OPEN ACCESS

Scholars International Journal of Chemistry and Material Sciences

Abbreviated Key Title: Sch Int J Chem Mater Sci
ISSN 2616-8669 (Print) |ISSN 2617-6556 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://saudijournals.com

Original Research Article

Extraction, Compositional Analysis and Therapeutic Studies of Essential Oils Obtained From The Leaves of *Ocimum gratissimum And Leucas martinicensis.*

Uba B[1,2*], Umar M[1], Aminu HT[1]

[1]Department of Chemistry Yobe State University Damaturu-Nigeria
[2]Desert Reseach Monitoring and Control Center Yobe State University Damaturu-Nigeria

DOI: 10.36348/sijcms.2021.v04i11.001 | **Received:** 26.07.2021 | **Accepted:** 30.08.2021 | **Published:** 07.12.2021

***Corresponding author:** Uba B

Abstract

The composition and therapeutic values of the essential oils obtained from the leaves of *O. gratissimum* and *L. martinicensis* was investigated. The GC-MS results reveals the presence of bioactive compound such as eucalyptol (10.99%), estragole (48.52%) and linalool (24.49%) in *O. gratissimum* while for the *Leucas martinicensis* its reveals the presence of diazoprogesterone (0.15%), Isoaromadendrene epoxide (0.10%) and beta.-copaene (4.37%). Oxalic acid, oleic acid, p-Menth-8(10)-en-9-ol, and Caryophyllene was detected in the essential oils of both plants. The IR-spectroscopy results confirmed the functional group of most of the compound identified. Test for tannins, flavonoids, steroids, cardiac glycoside, alkaloids terpenoid, and anthraquinone were positive in methanolic and aqueous extracts of both plants. The in vitro antimicrobial screening of the essential oils against *S. aureous, E.coli, S. pneumonia, K. pneumonia, A. niger and C. albicans* showed that they are potential antimicrobial agents. The DPPH scavenging activity of essential oils showed that *L. martinicensis* with an IC_{50} of (145.7ug/ml) have higher antioxidant activity than essential oil obtained from the leaves of *O. gratissimum* with the IC_{50} of (158.3ug/ml) but their activities is lower than the standard with IC_{50} of (134.3ug/ml) and (145.7ug/ml) respectively.

Keywords: *Oil, Ocimum Gratissimum, Leucas Martinicensis*, Antioxidant, Antimicrobial.

INTRODUCTION

Medicinal plants has impacted positively on human in many aspects such as the digestive system, nervous system, respiratory system, immune system, circulation, muscles and joints *(*Ogwuche, and Edema, 2020**)**. The potentials of medicinal plants are due to the presence of phytochemicals; among which are the essential oils. Essential oils have been reported to be of great benefit to human and animals as its help protect their body from the onslaught of pathogens (Ogwuche, and Edema, 2020**)** Essential oil is a natural product extracted from aromatic plants and are mostly presence in the roots, stems, leaves, flowers, or fruits . While few are obtained from animal sources e.g. musk and civet oils. Micro -organisms like mosses, liver wart, sea weed and fungi also produce essential oils (Bergougnoux *et al*., 2007).

Free radicals was found to be a product of normal metabolism and it is well known that Radicals cause molecular transformations and gene mutations in many types of organisms while Oxidative stress is major cause of many diseases (Storz and Imlay, 1999). Antioxidants are considered as possible protection agents reducing oxidative damage of human body. Therefore, many researchers have become more interested in natural sources which could provide active components to prevent or reduce its impact on cell (Ulubelen *et al*., 1995). Many herbal and plant infusions frequently used in domestic medicine have antioxidative and pharmacological properties connected with the presence of phenolic compounds (Prabhu *et al.*, 2009, Cody *et al.*, 1986). A direct relationship has been found between the phenolic content and antioxidant capacity of plants (Javanmardi *et al.*, 2003).

Ocimum gratissimum has been used extensively in the traditional system of medicine in many countries. In the North east of Brazil, it is used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion (Rabelo *et al.*, 2003). In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea (Effraim *et al.*, 2003). In the Savannah areas decoctions of the leaves are used to treat mental illness

(Akinmoladun *et al.,* 2007). *O. gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh (Ijeh *et al.,* 2005). Brazilian tropical forest inhabitants use a decoction of *O. gratissimum* roots as a sedative for children (Cristiana *et al.,* 2006)**.** People of Kenyan and sub Saharan African communities' use thiplant for various purposes like viz., the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, they are also used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum (Matasyoh *et al.,* 2007) . In India, the whole plant has been used for the treatment of sunstroke, headache, and influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity (Taˆnia *et al.,* 2006, Prajapati *et al.,* 2003). The tribals of Nigeria use the leaf extract in treatment of diarrhoea, while the cold leaf infusions are used for the relief of stomach upset and haemorrhoids (Kabir *et al.,* 2005).

Leucas martinicensis (Jacq.) R. Br. belongs to family Lamiaceae and commonly called Whitewort which grows widely in the Northern part of Nigeria. It has been used traditionally to treat inflammatory conditions, rashes, diarrhoea, epilepsy and convulsions. Different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Timothy et *al.,* 2016). The present study was to investigate the composition and therapeutic value of the essential oil obtained from the leaves of *Ocimum gratissimum and Leucas martinicensis*

MATERIAL AND METHOD

All chemicals and reagents were of analytical grade purchased from sigma Aldrich and used without further purification. The four (4) bacterial species (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae)*, as well as the fungus species (*Aspergillus niger*, *Candida albicans*) was obtained from Yobe state university teaching hospital and Identified at the Microbiology Department of the Yobe State University.

Extraction of essential oils

500g of the pulverized from each of the fresh samples was subjected to steam distillation in a modified steam distiller (as modified by Runde *et al*., 2015) according the British pharmacopoeia (BP) method. The time taken for the isolation of the oil was 4 hours (Kubmarawa *et al*., 2013).

Phytochemical screening

Phytochemicals screening of all the evaporated solvent extracts and Test for saponins, tannins, flavonoids, cardiac glycoside, alkaloids, phenols and anthraquinone were carried out in all the fraction in accordance with the standard procedure as described by (Harborne, 1973).

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis was performed on a J and W Scientific gas chromatography directly coupled to the mass spectrometer system (model GC Agilent technologies 7890A, Agilent technologies MSD 5975C), 5 % phenyl methyl silox: 469.56 509 packed capillary column (30M x 250µm) was used under the following conditions: ovum temperature 50 ^{0}C for 1 min, then raised within intervals of 10 ^{0}C/min to 200 ^{0}C for 1min, and 20 ^{0}C/min to 300 ^{0}C for 2 min. Injector temperature was 230 ^{0}C while the carrier gas was Helium flowing at the rate of 1ml/min, the volume of the injected sample was 0.2µL of diluted oil in hexane, splitless injection techniques was used and the ionization energy was 70ev in the electron ionization (EI) mode. Ion source temperature was 230 ^{0}C while the scan mass range of M/Z 60-335 was used. The constituents of the essential oils was identified based on comparison of the retention indices and mass spectra of most of the compounds with data generated under identical experimental conditions by applying a two dimensional search algorithm considering the retention index as well as mass spectral similar with those of authentic compounds available in NBS75K andNIST08 Libraries. The retention indices (RI) are in relation to a homologous series of nalkanes on the GC column under the same chromatographic condition. Relative concentration was obtained by peak area normalization as describe by (Ramzi *et al*., 2013).

