



**Phytochemical Screening, Thin-Layer Chromatography and Free Radical Scavenging Activity of *Ocimum gratissimum* Leaves (*Lamiaceae*)**

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**Abstract**

*Ocimum gratissimum* (family: *Lamiaceae*) is an aromatic plant that is used in traditional medicine for the treatment of many disorders such as: headache, dysentery infections, diarrhea, skin diseases, mental, pneumonia and epilepsy. In my search of pharmacologically active compounds, I carried out phytochemical screening on three different extracts: hexane, dichloromethane and methanol respectively using standard procedure. There was found to contain alkaloids, saponins, flavonoids, steroids, glycosides, carbohydrates (in methanolic and DCM extract); steroids, glycosides and carbohydrates (in hexane extract) and protein in methanolic extract. The thin-layer chromatography (TLC) studies showed the presence of various phytochemicals in the extracts. All the extracts (methanol, DCM, hexane) of *Ocimum gratissimum* leaves possessed antioxidant and free radical scavenging activities using DPPH assay.

**Key words:** *Ocimum gratissimum*,; Secondary metabolite; DPPH Free radical; Antioxidant; Thin layer chromatography.

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**1.0 INTRODUCTION**

Traditional medicine mostly from plant source have been used by the early man to cure and prevent diseases. *Ocimum gratissimum* is one of the numerous plant used in traditional medicine in many part of the world including Africa

specifically Nigeria and Kenya. It is an aromatic perennial herb belonging to the family *Lamiaceae*. The Hausa people in the Northern part of Nigeria call it Daddoya, other names of the plant include: Efinrin (Yoruba), African Basil (English), Vana tulsi (India) etc. The decoction of

the leave is used for treatment of mental illness and gonorrhoeal infection in Congo (Abdurrahman, 1992). The leaves are also used as laxative, relief for headache, fever, diarrhea, dysentery and convulsion (Adebolu and Oladimeji 2005; Akinjogunla *et al.*, 2011). In India it is used in the treatment of Urinary stones diseases, nephrotoxicity and other related problems (Varma and Argawal, 2014; Efiog and Ebong, 2014). Other studies on the plant shows that it can be used in the treatment of upper respiratory tract infections, ophthalmic, skin diseases, cough and conjunctivitis (Adebolu and Oladimeji, 2005). It is also used in the treatment of epilepsy (Agbogidi *et al.*, 2014). Qualitative phytochemical analysis of methanol and ethanol extracts of *Ocimum gratissimum* leaves revealed the presence of alkaloids, tannins, flavonoids, carbohydrates and terpenoids (Gupta *et al.*, 2011). Petroleum ether and chloroform extracts of the leaves shows the presence of alkaloids, flavonoids and terpenoids (Gupta *et al.*, 2011). Koche *et al* (2012) studied the phytochemical screening of the leave and revealed the presence of antimicrobial active agents such phenolics, glycosides, tannins, steroids and alkaloids. Analysis on the leaves revealed that they are rich in essential oils especially thymol others include saponins, steroids, camphor, estrajol and citral (Uhegbu, *et al.*, 2012). Antibacterial studies of aqueous and ethanol extracts were found to

## 2.0 MATERIALS AND METHODS

### 2.1 Collection and Identification of the plant

The leaves (together with flowers) of *Ocimum gratissimum* were collected in December, 2014, from Katsina local government area of Katsina state. The plant was identified as *Ocimum gratissimum* by a taxonomist in the Department of Biological Sciences, Umaru Musa Yar'adua University Katsina.

### 2.2 Drying of sample

The collected leaves of *Ocimum gratissimum* were air dried under shade, and grounded into powder using pestle and mortar. The powder stored into a rubber container until it is required for use.

exhibit selective inhibition against *E. coli* and *S. aureus* isolates at  $2\pm 0.01$  and  $10\pm 0.10$  zones of inhibition respectively (Obinna *et al.*, 2009). Opara *et al* (2012) studied the effect of aqueous extract of the leaves on adult rabbits and suggested that the extract can be used up to 40% level to increase their immunity and serum protein level. Uhegbu *et al* (2012) studied the hepato protective activity of methanolic extract of the leaves and concluded that the extract is a potent analgesic and anti-hepatotoxic agent. It has been reported that the plant have showed high antiviral indices against HIV1 and HIV2 (Ayissi and Nyadedzor, 2003). As typified by its total phenolic content, free radical scavenging activity and reducing power, the plant is highly antioxidant (Obho, 2006). The volatile oil of the leaves also showed good antioxidant activity when used in scavenging DPPH radical (Adesegun *et al.*, 2013).

