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FEATURE ARTICLE:
**Therapeutic Drug
Monitoring: A Golden
Opportunity for
Pharmacists**

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The Pharmaceutical Society of Kenya (PSK) is a representative organization that was formed enabling Pharmacists' to employ their professional expertise in the care of patients.

Established in 1964, PSK has its roots in the Pharmaceutical Society of East Africa, which was registered in 1950. Since its formation, PSK continues to promote a common standard for professional conduct and code of ethics for its members, as well as advocate for the welfare of Pharmacists.

EDITORIAL

REPORTING OF ADVERSE DRUG REACTIONS

*Prof. Apollo O. Maima, PhD
Editor-in-Chief, Pharmaceutical Journal of Kenya*

Antimicrobial Resistance (AMR) can be defined as the ability of a microorganism to multiply or persist in the presence of an increased level of an antimicrobial agent relative to the susceptible counterpart of the same species. It bears the greatest potential risk in the current management of infectious diseases.

Exposure to antimicrobials is the major cause of the emergence and rise of AMR. Research on the consumption of antimicrobials, in outpatient settings of 26 European countries, showed that AMR rates were higher in countries which consumed more antimicrobials. The abuse and overuse of antimicrobials has been phenomenal for decades, exacerbating the development of AMR, and rendering some infections untreatable using existing antimicrobials. This escalation of AMR has led to ineffective treatment, prolonged epidemics, increased morbidity and mortality

It is estimated that without adequately effective interventions, especially policy changes, AMR associated human global mortality will increase from 700,000 in 2014 to over ten million by 2050. In the process, the global economy may lose more than USD 6 trillion dollars, almost 4% of Gross Domestic Product, annually by 2050 because of AMR if not controlled. By 2030, 24 million more people may be forced into extreme poverty, hunger and malnutrition because of AMR, and many will be from low income countries. Because of the burden of infectious diseases, low-income countries like Kenya are more vulnerable to hardships, hence most affected by AMR, putting the achievement of Sustainable Development Goals (SDG's) in peril. Resistant bacteria are a global problem that requires a holistic and multi-sectoral (Global One Health) solution. Changes in agricultural production practices can help keep present antimicrobials working. Antimicrobials used in animals are similar to those used in humans; bacteria in humans, animals or the environment may spread from one species to the other, and from one country to another.

The 2016 United Nations General Assembly resolution and the 2017 Berlin Declaration of the G20 Health Ministers are signs that AMR is being taken seriously. International governments and agencies through the World Health Organization (WHO) in 2015 also took steps towards containment of AMR and member states agreed to develop action plans by 2017. Many countries, Kenya included, largely met this deadline. However Kenya, like many other low and medium income countries (LMIC's), has not yet started implementing appropriate policies to contain amplification of AMR, and reduce overuse and misuse of antimicrobials. The World Health Organization (WHO), Food

and Agriculture Organization (FAO), and World Organization for Animal Health (OIE), the Tripartite Partnership, are jointly primed to minimize the emergence and spread of AMR. The partnership supports the Global Action Plan (GAP) on AMR with a strategic plan on AMR; to provide the international standards to guide prevention and containment. It has led the global campaign on AMR and initiated country self-assessments on AMR to monitor progress with implementing their National Action Plans (NAP) on AMR.

Challenges abound in addressing AMR through government policies due to limited political commitment, low awareness and weak stakeholder engagement. Most governments globally also suffer limited capacity to implement policies because of poor technical capacity and financial resources. Kenya has developed a number of multi-sectoral policies in its attempt to contain AMR. They provide for what appear to be clear roadmaps for coordinated efforts to set national standards for minimizing transmission of AMR and associated infections. They should culminate in the formulation and establishment of a national integrated surveillance system for antimicrobial resistance by the National Policy for the Prevention and Containment of Antimicrobial Resistance. However, there is no evidence of much activity on the ground towards the implementation. In fact, due to lack of systematic surveillance, the actual burden of AMR in Kenya is unknown.

