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PROXIMATE COMPOSITION, AMINO ACID PROFILE, CHEMICAL COMPOSITION, *IN-VITRO* ANTIOXIDANT AND ANTI-DIABETIC ACTIVITIES OF *Ocimum gratissimum* ETHYL ACETATE STEM BARK EXTRACT

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Abstract

Ocimum gratissimum is a widely recognized medicinal plant known for its diverse therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, and antidiabetic effects. Its rich content of bioactive compounds has made it a staple in traditional medicine for managing infections, digestive disorders, and general wellness. This study investigates the nutritional composition, amino acid profile, antioxidant potential, enzyme inhibitory activity, and phytochemical constituents of *O. gratissimum* stem extract, providing scientific insight into its medicinal relevance. The proximate composition revealed carbohydrate having the highest percentage abundance of 53.69%. This is followed by fiber (12.50%), protein (12.26%) and moisture content (7.40%), ash content (6.27%), while fats have the least composition which accounted for 4.92%. Amino acid profile revealed abundant essential and non-essential amino acids, notably Isoleucine (2.94 g/100g) and Alanine (2.26 g/100g). *In vitro* Total Antioxidant Capacity assay demonstrated significant antioxidant activity, with ascorbic acid equivalent values increasing from 0.31 to 0.52 mg/ml, while the *In vitro* Nitric Oxide Scavenging Assay showed a progressive increase in the %NO Scavenging from 42.48% to 55.03% confirming significant radical scavenging ability. The extract also showed strong alpha amylase (64.29%) and alpha glucosidase (67.50%) inhibition. GCMS identified 35 bioactive compounds, with Erucic acid (with a peak area of 4.59%), D-carvone (5.78%), Oleic Acid (8.90%), Cyclopropane (17.01%) and 9 borabicyclo compounds (28.30%), having the most prominent peak areas. These results suggest the extracts potential in nutritional and therapeutic applications.

Keywords: *Ocimum gratissimum* stem bark, Essential and non-essential amino acids, Antioxidants, Alpha amylase, Alpha glucosidase

INTRODUCTION

The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: preserved monuments, written documents and even original medicines. Hence, plants have been employed as antioxidants, anti-diabetic, anti-inflammatory, anti-carcinogenic, antimicrobial and antioxidant drugs (Uadia et al., 2023; Ogbeide *et al*., 2022). The use of natural bioactive compounds from medicinal plants in the treatment of diseases dates back to human origin (Ogbeide and Aghedo, 2022). Millions of chemical compounds with therapeutic values are currently being synthesized in the laboratory, but plant extracts remain the greatest sources of new drugs (Ogbeide *et al.,* 2018). Plants of medicinal relevance are known to contain bioactive chemicals such as essential oils, flavonoids, saponins, tannins, terpenoids and alkaloids with healing properties (Ogbeide and Akhigbe, 2019). Plants synthesize hundreds of these chemical compounds for various functions, including defense and protection against insects, fungi, diseases, parasites (Francois *et al.,* 1999) and herbivorous mammals (Gershenzon and Ullah, 2022; Ugbogu *et al.,* 2021) and these chemicals are called phytochemicals. *Ocimum gratissimum*, also known as scent leaf, African basil or clove basil, family Lamiaceae, is widely distributed in tropical and warm temperate regions especially India and West Africa (Prabhu *et al.,* 2009).

It is one of the discovered medicinal plants with the potential to serve as an alternative therapy for the treatment of various ailments (Ogbeide and Uadia, 2022).

O. gratissimum is a perennial and odoriferous shrub which is about 1-3 m tall with an erected stem, and is branched, round-quadrangular, and woody at the base. The leaves are opposite, ovate-lanceolate, and have a strong clove-like aroma. The plant bears small white or purplish flowers arranged in spikes and are propagated through seeds or stem cuttings (Orwa *et al.,* 2009). It is known by various names in different parts of the world: "Efirin" by the Yorubas, "Nchanwu" by the Igbos, "Daidoya" by the Hausas, "Ebe-amwonkho" in Edo, "Anyeba" in Igalaand "Ntong" in Efik.