The leaves also have some culinary uses: spice to flavor foods and meat (Uhegbu *et al.*, 2012). Recent studies on the plant suggest that it can be used as a mosquito repellent if kept inside a room (Okoye and Madumelu, 2013). The present work is designed to focus on the investigation of phytochemical constituents, thin layer chromatography and qualitative free radical scavenging activities of *Ocimum gratissimum* leaves using three different extracts (methanol, dichloromethane and hexane).

### 2.3 Extraction of plant material

The powdered leaves (150g) of *Ocimum gratissimum* was extracted with the aid of soxhlet extractor using  $400\text{cm}^3$  of hexane for 3 hours, the marc was dried and weighed. The Marc (145g) was extracted with soxhlet extractor using  $400\text{cm}^3$  of methanol for 3 hours. The methanol extract was partitioned with separating funnel using  $400\text{cm}^3$  of dichloromethane to obtain dichloromethane fraction. The %yield of extract and fraction of the leaves were calculated.

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{initial weight of the sample}} \times 100$$

## 2.4 Phytochemical Screening

The phytochemical screening was carried on hexane extract, dichloromethane fraction and methanol fraction in order to test for the following secondary metabolites: saponins, alkaloids, flavonoids, steroid, proteins, carbohydrates, anthraquinones, and cardiac glycosides using appropriate reagent and standard procedures.

## 2.5 Thin Layer Chromatography

### a) TLC Plate

The TLC plate used for the separations was made of silica gel supported on aluminum (TLC silica gel 60F<sub>254</sub> 20 × 20 cm) obtained from Merck Germany.

### b) TLC Separation

Thin layer chromatography was carried out using TLC precoated plate (TLC silica gel 60F<sub>254</sub>) by ascending technique. Spotting was done normally using capillary tube and developed in a chromatographic tank at room temperature. TLC separations were conducted using different solvent systems as follows:

- i) Hexane extract

The solvent system that best resolved the extract was ethylacetate/hexane (1:4).

- ii) Dichloromethane fraction  
The solvent system that best resolved the fraction was ethylacetate/hexane (4:1).
- iii) Methanol fraction  
The solvent system that best resolved the fraction is methanol/ethylacetate/hexane (3:1:1).

### c) Visualization of TLC Plates

The chromatograms were observed first under UV light and then stained with 5% H<sub>2</sub>SO<sub>4</sub> to detect different spots and the retention factor for each chromatogram spot was calculated by taking the ration of the total distance moved by the each spot to that moved by the solvent.

$$R_f = \frac{\text{Distance move by spot front}}{\text{Distance move by solvent front}}$$

## 2.6 Qualitative free radical activity

For the qualitative free radical scavenging activity, DPPH solution (DPPH powder dissolved in methanol) was sprayed on the chromatograms. Formation of yellow or white spots against purple background is due to the presence of a free radical scavenging compound.

## 3.0 RESULTS

Table 1: Result of the phytochemical screening of the leave extracts of *Ocimum gratissimum*

Phytochemicals parameters	Extracts Inference		
	Hexane	DCM	Methanol
<b>1. Alkaloids</b>			
* Mayers test	N.D	++	+++
* Dragendroffs test	N.D	++	+++
<b>2. Saponins</b>			
* Frothing test	N.D	+++	+++
<b>3. Flavonoids</b>			
* NaOH test	N.D	++	+++
* Acid test	N.D	+	++
<b>4. Steroids</b>			
* Libermann test	+++	+	++
* Salkowskis test	+++	+	+
<b>5. Carbohydrates</b>			
* Molish test	++	++	++
<b>6. Cardiac glycoside</b>			
* Kella killiani test	+++	+	++

**7. Protein test**

* Xanthoproteic test	<b>N.D</b>	<b>N.D</b>	+
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**8. Anthraquinones**

* Borntragers test	<b>N.D</b>	<b>N.D</b>	<b>N.D</b>
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**Key:** +++ = Present in high concentration, ++ = present in moderate concentration, + = slightly or sparingly present, **N.D** = Note detected.

**3.1. Thin Layer Chromatography**

The result of the TLC separation of the various fractions with their Rf values are represented in Table 2, 3 and 4 respectively.

