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Proximate and Chemical Characterization of *Ocimum gratissimum* Plant Grown Using Different Manure

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TTA and OSO designed the study, performed the statistical analysis, wrote the protocol and managed the analyses of the study. Authors SAO and LOO wrote the first draft of the manuscript. Author OOA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The proximate, minerals, and phytochemical composition of *Ocimum gratissimum* plant (OGP) that grew with different manure were investigated. OGseeds were planted in experimental plots in a Randomized Complete Block Design (RCBD) laid out in Oyo State College of Agriculture, Igboora with three replicates having plot size 3 m x 3 m of four treatments namely; Treatment A-No fertilizer or manure -(control), B- Poultry manure, C- N:P:K 15:15:15 and D-Compost manure respectively. Other parameters studied were planted height, leave areas, number of leaves and plant girth. The pre-soil test carried out indicated that the soil had organic matter (OM) content of 1.56%, total N 0.11% and available P 16.92 mg/g. This low level of nutrients justified the need for fertilizer

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application for maximum production. It could be concluded that there was significant ($p<0.05$) differences in other parameters considered from 3 to 9 Weeks After Planting (WAP) on all the agronomic characters. Furthermore, the proximate and minerals of the OGP went in favour of Compost raised OGP with highest values of significance. Followed by Poultry manure plant while N: P: K and No-manure treatments had the least ($P>0.05$) values respectively. The phytochemicals of the OGP which include Alkaloid, Flavonoid, Saponin and others were also high ($P<0.05$) in values on Compost and Poultry manure and the least value were observed in N. P. K and Control plants.

Keywords: Ocimum gratissimum; manure; phytochemical; proximate and minerals.

1. INTRODUCTION

Ocimum gratissimum is one of the medicinal and nutritional plants which curative properties lie majorly in the bioactive phytochemical constituents [1], vitamins, minerals and anti-microbial constituents present in the plant tissues [2]. It is a herbaceousplant belonging to the *Lamiaceae* family, germinate naturally in tropical areas of Africa and Asia, and is the most abundant of its genius *Ocimum*. The common names of the plant are Basil Fever plant or Tea bush and vernacular names include Tanmotswangiwawagi (Nupe), Daidoyatagida (Hausa), Efinrin (Yoruba) and Nehonwu (Igbo) [3]. It is woody at the base and has an average height of 1-3 m. The leaves are broad and narrowly ovate, usually 5-13 cm long and 3-9 cm wide. It is a scented shrub with lime-green leaves [4]. The plant usage is found in food spicing and a is good medication for the treatment of malaria, diabetes, abdominal pain, pneumonia, cough catarrh, respiratory and urinary tract infections, fever, diarrhea, conjunctivitis, oral wounds, tooth infection, convulsion, rheumatism, eczema, piles and also use as a repellant [5]. The roots are used as sedative for children [6]. High anti- nutrient contents hinder essential elements absorption for body metabolism, hydrothermal process reduces this content and hence food nutritional quality, digestibility and palatability are enhanced [7].

Soil infertility, a challenge faced by farmers is being abated by the use of fertilizer. Fertilizers are organic or synthetic substance usually added or spread onto soil to increase its ability to support plant growth [8].

Fertilizer application is a good agricultural practice but its effects on plant growth, development, quality and yield is a function of the type of soil, crop and socio-economic condition of the area of the farmland [9]. Therefore, to study the comparative effects of different fertilizer on soil fertility, plant growth and quality, this research study was aimed at analyzing proximate and chemical characteristics of the *Ocimum gratissimum* raised using different manure in Ibarapa zone of Oyo State in Nigeria.

2. MATERIALS AND METHODS

2.1 Materials

Ocimum gratissimum seeds, experimental site, N.P.K fertilizer, compost manure, poultry manure, muffle furnace, soxhlet extractor, atomic absorption spectrophotometer, kjeldahl apparatus, mesh and Whatman No.1 filter paper.