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Effects of UV, Red and Sun Light on the Stability of Phytochemicals, Antioxidant and Antimicrobial Activity in the Rhizomes of *Zingiber officinale* (Zingiberaceae)

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Abstract

Phytochemicals have been reported to be degraded by environmental factors such as heat, light and oxygen. *Zingiber officinale* (Rosc.) is widely consumed worldwide with remarkable medicinal properties due to its phytochemicals. This study investigates the effect of exposure to sun, red and ultraviolet light of *Z. officinale* for 168 hours on the stability of the phenols, flavonoids, saponins, antioxidant and antimicrobial activity of this plant. Total phenolic and flavonoids content was determined using Folin-Ciocalteu reagent with gallic acid (GA) as standard and Colorimetric aluminium chloride method with quercetin as standard respectively and total saponin was also determined. The antioxidant and antimicrobial analysis of the irradiated rhizomes extracts were carried out using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and agar diffusion methods. The total phenolic, flavonoid and saponin content before exposure were 1.30±0.019 mg/g GA, 1.27±0.351 mg/g and 28.4±7.35% respectively while after exposure the values ranged from 1.21-1.40 mg/g GA for phenols, 0.981-2.0 mg/g of quercetin for flavonoid and 22.4-33.2% for saponins content respectively. The IC₅₀ before exposure to the different light sources was 0.2025 mg/mL while after exposure the IC₅₀ ranged from 0.0024 - 0.9262 mg/mL. Exposure to sunlight caused changes in the phytochemicals present as well as its antioxidant activity. Upon irradiation with the different light sources, there was significant increase (P<0.05) of DPPH radical scavenging activity as well as decrease in activity against *Staphylococcus aureus* 25913, *Staphylococcus aureus* 41, *Escherichia coli* and *Candida albicans*. These changes may be due to degradation of the phytoconstituents. Appropriate storage of herbal materials is necessary to prevent phytodegradation.

Key words: *Zingiber officinale*, radiation, antioxidant assay, Antimicrobial assay, phytochemical assay.

Introduction

The characteristics and effects on human health of natural bioactive compounds especially from plant sources have been investigated extensively [1]. Light is one of the most important variables among various environmental factors affecting the concentrations of phytochemical in plants [2]. For example, blue light significantly enhance the contents of total phenolics and total flavonoids in buckwheat sprouts grown under blue light [3]; UV-A induction of anthocyanins accumulation was observed in lettuce [4] and ascorbic acid in Komatsuna and lettuce [5]. Sunlight, ultraviolet (UV) light and visible light are known to accelerate autoxidation processes by triggering the hydrogen abstraction that results in the formation of alkyl radicals [6].

Zingiber officinale is a flowering plant, known commonly as Ginger and belongs to the family Zingiberaceae. Its rhizome has been used worldwide as a spice and food flavoring agent. It grows annual stems about 1 metre tall bearing yellow flowers and narrow green leaves; it's also a herbaceous perennial. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines for the treatment of rheumatism, asthma, catarrh, gingivitis, nervous diseases, stroke, toothache, constipation and diabetes [7].

The important active constituents in Ginger rhizomes include: essential oils (zingiberole, citral, borneol, citronellol, zingiberene, camphene, cineole, bisabolene, phellandrene, geraniol, linalool, limonene and camphene), phenols (gingerol and zingerone), vitamins (vitamin C, vitamin B6), minerals (calcium, magnesium, phosphorus, potassium and

sulfur), oleoresins, proteolytic and enzymes [8]. In Nigeria, this plant is being exposed to sunlight directly by street hawkers, traditional health practitioners (in bottles) and in combination with other products at times for more than two weeks. This study aimed at evaluating the effect of prolonged irradiation of red light (RL), ultraviolet (UV) and sunlight (SL) on powdered rhizomes of *Zingiber officinale* (RZO) and the effect on phytochemicals, antioxidant and antimicrobial activities.

Materials and Methods

Chemicals and Equipment

All chemicals and solvents used in this experiment were of analytical grade. Water bath (Fisher Scientific Company, USA), analytical weighing balance (Ohaus, USA), Electro-Heating Standing Temperature Air Dry Oven, UV-visible Spectrophotometer (Hewlett Packard), UV lamp and Red bulb (60W), Incubator, Autoclave, Sterile petri dishes were used.