In Nigeria, the leaves of *O. gratissimum* are used in making pepper soup, local rice, beans and many other delicacies. *O. gratissimum* is a useful insect repellant in other crops as it repels the thrips, "Thrips tabaci" (Ogbeide and Uadia, 2022). Scientific reports have shown that *O. gratissimum* has a wide range of bio active compounds such as flavonoids and poly phenols (Venuprasad *et al.,* 2014; Irondi *et al*., 2016) and essential oils with several beneficial effects (Benitez *et al.,* 2009; Melo *et al.,* 2019).

O. gratissimum is used in the treatment of a variety of conditions such as; aches, fever, inflammation, diarrhea, anaemia, fungal and bacterial infections (Shedoeva *et al.,* 2019; Ogbeide and Uadia, 2022). *O. gratissimum* possesses calcium and magnesium, both

of which work in lowering blood sugar (Okoduwa *et al.*, 2017). It also aids the heart to function properly by removing toxic substances and cholesterol which the body does not need (Ademiluyi *et al.*, 2023). Despite the increasing global interest in natural remedies and medicinal plants, the scientific exploration of the *O. gratissimum* stem bark remains limited as compared to its leaves. Hence the aim of this study was to evaluate the nutritional, chemical, antioxidant, and anti-diabetic properties of *Ocimum gratissimum* stem bark extract, in order to explore its potential medicinal and therapeutic applications.

MATERIALS AND METHODS

Plant Collection

Fresh stems of *Ocimum gratissimum* were obtained from a garden in Sapele road, Benin City, Edo State, Nigeria. The sample was identified and authenticated by a botanist at the University of Benin's Plant Biology and Biotechnology Department and given the voucher specimen number UBH-0333.

Plant Preparation and Extraction

The stems were rinsed, chopped into pieces and air dried for two weeks and ground to a coarse powder with an electrical blender. The powdered stem sample weighed 180 g. the extraction technique applied was the maceration technique using ethyl acetate as a solvent. The stem was soaked in 0.8 L of the solvent for 72 hours with intermittent stirring. It was filtered and the filtrate concentrated using a rotary evaporator at a regulated temperature of 45°C. The weight of the concentrate of the stem sample was taken to be 11.51 g. Afterwards, the extracted yield was calculated in percentage as in the equation below:

$$\% \text{ Extraction yield (w/w)} = \frac{Weight\ of\ dried\ extract}{Weight\ of\ sample\ used\ for\ extraction} \times 100 \quad (1)$$

Chemicals and reagents used for the assays

Ethyl Acetate, Conc. H_2SO_4, Distilled water, Sodium phosphate buffer, Ammonium molybdate, sodium nitroprusside, phosphate buffer saline (PBS), griessreagent, hexane, alkaline phenate, sodium potassium tertarate, sodium hypochlorite (bleach), sodium sulphate and calcium sulphate.

Proximate Composition

To determine the moisture content, ash content, crude protein content, crude fat, crude fiber and total carbohydrate content of *O. gratissimum* stem bark was done using standard laboratory methods (AOAC, 1990, Abdullahi *et al.,* 2025). The proximate parameters expressed in percentage were obtained by taking the average values twice in triplicates.

Amino Acid Profile

The concentration of each of the assayed amino acids was determined according to Dangambo *et al.* (2022) and Elkin *et al.* (1980). The assay involved acid hydrolysis with 6 M HCl at 110°C for 24 hours. After hydrolysis, it was filtered and the filtrate was made up to 25 mL with pyridine. A standard solution containing 1.25 µmol/mL of each amino acid in 0.1 M hydrochloric acid was created. A standard solution containing 50 µL of sample solution was pipetted into a 10 × 5 mm tube and dried in vacuum at 65°C. To the residue, 30 µL of methanol-water-Phenylisothiocianate (2:2:1 [v/v]) was added and then removed in vacuum at 65°C.

