Vol. 23 (2020) 121–133

Effects of Ultra-Violet, Red and Sun Light on the Stability of Phytochemicals, Antiradical and Antimicrobial Activity of *Ocimum gratissimum* Linn. (Lamiaceae)

NGAITAD S. NJINGA[1]*, MOJI T. BAKARE-ODUNOLA[1], SA'AD T. ABDULLAHI[1], FEHINTOLA F. ABDULMAJEED[1], RONKE H. BELLO[2], RIHANAT A. SAADU[3], MUSTAPHA ALIYU[3], GODSHELP O. EGHAREVBA[4], BAMIDELE O. DAVID[1] AND ABIODUN O. SHITTU[5]

[1]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria.*
[2]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria.*
[3]*Faculty of Pharmaceutical Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria.*
[4]*Industrial Chemistry Programme, Department of Physical Sciences, College of Pure and Applied Sciences, Landmark University, PMB 1001, Omu-Aran, Kwara State, Nigeria.*
[5]*Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria.*

The content and stability of phenols, flavonoids, saponins and alkaloids in *Ocimum gratissimum* upon exposure to sunlight, red and ultra-violet (UV) radiation over a period of 168 h was investigated as well as the effect of irradiation on its antiradical and antimicrobial activities. Total phenolic, flavonoids, saponins and alkaloid contents were determined using standard methods while antiradical and antimicrobial analyses were determined using 1,1-diphenyl-2-picrylhydrazyl and agar diffusion methods, respectively. Exposure to sunlight significantly increased ($p<0.05$) the total phenolic and alkaloid contents. Ultra-violet and sun light significantly decreased the total flavonoid and saponin content, while all the three forms of radiation significantly increased antiradical activity as well as antimicrobial activity against *Staphylococcus aureus*. Red and UV light significantly increased the activity against *S. aureus* while UV light increased activity against *Escherichia coli.* These findings suggest photochemical instability with increased antiradical and antimicrobial activities of the methanol extracts of *O. gratissimum* after irradiation.

KEY WORDS: *Ocimum gratissimum*, extract stability, sunlight, ultraviolet light, antimicrobial assay, antiradical activity

INTRODUCTION

Scientific community has shown considerable interest in the study of plant materials as sources of new compounds with antimicrobial and antioxidant principles. The major challenges in herbal medicines are determining the overall quality, safety and efficacy of the herbal products. Many plant phytochemicals exhibit photosensitization when exposed to ultra-violet (UV) or visible light.

Ocimum gratissimum L, commonly known as clove basil, has been reported to undergo light enhanced photosensitization as well as antimicrobial activity [1]. It also exerts antiviral activity against both type 1 and type 2 human immunodeficiency virus (HIV-1 and HIV-2), anti-diarrheal effects in experimental animals, hypoglycemic activity in rats and improved phagocytic function without affecting the humoral or cell-mediated immune system [2,3].

*Author to whom correspondence may be addressed: ngastanjin@yahoo.com.

High-performance liquid chromatographic analysis of *O. gratissimum* revealed the presence of phenolic compounds such as rutin, ellagic acid, myricetin and morin [4]. LC-ESI–MS/MS analysis of this plant also revealed the compounds sinapic acid, rosmarinic acid, methyl eugenol, luteolin, apigenin, nepetoidin A, xanthomicrol, nevadensin, hymenoxin, salvigenin, apigenin 7,4′-dimethyl ether, palmitic acid, basilimoside, 2,3-dihydroxyolean-12-en-28-oic acid, methyl acetate and oleanolic acid were detected [4,5]. Sinapic and rosmarinic acids, and myricetin, have antimicrobial activities [6,7,8]. Rutin has been reported to enhance antimicrobial activity [9] while sinapic and rosmarinic acids, as well as rutin, have antioxidant activity [10, 11, 12].

Previous studies have shown that post-harvest storage conditions and processing may be affected by climatic conditions, cultivation techniques and genotype [13]. These factors may alter the phytochemicals present as well as biological activities. Gamma radiation of non-stored almond hull was shown to slightly decrease the total phenolic and flavonoid content but maintained antioxidant activity. On the other hand, irradiation of stored almond hull increased total phenolic content but neither affected the flavonoid content nor antioxidant activity [14]. Therefore, the concentration of plant phytochemicals might vary under certain conditions such as exposure to irradiation and storage temperature. This study aimed at evaluating the effect of prolonged irradiation of red light, ultraviolet (UV) and sunlight on phytochemical composition as well as antioxidant and antimicrobial activities of powdered *Ocimum gratissimum*.

EXPERIMENTAL

Chemicals and reagents

Methanol, aluminium chloride, gallic acid, Folin-Ciocalteu's phenol reagent, sodium carbonate, sodium chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin, potassium ferricyanide, tannic acid, iron (III) chloride, acetic anhydride, sulphuric acid, sodium hydroxide, magnesium ribbon, hydrochloric acid, glacial acetic acid, chloroform, Mayer's and Wagner's reagents were form Sigma Aldrich (Gillingham, UK). All chemicals and solvents were of analytical grade. Other reagent used include Mueller Hinton agar, Sabouraud's dextrose agar and nutrient agar were obtained from Oxoid (Basingstoke, UK).