ANTIMICROBIAL ACTIVITIES

The sterilized was autoclaved at 121^{o}C for 15 min medium was inoculated with the suspension of ($5x10^{-5}$ cfu/ml) of the microorganism (matched to McFarland turbidity standard) and poured into a petridish to give a depth of 3–4 mm. Three wells was made containing different concentrations of (100, 200 and 300 ppm in methanol) of both essential oils. The plates were pre-incubated for 1 hr at room temperature and incubated at 37^{o}C for 24h and 48hrs for antibacterial and antifungal activities respectively. ciproxoflaxacin (500mg) and ketoconazole (200mg) was used as standard. After the incubation period, the plates were observed for zones of inhibition in (mm). The procedure was carried out in triplicated to minimized error (Uba *et al*., 2020)

DPPH radical scavenging activity

The ability of the extract to scavenge DPPH radical was determined according to the method described by Ramzi *et al*. (2013) with little modification. The essential oil was dissolved in methanol, and various concentrations (5, 10, 25, 50, 100, 200, and 400 µg/mL) were used. The assay mixture contained in a total volume of 1 mL, 500 µL of the oil, 125 µL prepared DPPH (1 mM in methanol), and 375 µL solvent (methanol). After 30 min incubation

at 25 °C, the decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was calculated from the equation:

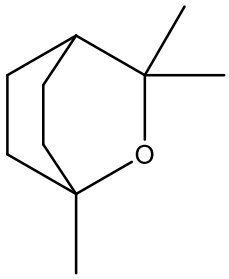
% of radical scavenging activity = Abscontrol – Abssample/Abscontrol × 100

The sample concentration providing 50 % inhibition (IC50) was calculated by plotting inhibition Percentages against concentrations of the sample. .

RESULT AND DISCUSSION

Table-1: GC-MS result of essential oil obtained from leaf of *Ocimum gratissimum*

S/N	Compound	Molecular formula	Molecular weight	Retention time (min)	Area (%)
1	Eucalyptol	$C_{10}H_{18}O$	154.249	3.453	10.99
2	3,7-dimethyl-1,6-Octadien-3-ol, linalool	$C_8H_{18}O$	182.259	5.942	24.49
3	Estragole	$C_{10}H_{12}O$	148.20	8.574	48.52
4	Anethole	$C_{10}H_{12}O$	148.205	11.206	0.58
5	1(2H)-Naphthalenone	$C_{10}H_8O$	144.17	11.630	0.21
6	Bicyclo[3.1.1]hept-2-ene	C_7H_{10}	94.15	11.853	1.91
7	Caryophyllene	$C_{15}H_{24}$	204.357	12.843	0.44
8	gamma.-Muurolene	$C_{15}H_{24}$	204.351	13.060	0.73`
9	4-(1,1-dimethylethyl)-Phenol	$C_{20}H_{26}O$	282.4	17.266	0.01
10	Dodecanoic acid	$C_{12}H_{24}O_2$	200.322	17.804	0.01
11	Benzothiazole	C_7H_5NS	135.186	18.313	0.00
12	7H-Purin-6-amine	C_6H_7N	149.153	18.439	0.00
13	Heptadecanoic acid	$C_{17}H_{24}O_2$	270.45	19.721	0.05
14	2-Piperidinone	C_5H_9NO	99.133	22.050	0.04
15	Oleic Acid	$C_{18}H_{34}O_2$	282.47	23.228	0.04
16	isobutyl tetradecyl ester	$C_{20}H_{54}O_4$	454.726	23.703	0.03
17	Oxalic acid	$C_2H_2O_4$	90.03	24.035	0.03
18	Aspidospermidin-17-ol	$C_{23}H_{30}N_2O_5$	414.5	25.065	0.01
19	p-Menth-8(10)-en-9-ol	$C_{10}H_{18}O$	154.25	26.358	0.01



(Eucalyptol)

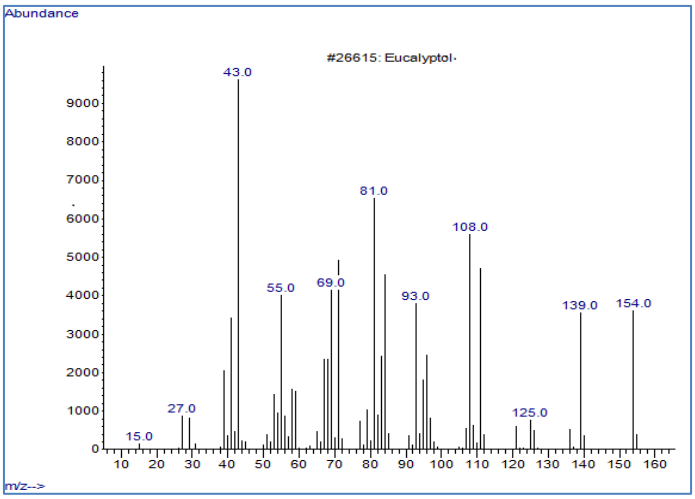
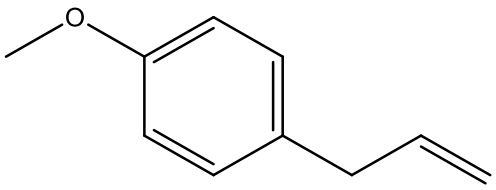


Fig-1: Mass spectra of Eucalyptol



(Estragole)