Next, 30µL of the derivatizing reagent methanol-water-Phenylisothiocianate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C, pending analysis. Prior to injection, 150 µL of diluent consisting of 5mM sodium phosphate with 5% acetonitrile was added to each tube. Chromatography was carried out at a constant temperature of 30°C using a gradient elution as follows: **Eluant A** was an aqueous buffer prepared by adding 0.5 mL/L Triethylamine to 0.14M sodium acetate and titrating it to pH 6.20 with glacial acetic acid. **Eluant B** was acetonitrile-water (60:40 [v/v]).

Antioxidant Assay

Total Antioxidant Capacity (TAC)

The Total Antioxidant Capacity (TAC) of the stem extract in different extracting solvents (absolute ethanol, 70% and 50% ethanol) were determined by the phosphomolybdate method according to Jayaprakasha *et al.* (2002). An aliquot (30 ml) of different concentrations (20, 40, 60, 80 and 100 mg/ml) of the stem extracts were mixed with 3ml of the reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes were then capped with aluminium foil and incubated in a boiling water bath at 95ºC for 90 minutes. The reaction mixture was then allowed to cool to room temperature and the absorbance of the solutions were measured at 695 nm against a blank containing 3ml of reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) and the appropriate volume of the dissolved solvents. The blank was incubated under the same conditions as the test samples. Ascorbic acid was used as a standard reference to compare the activities of the extracts. The results are expressed as Ascorbic Acid Equivalent (AAE) and it is derived by plotting a calibration curve using the standard antioxidant (ascorbic acid) by plotting Absorbance (y-axis) against concentration (x-axis). From the equation of the line, **"y=mx +c"**, the absorbance of the sample is compared to the absorbance of the standard, giving the AAE.

Nitric Oxide Scavenging Assay

The extent of inhibition of Nitric Oxide radical generation in vitro was followed as per the method reported by (Sarwar *et al.,* 2015). The reaction was initiated by adding 0.5 ml of the samples (50 mg), 2 ml of sodium nitroprusside and 0.5 ml of phosphate buffer saline (PBS). A control sample was also prepared by adding the same reagents but without the sample. The solutions were placed in an incubator at 25^0C for 30 minutes. 0.5 ml of Greiss reagent (a mixture of sulfanilamide, N-1-naphthylethylenediamine dihydrochloride <NED>,

and an acid, such as phosphoric acid) was added to each test tube and incubated again for another 30 minutes. The absorbance of the samples was then measured in a spectrometer at 546 nm. The percentage NO Scavenging activity was calculated by applying equation 2.

$$\%\text{NO SCAVENGING} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100 \quad (2)$$

Anti-diabetic Assay

Alpha Amylase Inhibition Assay

The alpha-amylase inhibitory test was performed using the standard method with minor modification (Ademiluyi *et al*., 2013). A volume of 250µl of extract or acarbose (1-300 mg/ml) was mixed with 250 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase at a concentration of 0.5 mg/ml. The mixture was pre-incubated at for 10 minutes. Then, 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added and incubated at 25° C for another 10 minutes. The reaction was stopped by adding 500µl of dinitrosalicylic acid (DNS). The tubes were then incubated in a water bath at 95°C for 5 minutes and cooled at room temperature followed by dilution with 5 ml distilled water. The optical density was measured at 540 nm. The inhibitory activity on alpha amylase was calculated as percentage inhibition, using equation 3.

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \quad (3)$$

Alpha Glucosidase Inhibitory Assay

The ability of extracts to inhibit the activity of α-glucosidase was assessed according to (Shai *et al*., 2011). A reaction mixture containing 50 µl phosphate buffer (20 mM, pH 6.9), 10 µl α-glucosidase (1 µ/ml), and 20 µl of varying concentrations of extracts (0.0, 40.0, 80.0, 120.0 mg/ml) was incubated at 37°C for 20 minutes. Then, 20 µL P-nitrophenylglucopyranoside substrate solution (pNPG, 3 mM) was added and incubated further at 37°C for another 20 minutes. The reaction was stopped with 1 ml of Na_2CO_3 (1 M). The α-Glucosidase activity was determined by measuring the absorbance of the released p-nitrophenol from pNPG at 405 nm. Without test samples was set up as a control. The percentage inhibition was calculated as in equation 4.

