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Proximate composition, mineral, vitamins and antioxidant activities of *Cymbopogon citratus* /dc/ stapt (lemon grass) and *Oregamun vulgare* (linn) leaves extracts harvested in Jos, North-Central Nigeria

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Abstract

There many factors that affects the physico-chemical features of plants, including, weather and climatic factors (lightintensity temperature, rainfall), pH, organic matter content, available nutrients etc. thus, the source of a plant plays a big role in its chemical and nutritive content. This study aimed to investigate the proximate composition, mineral, vitamins and antioxidant activities of *Cymbopogoh citratus* and *Oregamun vulgare* crude leave extracts (CCCLE and OVCLE), harvested in Zaramaganda, Jos, North-Central Nigeria.

Fresh leaves of *C. citratus* and *O. vulgare* were harvested in Zaramaganda, Jos, North-Central Nigeria, prepared and extracted via cold water Soxhlet extraction. Chemical and nutritive composition of the two plant extracts were carried out by way of proximate analysis and atomic absorption spectrophotometry (AAS) while antioxidant activity was determined by radical scavenging assay using DPPH (1,1-Diphenyl-2-pierylhydrazyl) as an artificial free radical activity.

Proximate analysis revealed varied proportions of the content in the investigated plants. The highest % by mass was; moisture content 7.33%, crude protein 16.25% and crude fibre 1.78% respectively in OVCLE while ash (12.54%) and fat (23.54%) content were highest in CCCLE. Mineral analysis revealed higher concentration of calcium, sodium, potassium, zinc, manganese, and iron in CCCLE than OVCLE in mg/100 g. Similarly, the vitamin A, C and E content were highest in CCCLE than OVCLE in mg/g. The inhibitory concentration at fifty percent (IC_{50}) values on the effect of aqueous extracts of OVCLE and CCCLE on DPPH scavenging activity showed that CCCLE (IC_{50}=131.8 µg/mL) had the higher activity than OV (IC_{50}=154.1 µg/mL) as compared to the standard, Ascorbic Acid (IC_{50}=49.13 µg/mL). The crude aqueous extract of *C. citratus* and *O. vulgare* harvested from Zaramaganda, Jos, North-Central Nigeria are good source of Vitamins (A, C and E), Potassium, Sodium, Calcium, Zinc and little of Manganese and Iron, also with good moisture content, ash

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content, crude fibre, lipid content and crude fat values. Furthermore, both plants were moderate in reducing DPPH radical levels and thus suggested for cancer patients.

Keywords: Proximate composition; Mineral; Vitamins; Antioxidant activities; Jos-Nigeria; Plant location; *C. citratus*; *O. vulgare*

1. Introduction

The use of herbal/natural remedies has generated lots of controversies especially within circle of "orthodox" medicine practitioners with lots of questions surrounding safety, also amidst the misconception that natural remedies are not toxic and are devoid of adverse effects as recorded by both underdeveloped, developing and highly developed countries [1], [2]. A salient point that is often raised from the scientific view point is the fact that, a toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose, thus, toxicity results from animals will be crucial in definitively judging the safety of medicinal plants if they are found to have sufficient potential for development into pharmacological products [3]. Owing to the fact that the basic premise that pharmacology is simply toxicology at a lower dose, the use of oral acute toxicity testing with Wistar albino rats in order to evaluate natural remedies for different pharmacological activities has been made possible [4]. The use of natural products has increased across the globe over the years and better ways of preparations of the products with monitoring by government and various agencies.

Origanum vulgare is widely distributed in the world including the Mediterranean, Irano-Turanian and Euro-Siberian regions [5]. **Oregano**, (*Origanum vulgare*), also called **origanum** or **wild marjoram,** aromatic perennialherb of the mint family (Lamiaceae) known for its flavourful dried leaves and flowering tops with all varieties containing essential oil also with thymol and carvacrol as the principal components [6].In a study on a culinary species oregano, Cu-NPs was found to modify Mg, Fe, Mn and Ca concentrations in roots and leaves by increasing the root uptake of Ca and Mg, and reducing Mn concentrations in both roots and leaves of *Origanum vulgare* [7]. The rich medicinal properties of its various parts have been explored as antimaliral agent [8], antimicrobial agent [9], anti-cervical cancer [10] etc. Cymbopogon, better known as lemongrass, is a genus of Asian, African, Australian, and tropical island plants in the grass family. Some species are commonly cultivated as culinary and medicinal herbs because of their scent, resembling that of lemons with some of its health benefits including maintaining the optimum cholesterol levels, managing type 2 diabetes, promoting healthy skin, aromatherapy and combating fatigue, anxiety, and body odor [11].Proximate analysis can be defined as a technique to measure the chemical properties of a compound based on four particular elements: moisture content, fixed carbon, volatile matter and ash content [12]. In another way, it is used for estimation of the quantitative of food and food substance including moisture, crude protein, total fat, total carbohydrate, and dietary fiber.