2.2 Methods

2.2.1 Experimental land design of *Ocimum gratissimum* plants

The experimental layout was Randomized Complete Block Design with three replicates having plot size 3 m x 3 m treatments. The experimental soils were randomly sampled at depth 30 cm. These samples were bulked, air dried and sieved using 2 mm mesh before physico and chemical analysis. No fertilizer or manure (Control), Poultry manure (five months decomposed chicken droppings) and Compost (farmyard manures) were applied at 5 tonnes/ha while the N.P.K fertilizer was applied at 120 kg/ha according to Adeniyan et al. [10].

2.2.2 *Ocimum gratissimum* plant cultivation

Poultry manure and compost were applied a week before planting to enhance nutrient use efficiency by the crop while the N.P.K fertilizer was applied a week after transplanting. The plots were weeded manually whenever necessary throughout the experiment and harvested 70^{th} days after planting. Growth parameter considered at 3-9 weeks after planting (WAP) includes plant height, number of leaves, leaf area and plant girth, this is in reference to Adeniyan et al. [10].

2.3 Proximate Analysis

Moisture content determination was carried out by drying 1 g of each sample in an oven at 105°C until constant weights were obtained. Ash Content was analyzed by heating 2 g of the sample in a muffle furnace at 600°C until it ashed completely. Crude fat was obtained by soxhlets extraction with petroleum ether at 60°C boiling range for 6 hrs. Determination of crude fibre was achieved by boiling 2 g of the residue obtained from fat extraction with dilute tetraoxosulphate (vi) acid (H_2SO_4) and sodium hydroxide (NaOH), then washed with methylated spirit, thrice with petroleum ether and finally ashed. Crude protein content was estimated using kjeldahl techniques by digesting the samples in hot concentrated tetraoxosulphate (vi) acid (H_2SO_4) and distilled in alkaline medium into a boric acid (H_2BO_3) and finally titrated with hydrochloric acid (HCl), then % N was converted to the percentage crude protein by multiplying by 6.25. The carbohydrate content was calculated by difference, all as described by AOAC [11].

2.4 Elemental analysis

2 g of each sample were subjected to 6 hrs heating at 600°C in a muffle furnace until the ash turns to white completely, it was then washed with 10 ml 0.1M of HCl into a volumetric flask. Warmed to avoid frothing and filtered with Whatman No 42 (ashless) filter paper. Distilled water was added to the filtrate to make it up to 100 mL for mineral determination. Samples for heavy metals were collected separately and acidified with concentrated nitric acid to keep the metals in solution and to minimize their absorption to the walls of the sample bottle. Atomic Absorption Spectrophotometer (AAS) was used to determine the level of metals in the samples [12].

2.5 Phytochemical Screening

2.5.1 Tannins determination

According to Swain [13] method of tannis determination, 20 mL of 50% methanol was added to 0.2 g weight of each sample in a beaker. The mixture was covered with paraffin and placed in a water bath of temperature range between 77°C - 80°C for 1 hr, stirred thoroughly and filtered using Whatman No.1 filter paper into a 100 mL volumetric flask. This was made up to mark with distilled water and thoroughly mixed. 2.5 mL of Folin-Denis reagent, 10 mL of 17% Na_2CO_3 and 20 mL of distilled water was added and properly stirred and mixed with 1 mL of sample extract pipette into 50 mL volumetric flask. The mixture was made up to mark with distilled water, vigorously mixed and left standing for 20 mins when a bluish-green colouration developed. Standard Tannic Acid solutions of range 10 mg/L were treated similarly as 1 mL of sample above. Tannic Acid Standard solutions and the samples absorbances were read after colour development on a Spectrophotometer at a wavelength of 760 nm. Percentage tannin was calculated.