Methods

Collection and Treatment of Plant Materials

Fresh RZO were obtained from Oja-oba market, Ilorin, Kwara state in March, 2016 and was authenticated at the herbarium section of the Department of Plant Biology, University of Ilorin, Nigeria by a botanist. A voucher number: UILH/001/984 was deposited for future references. After identification, the rhizomes were dried in an oven at 35°C for 5 days, pulverized and stored in a polythene bag for further use.

Exposure to Light

The method of Bakare-Odunola et al. [9] was adopted for the exposure to the light sources. One hundred and seventy five grams (175 g) of each of the powdered rhizomes of the plant was weighed into three flat trays labelled UV, RL and SL. Two of the trays were each placed in two cartons affixed with a UV lamp in one and RL in the other, for the light exposure at room temperature. The third tray was exposed to sunlight. Seventeen grams (17g) of the powdered samples was weighed out from each of the light sources at time intervals of 1, 2, 3, 6, 12, 24, 48, 72, 144 and 168 hours for analysis.

Extraction

Extraction of the plant samples was done separately before exposure and after exposure at each of the time intervals for the three light sources. The exposed powdered rhizomes and the unexposed were extracted with 100 mL of 99.5% methanol by the maceration method and decanted after 48 hours. The solvent in the extract was allowed to evaporate over a water bath at 40°C. The percentage yield was calculated and thereafter preserved for further use.

Qualitative Phytochemical Analysis of RZO

The methanolic extracts were tested for the presence of alkaloids, flavonoids, saponin, steroid, terpenoids on the unexposed and exposed sample using the standard procedures [10].

Determination of total phenolics

Total phenolic contents were evaluated using Folin Ciocalteu colorimetric method [11] with some modifications. A solution of 0.028 mg of the extract was made up to 1 mL with methanol and then mixed with 1 mL Folin-Ciocalteu reagent previously diluted with water (1:9 v/v). After 5 minutes, 0.8 mL of 7% Na₂CO₃ solution was added with mixing. The tubes were shaken for 5 seconds and allowed to stand for 30 min at 40°C in an oven for colour development. Absorbance was then 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 different concentrations 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 GA equivalent/1mg of extract using the following equation based on the calibration curve: $y = 1.99x$, $R^2 = 0.93$, where y was the absorbance and x was the concentration.

Determination of total flavonoids

Colorimetric aluminum chloride method was used for flavonoid determination of each extract [12]. Equivalent of 0.3mg from a stock solution of the extract was diluted to 1.4 mL with methanol. The solution was mixed with 1.5 mL of 2% aluminum chloride. After 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 different concentrations of 0.06 to 0.18 mg/mL was used as standard. All tests were performed in triplicates. Total flavonoid contents were calculated as quercetin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.0.23x$, $R^2 = 0.9424$, where y was the absorbance and x was the concentration.

Saponin determination

The method by Ezeonu and Ejikeme [13] with some modifications was used for saponin determination. One hundred grams (100 mg) of the extract was dissolved in 4 mL of methanol and separated in two equal halves in two different separating funnels. In each separating funnel, 5 mL of distilled water was added into it, then 4 mL diethyl ether was also added and shaken vigorously. The ether layer was discarded, while the purification process was repeated. Four millilitres (4 mL) of n-butanol was added and shaken, then allowed to stand for separation after which the n-butanol layer was then collected. This process was repeated five times. The n-butanol extracts were washed twice with 2 mL of 5% aqueous sodium chloride after which it was dried over a water bath at 40°C and the % saponin content was calculated. The test was carried out in duplicate.

Assay of DPPH scavenging activity

The Assay of DPPH radical-scavenging activity of the test extracts was examined as described by Aryal et al. [12] with some modifications. Different concentrations (0.03- 0.1 µg/mL) of each extract were added, to an equal volume, to methanolic solution of DPPH (100 µM). The mixture was allowed to react at room temperature in the dark for 30 minutes. Vitamin C was used as standard control while a

mixture without the extract was taken as blank. Two replicates were made for each test sample. After 30 minutes, the absorbance was measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

$$\frac{\text{Absorbance (DPPH)} - \text{Absorbance (Extract)}}{\text{Absorbance (DPPH)}} \times 100$$

IC₅₀ values denotes the half effective concentration of sample which is required to scavenge 50% of DPPH free radicals. The IC₅₀ values 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 assay

Staphylococcus aureus (clinical isolate 41 and Typed culture 25913), (*Escherichia coli*. Typed culture 35928) and the fungus *Candida albicans* were used in this study in the antimicrobial assay. These organisms were collected from the Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutical Microbiology and Biotechnology University of Ilorin, Nigeria.

