Original research article

Antibacterial and norfloxacin potentiation activities of *Ocimum americanum* L. against methicillin resistant *Staphylococcus aureus*

Sunday O. Oyedemi[1], Blessing O. Mbaebie-Oyedemi[2], Roger M. Coopoosamy[1] Jose M. Prieto[2], Paul Stapleton[2] and Simon Gibbons[2*]

[1]Department of Nature Conservation and Ethnobotany, Mangosuthu University of Technology P. O. Box 12363 Jacobs, 4026, Durban, South Africa

[2]Research Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom

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*****Author to whom correspondence should be addressed; simon.gibbons@ucl.ac.uk

T:0044(0)2077535913

F:0044(0)2077535964

Abstract

Staphylococcus species are among the most common resistant bacteria associated with the major cause of human ailments. The crude methanol extract from *Ocimum americanum* (OA) leaves was tested alone or in combination with norfloxacin (NOR) against strains of *Staphylococcus aureus* using the broth microdilution assay. The cytotoxicity of the OA extract was also evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent assay on a HepG2 hepatocarcinomal cell line. Whilst the plant extract exhibited a mild to poor antibacterial activity against our panel of bacteria, the antibiotic activity of norfloxacin at one-quarter MIC was enhanced by 2-4 fold in the presence of one-half MIC of OA extract against SA-1199B that over expresses the NorA efflux pump and against MRSA-274829 with synergistic effect. These positive interactions were confirmed using a time-killing test; the combination therapy completely attenuated the bacterial count of SA1199B but yielded a 4.2-$\log_{10}$-CFU/mL decrease for MRSA274829 after 24h incubation. The OA extract strongly depleted DPPH* (IC_{50}: 146.5µg/mL), LOI (152 µg/mL), PGI (47.6 µg/mL) and FRAP (122.75 µmolFe(II)/g) which is partly explained by the FRAP activity due to its richness in phenolic compounds. Furthermore, the OA extract showed a non-toxic effect on the HepG2 cells having an IC_{50} value of 378.0 µg/mL. These findings therefore support the folkloric use of *Ocimum americanum* at least in part for the treatment of infectious and free radical stress-related diseases.

Keywords: *Ocimum americanum*; polyphenols, HPLC fingerprint; cytotoxicity; MRSA; resistance modifier

1. Introduction

The global distribution of multidrug methicillin-resistant staphylococci is increasingly limiting the effectiveness of current antibiotic therapeutic agents due to acquired resistance to drugs (Nascimento et al., 2000). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most problematic clinically-relevant pathogens due to its intrinsic virulence and ability to adapt to various environmental conditions (Waldvogel, 2000). It is a leading cause of nosocomial resistance despite the availability of effective antimicrobials (Luzzaro et al., 2002). The high prevalence of infectious diseases caused by MRSA bacterial strains has motivated the search for new antibacterial agents with the capacity to circumvent resistance mechanisms or to modulate the activity of older antimicrobials (Sibanda and Okoh, 2007). Medicinal plants remain a natural reservoir of biologically active metabolites with unique chemistry that may perhaps hold a solution to the development of new drugs and to alleviate many side effects often related with modern medicines. However, only a small number of these botanicals has been evaluated and received rigorous scientific evaluation to assess their efficacy.

Ocimum americanum L (Lamiaceae) also known as *Ocimum canum* is generally distributed through India and tropical Africa (Khare, 2007). The leaves are tiny, elliptic lanceolate, faintly toothed and gland-dotted and are endowed with an aromatic essential oil. The plant is locally known as "Efinrin elewe dudu" in south-west region of Nigeria where its leaves is prepared as decoction or infusion for the management of piles, dysentery, fever, coughs, cold, skin diseases, diabetes, indigestion, diarrhoea, toothache, migraine and microbial infections (Ekundayo et al., 1989). It is also used in the preparation of local soup local among the Yoruba tribe and local condiment possibly due to its aromaticity (Bassole et al., 2005). Recent studies show the hepatoprotective and antioxidative capacities of the ethyl acetate, *n*-butanol, hydroalcoholic and acetone extracts from the leaves of *Ocimum americanum* (Aluko et al., 2012; 2013; Oboh, 2008). So far, the main chemical constituents isolated from OA leaf extracts include methyl cinnamate, methylheptenone, d-camphor, citral, ocimin, linalool, nevadensin, beta-sitosterol, betulinic, ursolic, oleanolic acids and pectolinarigenin-7-methyl ether (Ekundayo et al., 1989), which are mostly volatile. Berhow et al. (2012) identified rosmarinic acid and caffeic acid derivatives in the aqueous leaf extract of *Ocimum americanum* with diverse therapeutic potentials. The majority of the studies conducted on this botanical reported some biological activities of its essential oil but there is a paucity of scientific data existing on the antibacterial