2.5.2 Saponin determination

Based on the method of Obadoni and Ochuko [14], 5 g of each plant samples were weighed and 100 mL of 20% ethanol was added and heated over a hot water bath at 55°C for 4 hrs with continuous stirring to ensure uniform mixing. The filtrate and the residue were re-extracted with another 100 mL of 20% ethanol. The combined extracts were concentrated to 40 mL on a water bath at 90°C. The concentrate was transferred into a separating funnel and 20 mL of diethyl ether was added and mixed vigorously. The aqueous layer was recovered while the ether layer was discarded. The aqueous layer was allowed to undergo this purification process again and about 30 mL of n-butanol was added. The combined n-butanol extracts were washed two times with 10 mL of 5% aqueous sodium chloride and heated in a water bath, after evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage of the sample.

2.5.3 Alkaloid determination

This was determined by the method described by Obadoni and Ochuko [14], 5 g of each sample was weighed and addition of 200 mL of 20% acetic acid in ethanol was done and allowed to stand for 4 hrs. The mixture was filtered and the filtrate (extract) was concentrated on a water bath to evaporate 0.25 of the original volume. NH_4OH was added drop wise to the extract until the alkaliod precipitation was formed. The precipitate was collected by filteration after the whole solution has been left standing for a while to settle. The residue (alkaloid) was dried and weighed.

2.5.4 Total flavonoid determination

Using a standard method described by Boham and Kocipai [15], 100 mL of 80% aqueous

methanol was used to extract 10 g of the samples at room temperature. Whatman filter paper No. 42 (125 mm) was then used to filter the solution. The filtrate was transferred into crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.5.5 Steroids determination

100 mL of distilled water was added to 5 g of each plant sample and mix to achieve homogeniuty. The filtrate from the homogenized mixture was eluted with normal NH_4OH solution of pH 9. Ice-cold acetic anhydride of 3 mL was added to the mixture of 2 mL each of the eluents and chloroform in the flask and 2 drops of conc. H_2SO_4 were gently added to cool. Standard sterol solution was prepared and treated as described above. Spectrophotometer was used to measure the absorbances of standard and prepared samples at 420 nm. This method was described by Okeke and Elekwa [16].

2.5.6 Determination of terpenoids (salkowski test)

5 mL of each plant sample extract was mixed in 2 mL of chloroform, and 3 mL of conc. H_2SO_4 was cautiously added to form a layer. The presence of terpenoids was indicated by a reddish brown coloration formed at the interface [17].

2.5.7 Cardiac glycosides determination

0.5 g of each sample was refluxed in methanol for about 2 hrs. The extract obtained was treated with HCl, then further re-extracted using water and dried under anhydrous Na_2SO_4.The weight of the extract was calculated as percentage of the sample, modified method of Siddique et al. [18].

2.5.8 Oxalate determination

Oxalate extraction was done with dilute HCl at 50°C and was first treated with ammonium hydroxide and glacial acetic acid and re- treated with $CaCl_2$ solution. Precipitate of calcium oxalte formed was solubilized with hot dilute H_2SO_4 and titrated against $KMnO_4$ as equivalent to 2.2 mg of oxalate. A method described by Munro and Basir [19].

2.6 Statistical Analysis

Data from parameters investigated were analyzed in a completely randomized design using one way Analysis of variance (ANOVA) procedure.

3. RESULTS AND DISCUSSION

Results of the pre-soil analysis (Table 1) revealed the organic matter content of 1.56%, total nitrogen 0.11%, available phosphorus 16.92 mg/g and potassium 1.14%. The soil was low in organic matter, nitrogen and calcium; this justified the need for fertilizer application for maximum yield. Table 2 revealed the total aggregates of the pre-soil analysis, gave sand, silt and clay ratio to be 78.4:11.6:10% respectively which indicated that the soil is sandy loam. However, the proximate analysis of manure (Table 3) indicated that compost had highest value of total nitrogen content of 3.35% and average phosphorus of 21.47 mg/g compared with poultry manure which had 3.31% and 12.31 mg/g respectively and higher values of potassium, 9.50 cmol/Kg. The ability of the compost to perform better may be due to appropriate decomposition of the compositing materials and mineralization of nitrogen [20].