Antioxidants are compounds that *inhibit oxidation*, a chemical reaction that can produce free radicals. Antioxidant activity of bioactive macromolecules is important in regulating the redox state of the body and thereby, reducing the damage caused by diseases or drugs. Several studies have confirmed the antioxidant activity and immunostimulatory activities of polypeptides extracted from natural sources. Pedro et al, asserted that, antioxidants are well-known compounds that can play a significant role against these disorders, eliminating harmful ROS excess, and inhibiting or delaying molecular oxidation [13]. Also, vitamins and minerals are essential for bodily functions such as helping to fight infection, wound healing, making our bones strong and regulating hormones. Vitamins and minerals can cause toxicity if consumed in large amounts. In other words, vitamins and minerals are essential for bodily functions such as helping to fight infection, wound healing, making our bones strong and regulating hormones however, these can cause toxicity if consumed in large amounts [14]. Some studies have documented that plant species diversity is affected by environmental parameters, such as temperature, humidity, solar radiation, and soil nutrients [15].

This present study aimed to evaluate the proximate composition, mineral, vitamins and antioxidant activities of *Cymbopogoh citratus* /dc/ stapt (lemon grass) and *Oregamun vulgare* (linn) leaves extracts harvested in Jos, North-Central Nigeria.

Image *of Oreganum vulgare* [16]

Image *of Cymbopogon cytratus* [17]

Figure 1 Matured plants of *Origanum vulgare* and *Cymbopogom citratus*

2. Material and methods

2.1. Plant preparations and extraction procedures

Fresh plant samples of *Cymbopogon cytratus* and *Origanum vulgare* were collected from Bukuru area of Jos-South LGA, Plateau State Nigeria. These were identified at the Department of Plant Science of the University of Jos, Nigeria, after which fresh leaves same plants were air-dried and pulverized. Following these, 400 g powder each of both plants were weighed into separate container and 3 litres of distilled water was added, left for 72hrs with subsequent shaken and thus extracted by maceration in cold water at room 25-33 ºC for 72 hours in the Department of Plant Science and Biotechnology of the University of Jos, Nigeria. The mixture was filtered and the filtrates concentrated in vacuum using Rotary Evaporator to obtain the crude aqueous extracts. The percentage yield of the extracts was estimated and crude extracts stored in the refrigerator at 4 °C until needed for analysis. Percentage yield of *Cymbopogon cytratus* crude leave extract (CCCLE) and *Origanum vulgare* crude leave extract (OVCLE) were also determined using the formula below:

Percentage yield = Extract + container - empty container/ Sample weight X 100

These were done according to the methods of Yusuf Omope *et al*., [10] and Chanda *et al*., [18].

2.2. Proximate Analysis of CCCLE and OVCLE

These analyses were done using the method of Association of official analytical chemist (AOAC) [19] and Pearson composition and analysis of food.

2.2.1. Moisture content determination

Two grams (2 g) of the extracts were weighed and placed in a crucible of constant weight. These were placed in an oven at 105^{o}C then dried; the weights were measured carefully to get a constant weight. The loss in weight indicates the moisture content.

2.2.2. Ash content determination

Crucibleusedforashcontentdeterminationwereweighedanddriedinahotair oven at 110 ^{o}C to a constant weight for the two plants. Two grams (2 g) of eachextractwasweighedandplacedinthecrucibleandweightofthecrucibleandextract was taken. This was placed in a furnace and ignited for 3 hrs at 55 ^{o}C till the samples have a cotton wool-like texture; it was cooled in a desiccator and weighed with a digital balance.

