*Nigerian Research Journal of Chemical Sciences* **(ISSN: 2682-6054)**
**Volume 9, Issue 1, 2021**

# Determination of Anti-nutritional Contents and IR Spectra of *Ocimum basilicum* and *Ocimum gratissimum* Plant

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## ABSTRACT

Plants are sources of nutrients and also have medicinal value that keeps the human body healthy. Anti-nutrient is one of the factors that reduce the content of nutrients in plants. The aim of the research is to determine the anti-nutritional factors and organic contents of *Ocimum basilicum* and *Ocimum gratissimum* plant leaves using FTIR spectrometer. The results showed that *O. basilicum* leaves had high percentage content of tannins and saponin (3.50 and 5.35% respectively) and *O. gratissimum* leaves had alkaloid and oxalate contents of 4.50 and 1.02% respectively. Both plants leaves had same content of phytate (0.46%). The n-hexane extracts of *O. basilicum* and *O. gratissimum* leaves showed the presence of carboxylic acid, alkane, aldehyde, amine, aromatics, ether, alkyl halide and alkene groups except alkyne group seen in *O. basilicum* and ester group seen in *O. gratissium* leaves. The methanol extracts of *O. basilicum*and *O. gratissimum* leaves showed presence of alcohol, alkane, aromatics, ether, aromatic phosphate and alkyl halide groups except alkyne group seen only in *O. basilicum* and aldehyde and alkene seen only in *O. gratissumum* leaves in the FTIR spectra. Both plants leaves showed low content of anti-nutrition factors and also the FTIR spectra revealed most of the organic components found in each extracts of the pants which are the characteristic component of the plant leaves.

**KEYWORDS:** Anti-nutritional factors, FTIR spectra, Methanol and n-hexane extracts, *Ocimum basilicum* and *Ocimum gratissimum* leaves

## INTRODUCTION

Anti-nutrients are plant contents or synthetic compounds which inhibit the human body ability to absorb essential nutrients. They are factors that cause problems to the human body system especially when they are in high concentrations. The anti-nutritionals do not affect the plant that contained it but affect the human body when consumed. Anti-nutritional factors reduce nutrient intake, digestion, absorption and utilization or produce other adverse effects; also reduce growth and healing by affecting the availability of nutrients to the body [1]. Anti-nutritional factors such as alkaloids, phytates, tannins, saponins and oxalates, have an adverse effect on health through inhibition of protein digestion and growth [2]. They reduce iron and zinc absorption [3].

Fourier Transform infrared (FTIR) is an Infra Red (IR) instrument used in determining of organic components such as chemical bond as well as organic contents. FTIR is one of the important analytical techniques for researchers [4]. *Ocimum* (Scent) leaf is an important nutritious plant commonly used in Nigeria and other parts of the world. In the northern part of Nigeria the leaf is called "Daidoya" by the Hausas while in the southern part of Nigeria it is called "Nchanwu" by the Igbos [5].

*Ocimum basilicum* and *Ocimum gratissimum* leaves contain nutrients such as protein, carbohydrate, fiber, fats etc and also essential elements which is good for the human body [6].

The aim of this study is to determine their anti-nutritional content and also identify the functional groups present in the extracts of the leaves.

## MATERIALS AND METHODS

### Collection, identification and preparation of plant samples

Fresh samples of *Ocimum basilicum* leaves were collected from Government Reserved Area (GRA) along Damboa road, and 707 Housing estates at Maiduguri town, Borno state in North-Eastern Nigeria.*Ocimum gratissimum* plant leaves were collected at Nsukka and Awgu towns in Enugu state in South-Eastern part of Nigeria. Both leaves were examined and authenticated at the herbarium laboratory in the Department of Biological Sciences, Ahmadu Bello University, Zaria. The *voucher* number, 1285 for *Ocimum gratissimum* and 044 for *Ocimum basilicum* were given to the plants. The fresh samples of the scent leaves were washed with distilled water and dried at room temperature separately for 3 days. The two samples were then crushed in a mortar

separately. The resulting powder was sieved, weighed and stored in black polyethene leather for further analysis.