$$\% \text{ Inhibition} = \frac{1 - \text{Abs of sample}}{\text{Abs of control}} \times 100 \quad (4)$$

GC-MS Analysis

The GC-MS analysis was carried out as described by previous authors. An Agilent Technologies GC System equipped with a mass selective detector was used. Separation was achieved using a HP-5MS Capillary column (30 m x 0.25 mm i.d., 0.25 um film thickness). The carrier gas used was helium at a constant flow rate of 1.0 ml/min; injection volume of 1.0 ul (split ratio 10:1), injector temperature of 250°C, Initial Oven temperature of 60°C which was held for only 2 minutes then increased to 280°C for 10 minutes. The ionization mode of the mass spectrometer had an Electron Impact of 70 eV with a mass scan range of: m/z 50-600. The source temperature was 230°C and the quadrupole temperature was 150°C (Iyasele *et al.,* 2022; Selvaraju *et al.,* 2021; Sparkman *et al.,* 2011)

RESULTS AND DISCUSSIONS

The results obtained from the proximate composition, amino acid profile, chemical composition, in vitro antioxidant and anti-diabetic assays of the stem bark extract of *O. gratissimum* are presented below.

Percentage Yield of the Extract

The percentage yield of the stem bark extract of *O. gratissimum* obtained was 6.39%.

Proximate Composition

The result of the proximate composition (both qualitative and quantitative) of *O. gratissimum* stem bark extract is presented in table 1. Quantitatively, carbohydrate has the highest composition which accounted for 53.69%. This is followed by fiber (12.50%), protein (12.26%) and moisture content (7.40%), ash content (6.27%), while fat has the least composition which accounted for 4.92%.

Table 1: Proximate analysis of the stem bark extract of *O. gratissimum*

Parameters	Percentage %
Moisture	7.40
Ash	6.27
Crude Protein	12.26
Crude Fat	4.92
Crude Fiber	12.50
Carbohydrate	53.69

Amino Acid Profile

Table 2: Essential and Non-Essential Amino acids present in *O. gratissimum* stem bark extract

Essential Amino Acid	Concentration (g/100g)	Non-essential Amino Acid	Concentration (g/100g)
Isoleucine	2.94	Alanine	2.26
Threonine	1.32	Serine	1.25
Valine	0.54	Proline	1.13
Lysine	0.30	Arginine	0.75
Leucine	0.25	Glutamate	0.28
Phenylalanine	0.21	Aspartate	0.20
Tryptophan	0.13	Cystine	0.16
Histidine	0.09	Tyrosine	0.06
Methionine	0.08	Glycine	0.03

The results in Table 2 revealed a diverse composition of both essential and non-essential amino acids, contributing to its overall nutritional and therapeutic value. The result can be compared with the amino acid profile of the *Acalypha indica* plant, (Ogbeide *et al.,* 2025). A total number of 19 amino acids were identified, with isoleucine (2.94 g/100g), alanine (2.26 g/100g), threonine (1.32 g/100g), serine (1.25 g/100g), proline (1.13 g/100g), arginine (0.75 g/100g) and valine (0.54 g/100g), being among the most abundant.