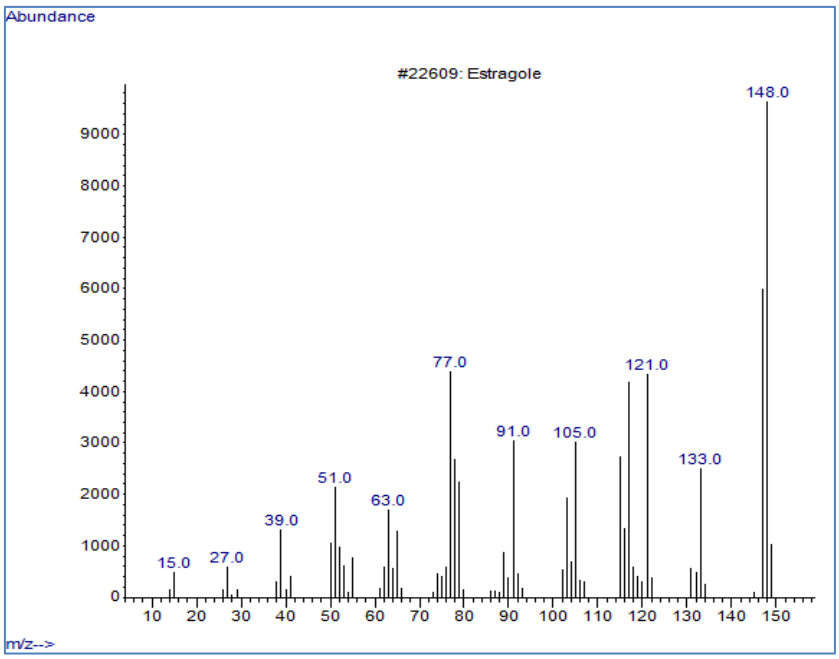
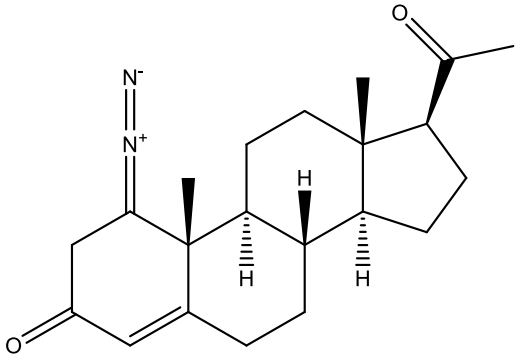


Fig-2: Mass spectra of Estragole

Table-2: GC-MS result of essential oil obtained from the leaf of *Leucas martinicensis*

S/N	Compound	Molecular formula	Molecular weight	Retention time (min)	Area (%)
1	3-methyl-Heptane	C_8H_{18}	114.2	6.846	17.0
2	1-Bromo-3,5,5-trimethylhexane	$C_9H_{19}Br$	207.151	9.244	9.58
4	Caryophyllene	$C_{15}H_{24}$	204.357	11.624	6.75
5	beta.-copaene	$C_{15}H_{24}$	204.36	12.568	4.37
6	2-Buten-1-ol, 3-methyl-, acetate	C_4H_8O	72.02	13.672	1.24
7	1,6-Cyclodecadiene	$C_{10}H_{16}$	136.1	14.863	0.77
8	Bicyclo[6.1.0]nonane	C_9H_{16}	124.22	14.863	0.77
9	octahydro-1,9,9-trimethyl-4-methylene	$C_{15}H_{24}$	24.3510	15.109	0.39
10	Tricyclo[6.3.3.0]tetradec-4-ene,10, 13-dioxo	$C_{14}H_{18}O_2$	218.29	15.469	0.94
11	1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	353	16.390	0.25
12	Diazoprogesterone	$C_{21}H_{30}N_2$	338.488	16.940	0.15
13	Hexadecanoic acid, palmitic acid	$C_{16}H_{32}O_2$	256.4	17.827	0.79
14	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220.351	18.828	0.10
15	Methyl 8,10-dimethyl-hexadecanoate	$C_{16}H_{34}O_2$	258.16	19.509	1.07
16	Oxalic acid	$C_2H_2O_4$	90.03	20.676	0.30
17	Oleic Acid	$C_{18}H_{34}O_2$	282.47	22.650	3.47
18	p-Menth-8(10)-en-9-ol	$C_{10}H_{18}O$	154.249	33.242	0.55
19	2-Piperidinone,	C_5H_9NO	99.131	22.868	1.85



(Diazoprogesterone)

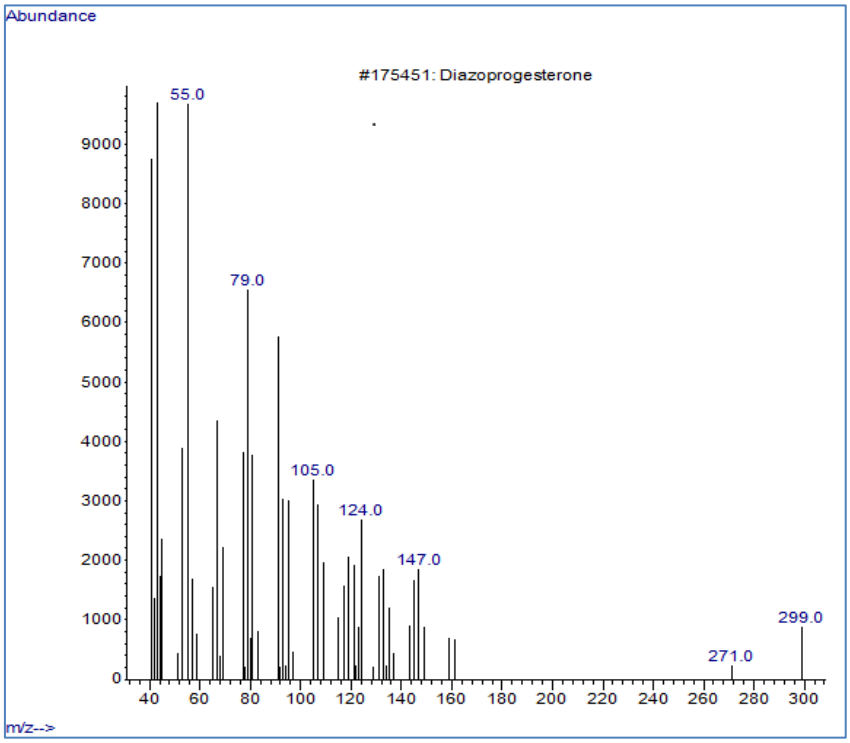
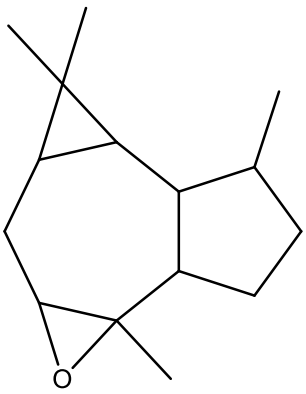


Fig-3: Mass spectra of Diazoprogesterone



(Isoaromadendrene)