Table 2: TLC separation of Hexane extract

Spot	Rf value
A	0.10
B	0.29
C	0.43
D	0.53
E	0.64
F	0.86

Solvent system: ethylacetate: hexane (1:4, v/v)

Table 3: TLC separation of DCM extract

Spot	Rf value
A	0.08
B	0.41
C	0.78
D	0.95

Solvent system: ethylacetate: hexane (4:1, v/v)

Table 4: TLC separation of methanol extract

Spot	Rf Value
A	0.07
B	0.80
C	0.91

Solvent system: methanol: ethylacetate: hexane (3:1:1, v/v)

**3.2 Qualitative free-radical scavenging activity against DPPH radical.**

The qualitative screening of free radical scavenging activity was determined by thin layer chromatography. A yellow or white spot against a purple background on the TLC plate indicates free radical scavenging activity. All the three extracts gave a positive result (Table 5).

Table 5: Qualitative free radical scavenging activity of the extracts against DPPH radical

Extracts	Results
Hexane extract	+
DCM	+
Methanol	+

#### 4.0 DISCUSSION

The plant *Ocimum gratissimum* is widely employed in folk medicine to treat a number of disease like upper respiratory tract infections, diarrhea, headache, ophthalmic and skin diseases, pneumonia, cough, fever, conjunctivitis, abdominal, oral and gynecological disorders (Gupta *et al.*, 2011). From the Table 1 steroids, glycosides and carbohydrates were present in all the extracts whereas anthraquinone is not detected in all the extracts. Proteins were not detected in DCM and Hexane extracts but found present in a minute amount in the methanol extract (Table 1). Methanol and DCM extracts showed the presence of most of the phytochemicals analyzed i.e. alkaloids, flavonoids, steroids, carbohydrates, saponins and glycosides (Table 1). The use of three solvents for extraction has revealed that phytochemical composition of the extracts varies with the solvent used. These phytochemicals are responsible for the pharmacological properties of *Ocimum gratissimum* leaves. Alkaloids plays some metabolic role and control development in living system and also in protective functions in animals, they are also used as medicine (Ugochukwu *et al.*, 2013). Flavonoids have been reported to exert multiple biological effects including free radical scavenging activities, antioxidants, anti-inflammatory etc. (Rai *et al.*, 2013).

#### 5.0 CONCLUSION

The result of preliminary phytochemical screening of *Ocimum gratissimum* leaves of this study revealed the presence of alkaloids, steroids, saponins, flavonoids, glycosides, carbohydrates and proteins. The qualitative free radical

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#### 7.0 REFERENCES

Abdurrahman F. (1992) Studies on Natural products the moraceae in African traditional medicine and management of psychiatry in Borno state. M.Sc Thesis University of Maiduguri. Maiduguri Pp 89-95.

Thin layer chromatography (TLC) is the simplest, sensitive and cheapest method of detecting plant constituents (IUPAC, 1999). Hexane extract chromatogram using solvent system 1:4 ethylacetate and hexane produces a good six spots with Rf values of 0.10, 0.29, 0.43, 0.53, 0.64 and 0.86 (Table 2). The chromatogram of DCM extract using solvent system 4:1 ethylacetate and hexane produces four spots with Rf values of 0.08, 0.41, 0.78 and 0.95 (Table 3) while three spots were observed in the chromatogram of methanolic extract using solvent system 3:1:1 methanol, ethylacetate and hexane, the Rf values calculated are 0.07, 0.80 and 0.91 (Table 4).

Another use of thin layer chromatography (TLC) for biological analysis is as a means for discovering antioxidant property of a plant (IUPAC, 1999). This is achieved by spraying DPPH radical solution on chromatogram obtained from TLC analysis. Antioxidants reduce the DPPH radical producing yellow spot on purple background. On spraying DPPH radical solution on the chromatograms, all the three extract gave a positive result by producing yellow spot on purple background (Table 5). This degree of discolouration indicates the scavenging potential of the antioxidant compounds of the leaves (Hemalatha *et al.*, 2010).

scavenging analysis against DPPH radical showed that *Ocimum gratissimum* leaves possessed antioxidant and free radical scavenging compounds.