Antioxidant Assays

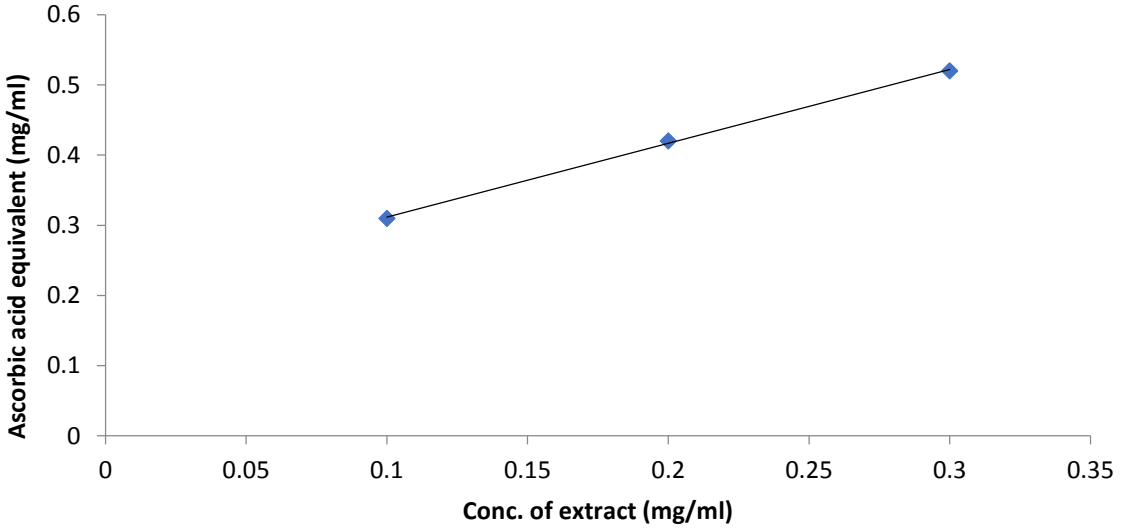

Figure 1: Phosphomolybdenum TAC assay of *O. gratissimum* stem extract

Phosphomolybdenum TAC assay of *O. gratissimum* stem extract showed an appreciable increase for the standard (ascorbic acid) and the stem extract. From the results, the antioxidant capacity of *O. gratissimum* stem extract expressed as AAE, increased with increase in concentration (Figure 1). The AAE value was determined by applying the following formula:

$$AAE = \frac{\text{Abs of sample} - \text{Intercept}}{\text{Control}} \quad (5)$$

From the calibration curve, the slope was gotten to be 0.85, while the intercept was gotten to be 1.93. The TAC of the stem extract was found to range between 0.31- 0.52 mg AAE/ml, which is considered moderate when compared to related studies on medicinal plant extracts that reported low AAE values ranging from 0.001 - 0.005 mg AAE/ml and high AAE values from 0.74 mg AAE/ml and above. (Tourabi *et al*., 2023). These findings supported the presence of potent antioxidant phytochemicals in the stem extract, making it a potential natural antioxidant source.

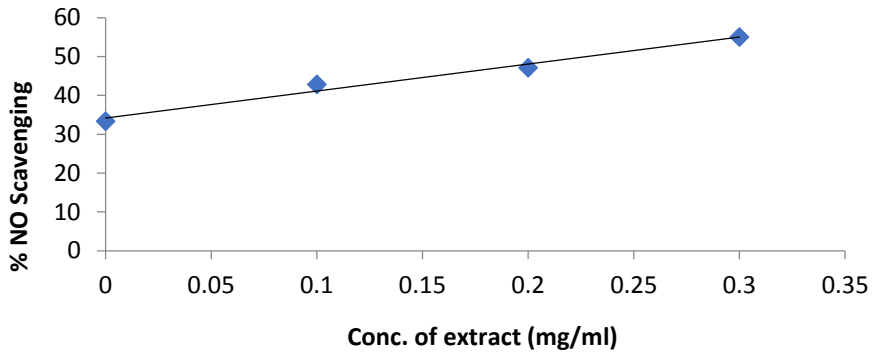

Figure 4: Nitric Oxide scavenging assay of stem extract of *O. gratissimum*

From Figure 2, the results showed that the stem extract of *Ocimum gratissimum* exhibited nitric oxide (NO) scavenging activity at all tested concentrations (0.1, 0.2 and 0.3 mg/ml). It was observed that the % NO scavenging increased with increasing concentration, indicating a dose-dependent antioxidant response (Figure 2). At 0.3 mg/ml, the extract showed the highest % NO scavenging activity of 55.03 % and the lowest % NO Scavenging activity was recorded at 0.1 mg/ml with a value of 42.86%. The %NO Scavenging assay is generally considered high when it exceeds 60% and low when it is below 30% (Adebayo *et al.,* 2019). Overall, the stem extract demonstrated appreciable nitric oxide scavenging capacity, supporting its potential as a natural antioxidant source.