Equipment

Water bath (Fisher Scientific, Pittsburgh, PA, USA), analytical weighing balance (Ohaus, Parsippany, NJ, USA), Electro-heating Standing temperature air-dry oven (Genlab, model OV/50/DIG-R38, Cheshire, UK), UV-visible spectrophotometer (Hewlett Packard, Palo Alto, CA, USA), UV lamp and red bulb, incubator (Microfield, SM9052, England) and autoclave (Cole Parmer, England), were used in this study.

Collection and treatment of plant materials

Fresh *O. gratissimum* leaves were obtained from Oja-Oba market, Ilorin, Kwara State in the month of March, 2016 and authenticated at the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria, where a voucher specimen (UILH/001/984) was deposited. The leaves were removed from their stalks, oven-dried at 35ºC for 2 days, and pulverized into fine powder with the aid of a blender. The powdered material was stored in a polythene bag at ambient temperature away from direct sunlight until use.

Exposure to irradiation

The method of Bakare-Odunola *et al.* [15] was adopted for the exposure to the light sources. For this purpose, 175 g each of the powdered leaves were weighed into three flat trays. Two

of the trays labelled UV light and red light were each placed in chambers flooded with UV lamp and red light, respectively. The third tray was exposed directly to sunlight. About 17 g of the powdered samples was weighed out from each tray at intervals of 1, 2, 3, 6, 12, 24, 48, 72, 144 and 168 h for analysis.

Extraction

Extraction of the plant samples was done separately at each of the time intervals for the three light sources. The exposed powders were macerated in 100 ml of methanol at room temperature and decanted after 48 h. The solvent in the extract was evaporated first using rotary evaporator and then over a water bath at 40°C. The same procedure was repeated for the unexposed leaf sample. The percentage yield of the extracts was determined before they were stored in the refrigerator until use.

Phytochemical analysis

Qualitative phytochemical analysis of *Ocimum gratissimum*

The methanolic extracts were subjected to phytochemical screening to identify the chemical constituents. Tests for the presence of alkaloids, flavonoids, saponins, steroids and terpenoids on the unexposed and exposed samples were conducted using standard procedures [16].

Determination of total phenolic content

Total phenolic content of the methanolic extracts was evaluated on each extract with Folin-Ciocalteu's phenol reagent according to the methods of Nabavi *et al.* [17] with some modifications. The extract (15 mg) was dissolved in 5 ml of methanol to make the stock solution. An aliquot of the stock solution (0.5 ml) was diluted with methanol to 2 ml. A volume equivalent to 0.028 mg extract was taken out of this solution, made up to 1 ml with methanol and mixed with 1 ml of Folin-Ciocalteu's reagent previously diluted with water (1:9 v/v). After 5 min, 0.8 ml of 7% Na_2CO_3 solution was added with mixing. The tubes were shaken for 5 sec and allowed to stand for 30 min at 40°C in an oven for colour development. Absorbance was measured at 765 nm using UV-Vis spectrophotometer. Samples of extract were evaluated at a final concentration of 0.01 mg/ml. Gallic acid at a concentrations range of 0.01 to 0.07 mg/ml was used as standard. All tests were performed in triplicates. Total phenolic content was expressed as mg/g gallic acid equivalent per 1 mg of extract using the calibration curve regression equation.

Determination of total flavonoids

Colorimetric method was used for flavonoid determination of each extract according to the methods of Sharmin *et al*. [18] with some modifications. About 15 mg of the extract was dissolved in 5 ml of methanol to make stock solution. A 0.1 ml aliquot of the solution was diluted with 1.4 ml of methanol. The resulting solution was mixed with 1.5 ml of 2% aluminum chloride. After standing for one hour at room temperature, the absorbance was measured at 420 nm. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Quercetin at concentration range of 0.06 to 0.18 mg/ml was used as standard. All tests were performed in triplicates. The total flavonoid content was calculated as quercetin equivalents (mg/g) using the calibration curve regression equation.

Saponin determination

The saponin content in each plant extract was estimated as described by Ejikeme *et al.* [18]. Fifty milligrams of the extract were dissolved in 1 ml of methanol and separated in two equal halves in two test tubes. Into each test tube were added 2 ml of distilled water and 2 ml diethyl ether, and shaken. The ether layer was discarded

and the purification process repeated. Thereafter, 2 ml of n-butanol was added, the mixture shaken, allowed to stand for separation, and the n-butanol layer collected. This process was repeated five times. The n-butanol extracts were washed twice with 2 ml of 5% aqueous sodium chloride after which they were dried over a water bath at 40°C. After evaporation, the samples were dried in the oven at 50°C to a constant weight. The test was carried out in duplicates. The saponin content was calculated using Equation 1.