2.2.3. Crude protein determination

One gram(1g) each of CCCLE and OVCLEsamplewasweighedinto2kjeldahlflasksand0.1gm of $Ca_2SO_4$and added into each flask with 20 ml Concentrated H_2SO_4. Each flaskwas slanted on kjeldahl heating mantle in the fume cup board. Digestion continued until there was a color change from black to bluish green signifying completion of digestion, set up against blank, digests removed and allowed to cool, diluted with water and made up to200 ml on ice. After these, aliquot (50ml) of each digest was poured into a distillation flask, then, 30 ml of NaOH was carefully layered into solution in order to

make It a strong alkaline. Following, 0.1NH_2SO_4(50ml) and 2drops of methyl red as an indicator, the distillate was titrated with 0.1M NaOH in the burette for each plant extract and blank and % of crude protein was calculated.

2.2.4. Lipid content determination

Foreach of CCCLE and OVCLE, 1 gm of sample was weighed into a thimble of known weight and150ml of petroleum ether (60-80 ^oC) were poured into 250 ml conical flask using measuring cylinder. The soxhlet extractor where the sack and its content had been introduced was fitted and solvent boiled under reflux. The extraction process lasted for 8 hours, sack with its content were removed dried in an oven for2 hours and then weighed with a digital balance.

2.2.5. Crude fibre determination

A total of 5 g each of CCCLE and OVCLE powder samples plus 200 mL (1.2%) H_2SO_4 was heated for 30 minutes and filtered via a buchner funnel after which the residue was washed with distilled H_2O until it was acid- free. Then, 200 mL (1.25%) was used to boil the residue 30 minutes and also filtered and washed severely with distilled H_2O until it was alkaline-free and rinsed once with HCl (%), twice ethanol and finally petroleum ether trice. The residue was dried in a crucible, placed inside an oven ()105oC overnight. Following this, cooled in a desiccator and ignited in a muffle furnace at 550oC for 90 minutes to and finally weighed to obtain the crude fibre. [19].

2.3. Vitamin content of CCCE and OVCE

2.3.1. Determination of vitamin C content

The ascorbic acid content was determined using 2, 6, dichloroindophenol (DIP)method with slight modification according to the method of Yen and Chen [20]. Sample of extract (1 mg) was dissolved in10ml of1%metaphosphoricacid and filteredusingWhatmannNo.1filterpaper, then1 ml of this filtrate was added with 9 ml of 50 µM DIP and incubated at room temperature for 15 seconds. The developed color was measured at 515 nm. The analysis was performed in triplicate and the results were expressed as ascorbic acid equivalent.

2.3.2. Determination of vitamin E content

Vitamin E was evaluated in the aqueous extract following the method of Prieto *et al*., [21]. An aliquot of 0.1 ml of extract (10 mg/ 2 ml) was mixed with 1 ml of reagent solution (0.6Msulfuricacid ,28mM sodium phosphate and 4m Mammoniummolybdate) and incubated at 37°C for 90 min with vigorous shaking. Absorbance of the aqueous phase at695 nm was measured against the appropriate blank. A typical blank contained 1 ml of reagent solution and 0.1 ml of the respective solvent, incubated under the same conditions as the samples. The analysis was performed in triplicate and the vitamin E content was expressed as α-tocopherol equivalents.

2.3.3. Determination of vitamin A content

Vitamin A was determined by using Godwin *et al*., method [22].1Moftheanalyzed liquid was measured into the test tube (I)and centrifuged with a tights topper for300 rpm for 5 minutes. 1m of KOH solution was added and shook vigorously for a minute. The mixturein the test tube was heated in the water bath at 60^{0c}. for 20minutes and allowed to cool, then, 1 mlof Xylene was added into the mixture, also shook vigorously for another 1 minute while, the mixture was centrifuged for 1500g rpm for 1 minute and the supernatant was collected into separate tube (II)AbsorbanceA1 was measured at 335nm against xylene AbsorbanceA2 was measured after allowing the test tube 11 to pass through UV light for 30 minutes VitaminA content was then calculated by the formular below:

$$Cx+(A1-A2).22.23$$

2.4. Determination of Mineral content of CCCLE and OVCLE

This was done by using atomic absorption spectroscopy for six elements - potassium, sodium, calcium zinc, manganese and iron in order higher value respectively according to the method of Godwin *et al*., [22].