**Anti-nutritional determination**

The anti-nutritional contents were determined forphytate, tannins, alkaloid, saponin and Oxalate contents in percentage.

**Phytate content**

About 4 g of sample was soaked in 100 ml of 2% HCL for 3 hr. Exactly 25 ml of the sample filtrated was dispensed into a conical flask and 5 ml of 0.3 ml ammonium thiocyanate solution was added. The mixture was then titrated with Iron(III) chloride solution until a brown-yellow colour persisted for 5min was obtained [7].

%Phytate = Titre x 1.95 g of Titrephytate x Iron equivalent (0.00195 g/ml) x 100

**Tannin content**

About 2 g of sample was poured into a beaker containing 50 ml distilled water and heated to 60 $^{o}$C and filtered using whatman filter paper No. 1 and the residue discarded. Exactly 10 ml of 4% copper acetate solution was added to the hot filtered and boiled for 10 min. The precipitate was filtered and the filtered discarded. The residue on the filter paper was dried, scraped, weighed and poured into a pre-weighed crucible. The sample in the crucible was heated in a muffle furnace at 550 $^{o}$C, cooled in a dessicator and reweighed. The difference between the weight of sample before ashing and ash residue after incineration represent the tannin content [8].

$$\%\text{Tannin} = \frac{Weight\ of\ precipitated\ extract}{Weight\ of\ Original\ sample} \times 100$$

**Alkaloid content**

About 5 g of powered sample was dispersed in 50 ml of 10% acetate acid in methanol and allowed to stand for 4 hr. The mixture was filtered and the filtrate was evaporated to 15 ml of the original volume in a water bath. The Alkaloid was precipitated with drop of Ammonium hydroxide and the precipitate was recovered by centrifugation at 1000 rpm for 10 min, dried at 70 $^{o}$C for 30 min in the vacuum oven and weighed [9]. The following equation was used to calculate the content of alkaloid.

$$\%\text{Alkaloid} = \frac{Weight\ of\ dried\ precipitate}{weight\ of\ original\ sample} \times 100$$

**Saponin content**

About 2 g of sample was extracted using 100 ml of acetone and methanol separately for 3hr. The extract was recovered by removing the solvent using rotary evaporator and the extract was dried and weighed [10]. The percentage content of saponin content was calculated using the following equation.

$$\%\text{Saponin} = \frac{Dried\ extracts\ of\ sample}{Weight\ of\ sample} \times 100$$

**Oxalate content**

About 2 g of sample was dispersed in 190 ml of distilled water in 250 ml volumetric flask. Exactly 10 ml of 6M HCl was added, then digested at 100 $^{o}$C for 1hr, cooled and made to volume before filtration. The filtrate was precipitated with ammonium hydroxide and the precipitate was dissolved in 10 ml of 20% sulphuric acid. The solution was titrated with 0.05 M potassium permanganate [11]. The following equation was used to calculate the percentage oxalate content.

$$\text{Oxalate} = \frac{T\ x\ Vme\ x\ DF\ x\ 105}{ME\ x\ MF} \times 100$$

T = Titre value of potassium permanganate

Vme = Volume-mass equivalent (1ml of $KMnO_7$ = 0.00225)

DF = Dilution factor

ME = Molar equivalent of potassium permanganate.

MF = Mass of the sample used

**Sample Extraction**

The powdered sample of 250 g of each plant samples was successively extracted by adding 1000 $cm^3$ of n-hexane and methanol as solvent in soxhlet apparatus for 48hr. The resulting extract was filtered through a Whatman filter paper No.1 and solvent was removed by using a rotary evaporator and was subjected to freeze drying in a lyophilizer until dry extract was obtained.

**IR Determination**

The IR spectra were determined using FTIR machine Agilent Technologies Cary 630 model, 8 Resolution, 4000- 650 rangeHapp-Genzel and the spectra were interpreted using IR chart from www.scribd.com/document/341836490/05-chart-1-pdf site.