Alkaloid determination

One millilitre was taken out of the 50 mg/ml stock solution, diluted with 5 ml of methanol and separated into two equal halves. In each test-tube, 2 ml of acetic acid was added, allowed to stand for 24 h and filtered. Afterwards, it was placed on a water bath at 55°C and allowed to evaporate to one quarter of its original volume. Concentrated ammonium hydroxide was added dropwise until basic to litmus paper. The solution was filtered and the precipitate oven-dried at 50°C to constant weight. The collected precipitate was weighed and the alkaloid content calculated using Equation 2 [17].

Assay for radical scavenging activity

The DPPH radical-scavenging activity of the test extracts was examined as described by Coklar and Akbulut [21] with minor modifications. Different concentrations (0.03–0.1 µg/ml) of each extract were added to an equal volume of a methanolic solution of DPPH (100 µM). The mixture was allowed to react at room temperature in the dark for 30 min. Vitamin C was used as the standard control while a mixture without the extract was taken as blank. Two replicates were made for each test sample. After 30 min, the absorbance (A) was measured at 518 nm and converted into the percentage antioxidant activity using Equation 3. The EC_{50} values, which denote the concentration of sample required to scavenge 50% of DPPH free radicals, were calculated by nonlinear regression using graph pad prism. Where the abscissa represented the concentration of the tested plant extract and the ordinate represented the average percent of scavenging capacity from two replicates.

Antimicrobial study

Bacterial and fungal isolates

American type culture collection (ATCC) and clinical isolates microorganisms were obtained from the Pharmaceutical Microbiology Laboratory at the Department of Pharmaceutical Microbiology and Biotechnology, University of Ilorin, Nigeria. *Staphylococcus aureus* (clinical isolate 41 and ATCC 25913), *Escherichia coli* (ATCC 35928), and *Candida albicans* (clinical isolate), were used in this study.

Antimicrobial assay

The antimicrobial assay of *Ocimum gratissimum* extract was performed by deep-well agar diffusion method using Mueller-Hinton agar. Mueller-Hinton agar plates were prepared as specified by the manufacturer. The Mueller Hinton agar was inoculated separately with 10^7 colony forming units (CFU) of *S. aureus* and *E. coli* evenly spread on entire plate surface. The agar was punched using a cork-borer No 6, to form 4 wells at equal distance around the plates. The extracts (100 µL) at concentrations of 2.5 mg/ml were dispensed into the wells and left at ambient temperature for 30 min for pre-diffusion and then incubated at 37°C for 18–24 h. Extracts were dispensed into two wells while the other two were used for positive and negative controls. Dimethyl sulfoxide and gentamicin were used as negative and positive controls, respectively [22]. The diameter of inhibition zones was measured using a ruler in millimeters.

Antifungal activity of *Ocimum gratissimum* leaf extracts

The antifungal activity of *O. gratissimum* leaf extract was performed by deep-well agar diffusion method on Sabouraud's dextrose agar (SDA) plates. Sterile SDA was inoculated with 10^7 CFU of *C. albicans* culture and evenly spread on each plate. The agar was punched using a No. 6 cork-borer, making four wells at equal distance around the plates. The extracts (100 µL) at concentrations of 2.5 mg/ml were dispensed into the wells and left at ambient temperature for 30 min for pre-diffusion and then incubated at 25°C for 48–72 h. Extracts were dispensed into two wells while the other two were used for positive and negative controls. Dimethyl sulfoxide was used as the negative control, while gentamicin and fluconazole were the positive controls for antibacterial and antifungal screening, respectively [22].

Statistical analysis

Results were analyzed using Graph Pad prism 7 (GraphPad prism 6 software. Inc, USA), and the results were expressed as mean ± standard deviation. Differences of $p<0.05$ was taken as statistically significant.

$$\text{Amount of saponin (mg/g)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \quad \text{Equation 1}$$

$$\text{Amount of alkaloid (mg/g)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \quad \text{Equation 2}$$

$$\text{Percentage antioxidant} = \frac{\text{Absorbance (DPPH)} - \text{Absorbance (Extract)}}{\text{Absorbance (DPPH)}} \times 100 \quad \text{Equation 3}$$

RESULTS AND DISCUSSION

Yield of *Ocimum gratissimum* leaves

A significant increase in the percentage yield of methanolic extract of *O. gratissimum* was observed in the sample exposed to sunlight compared to the samples exposed to red and UV lights (Table 1). The relationship between the duration of exposure and the percentage yield was not linear. Similar trend was observed for percentage yields when exposed to red light and UV light while there was no statistically significant difference in the percentage yield between red light and UV light exposure across the period of exposure (Table 1).

Qualitative phytochemistry of *Ocimum gratissimum* leaves

The qualitative phytochemical screening of methanol extract of *O. gratissimum* showed the presence of alkaloids, saponins, flavonoids, tannins, phenols, steroids, and cardiac glycosides, while terpenoids were absent (Table 2). This report was in contrast to Akinmoladun *et al.* [23] who reported the absence of saponins in the methanolic extract of the leaves of *O. gratissimum* but presence in aqueous extracts. The study also reported the presence of terpenoids in both methanolic and aqueous extracts. This discrepancy could be caused by many factors including the time of collection, geographical region where the plant was collected and extraction procedure.