Adebolu T.T, Oladimeji S.A. (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhea causing bacteria

in south western Nigeria. *African Journal of Biotechnology* 4(7):682-684

Adesegun A.S, Sameul F.O, Anthony O.B, Nuruddeen O.A. (2013). Antioxidant and Inhibitory properties of essential oil of *Ocimum gratissimum* against extracellular protease of *Escherichia coli*. *IOSR Journal of pharmacy* 3(1):50-55.

Agbogidi O.M, Obi-Iyeke G.E, Olisa A.C.(2014) Health and food values of scent leave (*Ocimum gratissimum*). *Unique research Journal of food sciences* 1(3):021-027.

Akinjogunla O.J, Ekoi O.H, Odeyemi A.T. (2011). Phytochemical screening and in vitro antibacterial assessment of aqueous leaf extraction of *Vernonia amygdalina* (Asteraceae) and *Ocimum gratissimum* (Lamiaceae) on Moxifloxin resistance *Escherichia Coli* Isolate from clinical and Enviromental samples. *Journal Nature and science* 9(7):42-52.

Ayissi N.K, Nyadezor C. (2003). Comparative in vitro effects of AZT extracts of *Ocimum gratissimum*, *Ficus polita*, *Clausena anisata*, *Alchorne cordifoia* and *Elaeophorbia drupifera* against HIV 1 and HIV 2 infections. *Journal of antiviral Research*. 58:25-33.

Efiong E. E, Ebong P. E. (2014). *Ocimum gratissimum* and *Moringa Oleifera* ameliorates diabetic nephropathy in a synergistic manner similar to insulin chemical. *Journal of pharmaceutical and biological sciences* 2(2):158-165.

Gupta V. K, Singh J, Kumar R, Bhanot A. (2011). Pharmacognostic and preliminary phytochemical study of *Ocimum gratissimum* linn. *Asian Journal of plant sciences* 10(7):365-369.

Hemalatha S, Lathila P, Arulpriya P. (2010). Antioxidant activities of the extracts of the aerial roots of *Pothos aurea* (Linden ex Andre). *Scholars Research library De pharma chemica* 2(6):84-89.

IUPAC 1999. Strategy for the Biological and Chemical evaluation of plant extracts Retrieved May 2015, from

<http://www.iupac.org/symposia/proceedings/phuket97/hostettmann.html>

Koche D. K, Kokate P. S, Suradkar S. S, Pohadange D. G. (2012). Preliminary phytochemistry and antibacterial activity of Ethanolic extract of *Ocimum gratissimum* L. *Bioscience Discovery* 3(1):20-24.

Opara M. N, Iwuji T. C, Igwe I, N, Etuk, I. F Maxwell J. A. (2012). Haematological and biochemical responses of adult Rabbits to aqueous extract of *Ocimum gratissimum*. *Journal of physiology and pharmacology Advances*. 2(9):301-306.

Obho G. (2006). Antioxidant and antimicrobial properties of Ethanolic extract of *Ocimum gratissimum* leaves. *Journal of pharmacology and toxicology* 1:47-53.

Obinna C. W, Nwodo S. C, Olayinka O. A, Chinwe O. I, Kehinde O. O (2008). Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia Coli* and *Staphylococcus aureus*. *African Journal of food Science* 3(1):022-025.

Okoye E. I, Madumelu (2013). Phytochemical constituent of leaves of *Ocimum gratissimum* and production of mosquito coil. *Global research Journal of science* 2(2):62-66.

Rai V. M, Pai R. V, Kedilaya H. P, Hedge S. (2013). Preliminary photochemical screening of members of Lamiaceae family: *Leucas linifolia*, *Coleus aromaticus* and *Pogestemon patchouli*. *International Journal of pharmaceutical science* 21(1)131-137.

Ugochukwu C S, Uche I A, Onouha Ifeanyi (2013). Preliminary phytochemical screening of different solvent extracts of stem back and roots of *Dennetia tripetala* G. Baker. *Pelagia research Library Asian Journal of plant science and research* 3(3):10-13.

Uhegbu O.F, Elekwa I, Emmanuel I, Akubugwo G, chinyere C, Iweala E.E, (2012). Analgesic and hepatoprotective activity of methanolic extract of

*Ocimum gratissimum*. *Research Journal of Medicinal plant* 6(1):108-115.

Varma R, Agarwal K. (2014). *Ocimum gratissimum* L:A medicinal plant with promising antiurolithiatic Activity. *International Journal of pharmaceutical sciences and drug research*. 6(1):78-81.