The antimicrobial assay of RZO extract was performed by deep-well agar diffusion method using Mueller-Hinton agar (bacteria) and Saboraud dextrose agar (fungi). Agar plates were prepared as specified by the manufacturer. The agar was inoculated separately with 10⁷ CFU of test microbe culture and evenly spread on the entire surface of each plate. The agar was carefully punched using a cork-borer No 6, 4 wells were bored at equal distance around the plates, and the extracts were dispensed into the well's agar wells seeded with bacterial culture. The plates were left at ambient temperature for 30 minutes and then incubated at 37°C for 18-24 hours and 25°C for 48-72 hours for bacteria and fungi respectively. The diameter of inhibition zones was measured using a ruler in millimeters. The 3rd and 4th wells served as positive and negative controls. The negative and positive control well was filled with sterile dimethyl sulfoxide and gentamycin respectively [14].

Statistical Analysis

Results were analysed using Graph Pad prism 7 (GraphPad prism 6 software. Inc, USA), and the results were expressed as mean ± standard error of mean, (n=3). Values carrying * are significantly different (p<0.05) from the zero hour for each parameter.

Results

Percentage yield of RZO

The percentage yield of the extract after exposure to UV light was the highest followed by that exposed to RL while that exposed to SL was the least as shown in Figure 1.

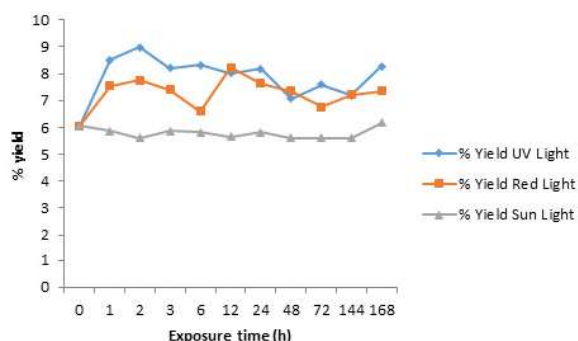


Figure 1. Percentage yield of the methanol extract of RZO before and after exposure to red light and ultraviolet and sun light from 0-168 hr.

Qualitative phytochemistry of RZO

The unexposed methanol extract of RZO was found to contain saponins, phenols, tannins, cardiac glycosides, flavonoids, alkaloids, steroids as shown in Table 1.

Table 1. Qualitative photochemistry of RZO leaves.

Phytochemical Constituent	Methanol
Alkaloids	+
Cardiac Glycosides	+
Flavonoids	+
Saponins	+
Steroids	-
Phenols and Tannins	+
Terpenoids	+

KEY: + = Presence; - = Absence.

Quantitative phytochemistry

The phenolic content of the RZO after exposure to UV was generally higher followed by that exposed to SL while that exposed to RL was the least (Figure 2). The highest flavonoid content was at the 24 hour exposure for UV, that for RL was at the second hour of exposure while that for SL was at the 168 hour of exposure as shown in Figure 2.

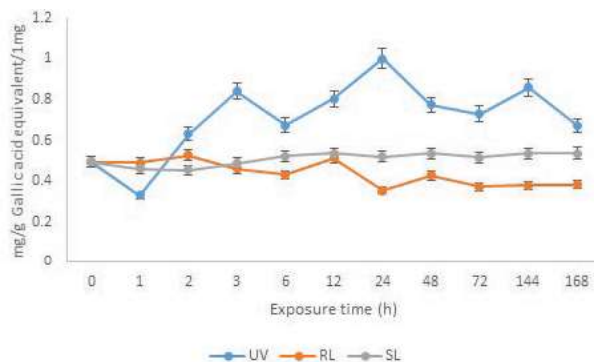


Figure 2. Phenolic Content of *Zingiber officinale* rhizome before and after exposure to UV, red and sun light.