Quantitative phytochemistry

Figure 1 illustrate the calibration curves used in the determination of the concentrations for phenols and flavonoids in *O. gratissimum* leaves. The phenolic, flavonoids and saponin content before and after exposure are represented in Tables 3, 4 and 5, respectively.

Tables 1: Percentage yield of the methanol extract of *Ocimum gratissimum* leaves before and after exposure to red, ultraviolet and sun light

Duration of exposure (hours)	Percentage yield (%)		
	Red light	Ultra violet light	Sun light
0	9.47	9.47	9.47
1	6.32	7.65	13.78
2	7.45	7.44	14.29
3	7.81	6.79	15.47
6	6.95	8.09	14.40
12	7.76	6.39	13.26
24	5.40	7.08	15.26
48	7.10	6.95	17.60
72	6.76	7.88	13.6
144	7.52	9.13	14.29
168	7.18	7.18	10.94

Table 2: Qualitative photochemistry of *Ocimum gratissimum* leaves

Phytochemical	Test	Methanol extract
Alkaloids	Wagner's Reagent	+
	Mayer's Reagent	+
Cardiac glycosides	Keller-Kiliani test	+
Flavonoids	Alkaline (NaOH) reagent test	+
	Shinoda test	+
Saponins	Frothing test	+
Steroids	Acetic anhydride + Conc. H_2SO_4	+
Phenols and tannins	$AlCl_3$ Test	+
Terpenoids	$CHCl_3 + H_2SO_4$	-

+ = Present; - = Absent.

Phenols

There was a gradual increase in the total phenolic content of the leaves of *O. gratissimum* when exposed to sunlight which was significant ($p<0.05$) at 24, 144 and 168 h post-exposure (Table 3). Conversely, a significant decrease ($p<0.05$) in the total phenolic content was observed on red-light and from 72 h to 168 h post-exposure to UV-light when compared with the unexposed at zero hour.

The results of the unexposed extract are consistent with those of Olamilosoye *et al.* [24] and Akinmoladun *et al.* [25] who reported total phenol content of 90.03 mg/100 g gallic acid and 5.68 mg/g gallic acid, respectively. The total phenolic content was lower than the 124.3±5.8 mg/g gallic acid reported by Igbinosa *et al.* [26]. The inconsistency could arise from differences in geographic location, climate and soil type. Generally, it was deduced that the longer the exposure time to red-light and UV light, the

lower the total phenolic content. However, the decrease in total phenolic content was more when exposed to red light compared to its exposure to UV-light. From the result, it is best to dry the leaves of *O. gratissimum* in sunlight when phenolic content is the secondary metabolite of interest.

Flavonoids

The association between the time of exposure and flavonoid contents was inconsistent (Table 4). However, the exposure to sunlight gave a significant ($p<0.05$) decrease in flavonoid content from one hour to 168 h of exposure when compared with red and UV light exposure. This indicates that sunlight enhances the rate at which flavonoid compounds breakdown, the longer the exposure the more the degradation of flavonoids, thus, reduced biological activity.

However, the trend in the flavonoid content between the red and UV light exposure were similar with no significant ($p<0.05$) difference except at 6 h where a significant ($p<0.05$) increase in flavonoid content was observed with the exposure to red-light. The decrease observed when exposed to UV light only became significant at 144 and 168 h exposure, respectively, while significant decrease ($p<0.05$) occurred at 168 h exposure time (Table 4). Previous study reported total flavonoid content of 15.57±0.56 mg/g quercetin [26], and 25.33±1.8 mg/g catechin [24] which is higher than the results of the unexposed extract in this study. The inconsistency could arise from differences in geographic location, climate and soil type.

Saponins

There was a significant ($p<0.05$) decrease in saponin content when exposed to UV light from 2 to 168 h except for 72 and 144 h where an increase was recorded (Table 5). Similarly, the saponin content when exposed to red-light significantly decreased from 24 h through to 168 h of exposure. There was also a significant ($p<0.05$) increase in saponin content at 2 and 3 h of exposure to sunlight which significantly ($p<0.05$) decreased thereafter.

Alkaloids

There was an increase in the alkaloid content with exposure to sunlight as shown in Figure 2. Some studies [25, 27] also reported the presence of alkaloids in the leaves of this plant. Alkaloids have been reported to have antibacterial activity through inhibition of nucleic acid synthesis, toxin production, and disruption of bacterial homeostasis [28, 29]. From the results, it shows that exposure to sun light could increase the antibacterial activity.

