro and micro minerals in *O. gratissimum* and *M. charantia* is presented in Fig. 2. The results showed that both plants contained essential nutrients that are relevant to the well being of the animal. It also showed that the leaves of both plants could be used as alternative sources of essential minerals in the diet. *M. charantia* leaf powder revealed higher zinc and calcium concentration compared to *O. gratissimum* leaf powder ($p < 0.05$). While iron and phosphorus concentrations were not significantly different ($p > 0.05$) between the two plants. Zinc is a mineral antioxidant needed to maintain the normal function of the immune [8]. In contrast, calcium is needed in animal nutrition to repair worn-out cells, form strong bones and teeth, and build red blood cells [33]. Iron is an essential trace element needed for haemoglobin formation, normal functioning of the central nervous system and the oxidation of carbohydrates, protein and fat [34]. The concentration of zinc, iron, calcium and phosphorus recorded in this study were relatively lower than that reported by Igbinsosa et al. [8] for *O. gratissimum* leaf and Bakare et al. [20] for *M. charantia* leaf. This difference could be attributed to the processing technique, geographical location, maturity at harvest, season and time of harvest, soil factor and plant genotype, among others [18,22].

4. CONCLUSION AND RECOMMENDATIONS

Findings from this present study have shown that *Momordica charantia* L and *Ocimum gratissimum* L. leaf powder contain appreciable amounts of nutrients and phytochemicals with intense antioxidant activity. With this, *M. charantia* L and *O. gratissimum* L. leaf powder could be a potential natural feed additive in animal nutrition. Although, further research is required to assess the efficacy of *M. charantia* L and *O. gratissimum* L. leaf powder as feed additives on animal performance and health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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