The flavonoid content of the RZO after exposure to RL was decreasing as the exposure time increased while that for UV increased up to the 12 hour exposure and then started decreasing as shown in Figure 3. That for SL also decreased upon exposure up to the third hour of exposure and

generally increased after (Figure 3). Generally the flavonoid content for exposure to SL was higher than that exposed to UV then last with that of RL (Figure 3).

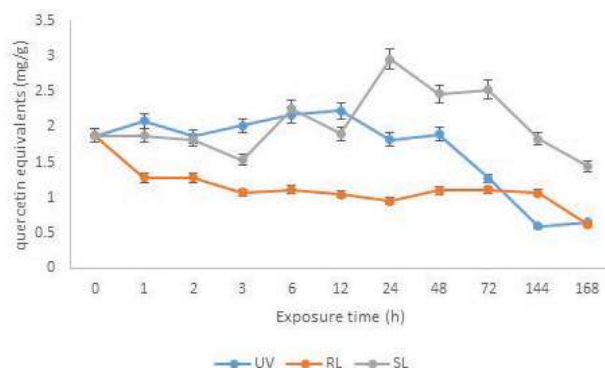


Figure 3. Flavonoid Content of *Zingiber officinale* rhizome before and after exposure to UV, red and sun light.

The highest and lowest percentage of saponin was at 2 h and 168 h of exposure respectively to RL and SL (Figure 4). A constant reduction was observed after 6 h and 72 h of exposure to RL and UV respectively.

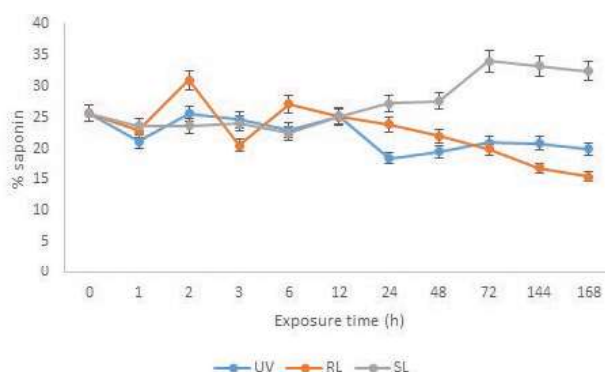


Figure 4. Saponin Content of *Zingiber officinale* rhizome before (0 hr.) and after exposure to UV, red and sun light.

Antioxidant Activity

The antioxidant activity of the RZO increased with increase in exposure to UV and RL while that exposed to SL decreased generally up to the 12 hour exposure time and then started increasing as the exposure time further increased shown in Figure 5.

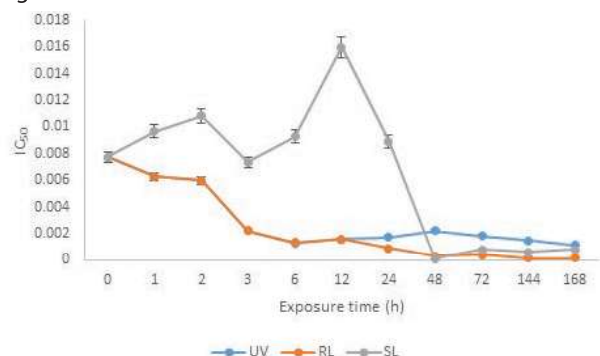


Figure 5. IC₅₀ of *Zingiber officinale* rhizome before and after

exposure to UV, Red and Sun light (UV = Ultraviolet light, RL= Red light, SL = Sun light; Data are mean $\pm$ SEM, (n=3).

Antibacterial Properties

The RZO extract before exposure showed antibacterial activity against *S. aureus* 25913. After exposure, the activity becomes inconsistent as shown in Figure 6. After the two hours exposure to RL, the activity against *S. aureus* 25913 was lost completely likewise upon exposure to UV light, there was no activity in the 1 hour exposure and later loss of activity from the 6 to 168 hour exposure time. Exposure to sunlight shown activity except at the 3, 12 and 24 hour exposure time (Figure 6).