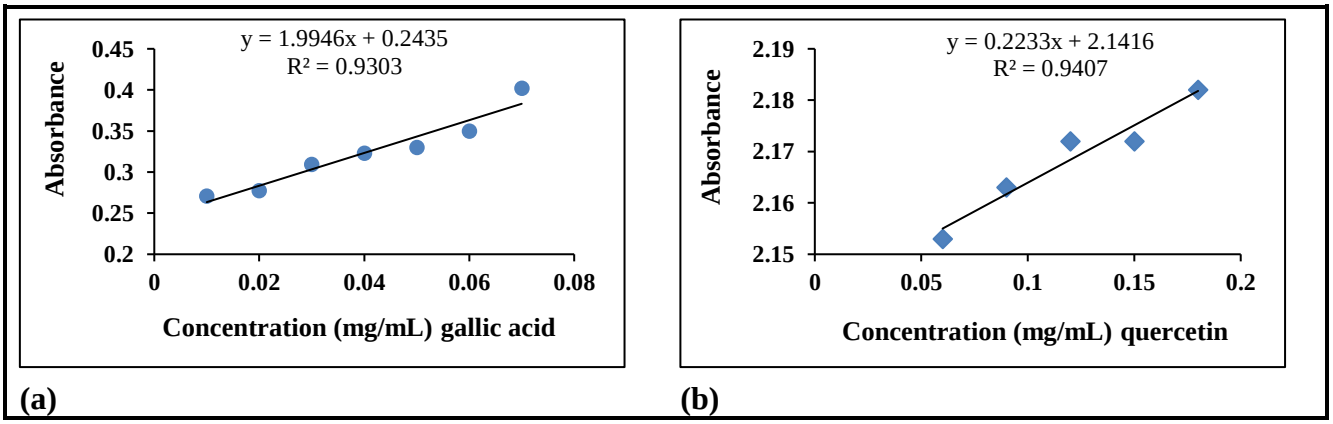

Figure 1. Folin-Ciocalteau gallic acid (a) and quercetin (b) calibration curves for determination of phenol and total flavonoids.

Table 3. Phenolic content of *Ocimum gratissimum* rhizomes before and after exposure to ultra-violet, red and sun light

Exposure time (h)	Phenolic content (mg/g gallic acid)										
	0	**1**	**2**	**3**	**6**	**12**	**24**	**48**	**72**	**144**	**168**
UV light	1.3733 ± 0.077[a]	1.318 ± 0.047[a]	1.312 ± 0.023[a]	1.356 ± 0.056[a]	1.238 ± 0.02[a]	1.204 ± 0.012[a]	1.219 ± 0.006 [a]	1.200 ± 0.01[a]	1.152 ± 0.008 [b]	1.149 ± 0.022[b]	0.841 ± 0.018[b]
Red light	1.3733 ± 0.077[a]	0.9073 ± 0.011 [b]	0.817 ± 0.014[b]	0.762 ± 0.027[b]	0.7117 ± 0.008[b]	0.689 ± 0.046[b]	0.655 ± 0.018[b]	0.417 ± 0.011[b]	0.326 ± 0.042[b]	0.65 ± 0.03[b]	0.8763 ± 0.042[b]
Sun light	1.3733 ± 0.077[a]	1.456 ± 0.001[a]	1.442 ± 0.002[a]	1.441 ± 0.01[a]	1.358 ± 0.006[a]	1.519 ± 0.001[a]	1.560 ± 0.007[b]	1.550 ± 0.016[a]	1.547 ± 0.003[a]	1.581 ± 0.011[b]	1.569 ± 0.002[a]

Data are mean ± SEM, (n=3). Values carrying superscripts different from the 0 h for each parameter are significantly different ($p<0.05$).

Table 4: Flavonoid content of *Ocimum gratissimum* before and after exposure to ultra-violet, red and sun light

Exposure time (h)	Flavonoid content (mg/g quercetin)										
	0	**1**	**2**	**3**	**6**	**12**	**24**	**48**	**72**	**144**	**168**
UV light	1.9143 ± 0.049[a]	1.858 ± 0.192[a]	1.838 ± 0.137[a]	1.770 ± 0.084[a]	1.564 ± 0.12[a]	1.930 ± 0.015[a]	2.004 ± 0.05[a]	1.915 ± 0.089	1.687 ± 0.16[a]	1.158 ± 0.041[b]	0.687 ± 0.025[b]
Red light	1.914 ± 0.049[a]	1.564 ± 0.064[a]	1.942 ± 0.02[a]	1.826 ± 0.039[a]	2.3017 ± 0.14[a]	1.887 ± 0.028[a]	2.057 ± 0.023[a]	1.855 ± 0.021	1.6753 ± 0.012[a]	1.767 ± 0.019[a]	1.5463 ± 0.067[b]
Sun light	1.914 ± 0.049[a]	0.880 ± 0.011[b]	0.480 ± 0.225[b]	0.670 ± 0.012[b]	1.280 ± 0.006[b]	0.887 ± 0.013[b]	0.963 ± 0.049[b]	0.7263 ± 0.018[b]	0.726 ± 0.002[b]	0.446 ± 0.026[b]	0.739 ± 0.01[b]

Data are mean ± SEM, (n=3). Values carrying superscripts different from the zero hour are significantly different ($p<0.05$).