2.5. Antioxidant assays of CCCLE and OVCLE

2.5.1. Radical scavenging assay1,1-Dipheyl-2-pierylhydrazyl (DPPH)

The free radical scavenging activity of freeze-dried and rotary evaporator dried plant extracts of *Cymbopogon citratus and Origanum vulgare* crude extracts were measured each in terms ofthehydrogen donated orradical scavengingabilityof theDPPH. The scavenging activity of the extract against DPPH radical was determined according to

the method of McCune and Johns [23]. Five hundred microliters(500µL) of 0.11mM methanolic DPPH were added to 500 µL of the extract and vitamin C, incubated at room temperature in the dark for 10 minutes. The absorbance of the blank (Ab) and samples (As)was measured at 517nm a spectrophotometer. A control was prepared as above without the sample and distilled water was used for baseline correction [24]. DPPH was calculated as [(Ab-As)]/ Ab × 100. The IC_{50}values for DPPH inhibition were determinedby fitting the percentage inhibitions calculated from absorbance data to a sigmoidal dose response curve using Origin 7.0 software. The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration [23].

3. Results and discussion

3.1. Proximate composition analysis of CCCLE and OVCLE

Table 1 Proximate composition of CCCLE and OVCLE

S/NO	Composition	% Dry Weight	
		Cymbopogon citratus	***Oreganum vulgare***
1	Moisture content	4.21	7.33
2	Ash content	12.54	10.24
3	Crude protein	15.05	16.25
4	Crude fiber	1.01	1.78
5	Fat content	23.54	21.12

Values are means of three independent analyses ±SD(n=3)

Proximate analysis was carried out in this study, this includes moisture content, ash content, crude fibre, lipid content and crude fat of *Cymbopogon citratus and Origanum vulgare*.

The low value of moisture content in the *C. citratus* extracts shows that the plants have capacity to prevent microbial attack and allows for high storage capacity. This result is in agreement with Nambiar and Hema [25] Ash content is generally identified as a measure of quality for the assessment of the functional properties of foods as it is the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid, etc.) and invariably taken as a measure of the mineral content of the original food. The actual amount of protein present in the *C. citratus* and *O. vulgare* extracts (15.05 and 16.25) respectively, show that the plant can form a part of human diet.

In terms of nutrient analysis of CCCLE and OVCLE, the quantitative estimation of vitamin content as presented in the Table 2 shows that, for *C. citratus*, vitamin E >vitamin C > vitamin A > and for *O. vulgare*, vitamin E > vitamin C > vitamin A.

Table 2 Vitamin Analysis Extracts of CCCLE and OVCLE

Composition	Concentration mg/gm of Extracts	
	C. citrates	***O. vulgare***
Vitamin A	130.05	120.16
Vitamin C	150.31	142.04
Vitamin E	214.13	194.05

Values are means of three independent analyses ±SD (n=3)

Vitamin E protects the body against a number of degenerative diseases such as a therosclerosis, aging and certain types of cancer. The moderate level of these molecules in plant extracts indicate that the extracts contain nutritional and medicinal value significantly. In this study, vitamin content was strongly correlated with antioxidant capacity. Vitamin

A is important for normal vision, the immune system, reproduction, growth and development. Vitamin A also helps the heart, lungs, and other organs work properly. This supports the observation by [26]. The results showed in Table 3 were in order of increase Vitamin A (13.05and120.16mg), Vitamin B (150.31 and 142.04mg) and Vitamin E (214.13 and 194.05mg).

The mineral analysis from this study revealed various major and trace elements shown in Table 3.

Table 3 Mineral Concentration of CCCLE and OVCLE

Composition	**Concentration mg/100gm**	
	C. citrates	***O. vulgare***
Calcium	34.40	25.30
Sodium	56.30	47.10
Potassium	60.20	52.90
Zinc	1.25	0.89
Manganese	0.94	0.63
Iron	0.95	0.01

Nutrient composition varies in different plants. Improvement of nutritional quality of food especially with respect to essential nutrient minerals, such as magnesium, iron sodium, calcium, potassium and zinc would be an important goal of plant preservation and cultivation. The human body requires number of minerals in order to maintain good health. The present study revealed that Potassium had the highest value, as indicated in the table 4. followed by sodium, calcium, zinc, manganese and iron which Is the lowest. 60.20 and 52.90, 56.30 and 42.10, 34.40 and 25.30, 1.25 and 0.89, 0.94 and 0.63 and 0.95 and 1.01 respectively (Table 3). These results were in consonance with the study reported by Geetha and Geetha [27] highlighting potassium as major and Iron as the lowest content.