In Table 4 significant ($P<0.05$) treatment effects were observed in all the agronomic traits considered. The replenishment of the depleted experimental soil nutrients was achieved through the application of different manure and this corresponds to report made by Adeniyan and Ojeniyi [21]. The experiment showed that compost based manure performed better in most of the characters considered than the use of poultry manure. The highest response was recorded with the addition of 120 Kg/ha of N.P.K. The observed trend showed that the inorganic fertilizer gave an instant response to the crop need compared to organic manure with slow release of nutrients. The above assertion agrees with work of Adeniyan et al. [10].

In Table 5 and Fig. 1, proximate compositions of OGP raised with different manure evaluated shows that the highest moisture content (%) with 78.76 value in TD-compost manure was significantly ($P<0.05$) difference to other treatments, this was followed by TB-poultry manure with 77.53 while the least value was found in the control (TA-no manure) with 77.17. The moisture contents were relatively high and the active ingredients are much higher in the fluid, therefore hindered micro-organisms growth and increase stored samples life span. This enhanced preservation thereby prevents early

spoilage [22]. Furthermore, as reported by Mlitan et al. [5], OG crude protein contents (%) contain 9.10, 9.80 and 9.22 respectively, which were higher in values than 7.00% reported by Isong and Idan [23], however, lower than 29.78% reported by Akindahunsi and Salawu [24] and 23.74% [25] while the crude protein (%) in this study were relatively lower 6.60 (TA-No manure), 8.17 (TB-Poultry manure), 7.97 (TC-N.P.K) and 8.33 (TD-Compost) than that reported by Mlitan et al. [5]. Though it was cleared that TD-Compost (%) and TB- Poultry manure (%) Crude protein were significantly (P<0.05) different to all other manure grown, OGP however, the least crude protein mean value was in TA-No manure (Control). The Ash content (%) mean values were TA-Control (5.93), TB-Poultry manure (7.00), TC–N.P.K (5.83) and TD-Compost (7.03). These Ash mean values were lower than the values reported by Pandey et al. [25] for *Ocimum gratissimum* leaves 22.84 and 15.09 by Antia et al. [26]. The values of the crude fat (%) for the leaves of OG reported by Mlitan et al. [5] were also relatively higher than the mean values reported in this research but almost equal to those reported as 4.80 according to Akindahunsi and Salawu [24] and 3.15 by Abolaji et al. [22].

Table 1. Pre-soil analysis of the experimental site

Element	Values
pH	6.76
T^{o}	28.4
Organic Matter (%)	1.56
Total Nitrogen (%)	0.11
Average Phosphorus (mg/g)	16.92
Potassium (cmol/Kg)	1.14
Sodium (cmol/Kg)	0.31
Calcium (cmol/Kg)	0.16
Magnesium (cmol/K g)	0.38