## RESULTS AND DISCUSSION

### Anti-nutritional Contents in *Ocimum basilicum* and *Ocimum gratissimum* Leaves

The result for the anti-nutritional contents showed that *O. basilicum*leaves found at 707 Housing estate in Maiduguri had percentage concentrations of tannins and saponin at 3.50% and 5.35% respectively, *O. gratissimum* leaves found at Nsukka town had percentage of alkaloid content at 4.20% and *O. gratissimum* leaves found at Awgu and Nsukka towns had percentage concentrations of oxalate content of 1.02% respectively. *O. gratissimum* leaves found at Nsukka town and *O. basilicum* leaves found at Damdoa road had the percentage concentration in phytate content of 0.46% respectively as shown in Figure 1.

Anti-nutritional factors are substances when present tend to reduce the availability of one or more nutrients [12]. Results showed that 707 Housing estate and Damdoa road had high percentage tannin content at 3.50 - 0.04% and 3.00 - 0.02% in *O. basilicum* leaves while Nsukka and Awgu towns had low tannin content of 0.40 - 0.04% and 0.45 - 0.01% in *O. gratissimum* leaves as illustrated in figure 1. Results also showed that 707 Housing estate and Damdoa road had high percentage mean saponin content of 5.35 - 0.06% and 2.50 - 0.03% in *O. basilicum* leaves while Nsukka and Awgu town had low saponin content of 0.19 - 0.02% and 0.12 - 0.02% in *O. gratissimum* leaves as shown in figure 1.Nsukka and Awgu towns had high oxalate content of 1.02 - 0.03% and 1.02 - 0.02% in *O. gratissimum* leaves while 707 Housing estate and Damdoa road had low oxalate content of 0.05 - 0.01% and 0.03 - 0.01% in *O. basilicum* leaves as shown in figure 1.Nsukka town had alkaloid content of 4.20 - 0.01% in *O. gratissimum* leaves followed by Damboa road of 3.20 - 0.07% in *O. basilicum* leaves while Awgu town and 707 Housing estate had low alkaloid content of 1.40 - 0.01% in *O. gratissimum* leaves and 1.12 - 0.01% in *O. basilicum* leaves respectively as illustrated in Figure 1.Nsukka town and Damboa road had same percentage mean phytate content of 0.46 – 0.01% in both *O. basilicum* and *O. gratissimum* leaves while 707 Housing estate and Awgu town had low phytate content of 0.44 - 0.02% in *O. basilicum* leaves and 0.34 - 0.01% in *O. gratissimum* leaves shown in Figure 1. The

results showed that both leaves of *O. basilicum* and *O. gratissimum* plant had low content of anti-nutritional factors which showed that the plant is good for consumption. It was reported that high content of nutrients was obtained in both leaves of *Ocimum basilicum* and *Ocimum gratissimum* plants [6].

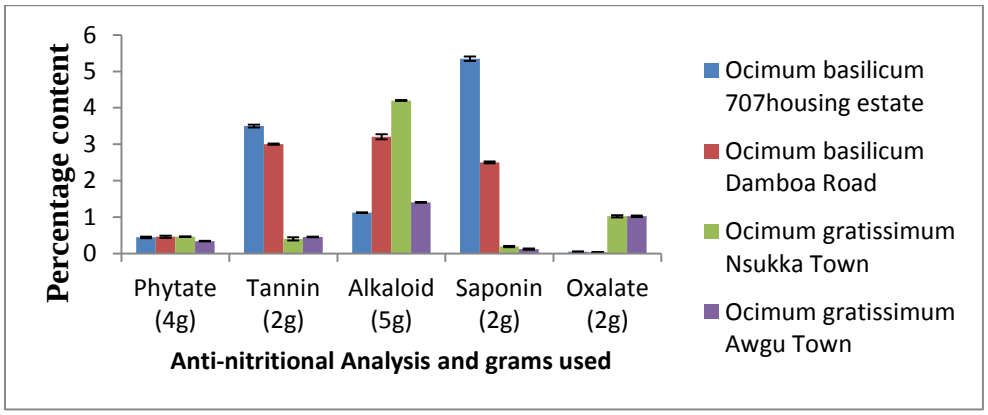


Figure 1: Anti-nutritional contents of *Ocimum basilicum* and *Ocimum gratissimum* leaves plant from the four locations.