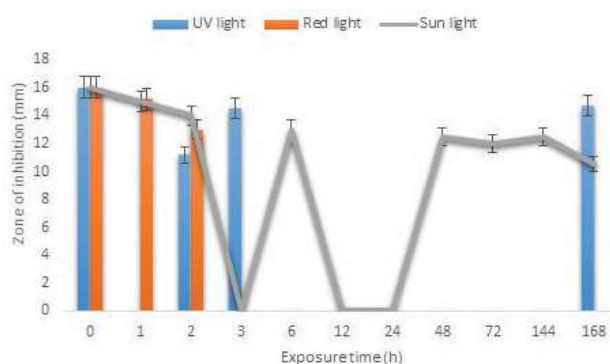


Figure 6. Effect irradiated RZO before and after exposure to UV, Red and Sun light on the susceptibility of *S. aureus* 25913(n=3)

The RZO extract before exposure showed antibacterial activity against *S. aureus* 41. After exposure to RL, the activity against *S. aureus* 41 was lost completely at the 1-2, 24 – 168 hours exposure time with activity only at the 3-6 hour exposure time. Exposure to UV and SL showed activity until 24 hour and thereafter, complete loss of activity (Figure 7).

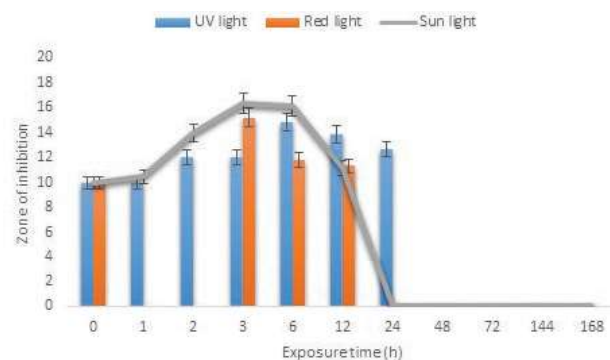


Figure 7. Effect irradiated RZO before and after exposure to UV, Red and Sun light on the susceptibility of *S. aureus* 41(n=3).

The activity against *E. coli* after exposure generally was less than that after exposure. Exposure to UV light showed activity up to the 48 hour exposure and loss of activity after 48 to 168 hour exposure. Likewise, exposure to SL showed activity up to the 24 hour and complete loss of activity after 24 hours onward. Exposure to RL showed activity only at the first and 24 hour exposure time (Figure 8).

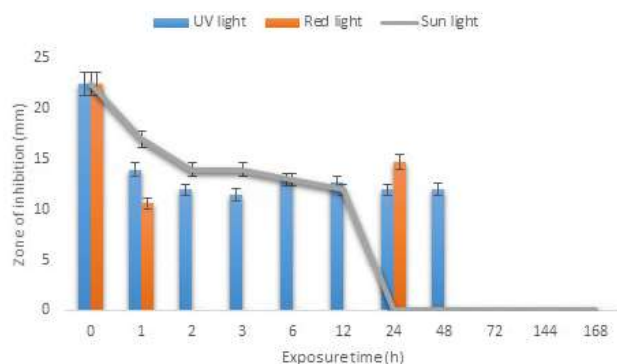


Figure 8. Effect irradiated RZO before and after exposure to UV, Red and Sun light on the susceptibility of *E. coli* (n=3).

Generally, the activity of RZO against *C. albicans* before exposure was higher than after exposure to all the different radiations. RL and SL showed activity throughout exposure while UV showed activity up to the 72 hour only (Figure 9).

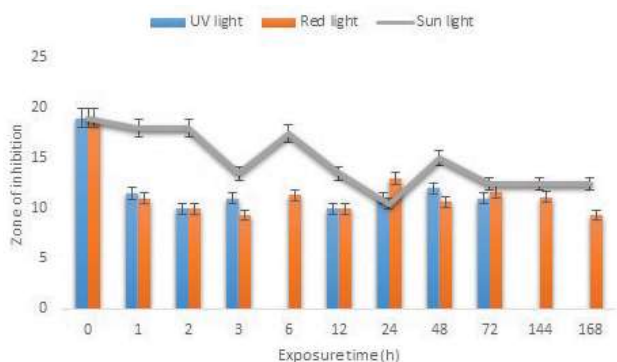


Figure 9. Effect irradiated RZO before and after exposure to UV, Red and Sun light on the susceptibility of *C. albicans* (n=3).