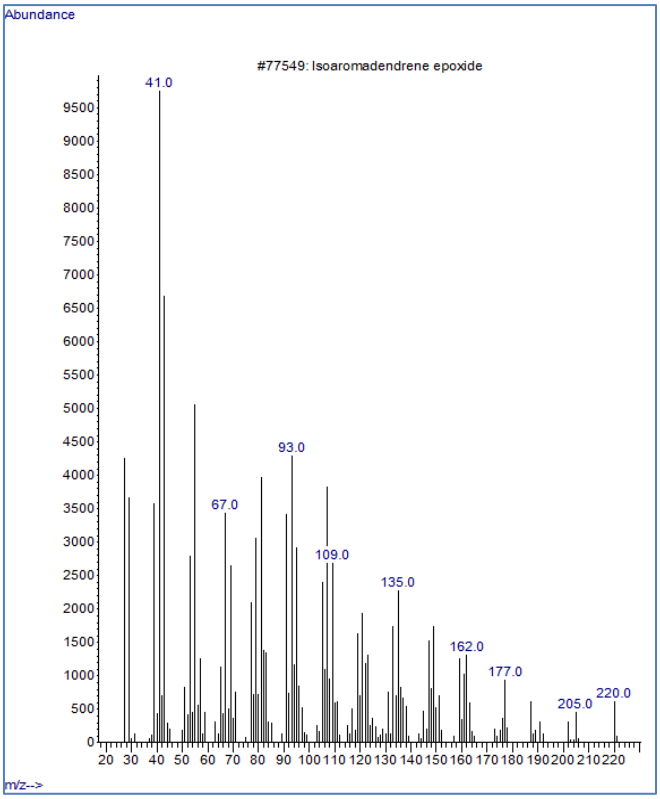


Fig-4: Mass spectra of asoaromadendrene

GCMS Result for the composition of essential oils obtained from the leaves of *O. Gratissimum* and *L. Martinicenses*

The GCMS result of essential oil obtained from the leave of *Ocimum gratissimum* reveals the presence of eucalyptol (10.99%), estragole (48.52%) and linalool (24.49%) in abundance. the presence of linalool was also reported by Joshi (2017) and for the presence of estragole and eucalyptol was also reported by pandiya (2012). while for the *Leucas martinicensis* its reveals the presence of diazoprogesterone (0.15%), Isoaromadendrene epoxide (0.10%) and beta.-copaene (4.37%) the essential oils of both leaves indicate the presence of oxalic acid, oleic acid, p-Menth-8(10)-en-9-ol, and Caryophyllene. As shown in table 1-2.

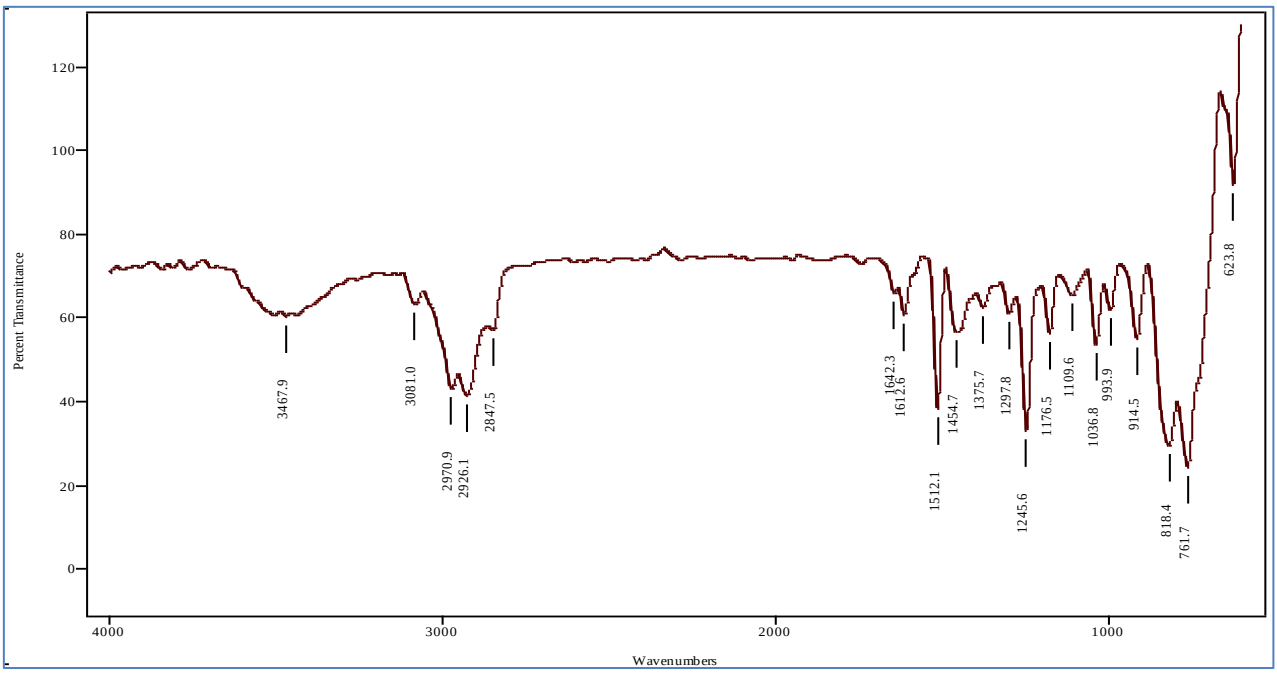


Fig-5: IR spectra of ocimum gratissimum

The IR-spectroscopy analysis carried out for *ocimum gratissimum* showed a broad absorption band at 3467.9cm^{-1} which could be assign to v(OH) vibrational frequency of carboxylic acid (pandiya, 2012). The band at 3081.0 cm^{-1} could be due to v (N-H) stretching frequency of primary amine. The appearance of band at 2970.9 cm^{-1} may be due to v(C-H) stretching frequency of alkane while 2847.0^{5}cm^{-1} assign to v(C-H) for aldehyde (Ogwuche and Edema, 2020). The vibrational frequency of 761.7 cm^{-1} could be assigned to benzene. 1512.1 cm^{-1}and 1454.7 cm^{-1} are due to v(C-C) aromatic stretching vibration while 1375.7 cm^{-1} appeared as result of v(C=C) aromatic ring stretching vibration. The appearance of absorption bands at 1176.5 cm^{-1} and 1297.8 cm^{-1} could be to phenolic v(C-O) and 2970.9 cm^{-1} methylene stretching vibration of v(C-H) respectively(Anjili *et al.*, 2021).