activity of OA leaf extracts against strains with characterised efflux-mediated mechanisms of resistance. The present study, therefore reports the antibacterial, bacterial-resistance modulation and time kill studies of *Ocimum americanum* against a panel of *Staphylococcus aureus* strains as well as cytotoxicity and HPLC-DAD fingerprints of its phenolic compounds.

2. Materials and Methods

2.1. Plants collections and preparation

The dried and powdered leaves of *Ocimum americanum* (OA) were obtained from a local farmland near Orin Ekiti South-Western Nigeria in May, 2011. It was identified and authenticated by Mr. Omotayo (herbarium curator) at the Department of Plant Science, University of Ado-Ekiti, Nigeria where the voucher specimen (OA-001) was deposited. Fifty grams of the dried powdered plant material was extracted in 1 L methanol on a mechanical shaker (Labotec Scientific Orbital Shaker, South Africa) for 48 h and the extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The volume was concentrated under vacuum at 40°C to recover the solvent and stored in the refrigerator at 6^{o}C for future use. The herbal preparation involved the boiling of a handful of broken leaves in approximately 1.5 L of water for 15 min, after which the decoction was strained and served. Methanol was chosen in this study to bridge the gap and as a good solvent for the extraction of phenolic compounds.

2.2. Bacterial strains

Staphylococcus aureus strains NCTC 12981 (methicillin-susceptible), NCTC-13373 (methicillin-resistant), MRSA- 274829, EMRSA-15 and the susceptibility testing standard *S. aureus* isolate ATCC-25923 (methicillin-resistant) were obtained from Dr Paul Stapleton, UCL School of Pharmacy. SA-1199B, kindly provided by Dr G. W. Kaatz, is a multidrug-resistant *S. aureus* strain that overexpresses the NorA efflux pump. EMRSA-15 is a major epidemic methicillin-resistant *S. aureus* (MRSA) strain prevalent in UK Hospitals (Richardson and Reith, 1993) and is notable for the presence of mecA gene which contributes to the resistance phenotype. The inocula of the test organisms were prepared using the colony suspension method (Oluwatuyi et al., 2004) with adjustment utilising a 0.5 McFarland turbidity standard followed by dilution to achieve 5×10^5 colony forming units per mL (CFU/mL).

2.3. Cells culture and Maintenance

The HepG2 cell lines (American Type Culture Collection) were obtained from Prof. Andreas Kortenkamp at the UCL School of Pharmacy. HepG2 cells were replenished with new growth medium every 2-3 days and sub-cultured when reaching 80% confluence. Cells were maintained in Dulbecco's Modified Eagles Medium (DMEM) (Invitrogen, UK) containing 10% fetal bovine serum (GIBCO 10010) and antibiotics [Penicillin (100 IU/mL) and streptomycin (100 µg/mL)] incubated at 37ºC in a 5% CO_2 humidified atmosphere. The cells were allowed to grow in the medium before harvesting for the MTT assays. The cell density was adjusted to 7.5 x 10^4 cells/mL before exposed to different concentrations of plant extracts prepared in 1 % v/v DMSO. The assays were performed in triplicate for each extract concentration while the mean values were calculated and compared with the controls.

2.4. Ferric reducing antioxidant power (FRAP)

The antioxidative capacity of the OA extracts to reduce ferric ions was measured using the method described by Benzie and Strain (1999). The FRAP reagent was prepared by mixing 20 mL acetate buffer (300 mM sodium acetate, pH 3.6), 2 mL TPTZ (tripyridyl-s-triazine) solution, with 2 mL of $FeCl_3$ solution and 2.4 mL of distilled water. The straw coloured solution was incubated in a water bath at 37ºC for 10 min. 10 µL of the sample was transferred into a 96 well plate and allowed to react with 250 µL of freshly prepared FRAP reagent in the dark. After 5 min, the absorbance was measured and recorded at 593 nm against a blank solution (distilled water instead of extract). Results were expressed as catechin equivalents (mg/g) using the equation from the calibration curve: $Y = 0.3329x$, $R^2 = 0.9763$ where x was the absorbance and Y the catechin equivalent (mg/g).