Table 2. Pre soil analysis

Soil	Texture
Sand	78.4
Silt	11.6
Clay	10
Sandy Loam	

The mineral composition in Table 6 (mg/g) of *O. gratissimum* plants were analysed accordingly, the Potassium (68.33 mg/g) in TD-Compost grown was significantly (P<0.05) difference to others, followed by mean values (65.33 mg/g) of TB-Poultry manure grown *O. gratissimum* and least value (61.6 mg/g) was shown in Control-No manure. The Sodium, Magnesium and Chlorine follows the same trend of significance (P<0.05). However, Iron, an important trace element for haemoglobin, useful in the normal functioning of the central nervous system and in oxidation of carbohydrates, protein and fat [27], varies in mean values as detected in this analysis with highest value of 6.50 mg/g and 6.17 mg/g recorded in TD-Compost and TB–Poultry manure *O. gratissimum* raised plants respectively while TA- Control has the lowest recorded mean value (5.87 mg/g). The values of Zinc content in this study seems low compare to Elegbede et al. [28] reports, however, Zinc is involved in the normal functioning of immune system [29] and is associated with protein metabolism. The leaves are good source of Zinc because it relatively contains quantity as recommended by RDA [30]. The Lead values in this study range between 0.10 mg/g – 0.13 mg/g, which are lesser than the suggested concentration range of 2 mg/g – 6 mg/g in the plant species [31]. This suggest that *O. gratissimum* does not contribute or rather cannot be used as a substitute for other blood forming leafy vegetables but can be used in boosting the immune system and are antioxidants micronutrient [32]. The Copper values in different OGPs grown manure ranges between 0.33 mg/g to 0.40 mg/g. It has been reported that copper deficiency could cause cardiovascular disorders as well as anaemia, bone disorder and nervous systems disorder. Also, Lead and Copper are highly toxic even at low concentrations. Finally, Cadmium concentration values in this study was 0.10 mg/g in all the treatments though tolerable amount, although Cadmium is considered non-essential elements of the human being and existence, even in small amounts may cause some health problems such as kidney failure and heart disease [1,33,34].

Table 3. Proximate analysis of organic materials

	Poultry manure	Compost (maize base)
% Total Nitrogen	3.31	3.35
Average Phosphorus (mg/g)	12.31	21.47
Potassium (cmol/Kg)	9.80	8.50
pH	8.74	9.32
Electric charge	7399	3125
Temperature (^{o}C)	27.1	27.2

Table 4. Growth parameters on leave number, plant growth, leaf area and plant height

	Leaf number			Plant growth			Leaf area			Plant height		
	3WAP	**6WAP**	**9WAP**	**3WAP**	**6WAP**	**9WAP**	**3WAP**	**6WAP**	**9WAP**	**3WAP**	**6WAP**	**9WAP**
CONTROL	8.33^{a}	13.20^{a}	31.00^{d}	0.20^{b}	0.37^{c}	0.53^{c}	0.79^{a}	3.95^{d}	7.97^{a}	1.33^{a}	1.80^{c}	9.38^{c}
PM	6.67^{a}	21.33^{a}	40.67^{c}	0.30^{ab}	0.53^{bc}	0.67^{bc}	1.80^{a}	10.33^{c}	12.04^{c}	2.17^{a}	5.03^{bc}	13.29^{b}
COMPOST	6.67^{a}	25.30^{a}	49.60^{b}	0.26^{b}	0.67^{ab}	0.80^{ab}	1.00^{a}	12.83^{b}	17.60^{b}	2.57^{a}	7.37^{ab}	15.30^{b}
N:P:K	6.33^{a}	26.67^{a}	63.67^{a}	0.37^{a}	0.77^{a}	0.97^{a}	1.34^{a}	15.09^{a}	25.97^{a}	3.53^{a}	8.97^{a}	21.65^{a}
SE	2.04	3.3	0.62	0.02	0.04	0.05	0.45	1.21	0.59	0.56	1.21	0.59
STD	0.81	0.70	1.05	0.01	0.06	0.50	1.24	1.62	0.46	0.62	1.41	0.91
LSD	3.20	3.05	3.20	3.20	3.20	3.10	3.20	3.20	3.05	3.20	3.20	3.10

Key: PM = Poultry manure; NPK 15:15:15; WAP = Week after planting
SE = Standard error, STD = Standard deviation and LSD = Least significant difference
abcd – Means on the same column with different superscripts are significantly different (P<0.05)

Table 5. Proximate analysis of *Ocimum gratissimum* plant raised with different manure

Parameters	TA (No manure)	TB (Poultry manure)	TC (N:P:K)	TD (Compost)
Moisture	77.17^{b}	77.53^{ab}	77.73^{ab}	78.76^{a}
Ash	5.93^{c}	7.00^{b}	5.83^{d}	7.03^{a}
Crude fibre	3.00	3.20	3.03	3.80
Crude protein	6.60^{d}	8.17^{b}	7.97^{c}	8.33^{a}
Ether Extract	2.20^{d}	3.17^{b}	2.23^{c}	3.33^{a}

abcd – Means on the same column with different superscripts are significantly different (P<0.05)