Table 5: Saponin content of *Ocimum gratissimum leaves* before and after exposure to ultra-violet, red and sun light

Exposure time (h)	Saponins content (%)										
	0	**1**	**2**	**3**	**6**	**12**	**24**	**48**	**72**	**144**	**168**
UV light	12.8 ± 0.001[a]	12.563 ± 0023 [a]	10.940 ± 1.057[b]	10.947 ± 0.058[b]	12.097 ± 0.130[b]	12.413 ± 0.344[a]	11.697 ± 0.101[b]	10.027 ± 0.092[b]	14.803 ± 0.170[b]	13.367 ± 0.098[b]	11.050 ± 0.453[b]
Red light	12.8 ± 0.001	12.853 ± 0.090 [a]	13.46 ± 0.098[a]	12.16 ± 0.312[a]	12.553 ± 0.141[a]	13.003 ± 0.061[a]	3.730 ± 0.006[b]	7.920 ± 0.196[b]	6.957 ± 0.038[b]	6.610 ± 0.179 [b]	6.300 ± 0.001[b]
Sun light	12.8 ± 0.001	12 ± 0.462[a]	16 ± 0.462 [b]	15.6 ± 0.693[b]	3.600 ± 0.231[b]	1.600 ± 0.001[b]	3.200 ± 0.001[b]	2.800 ± 0.231[b]	4.000 ± 0.462[b]	8.800 ± 0.462[b]	7.200 ± 0.001[b]

Data are mean ± SEM, (n=3). Values carrying superscripts different from the zero hour are significantly different ($p<0.05$).

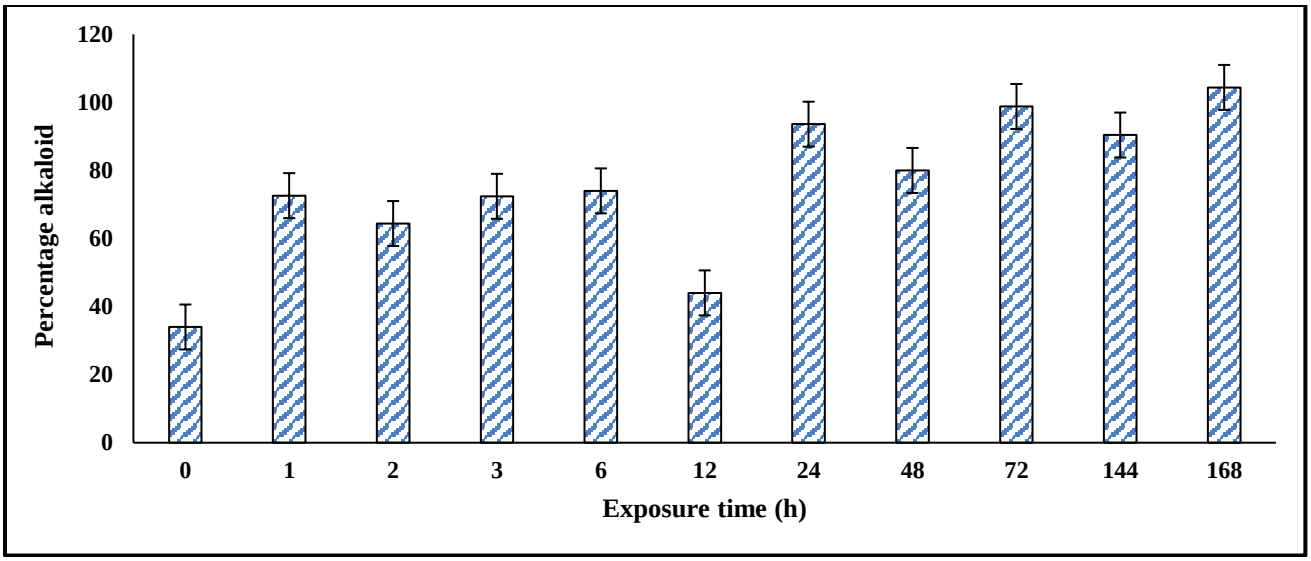

Figure 2. Alkaloid content of *Ocimum gratissimum* leaves before and after exposure to sun light.

Antiradical activity

The antioxidant activity of the methanolic extract of the leaves of *O. gratissimum* significantly ($p<0.05$) increased when exposed to the various sources of lights (UV, red and sun light) as reflected in the values of half-maximal inhibitory concentration (IC_{50}) obtained (Figure 3). The IC_{50} is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function thus the lesser the IC_{50} the more effective the extract. The antioxidant activities were enhanced upon exposure to sunlight in the first 24 h, followed by a decrease at 48 and 72 h post-exposure (Figure 3). There was a consistency in the antioxidant activities when exposed to red-light all through the period of exposure. Similarly, there was a consistent increase in antioxidant activity when exposed to UV light except at 144 h where reduced activity was noticed (Figure 3).