2.5. Free radical scavenging activity (FRSA) using the DPPH assay

The DPPH radical scavenging properties of methanol crude extracts from OA leaves were evaluated according to the method of Yan et al. (1998) with slight modification. Twenty five milligrams of the crude extract (3.125-100 µg/mL) were dissolved in methanol and 1.5 mL of this solution was added to 1.5 mL of 0.1 mM DPPH dissolved in DMSO. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was read at 517 nm after 2 min. The FRSA was calculated as $[A_o\text{-}A_1/A_o \times 100]$, where A_1 was the absorbance of 1.5 mL of the crude extract solution mixed with an equal volume of the DPPH solution whereas A_o was the absorbance of DPPH mixed with an equal volume of DMSO. The experiment was carried out in triplicate to determine the mean values. Caffeic acid was used as a reference standard.

2.6 Inhibition of lipid oxidation (LOI)

The inhibitory potential of methanol extracts from OA leaves against lipid peroxide formation was determined using a modified thiobarbituric acid-reactive species (TBARS) assay (Dharmananda, 2012). Egg homogenate (0.5 mL, 10% in distilled water, *v*/*v*) and 0.1 mL of the methanol leaves extracts from OA was mixed in a test tube and made up to 1 mL with distilled water. A volume of 0.05 mL $FeSO_4$ (0.07 M) was added to the above mixture and further incubated for 30 min, to induce lipid oxidation. Thereafter, 1.5 mL of 20% acetic acid (pH 3.5), 1.5 mL of 0.8 % *w*/*v* TBA prepared in 1.1 % *w*/*v* sodium dodecyl sulphate (SDS) and 0.5 mL of 20 % *w*/*v* TCA were added, vortexed and then heated in a boiling water bath for 60 min. To avoid non-MDA interference due to high anthocyanin formation, another set of samples were prepared in the same manner, incubated without TBA. After cooling, 5.0 mL of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the upper layer solution was measured at 532 nm. For the blank, 0.1 mL of distilled water was used instead of the extract. The percentage inhibition (% LOI) of peroxide formation by the plant extract was calculated using the equation (1-E/C) x 100, where C is the absorbance value of the fully oxidized control and E is change in absorbance of TBA present/absent.

2.7 Inhibition of protein glycation

Inhibition of albumin glycation by the OA extracts was done according to the method described by Matsuura et al. (2001). Six tubes were prepared for the blank and each of the extract concentrations (3, 6 and 12.5 µg/mL) was mixed with 400 µL of 1 mg/mL BSA and 90 µL of 1.11M glucose in 50 mM phosphate buffer (pH 7.4). Three of these samples were incubated in a 60°C water bath for 24 h with 10 µL of extracts or distilled water while another three tubes were kept at 4°C. After incubation, 100 µL of each sample was transferred to a new tube and 10 µL of cold 100% TCA was added to each tube. They were vortexed and centrifuged at 15000 × g for 4 min at 4°C. The pellet [containing advanced glycation end products (AGE)] was collected and dissolved in 400 µL of alkaline phosphate buffered solution (PBS). The fluorescence intensity was measured using a Multiscan MS microtitre plate reader with an excitation/emission wavelength of 370/440 nm.

2.8. Minimum inhibitory concentration (MIC) assay

The Minimum Inhibitory Concentration (MIC) of antibiotics and plant extract were determined against multidrug-resistant *Staphylococcus aureus* via the standard method of the Clinical

Laboratory Standards Institute (1994). *S. aureus* strains were cultured on nutrient agar (Oxoid) and incubated for 24 h at 37 °C prior to MIC determination. A volume of 100 µL of sterile Mueller-Hinton broth (MHB: Oxoid) containing 20 mg/L and 10 mg/L of Ca^{2+} and Mg^{2+} respectively was dispensed into 10 wells of a 96 well microtitre plate. The plant extract was dissolved in dimethyl sulfoxide (DMSO) and further diluted in MHB to give a stock solution. A volume of 100 µL of the antibacterial agent stock solution (2.048 mg/L) was serially diluted into each well and then mixed with 100 µL of standardized bacterial inocula to give a final concentration range from 512 to 1 mg/L. All procedures were performed in duplicate and the plates were incubated for 18 h at 37 °C. A volume of 20 µL of a 5 mg/mL methanol solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) was added to each well and incubated for 30 min. A blue coloration indicated bacterial growth. The MIC was recorded as the lowest concentration at which no visible growth was observed.