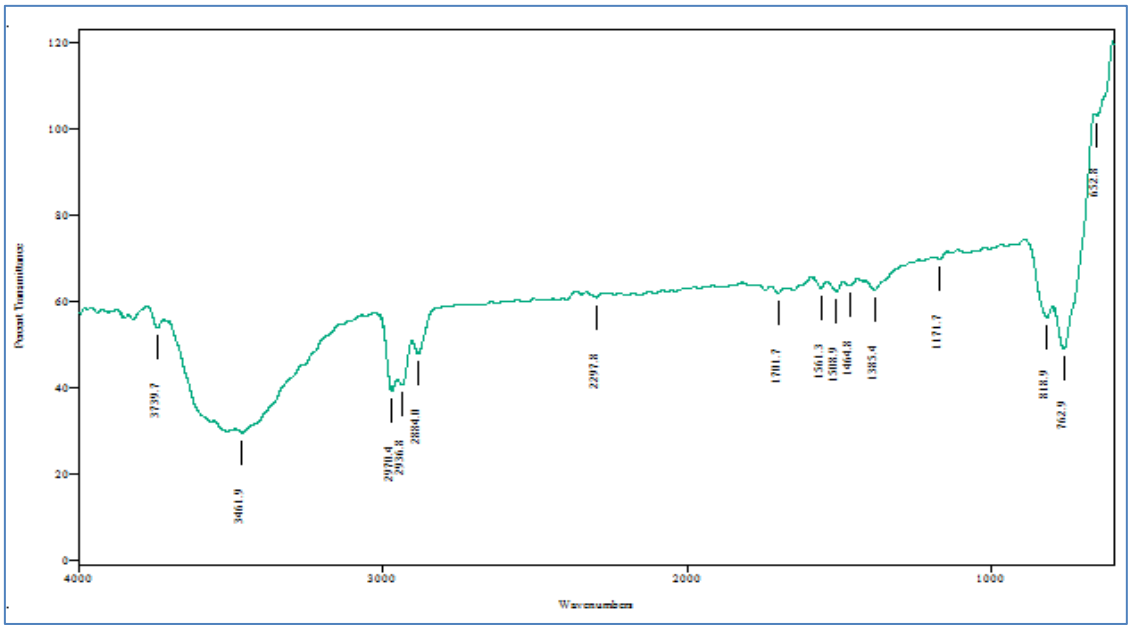


Fig-6: IR spectra of leucas martinicensis

The IR-spectroscopy analysis carried out for *leucas martinicensis* showed an absorption band at 3461.9cm^{-1} which could be assign to v(OH) vibrational frequency of carboxylic acid(Kadhim *et al*., 2016). The band at 3739.7 cm^{-1} could be due to v (N-H) stretching frequency of primary amine. The appearance of band at 2970.4 cm^{-1} may be due to v(C-H) stretching frequency of alkane while 2884.0 cm^{-1} assign to v(C-H) for aldehyde(Okereke *et al*., 2017). The vibrational frequency of 762.9 cm^{-1} could be assigned to benzene 1508.9 cm^{-1}, 1561.3 cm^{-1} and 1464.8 cm^{-1} are due to v(C-C) aromatic stretching vibration while 1385.4 cm^{-1} appeared as result of v(C=C) aromatic ring stretching vibration. The appearance of absorption bands at 1171.7 cm^{-1} and 2884.0 cm^{-1} could be to phenolic v(C-O) and methylene stretching vibration of v(C-H) respectively (Rajendran *et al*., 2020).

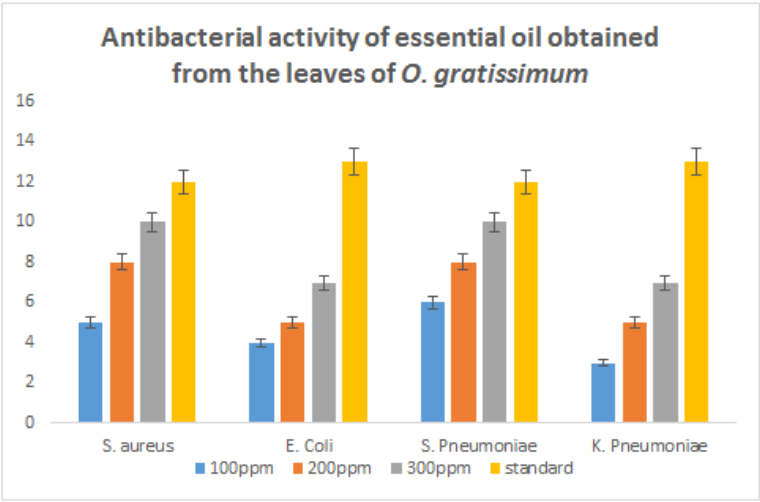


Fig-7: Antibacterial activity of essential oil obtained from O. gratissimum

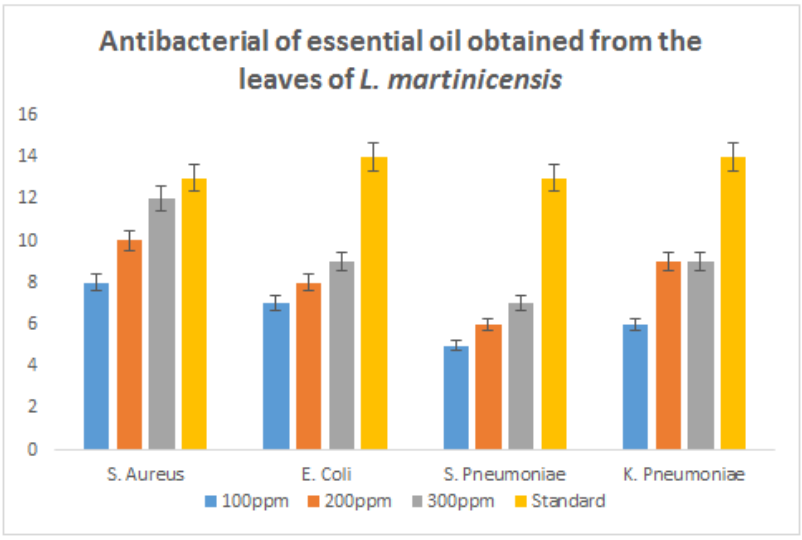


Fig-8: Antibacterial activity of essential oil obtained from leaves L.martinicensis

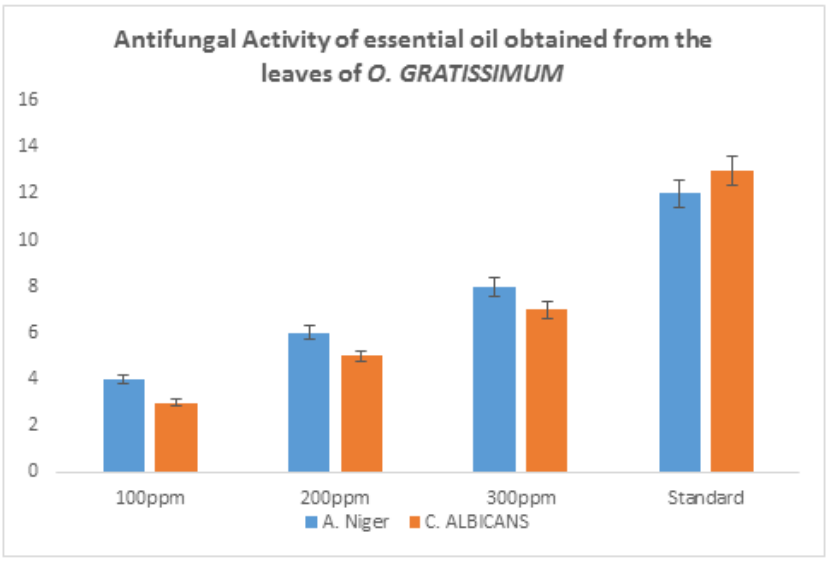


Fig-9: Antifungal activity of essential oil obtained from the leaves O. gratissimum