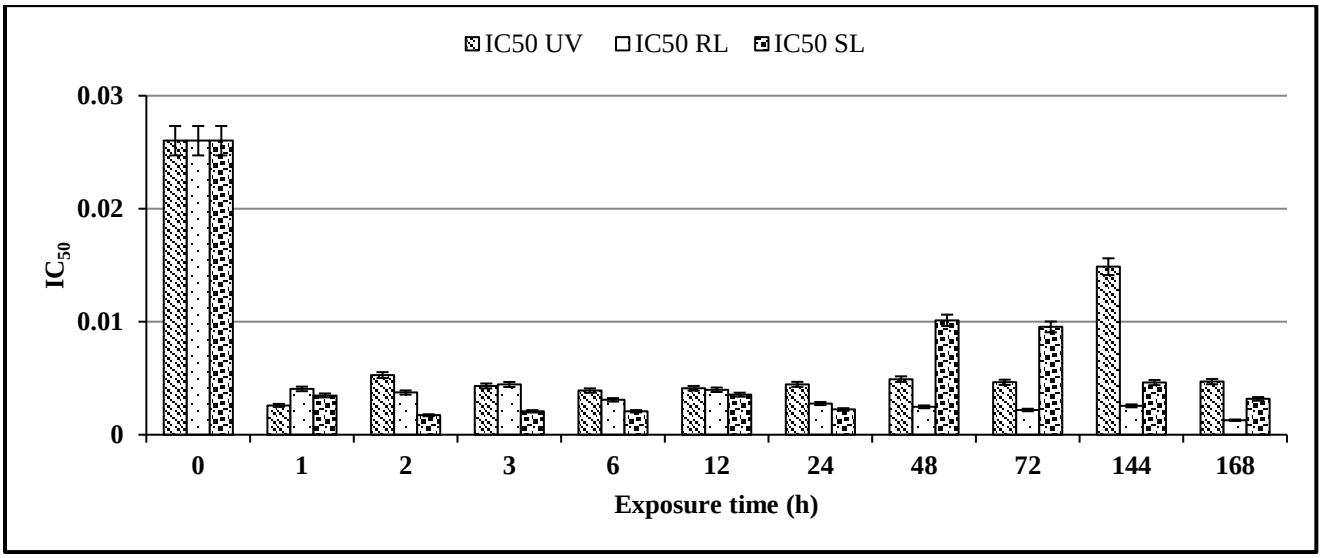

Figure 3. Half-maximal inhibitory concentrations of *Ocimum gratissimum* leaves before and after exposure to UV, red and sun lights. UV = Ultraviolet light, RL=Red light, SL = Sun light. Data are mean ± SEM, (n=3).

Antimicrobial activity

The activity against *S. aureus* of the extract exposed to the different lights shows an increase in activity when compared to the unexposed extract (Table 6). The activity of the exposed extract to sunlight against *E. coli* was not statistically different from that of the unexposed extract except at 12 and 24 h. For the extract exposed to red light, there was a statistically significant increase in activity after exposure though at 24 h and 168 h, there was loss of activity (Table 7). Likewise, for exposure to UV light, there was complete loss of activities at 2, 6, 48, 72, 144 and 168 h exposure time but where there was activity, it was significantly higher than that of the unexposed extract.

There was an inconsistency in antifungal activity on exposure to UV light. Activity against *C. albicans* was observed at the 2 and 12 h post-exposure only as shown in Table 8. For exposure to red light, there was activity only after 6 h which was significantly lower than the unexposed. On exposure to sunlight, there was no loss in activity but the activity trend was inconsistent being significantly lower at 144 h and higher at 168 h.

The percentage yield on the unexposed sample was consistent with the work of Awojide *et al.* [30] and similar to values reported in literature [31,32]. The increase in the total phenolic content upon exposure to sunlight could be due to degradation pathways that enhance UV absorption. The result of this study at 0 h (before exposure) is higher than 0.56 mg gallic acid/1 mg extract for total phenolic content, 0.14 mg quercetin/1 mg extract flavonoid reported in the literature [33].

Interestingly, the significant antioxidant activity observed across the period of exposure with various light treatments despite the inconsistency observed in the content of phenolic, flavonoids and saponins suggests synergistic activity of various metabolites in eliciting biological effects. Though there were variations in the content of these phytochemicals evaluated over the period of exposure to different light sources, these variations did not however reflect on the antioxidant activities of the extracts. Thus, it could be safe to infer that duration of exposure and sources of light (UV, red and sunlight) have significant effects on the phytochemicals and antioxidant activities of the methanolic extract of *O. gratissimum*.

Inadequate or improper storage and distribution of the products may cause photo deterioration and chemical decomposition resulting in reduced bioactivity [34]. Photodegradation is a major form of instability for herbal products as well as orthodox medicines. In the case of *O. gratissimum*, the situation is further complicated due to the multi-component nature of the products and the presence of unknown compounds [35, 36]. This could explain the inconsistencies in antioxidant as well as antimicrobial activity.

Photochemical degradation reactions can be induced in the presence of heavy metals, influence of light and oxygen or enzymes [37]. Herbal medicines from *Andrographis paniculata* were found to degrade photochemically. Some Chinese medications like *Tagetes patula, Anthemis nobilis* and *Rhus toxicodendron*, have also been reported to cause photo-induced contact dermatitis [38]. In another study, the amount of phenolic anthraquinone in *Aloe vera* sap was significantly decreased after irradiation of the *Aloe vera* sap with either sunlight or UV light [39]. Light has also been reported to accelerate the degradation of pseudohypericin and hypericin, causing a significant reduction in hypericin content of the *Hypericum perforatum* extract in solution [40].