2.9. Antibacterial modulation assay

The OA extract was evaluated as an antibiotic resistance modulator or modifier against *Staphylococcus aureus* resistant bacteria strains SA-1199B, NCTC 13373, NCTC 12981 and MRSA- 274829 in the presence or absence of norfloxacin or reserpine. Antibiotics or plant extracts were prepared in Mueller Hinton Broth (MHB) for synergistic combination with norfloxacin. Reserpine (20 µg/mL) a standard antibiotic modulator was used as the positive control. 100µL of norfloxacin or plant extract or reserpine was serially diluted across the plate, followed by adding 100 µL of each bacterial inoculum and then incubated at 37^{o}C for 18 h. A volume of 20 µL MTT (5mg/mL) was added to the overnight cultures to detect bacterial growth by a colour change from yellow to blue. The MIC was determined as the lowest concentration at which no growth was observed.

2.10. Time kill-kinetics of the extract-antibiotic combination

The combinatorial effects of methanol extracts and antibiotics against SA-1199B and MRSA 274829 using the time kill assay as a method of investigation was used with slight modification (NCCLS, 1999). For time-kill assays, bacteria were freshly sub-cultured in MHB until the exponential phase was reached, diluted to approximately 5×10^5 CFU/mL in MHB containing antimicrobial agents (extract and antibiotics) and incubated for 24 h at 37^{o}C with aliquots were taken from controls and the test samples at regular time intervals. To prevent drug carryover during serial dilution plating, aliquots were transferred to a recovery medium containing 3% Tween 80 and later plated on to nutrient agar in duplicate. The plates were incubated at 37^{o}C

for 24 h under aerobic conditions. The number of surviving organisms in the presence of the combination was counted and the mean counts (CFU/mL) for each treatment were expressed as a logarithmic reduction. Bacteriostatic and bactericidal activities were defined as < 3-$\log_{10}$ reductions and ≥ 3-$\log_{10}$ reductions in CFU/mL after 24 h of incubation, respectively, relative to the starting inocula.

2.11. MTT toxicity assay

The cytotoxicity of the herbal extract was determined using the MTT assay on human liver carcinoma cells as described by Mossman (1983). Briefly, HepG2 cells were seeded in 96-well plates with 1 x 10^4 cells in 0.1 mL of DMEM medium supplemented with 10% FBS and routinely cultured in a humidified incubator (37°C in 5% CO_2) for 24 h to reach full confluence. 100µL from each extract concentration were added, serially diluted (15.625-500 µg/mL) and then incubated for 24 h. After incubation, the medium was discarded and further incubated with 100 µL of tetrazolium dye (MTT) in 1 mg/mL PBS solution. The resulting dark blue formazan crystals formed due to mitochondrial activity after incubation was dissolved by adding 100 µL of DMSO into each well. The plate was then read on a microplate reader at 490 nm. MTT solution with DMSO (without cells and medium) acted as a blank control in the microplate reading whilst the PBS-treated cells served as a control of 100% viability.

2.12. HPLC/DAD analysis for phenolic compounds

The phenolic compounds in the leaf methanol extracts from OA were identified and characterized by HPLC-DAD according to the method described by Giner et al. (1993). All analyses were performed using a Hewlett-Packard Chemstation series 1100 chromatograph (Agilent, Palo Alto, CA, USA), coupled with a diode array detector. Ten milligrams of the plant samples were sonicated in HPLC grade methanol (1 mL) for 30 min. The resultant mixtures were centrifuged at 10000 g for 10 min and the supernatants were used for the HPLC/DAD analysis. The resulting solutions were filtered through a 0.22 µm filter and 10 µL aliquots of the filtrates were injected onto the HPLC system. This system comprised a RP Nova Pack C18 column (300 x 3.9 mm) packed with 4 µm particles and a pre-column containing the same packing material. The columns were eluted in two solvent systems; Solvent A (water/phosphoric acid, 0.1%) and solvent B (methanol). The solvent gradient was composed of A (75-0%) and B (25-100%) for 20 min, then 100% B for 4 min, then again at the initial conditions (75% A and 25% B) for 10 min. A flow rate of 1.0 mL/min was used at 30ºC. The UV spectral data for all peaks of different compounds were accumulated in 285 nm and

recorded using DAD. These compounds were identified by comparison of their retention times and spectra of each peak with those of known standards analysed in the same conditions.