Table 6. Elemental analysis of *Ocimum gratissimum* plant raised with different manure (mg/g)

Parameters	TA (No manure)	TB (Poultry manure)	TC (N:P:K)	TD (Compost)
K^{+}	61.6^{c}	65.33^{ab}	62.67^{b}	68.33^{a}
Na^{+}	787.00^{b}	823.33^{ab}	795.33^{b}	853.33^{a}
Ca^{2+}	348.67^{d}	363.33^{c}	395.33^{a}	383.33^{b}
Mg^{2+}	45.67^{b}	42.67^{c}	41.33^{c}	48.33^{a}
Cl^{-}	185.00^{b}	186.00^{b}	175.33^{c}	193.33^{a}
Fe^{2+}	5.87^{b}	6.17^{a}	5.87^{b}	6.50^{a}
Cu^{2+}	0.33	0.40	0.30	0.40
Zn^{2+}	4.00^{c}	5.20^{b}	4.90^{d}	5.90^{b}
Cd^{2+}	0.10	0.10	0.10	0.10
Pb^{2+}	0.10	0.12	0.10	0.13

abcd – Means on the same Column with different superscripts are significantly different (P<0.05)

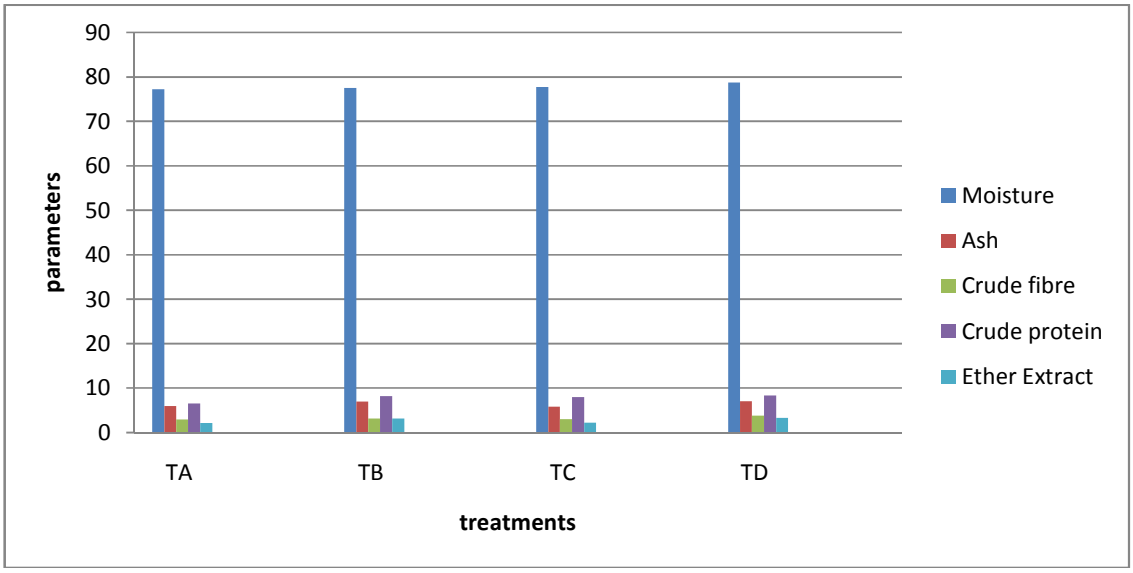

Fig. 1. Proximate analysis of *Ocimum gratissimum* plant raised with different manure

Table 7. Phytochemical component of *Ocimum gratissimum* plant raised with different manure