Table 1: IR absorbance of n-hexane extracts of *Ocimum basilicum* leaves and the quantified frequencies of functional groups.

| Sample Absorbance cm | Range of absorbance cm | Assignment | Functional group |
|---|---|---|---|
| 3369.5 | 3200-3400 | O-H Stretch | Carboxylic Acid |
| 2922.2 | 2850-3000 | C-H Stretch methylene | Alkanes/Alkyl |
| 2855.1 | 2850-3000 | C-H Stretch methylene | Alkanes/Alkyl |
| 2150.7 | 1725-1750 | C≡C Stretch | Alkynes |
| 1707.1 | 1685-1710 | C=O Stretch, Conjugated | Aldehyde |
| 1513.3 | 1510-1560 | N-H Bend, Secondary | Amines |
| 1453.7 | 1450-1600 | Amine | Aromatic |
| 1375.4 | 1370-1390 | C=C Aromatic ring Stretch | Alkane/Alkyl |
| 1237.5 | 1200-1275 | Methylene C-H Bend | Ether |
| 1162.9 | 1130-1190 | Methyl C-H Bend | Amine |
| 1080.9 | 1000-1350 | C-N Stretch, Amine | Alkyl halides |
| 1036.2 | 1020-1075 | C-F Stretch | Ether |
| 913.2 | 890-915 | =C-O-C Symmetric Stretch | Alkene |
| 834.9 | 790-840 | C-H Vinyl out of plane | Alkene |
| 719.4 | 715-725 | bend | Alkane |
| | | =C-H Bend | |
| | | $(CH_2)_n$ Bend | |

Table 2: IR absorbance of n-hexane extracts of *Ocimum gratissimum* leaves and the quantified frequencies of functional groups.

| Sample Absorbance cm | Range of absorbance cm | Assignment | Functional group |
|---|---|---|---|
| 3373.2 | 3200-3400 | O-H Stretch | Carboxylic |
| 2922.2 | 2850-3000 | C-H Stretch methylene | Acid |
| 2855.1 | 2850-3000 | C-H Stretch methylene | Alkanes/Alkyl |
| 1736.9 | 1735-1750 | C=O Stretch | Alkanes/Alkyl |
| 1688.5 | 1685-1710 | C=O Stretch, Conjugated | Esters |
| 1617.7 | 1580-1650 | N-H Bend, | Aldehyde |
| 1584.1 | 1450-1600 | C=C-C Aromatic ring | Amine |
| 1453.7 | 1450-1600 | Stretch | Aromatic |
| 1375.4 | 1370-1390 | C=C-C Aromatic ring | Aromatic |
| 1289.7 | 1250-1310 | Stretch | Alkane |
| 1226.3 | 1200-1275 | C-H Bend Methyl | Ester |
| 1177.8 | 1160-1210 | O=C-O-C Aromatic | Ether |
| 1110.7 | 1000-1350 | Stretch | Ester |
| 1036.2 | 1020-1075 | =C-O-C Stretch | Alkyl halides |
| 887.1 | 885-895 | O=C-O-C Stretch | Ether |
| 838.7 | 790-840 | (Aliphatic) | Alkene |
| 805.1 | 790-840 | C-F Stretch | Alkene |
| 719.4 | 715-725 | =C-O-C Symmetric | Alkene |
| 670.9 | 665-730 | Stretch | Alkane |
| | | =C-H Bend | Alkene |
| | | =C-H Bend | |
| | | =C-H Bend | |
| | | $(CH_2)_n$ Bend | |
| | | =C-H Bend | |

Table 3: IR absorbance of methanol extracts of *Ocimum basilicum* leaves and the quantified frequencies of functional groups.