Our findings is also in tandem with an earlier study by Nimenibo-Uadia and Nwosu, (2020), who reported rich in crude protein (15.86%), ash value (9.40%), crude lipid content (6.90%) crude fibre content (1.00%) carbohydrate content (66.54%) and moisture content (72.95%) as well as Calcium > Potassium > Magnesium > Phosphorus > Sodium > Iron > Copper from proximate analysis, atomic absorption spectrophotometry (AAS) and complexometric titration and biomineral analysis of Lemongrass (Cymbopogon citratus (DC) Stapf) grown in Ekosodin, Benin City, Nigeria [28].

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Several mechanisms have been proposed to be involved in the antioxidant activity such as hydrogen donation, termination of free radical mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions and elimination of peroxides. Owing to the complex reactive nature of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by only a single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity. The simplicity in the various free radical scavenging methods used in this study and its reproducibility was shall be depicted in the results. In Vitro Antioxidant Activity of the Plant Extracts

3.1.1. 1,1-Dipheyl-2-pierylhydrazyl (DPPH) of lemon grass and Oregano Extracts

The inhibitory concentration at fifty percent (IC_{50}) values on the effect of aqueous extracts of *O. vulgare* and *C. citratus* on DPPH scavenging activity showed that *C. citratus* (IC_{50}=131.8µg/mL) had the higher activity than *O.*

vulgare (IC_{50}=154.1 µg/mL) as compared to the standard; Ascorbic Acid (IC_{50}=49.13µg/mL) as shown in Figures 2-4.

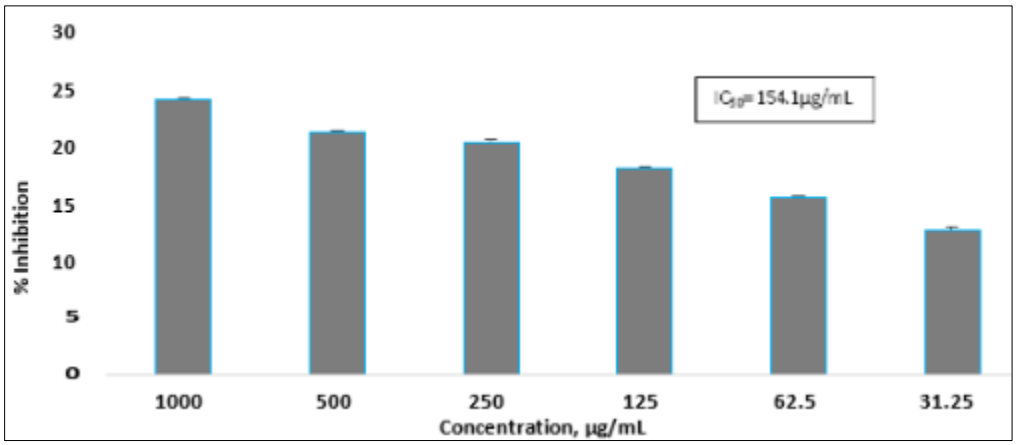

Figure 2 IC_{50} and Percentage Inhibition Values of DPPH Assay for Free Radical Scavenging Activity of Aqueous Extract of *O. vulgare* Leaf

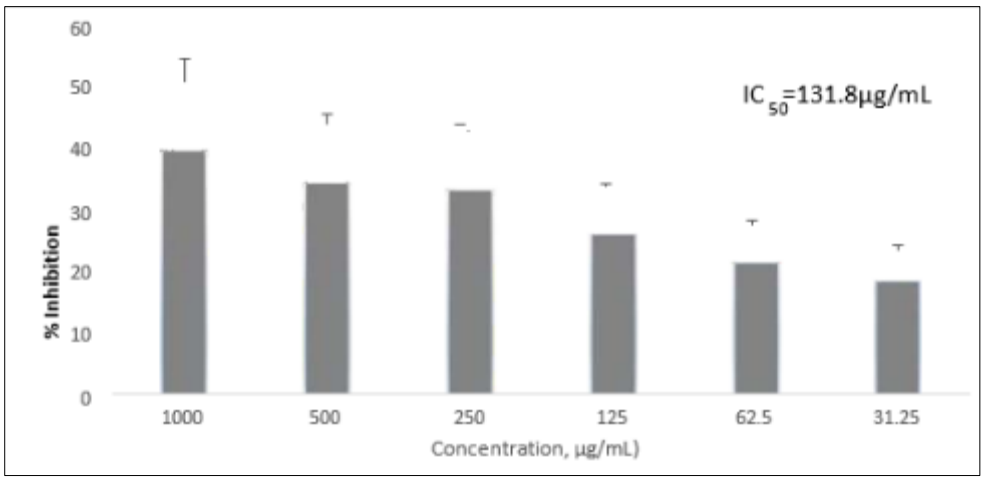

Figure 3 IC_{50} and Percentage Inhibition Values of DPPH Assay for Free Radical Scavenging Activity of Aqueous Extract of *C. Citratus*