Discussion

The relative increase in extractive value after exposure to UV and RL but not SL was noted (Figure 1). The differences in the extraction yield could be caused by several factors such as differences in solubility and polarity [15]. Saponins, phenols, tannins, cardiac glycosides, flavonoids, alkaloids, steroids were present in the methanol extract (Table 1) which is similar to literature [16].

The decrease in flavonoid content upon exposure to RL (Figure 3) shows that RL requires a shorter time to reduce total flavonoids. There was a significant reduction in samples exposed to UV after 48 h exposure.

The reduction in the total flavonoids when exposed to UV light may be due to UV induced effect, while the slight increase between 6-12 h may be due to photochemical transformation [23]. In a similar study carried out by Zvezdanović et al. [17] where the degradation of flavonoids (quercetin) was dependent on UV-photon energy input on quercetin. The flavonoid content after exposure to SL was inconsistent as shown in figure 3. Variability in the level of flavonoids could be as a result of photochemical

transformation that flavonoids may undergo. A photochemical transformation requires excitation of an electron from a ground state orbital to an excited state orbital, this is usually achieved via the absorption of light by a chromophore [18].

There was a significant increase in phenolic content for UV, while that for RL and SL was nearly constant throughout exposure (Figure 2). Various chemical reactions may have occurred such as the formation of intermediate products of phenol to cause a significant rise and fall in total phenolics when exposed to UV and RL. The difference in the phenolic content of *Z. officinale* when compared to those exposed to the three light sources could be caused by a change or degrade in the structure of polyphenols, resulting in marked changes in their concentration [19].

RL showed a greater reducing effect on total saponins as compared to UV while SL showed a greater % saponin content. Abiotic stress factors such as light and temperature can influence both the quality and quantity of saponin content [20]. A similar result was observed for saponin content in a study carried out by Mechthild in 1981 on exposing *Avena sativa* seedlings to RL.

The antioxidant activity of the extract of RZO exposed to UV light correlated with the total phenolic content indicating that phenolic compounds are dominant contributors of antioxidant activity of the extracts. The finding (implies) that phenolic compounds have the ability to scavenge the reactive oxygen species and are commonly accepted as the most vital antioxidative plant component [3]. On exposure to RL, high antioxidant activity was observed when compared to the unexposed (Figure 5) while that of SL was inconsistent. This could be as a result of lower levels in the phenolic content on exposure to RL and SL. This is possibly due to reduction of bioactive phenolic antioxidants in the extracts. Overall, all the irradiated extract exhibited significant DPPH radical scavenging activity as compared to the standard ascorbic acid with IC₅₀ 41.25 µg/mL [24]. RL showed a greater reduction effect on antioxidant activity which is in line with a study carried out by Samuolienė et al. [21] where red light was observed to negatively affect the antioxidant activity in Lettuce.

There was a decrease in antibacterial activity of the exposed extract against *S. aureus* 25913, *S. aureus* 41, *E. coli* and *C. albicans* (Figures 6-9). There was no activity *S. aureus* 25913 after the 6h for both red and UV light (Figure 6). All the exposed rhizomes lost activity against *S. aureus* 41 after the 48 h exposure (Figure 7). Exposure to red light causes loss of activity against *E. coli* after the 2 h exposure (Figure 8). The decrease in activity on exposure to RL correlates with the reduction in flavonoids. For the UV and SL, it might have been due to photo deterioration and chemical decomposition resulting in reduced bioactivity [22].

In conclusion, saponins, flavonoids and phenolics present in RZO become unstable on irradiation with UV, red light and sunlight, which has a negative effect on its antimicrobial activities but a positive effect in antioxidant properties. The

study revealed the possibility of photodegradation of phytochemicals. Thus herbal hawkers and traditional health practitioners needs to preserve their plant material under shade to prevent degradation and to obtain maximum benefit

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