| Sample Absorbance cm | Range of absorbance cm | Assignment | Functional group |
|---|---|---|---|
| 3291.2 | 2500-3500 | O-H stretch | Alcohol |
| 2922.2 | 2850-3000 | C-H Stretch | Alkanes |
| 2154.4 | 2100-2260 | C≡C Stretch | Alkynes |
| 1602.8 | 1580-1615 | C=C-C Aromatic ring | Aromatic |
| 1513.3 | 1580-1615 | Stretch | Aromatic |
| 1375.4 | 1370-1380 | C=C-C Aromatic ring | Alkane/Alkyl |
| 1155.5 | 1125-1205 | Stretch | Alcohol |
| 1032.5 | 1000-1350 | Methyl C-H Bend | Ether |
| 916.9 | 950-1225 | C-O Stretch | Aromatic |
| 864.7 | 850-995 | =C-O-C Symmetric | Aromatic |
| 820.0 | 810-840 | Stretch | phosphate |
| 779.0 | 750-850 | C-H plane bend Aromatic | Aromatic |
| | | P-O-C Stretch | Alkyl halides |
| | | C-H Bend p-di-substituted | |
| | | C-Cl Stretch | |

Table 4: IR absorbance of methanol extracts of *Ocimum gratissimum* leaves and the quantified frequencies of functional groups.

| Sample Absorbance cm | Range of absorbance cm | Assignment | Functional group |
|---|---|---|---|
| 3309.9 | 2500-3500 | O-H stretch | Alcohol |
| 2926.0 | 2850-3000 | C-H Stretch methylene | Alkane |
| 2855.1 | 2850-3000 | C-H Stretch methylene | Alkane |
| 1688.5 | 1685-1710 | C=O conjugated Stretch | Aldehyde |
| 1606.5 | 1450-1600 | C=C-C Aromatic ring | Aromatics |
| 1509.6 | 1450-1600 | Stretch | Aromatics |
| 1420.1 | 1410-1420 | C=C-C Aromatic Ring | Alkene |
| 1371.7 | 1370-1380 | Stretch | Alkane |
| 1248.7 | 1260-1350 | C-H Vinyl in plane bend | Alcohol |
| 1174.1 | 1125-1205 | Methyl C-H symmetric | Alcohol |
| 1032.5 | 1020-1075 | Bend | Ether |
| 864.7 | 850-995 | Secondary OH in-plane | Aromatic phosphate |
| 808.8 | 750-850 | Bend | Alkyl Halide |
| 771.0 | 750-850 | C-O stretch | Alkyl Halide |
| | | =C-O-C Symmetric Stretch | |
| | | P-O-C Stretch | |
| | | C-Cl Stretch | |
| | | C-Cl Stretch | |

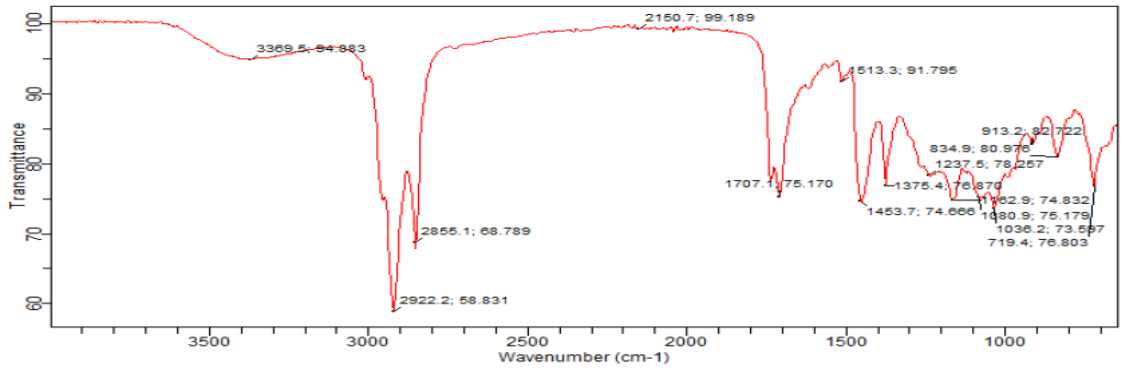


Figure 2: IR spectrum of n-hexane extracts of *Ocimum basilicum* leaves.