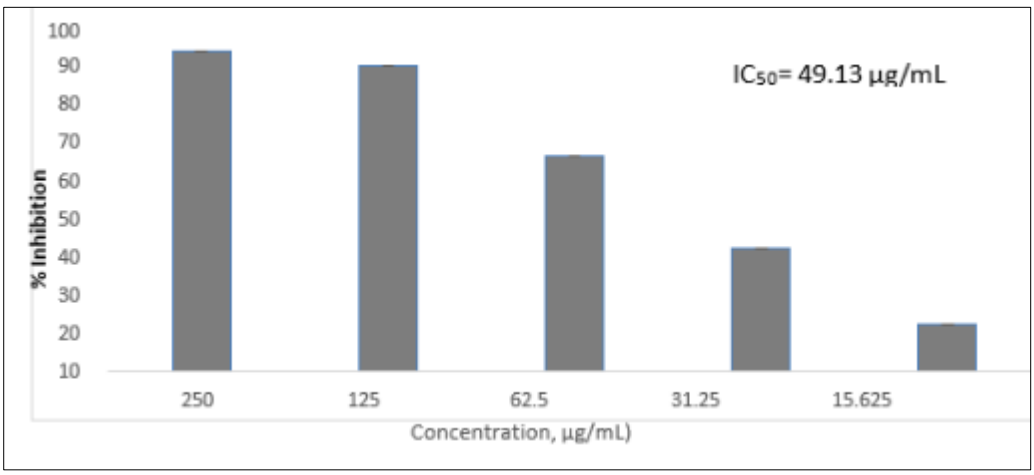

Figure 4 IC_{50} and Percentage Inhibition Value of DPPH Assay for Free Radical Scavenging Activity of Ascorbic Acid (Standard)

Oxidative stress plays an important role in mediating free radicals which have been linked with diseases such as atherosclerosis and cardiovascular complications, tumors, etc [29]. Addition of plants with high antioxidant properties would be of great need to scavenge free radicals when the primary defense systems of the body are weighed down by oxidative stress.

The *invitro* antioxidant activity of both *C. citratus* (IC_{50}=131.8µg/m l*and O. vulgare* (IC_{50}=154.1 µg/mL) aqueous crude extracts both falls within the moderate range in reducing DPPH radical levels following the classification of antioxidant intensity, including, strong (<50 µg/mL), active (50-100 µg/mL), moderate (101-250µg/mL), weak (251-500µg/mL) and inactive antioxidant (>500 µg/mL) according to by Jun et al., [30]. Furthermore, this study is also in line with the findings by (Han et al 2017) who reported that results of the DPPH free radical scavenging assay showed that the half maximal inhibitory concentration (IC_{50}) values of the essential oils were (0.332±0.040) mg/ml (leaves-flowers), (0.357±0.031) mg/ml (roots), and (0.501±0.029) mg/ml (stems), respectively, also, the results of reducing the power test also revealed that when the concentration exceeded 1.25 mg/ml, the leaf-flower oils had the highest reducing power; however, the stem oils were the lowest [31]. These supports *C. citratus* and *O. vulgare* as good anticancer agents.

4. Conclusion

The crude aqueous extract of *C. citratus and O. vulgare* harvested from Zaramaganda, Jos, North-Central Nigeria are good source of Vitamins (A, C and E), Potassium, Sodium, Calcium, Zinc and little of Manganese and Iron, also with good moisture content, ash content, crude fibre, lipid content and crude fat values. Furthermore, both plants were moderate in reducing DPPH radical levels and thus suggested for cancer patients.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors hereby declare no conflict of interest.

Statement of ethical approval

Ethical approval with reference number - AEC/02/107/21 was obtained from the Animal Ethics Committee of the National Veterinary Research Institution, Vom, Plateau State, Nigeria.

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