Anti-Diabetic Assay

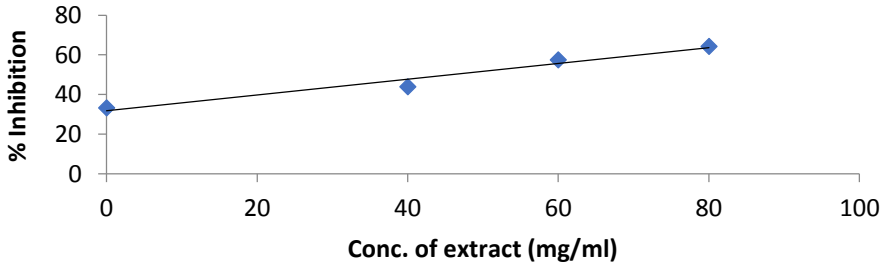

Figure 3: α-Amylase inhibition assay of stem extract of *O. gratissimum*

At concentrations 40.00 mg/ml, 60.00 mg/ml and 80.00 mg//ml, the percentage inhibition recorded were 44.00%, 57.45% and 64.29% respectively (Figure 3). This result corresponds to the alpha amylase assay performed on the stem of *Tamarindus indica* by Bhutkar and Bhise (2012), where the highest % inhibition gave a value of 63.54%. The highest inhibition was observed at 80.00 mg/ml. Based on the findings, these plant extracts may be highly useful in slowing down carbohydrate digestion and absorption, which can help manage diabetes by lowering post-meal blood sugar spikes. Further research is needed to explore the molecular pathways through which these plants and their active components influence glucose regulation.

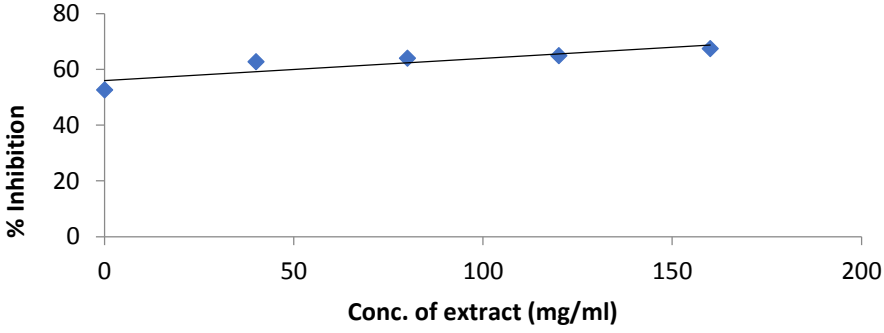

Figure 4: α-Glucosidase inhibition assay of stem extract of *O. gratissimum*

The α-glucosidase inhibitory potential of the stem extract of *O. gratissimum* was evaluated at concentrations ranging from 40mg/ml to 160mg/ml. The results revealed a concentration-dependent inhibition up to 160 mg/ml. The extract showed significant inhibitory activity when compared to the control (67.50% inhibition), corresponding with the value gotten by Lee *et al.* (2013), for the alpha glucosidase inhitory assay of *Neptunia oleracea* (67.4%), indicating its potential as a natural α-glucosidase inhibitor, which supports its ethno-medicinal use in the management of diabetes mellitus.

GC-MS Analysis

Table 3: Chemical compounds identified in the stem extract of *O. gratissimum* by GC-MS