Table 6: Effect of irradiation on *Ocimum gratissimum* on the susceptibility of *Staphylococcus aureus*

Exposure time (h)	0	1	2	3	6	12	24	48	72	144	168
UV light	11.5 ± 0.87[a]	18.5 ± 0.289[b]	0[b]	17.50 ± 0289[b]	16.00 ± 1.732[b]	17.50 ± 0.289[b]	0[b]	13.00 ± 0.00[a]	19.00 ± 0.577[b]	17.00 ± 0.00[b]	0[b]
Red light	11.5 ± 0.88[a]	15.0 ± 2.887[a]	18.00 ± 0.577[b]	18.00 ± 1.155[b]	17.00 ± 0.00[b]	19.00 ± 0.00[b]	15.00 ± 0.00[b]	18.50 ± 0.289[b]	15.00 ± 0.00[b]	17.50 ± 1.443[b]	16.00 ± 0.577[b]
Sun light	11.5 ± 0.89[a]	11.50 ± 0.866[a]	12.00 ± 1.155[a]	14.00 ± 0.577[a]	17.50 ± 0.289[b]	12.00 ± 0.00[a]	13.00 ± 0.00[a]	16.00 ± 0.577[b]	19.00 ± 0.577[b]	18.00 ± 0.00[b]	20.00 ± 0.00[b]

Data are mean ± SEM, (n=3). Values carrying superscripts different from the zero hour are significantly different ($p<0.05$).

Table 7: Effect of irradiation on *Ocimum gratissimum* leaves on the susceptibility of *Escherichia coli*

Exposure time (h)	0	1	2	3	6	12	24	48	72	144	168
UV light	12.00 ± 0.577[a]	17.50 ± 0.289[b]	0.00 [b]	11.50 ± 0.289[a]	0.00 [b]	12.00 ± 0.00[a]	14.50 ± 0.289[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]
Red light	12.00 ± 0.577[a]	15.10 ± 0.058[b]	17.00 ± 0.00[b]	15.00 ± 0.00[b]	14.25 ± 0.144[b]	15.15 ± 0.087[b]	0.00 [b]	17.00 ± 0.577[b]	16.5 ± 0.289[b]	15.50 ± 0.289[b]	0.00[b]
Sun light	12.00 ± 0.577[a]	11.00 ± 0.577[a]	11.00 ± 0.577[a]	10.00 ± 0.577[b]	10.50 ± 0.289[b]	13.00 ± 0.577[a]	14.00 ± 0.00[b]	12.50 ± 0.866[a]	12.00 ± 0.00[a]	11.50 ± 0.866[a]	10.50 ± 0.866[a]

Data are mean ± SEM, (n=3). Values carrying superscripts different from the zero hour are significantly different ($p<0.05$).

Table 8: Effect of irradiation on *Ocimum gratissimum* leaves on the susceptibility of *Candida albicans*

Exposure time (h)	0	1	2	3	6	12	24	48	72	144	168
UV light	13.50 ± 0.289[a]	0.00 [b]	15.92 ± 0.058[b]	0.00 [b]	0.00[b]	12.00 ± 0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]
Red light	13.50 ± 0.289[a]	0.00[b]	0.00[b]	0.00[b]	9.00 ± 0.00[b]	11.25 ± 0.144[b]	12.00 ± 0.00[a]	10.25 ± 0.144[b]	11.05 ± 0.029[b]	10.15 ± 0.087[b]	9.20 ± 0.115[b]
Sun light	13.50 ± 0.289[a]	11.50 ± 0.289[b]	15.50 ± 0.289[b]	13.50 ± 0.866[a]	13.50 ± 0.866[a]	14.50 ± 0.289[b]	14.50 ± 0.866[a]	14.50 ± 0.289[b]	15.17 ± 0.167[b]	9.79 ± 1.809[a]	16.50 ± 0.289[b]

Data are mean ± SEM, (n=3). Values carrying superscripts different from the zero hour are significantly different ($p<0.05$).

The results of this study are in agreement with literature precedence in which the antimicrobial activity of irradiated dry, aqueous and methanolic samples of *Zanthoxylum zanthoxyloides* revealed a total loss of activity for sunlight irradiated samples against *E. coli* [41]. Studies showed that thymol exhibited high antibacterial against a wide number of bacteria and fungi including *C. albicans* (human isolate). The antibacterial activity of carvacrol has been attributed to its effects on the structural and functional properties of cytoplasmic membrane. γ-Terpinene has been reported to have activity against *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *Salmonella enteritidis* CMCC (B) 50041 [42]. There is possibility of photochemical degradation of the active compounds in *O. gratissimum* like carvacrol, thymol and γ-terpinene which might have resulted in structural modifications of functional group(s) required by the compounds for the activity against *C. albicans* and *E. coli*.

CONCLUSION

This study revealed the photochemical instability of the methanol extracts of *O. gratissimum*. Irradiation with sun light increased the alkaloids content. Irradiation with UV, red and sun light inconsistently affects the activity of *O. gratissimum* against *S. aureus, E. coli* and *C. albicans* at times with complete loss of activity. Irradiation with the three light sources enhance the antioxidant activities of the leaves. Thus, irradiation of *O. gratissimum* leaves with sunlight, UV and red light affects its phytochemicals, antioxidant and antimicrobial activities.

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