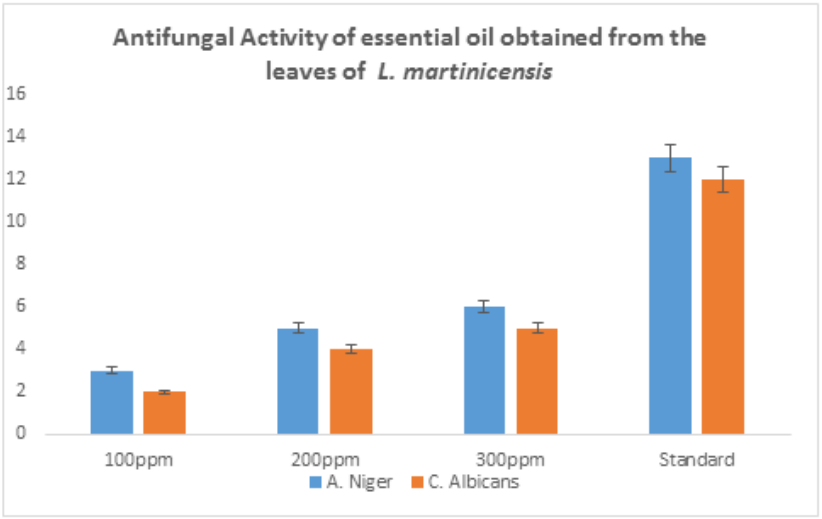


Fig-10: Antifungal activity of essential oil obtained from the leaves L.martinicensis

The antibacterial activity of the essential oils at various concentrations showed significant activities on the tested organism at all concentration but the activities increase with the increase in concentration of the essential oil (Lexa *et al.,* 2007). The essential oil obtained from the leaves of *L. Martinicensis* showed higher activity on *Staphylococcus aureus, Klibsiella pneumoniae and Escherichia Coli* with inhibitory zone ranging from 8mm-12mm, 6mm-9mm and 7mm-9mm respectively as compared with that of *O. gratissimum* (Timothy *et al*., 2017). In other hand the essential oil obtained from the leaves of *O.gratissimum* showed higher activity on *Streptococcus pneumonia* with inhibitory zone ranging from 6mm-10mm. the essential oil of both plants showed low activity as compared with the standard (streptomycin 500mg).the higher antibacterial activity of the essential oil obtained from the leaves of L. Martinicensis could be associated with the presence of monoterpenes, aromatic ring and phenol groups which are capable of forming hydrogen bond with the active sites of target enzyme cause the death of organism (molu *et al.*, 2017). Other compounds such as alcohols, aldehydes, and esters also contribute to the antibacterial effect (Belletti *et al.,* 2004). As shown in figure 7-8.

The antifungal activity of the essential of the both plant showed activity on the tested fungus but the activities differs as the essential oil of *O. gratissimum* showed higher inhibition zone of 4mm-8mm and 3mm-7mm *Aspergillus niger to Candida albicans* respectively as compared with essential oil of *L. martinicensis* with inhibition zone of *Aspergillus niger* 3mm-6mm and Candida albicans 2mm-5mm. the activity of the standard (ketoconazole 200mg) is higher than the essential oil of all plants and the activity increase with increase in concentration of the essential oil. The better activity of O.gratissimum essential oil is due to higher linalool content and it is believed that Linalool can cause protein denaturation and dehydration of vegetative cells, which causes the death of microorganisms in contact with the EO (Camargo and Vasconcelos, 2014). As shown in figure 9-10.

Table-3: Phytochemicals screening results in methanolic and aqueous leave extract of *O. gratissimum*.

Phytochemicals	Methanolic extract	Aqueous extract
Saponins	-	+
Tannins	+	+
Flavonoids/Terpenoids	+	+
Cardiac glycoside	+	+
Alkaloids	+	-
Steriod	+	+
Anthraquinone	-	+

Table-4: Phytochemicals screening results in methanolic and aqueous leave extract of *L. martinicensis*

Phytochemicals	Methanolic extract	Aqueous extract
Saponins	+	+
Tannins	+	-
Flavonoids/Terpenoids	+	+
Cardiac glycoside	+	+
Alkaloids	+	-
Steriod	+	+
Anthraquinone	-	+

The phytochemical screening results of *O. gratissimum* and *L.martinicensis* of methanolic and aqueous leaves extract reveals the prensence of tannins, flavonoids, steroids, cardiac glycoside, alkaloids and steroids a in methanolic extracts of both plant (Akiyarma *et al.,* 2001) while anthraquinone is present in methanolic leave extract of L. martinicensis and absent in *O gratissimum.* For the aqueous leaves extract it showed the presence of saponins, flavonoid, terpenoid, cardiac glycoside, anthraquinone and steroids. Tannins are absent in L.martinicensis leave while alkaloids are absent in both leaves (Akinmoladun *et al*., 2007: Musa, 2010). The present of phytochemicals may be responsible for the biological activity of both leaves (Devendran *et al., 2011)*. The presence of saponnins in the essential oil of both plant suggest the therapeutic value of the oil due to the fact that saponnin are used as antinutrients in human diet for controlling cholesterol (Asl and Hosseinzadeh, 2008). Tannins is among the organic compound that is reported to possessed remarkable antiviral, antibacterial and antiparacetic activities hence it is responsible for therapeutic value of the investigated essential oils (Akiyarma *et al.,* 2001). As shown in table 3-4.

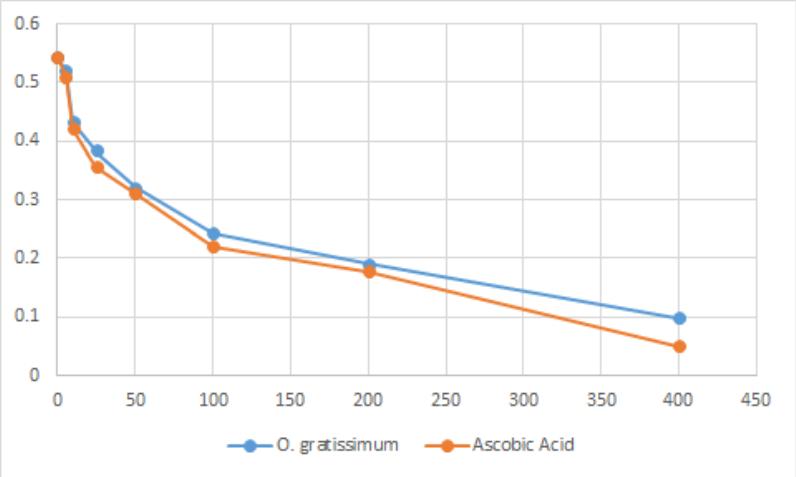


Fig-11: Graph of absorbance against concentration of *O.gratissimum* essential oil and ascobic acid