Parameters	TA (No manure)	TB (Poultry manure)	TC (N:P:K)	TD (Compost)
Alkaloid	1299.67^{b}	1313.33^{ab}	1331.00^{ab}	1356.67^{a}
Saponin	475.00^{a}	473.67^{a}	462.67^{c}	468.33^{b}
Flavonoid	1332.67^{b}	1345.00^{b}	1245.00^{c}	1431.67^{a}
Tannis	835.33^{b}	875.33^{ab}	860.00^{ab}	935.00^{a}
Terpenoid	1868.33	1875.33	1815.00	1878.33
Oxalate	1.37^{d}	1.45^{b}	1.38^{c}	1.51^{a}
Steroid	11.67^{b}	9.33^{d}	10.00^{c}	12.67^{a}
Cardiac glycoside	5.90^{c}	7.71^{a}	6.60^{b}	7.83^{a}

abcd – Means on the same Column with different superscripts are significantly different (P<0.05)

Table 7 showed the phytochemicals of OGPs raised with different manure. The plants contained terpenoid which has excellent activity against *Bacillus subtilis*and *Staphylococcus aureus* and lesser activity against gram-negative bacteria as well as *Candida albicans*. Alkaloids have been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and also possess antihypertensive, antifungal, anti-inflammatory, antifibrogenic effect [35]. The presence of alkaloids in this different manure grown OGPs, especially compost manure make them recommendable for a patient with the above listed challenges. Tannin is non-toxic and can generate physiological responses in animals that consume them [36] but toxic to filamentous fungi, yeast and bacteria. However, the quantity discovered in this study had the highest quantity in compost grown followed by poultry and least was found in no manure. Saponins showed a positive result in the leaves of *O. gratissimum*, in the order of TA-Control (475.00 mg/g), TB-Poultry manure (473.67 mg/g), TD-Compost (468.33 mg/g) and TC–N.P.K (462.67 mg/g). This compound has been reported to kill or inhibit cancer cells without killing the normal cells in the process [37]. Steroid also showed a positive result in OG leaves, TD-Compost (12.67), TA-Control (11.67), TC–N.P.K (10.00) and TB-Poultry manure (9.33) which are of importance in pharmacy as sex hormones [38], promote immune function in the skin and also reduce inflammation [39]. In this study, the leaves of OG in all treatments also contained flavonoid which modifies the body's reaction to allergens, virus and carcinogens. It has been reported to show anti-inflammatory and antimicrobial activity [40]. Akubugwo et al. [41] reported the presence of flavonoids in *A. hybridus*. Cardiac

glycoside showed positive result in the leaves, Taiwo et al. [42] justifies the plants in treatment and management of hypertension. The Oxalate content of the OG for all the treatments are TD-compost (1.51 mg/g), TB-Poultry manure (1.45 mg/g), TC-N: P: K (1.38 mg/g) and TA-Control (1.37 mg/g) respectively. These values are higher than *Musa sapentum* with 0.72 mg/g [43] and almost equal to *Butyrospermum parkii* with 1.5 mg/g [38], *Spondias mombin* of 0.9 mg/g [44] and lower than the reported values for cotton leaves of 22 mg/g [42].

4. CONCLUSION

OG plant grown using different manure in Ibarapa zone of Oyo State in Nigeria is a rich source of proximate, minerals and phytochemicals. It is a potential source of nutrients, drugs, good health and longevity. The study also verified the usefulness of this OG for nutritional purposes and partly explains its uses in herbal medicine. The agronomy traits of OG raised with N: P: K fertilizer had the highest significant values. Also, the proximate and minerals analysis went in favour of compost raised OGP followed by poultry manure plant while the N: P: K and No manure treatment had the least values. The phytochemical values of OGP in compost manure were the highest, followed by poultry manure while the least values were observed in N: P: K and Control grown plant samples respectively. This research conclusively proved that soil fertility can be improved through the application of organic and inorganic manure. Consequently, most of the analysis carried out favoured of OG raised with compost manure. However, OG samples in this study can be used for target purpose because the levels of anti-nutrient do not reach lethal dosages. AOAC [45] and hence allow absorption of essential elements for body metabolism [7].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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