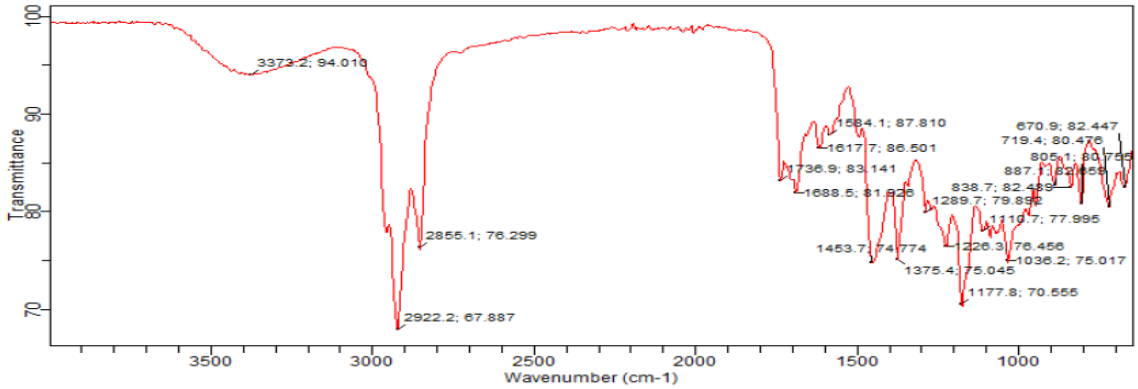


Figure 3: IR spectrum of n-hexane extracts of *Ocimum gratissimum* leaves.

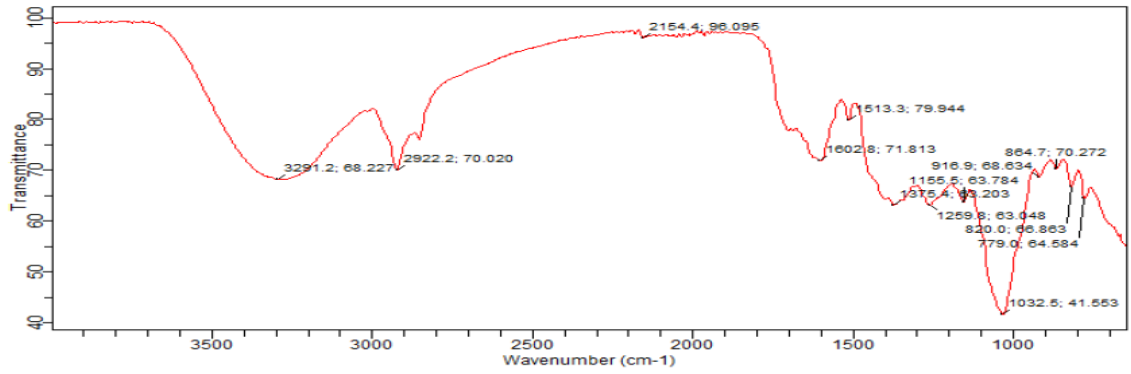


Figure 4: IR spectrum of methanol extracts of *Ocimum basilicum* leaves.