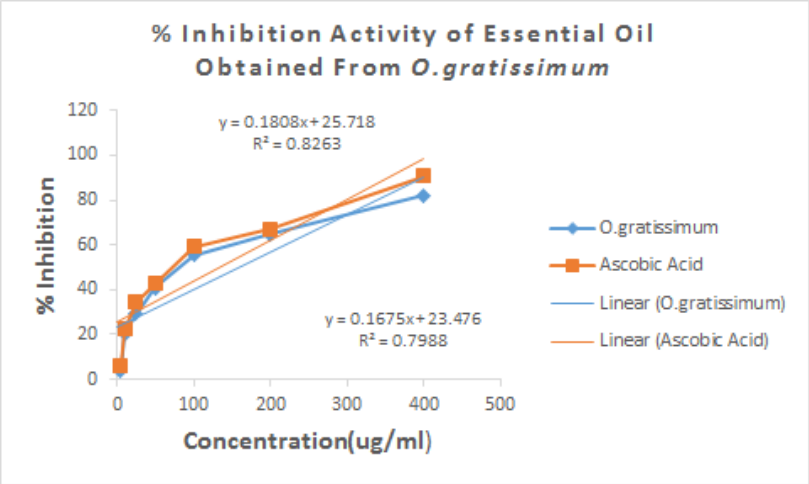


Fig-12: % Inhibition Activity of Essential Oil obtained from the leaves of *O.gratissimum*

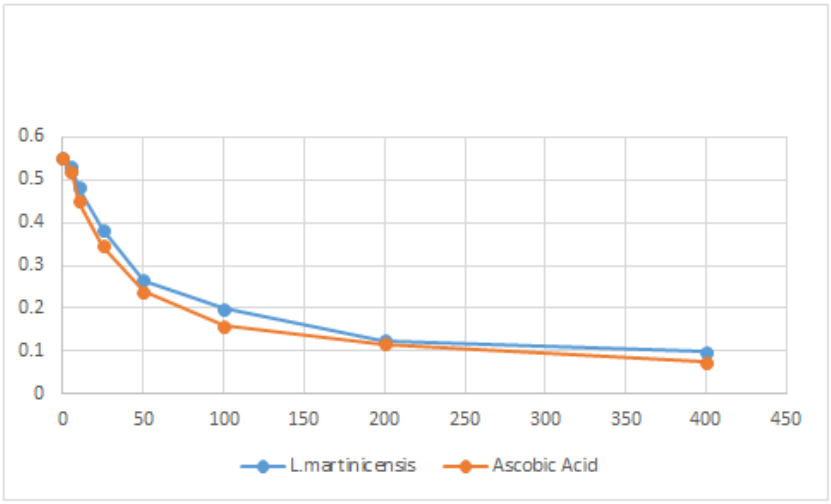


Fig-13: Graph of absorbance against concentration of *L.martinicensis* essential oil and ascobic acid

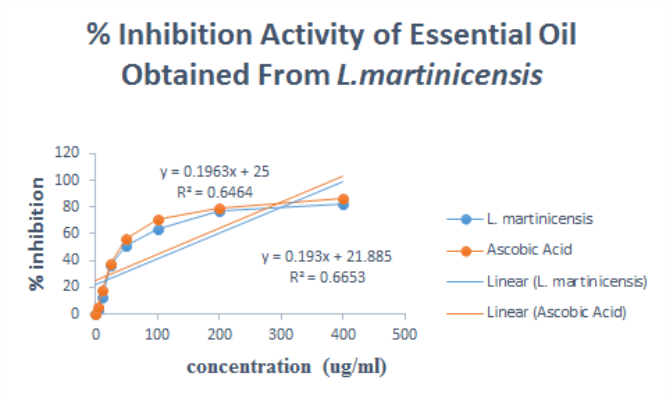


Fig-14: % Inhibition Activity of Essential Oil obtained from the leaves of *L.martinicensis*

The result of the DPPH scavenging activity of essential oils is shown in Figure 11-14. Both showed a dose dependent antioxidant activity. The *O. gratissimum* activity of was remarkably higher than that of *L. martinicensis* at lower concentrations but at higher concentration *L. martinicensis* showed higher activity as compared with that of *O.gratissimum*. The IC_{50} of *L. martinicensis* were (145.7ug/ml) while that of *O. gratissimum* were (158.3ug/ml) which indicate that *L. martinicensis* has higher antioxidant activity than essential oil obtained from the leaves of *O.gratissimum* but their activity is lower than the standard with IC_{50} of (134.3ug/ml) and (145.7ug/ml) in *O. gratissimum and L. martinicensis* respectively. The higher antioxidant activity of *L.martinicensis* could be attributed to the presence phenolic compound and their hydrogen ability (Ciz *et al.*, 2010).

CONCLUSION

The results of these findings reveals that essential oils obtained from the leaves of *Ocimum gratissimum and L. martinicensis* contain bioactive compound that have antimicrobial and antioxidants activity which justify the use of this oils as a therapeutic agents.

ACKNOWLEDGEMENTS

The authors acknowledge Tertiary Education Trust Fund (TET-FUND) Abuja-Nigeria and Management of Yobe State University Damaturu Nigeria for sponsoring the research. We also appreciated the contributions of Chemistry Department.

REFERENCES

- Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M., & Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. *Scientific Research and Essays*, *2*(5), 163-166.
- Adebolu, T.T., Salau, A.O. (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. *Afr J Biotech*, *4*; 682-4.
- Anjili Ezekiel, M., Uba Sani, Anjili Jeremia., & Kizito, I. G. (2021). Determination of Anti-nutritional Contents and IR Spectra of *Ocimum basilicum* and *Ocimum gratissimum* Plant, *Nigerian Research Journal of Chemical Sciences* (ISSN: 2682-6054) Volume 9, Issue 1.
- Asl, M. N., & Hosseinzadeh, H. (2008). Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *22*(6), 709-724.
- Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., & Iwatsuki, K. (2001). Antibacterial action of several tannins against Staphylococcus aureus. *Journal of antimicrobial chemotherapy*, *48*(4), 487-491.
- Belletti, N., Ndagijimana, M., Sisto, C., Guerzoni, M. E., Lanciotti, R., & Gardini, F. (2004). Evaluation of the antimicrobial activity of citrus essences on Saccharomyces cerevisiae. *Journal of Agricultural and Food Chemistry*, *52*(23), 6932-6938.
- Bergougnoux, V., Caissard, J. C., Jullien, F., Magnard, J. L., Scalliet, G., Cock, J. M., ... & Baudino, S. (2007). Both the adaxial and abaxial epidermal layers of the rose petal emit volatile scent compounds. *Planta*, *226*(4), 853-866.
- Harborne, J. B. (1986). Nature, distribution and function of plant flavonoids. *Plant Flavonoids in Biology and Medicine, Buffalo, New York (USA), 22-26 Jul 1985*.
- Freire, C. M. M., Marques, M. O. M., & Costa, M. (2006). Effects of seasonal variation on the central nervous system activity of Ocimum gratissimum L. essential oil. *Journal of ethnopharmacology*, *105*(1-2), 161-166.
- Camargo, S. B., & de Vasconcelos, D. F. S. A. (2014). Atividades biológicas de Linalol: conceitos atuais e possibilidades futuras deste monoterpeno. *Revista de Ciências Médicas e Biológicas*, *13*(3), 381-387.
- Číž, M., Čížová, H., Denev, P., Kratchanova, M., Slavov, A., & Lojek, A. (2010). Different methods for control and comparison of the antioxidant properties of vegetables. *Food Control*, *21*(4), 518-523.
- Devendran, G., & Balasubramanian, U. (2011). Qualitative phytochemical screening and GC-MS

analysis of Ocimum sanctum L. leaves. *Asian J Plant Sci Res*, *1*(4), 44-48.