S/N	Retention Time (mins)	Compound Name	Peak Area (%)
1	5.37	1-Fluorononane	0.61
2	5.47	Benzene, 1-ethyl-2-methyl-	0.99
3	5.62	1,3-cyclohexanediamine	0.35
4	5.67	Mesitylene	0.50
5	11.58	Levo-menthol	2.19
6	12.12	α-Terpineol	0.63
7	12.27	Cyclohexanol	0.96
8	13.02	Carveol (cis and trans forms)	0.91
9	13.40	Carveol	3.36
10	13.51	D-carvone	5.78
11	13.59	Cyclopropane	17.01
12	13.62	9-Borabicyclo compounds	28.30
13	14.84	Cyclohexanol derivatives	0.45
14	16.82	Acetate derivatives	0.70
15	18.22	Caryophyllene	0.26
16	22.76	Hexadecane	0.24
17	22.90	Dodecanoic acid (Lauric acid)	0.48
18	23.32	Epibubenol, Dodecanoic acid	0.53
19	29.54	Hexadecanoic acid, methyl ester (Methyl palmitate)	0.90
20	31.11	Octadecadienoic acid (Linoleic acid derivatives)	0.34
21	31.16	Octadecenoic acid (Methyl oleate)	2.90
22	31.34	Methyl stearate	0.78
23	31.64	Isopropyl tetracosyl ether, Nonyl ether derivatives	2.26
24	31.69	Tert-Hexadecanethiol, Octadeceenoic acid derivatives	0.73
25	31.77	1-Nonadecene, 9-Tricosene	3.75
26	31.87	Cis-Vaccenic acid, 9-Octadecenoic acid, Ethanol esters	1.06
27	31.93	Oleic acid, ethanol esters, Cyclopropane derivatives	2.65
28	32.91	1-Docosene, 9-Tricosene	0.06
29	33.74	Oleic acid, Vaccenic acid, Cyclohexane derivatives	8.90
30	33.98	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	0.46
31	33.90	13-Octadecenoic acid esters	0.36
32	33.94	Oleic acid, Bis(2-ethylhexyl) phthalate	1.75
33	36.04	Erucic acid, cis-Vaccenic acid, Oleic acid	4.59
34	36.10	9-Octadecenoic acid esters, Oleic acid	0.54
35	36.10	13-Octadecenoic acid	3.72

The GC-MS analysis of the methanolic stem extract of *Ocimum gratissimum* revealed a diverse array of 35 phytochemical constituents, encompassing monoterpenes, sesquiterpenes, fatty acids, esters, alcohols, and hydrocarbons. The study showed the presence of borabicyclo compounds (28.30%), cyclopropane (17.01%), Oleic Acid (8.90%), D-carvone (5.78%) and Erucic acid (4.59%) as major compounds. These compounds collectively contribute to the plant's traditional medicinal applications, particularly its antioxidant, anti-inflammatory and antimicrobial properties.

It is interesting to note that in earlier reports (Joshi, 2017), of the compounds present in the essential oil of the leaf of the same plant, the compound caryophyllene (14.2%) was reported in high amounts from cultivated plants of *O. gratissimum.* However in this report on the stem extract, the amount of caryophyllene (0.26%), was noticeably lower. Also in the report of Adefolalu *et al.* (2015), Terpineol had a peak area of (1.13%), however in this report, terpineol had a peak area of (0.63%).

CONCLUSION

This research successfully explored the nutritional and medicinal potential of the stem extract of *Ociumum gratissimum* through a series of biochemical and phytochemical evaluations. The proximate analysis revealed that the plant stem possesses appreciable amounts of essential macronutrients, suggesting its potential contribution to dietary needs. The amino acid profiling confirmed the presence of vital amino acids required for various physiological and metabolic functions.

Furthermore, the antioxidant examinations indicated strong radical scavenging activity, highlighting the plant's potential to combat oxidative stress-related diseases. The anti-diabetic assays, particularly the inhibition of alpha-amylase and alpha-glucosidase enzymes, indicated the extracts promising role in managing postprandial hyperglycemia and by extension, Type 2 diabetes. Finally, the GC-MS analysis revealed the presence of several bioactive comounds with known pharmacological and therapeutic properties, further supporting the traditional use of *Ocimum gratissimum* in herbal medicine. Overall, the findings from this study underscore the plant's nutritional, therapeutic, and pharmacological relevance, especially in the development of functional foods and natural drug formulations. Further studies involving isolation, characterization, and in vivo examination of its bioactive components are recommended to validate and harness its full medicinal potential.

Authors' Contributions

OU conceptualize and design the research work. UJO reviewed the manuscript critically for important intellectual content. OMK developed the study methods and wrote the manuscript. OKO supervised the analytical works and writing of the manuscript with final approval of the version of manuscript to be submitted.

Conflict Of Interest

We have no conflicts of interest to disclose

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