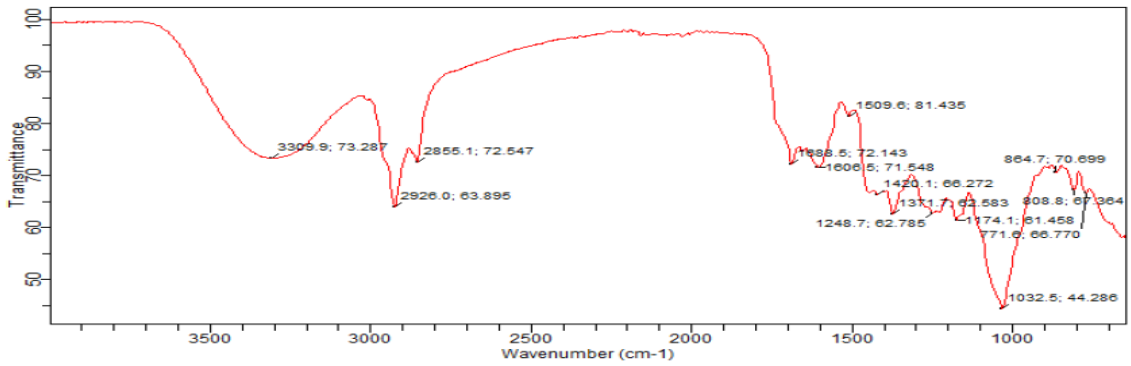


Figure 5: IR spectrum of methanol extracts of *Ocimum gratissimum* leaves.

The IR spectrum of the n-hexane extract of *Ocimum basilicum* leaves shows the presence of one carboxylic acid, four alkanes, one alkyne, one aldehyde, one aromatic, two amines, one alkyl halide, two ethers and two alkenesas shown in Table 1 and Figure 2. The spectrum of n-hexane extracts of *Ocimum gratissimum* leaves shows the presence of one carboxylic acid four alkane, three ester, one amine, one aldehyde, two aromatic, two ether, one alkyl halide and four alkene functional groups as shown in Table 2 and Figure 3. The methanol extracts of *Ocimum basilicum* leaves shows the presence of two alcohols, two alkane, one alkyne, four aromatic, one ether, one aromatic phosphate and one alkyl halide as shown in Table 3 and Figure 4, while the methanol extracts of *Ocimum gratissimum* leaves shows the presence of three alcohol, three alkane, one aldehyde, two aromatics, one alkene, one ether, one aromatic phosphate, and two alkyl halide functional groups as shown in Table 4 and Figure 5.

The n-hexane extracts of both leaves of *Ocimum basilicum* and *Ocimum gratissimum* plants show similarity of the interpreted IR absorbance's functional groups except ester functional group in *Ocimum basilicum* leaves and alkyne in *Ocimum gratissimum* leaves as shown in Tables 1 and 2, and Figures 2 and 3. Also the methanol extracts leaves of *Ocimum basilicum* and *Ocimum gratissimum* plants show similarity of the interpreted functional groups except aldehyde and alkene in *Ocimum basilicum* leaves and alkyne in *Ocimum gratissimum* leaves as shown in Tables 3 and 4, and Figures 4 and 5. It was reported that both n-hexane and methanol extracts of *Ocimum basilicum* and *Ocimum gratissimum* leaves had good antioxidant properties [13].

## CONCLUSION

The anti-nutritional contents and IR spectra identification of *Ocimum basilicum* and *Ocimum gratissimum* leaves were investigated. The anti-nutritional factors showed that *O. gratissimum* leaves had high content of alkaloid and oxalate. *O. basilicum* leaves had high content of tannin and saponin. Both leaves had the same content of phytate. Also all the contents of the anti-nutritional factors analyzed were low which means that both leaves may not have negative effect on human health when they are consumed. The IR spectra were determined using n-hexane and methanol leaf extracts of *O. basilicum* and *O. gratissimum* plants. The n-hexane extracts of both leaves showed presence of carboxylic acid, alkane, aldehyde, amine, aromatics, ether, alkyl halide and alkene groups except alkyne group in *O. basilicum* and ester group in *O. gratissium* leaves. The methanol extracts of *O. basilicum* and *O. gratissimum* leaves show presences of alcohol, alkane, aromatics, ether, aromatic phosphate and alkyl halide groups except alkyne group in *O. basilicum* and aldehyde and alkene in *O. gratissumum* leaves.

*Ocimum basilicum* and *Ocimum gratissimum* leaves showed a low content of anti-nutritional factors analyzed.Therefore the leaves will have no negative effects on consumption by human and animals, and hence it will be good to add the plant leaves in our daily diet.

**Acknowledgement**

I wish to thank the lab technicians, Mal Hamza, Muti-user Lab, Mr Alhamdu, Mr Oche and Colic of Chemistry Department, Ahmadu Bello University, Zaria, for their support, encouragement and assistance in carrying out the analysis.

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