- Effraim KD, Jacks TW, Sodipo OA. Histopathological studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of rabbit. Afr J Biomed Res 2003; 6: 21-5.
- Harborne, J.B. (1973). Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapman and Hall, London.
- Ijeh, II., Omodamiro, O.D., Nwanna, I.J. (2005). Antimicrobial effects of aqueous and ethanolic fractions of two spices, *Ocimum gratissimum* and *Xylopia aethiopica*. Afr J Biotech; 4:
- Javanmardi, J., Stushnoff, C., Locke, E., & Vivanco, J.M. (2003)**.** Antioxidant activity and total phenolic Content of Iranian *Ocimum accessions*. Food Chem. 83: 547-550.
- Joshi, R. K. (2017). GC—MS analysis of the essential oil of Ocimum gratissimum L. growing desolately in South India. *Acta Chromatographica*, *29*(1), 111-119.
- Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C., & Fasure, K. A. (2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. *BMC complementary and alternative medicine*, *5*(1), 1-7.
- Kubmarawa, D., Ajoku, G. A., Enwerem, N. M., & Okorie, D. A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology*, *6*(14).
- Matasyoh, L. G., Matasyoh, J. C., Wachira, F. N., Kinyua, M. G., Muigai, A. W. T., & Mukiama, T. K. (2007). Chemical composition and antimicrobial activity of the essential oil of Ocimum gratissimum L. growing in Eastern Kenya. *African journal of biotechnology*, *6*(6).
- Matasyoh, L. G., Matasyoh, J. C., Wachira, F. N., Kinyua, M. G., Muigai, A. W. T., & Mukiama, T. K. (2007). Chemical composition and antimicrobial activity of the essential oil of Ocimum gratissimum L. growing in Eastern Kenya. *African journal of biotechnology*, *6*(6).
- Kadhim, M. J., Sosa, A. A., & Hameed, I. H. (2016). Evaluation of anti-bacterial activity and bioactive chemical analysis of Ocimum basilicum using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. *Journal of pharmacognosy and phytotherapy*, *8*(6), 127-146.
- MUSA, T. L. (2017). Pharmacognostic and antiulcer studies on the leaf of leucas martinicensis (JACQ) R. Br.(LAMIACEAE).
- Mohr, F. B. M., Lermen, C., Gazim, Z. C., Gonçalves, J. E., & Alberton, O. (2017). Antifungal activity, yield, and composition of Ocimum gratissimum essential oil. *Genet. Mol. Res*, *16*(1), 1-10.
- Ogwuche, C. E., & Edema, M. O. (2020). GC-MS and FTIR Characterization of essential oil from the fresh leaves of Pandanus candalabrum obtained from Bayelsa state, Nigeria. *Nigerian Journal of Chemical Research*, *25*(1), 1-10.
- Okereke, S. C., Arunsi, U. O., & Nosiri, C. I. (2017). GC-MS/FT-IR screening of Xylopia aethiopica (Dunal) A. rich fruit. *African Journal of Biochemistry Research*, *11*(3), 12-17.
- Pandiyan, A. (2012). *Chemical Profiling and Biological Activity on Leaves of Ocimum Gratissimum Grown in Tamilnadu* (Doctoral dissertation, Adhiparasakthi College of Pharmacy, Melmaruvathur).
- Prabhu, K. S., Lobo, R., Shirwaikar, A. A., & Shirwaikar, A. (2009). Ocimum gratissimum: A review of its chemical, pharmacological and ethnomedicinal properties. *The Open Complementary Medicine Journal*, *1*(1).
- Narayan, D. P., & Kumar, U. (2003). Agro's dictionary of medicinal plants. *Agrobios India*.
- Rabelo, M., Souza, E. P., Soares, P. M. G., Miranda, A. V., Matos, F. J. A., & Criddle, D. N. (2003). Antinociceptive properties of the essential oil of Ocimum gratissimum L.(Labiatae) in mice. *Brazilian Journal of Medical and Biological Research*, *36*, 521-524.
- Ramzi, A.M., Mansour, S.A., Mohammed, A.A., Adnan, J.A., & Jamal, M.K. (2013). GC and GC/MS Analysis of Essential Oil Composition of the Endemic Soqotraen *Leucas virgata Balf.* and Its Antimicrobial and Antioxidant Activities, *Int. J. of Mol. Sci.*, *14***(**11**)**, 23129-23139.
- Runde, M. (2005). Extraction, compositional analysis and therapeutic studies of essential oils rom eighteen medicinal plant (PhD thesis) Department of chemistry, Modibbo Adama University of technology yola
- Priya, R. R., Bhaduhsha, N., Manivannan, V., & Gunasekaran, T. (2020). Extraction and isolation of bioactive compounds from a therapeutic medicinal plant–sennaalata (L.) ROXB. *PalArch's Journal of Archaeology of Egypt/Egyptology*, *17*(12), 1569-1577.
- Storz, G., & Imlay, J.A. (1999). Oxidative stress. Current Opinion in Microbiology, 2: 188-194.
- Ueda-Nakamura, T., Mendonça-Filho, R. R., Morgado-Díaz, J. A., Maza, P. K., Dias Filho, B. P., Cortez, D. A. G., ... & Nakamura, C. V. (2006). Antileishmanial activity of Eugenol-rich essential oil from Ocimum gratissimum. *Parasitology international*, *55*(2), 99-105.
- Timothy, S. Y., Sugun, M. Y., Tata, F. Y., Milagawanda, H. H., & Ibrahim, A. W. (2016). Antibacterial and antiepileptic activity of ethanol extract of whole plant of Leucas martinicensis (Jacq.) R. *Int. J. Pharm. Pharm. Res*, *6*(3).
- Uba. B., Muhammad, C., Uba, A., Amali, A.M. (2020). Synthesis, characterization antibacterial and antifungal activities of Schiff base complexes of Ni (II) and Cu (II)
- Ulubelen, A., Topcu, G. Ü. L. A. Ç. T. I., Tan, N., Ölçal, S., Johansson, C., Üçer, M., ... & Tamer, Ş. (1995). Biological activities of a Turkish medicinal plant, Prangos platychlaena. *Journal of ethnopharmacology*, *45*(3), 193-197.