2.13. Statistics

Data analysis was done on Microsoft Excel to obtain descriptive statistics. Mean values were separated by the Duncan multiple test using SAS. The different levels of significance within the separated groups were analyzed using one way analysis of variance (ANOVA). Values were considered significant at $P<0.05$.

3. Results and discussion

Humans are constantly exposed to free radicals (FRs) such as hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{-\cdot}$) and nitric monoxide ($NO^{\cdot}$) via cosmic radiation, cellular metabolism and pollutants including cigarette smoke (Fridovich, 1999). These radicals are also generated in the human body as by products through diverse physiological and biochemical processes. However, the accumulation of FRs has been implicated in most studies as a major cause of oxidative damage to biomolecules such as lipids, proteins and DNA, which can subsequently lead to neurodegenerative diseases (Gilgun-sherki et al., 2001; Oyedemi and Afolayan, 2011). Epidemiological studies have shown that most diseases caused by these FRs can be ameliorated upon intake of natural antioxidants (Zandi et al., 2004). In this work, the antioxidative capacity of the methanol extract from *Ocimum americanum* leaves was assessed via its ability to reduce ferric 2, 4, 6-tripyridyl-s-triazine [TPRZ-Fe (III)] complex to the coloured ferrous form [TPTZ-Fe (II)]. The OA extract exhibited a strong antioxidative potential, with an IC_{50} value of 122.75 µmol Fe (II)/g higher than the standard BHT (75µmol Fe (II)/g) but significantly lower as compared to catechin (859.32 µmol Fe (II)/g (Table 1). In the same manner, a strong free radical scavenging activity (FRSA) was recorded having an IC_{50} value of 146.5 µg/mL, though markedly lower as compared to the standard caffeic acid (13.22 µg/mL). Due to the high phenolic components, it is possible that the extract donated electron or protons through the termination or suppression of free radical chain reactions (Fecka and Turek, 2008) into stable non-radical products which corresponds to the findings of Zhang et al. (2010) on some *Ocimum* species.

Lipid oxidation (LO) is well-recognised as the major outcomes of free radical-mediated process, in which oxidative damage is propagated to polyunsaturated fatty acids via lipid-derived radicals (alkoxyl and peroxyl). The oxidation of lipids alters the physicochemical

properties of membranes, resulting into severe cellular dysfunction such as cataract, rheumatoid arthritis and neurodegenerative disorders (Dharmananda et al., 2012). The inhibitory potential of the OA extracts and the standard drugs against TBARS production in the egg yolk homogenate induced by $FeSO_4$ is presented in Table 1. The results are concentration dependent having IC_{50} values of 0.36, 0.06 and 0.102 mg/mL for the OA, BHT and gallic acid, respectively. The OA extract exhibited a strong antioxidative capacity to prevent MDA formation possibly due to its richness in phenolic compounds via different mechanisms such as chain breaking or termination (Hatano et al., 1989). Additionally, a possible synergistic interaction between the molecular compositions could also influence the observed LOI activity. Although, the LOI activity of OA extract is considered lower as compared to the standard BHT or gallic acid, however, being a natural product it could help protecting human cells against oxidative damage without causing adverse reaction. This could also justify its traditional use for the treatment of oxidative stress related diseases in the region.

Reactive oxygen species (ROS) has been implicated in several human diseases through biomolecules damage and as a result alter cellular metabolism. The non-enzymatic interaction of ketone or aldehyde groups of reducing sugar with the free amino groups of proteins, lipids and nucleic acids resulting to the formation of advanced glycation end products (AGE). The generation of ROS during glycation diminishes oxidative defence system of protein via thiol groups reduction which has been implicated in the pathogenesis of diabetic micro and macro-vascular complications (Kiho et al., 2000). Here, the plant extract suppressed the intensity of the fluorescent conjugated BSA (advanced glycation products) in a concentration dependent manner, indicating its antioxidative potential. The percentage protein glycation in the medium was reduced to 72.8, 65.7, 58.8, 53.2 and 48% at 3, 6, 12.5, 25 and 50μg/mL, respectively for OA extract while that of aminoguanidine at 0.12 μg/mL, a known inhibitor of protein glycation process, was reduced to 12.5% (Table 1). The PGI activity demonstrated by the OA extract was expressed as the IC_{50} (47.6 μg/mL), an effective concentration at which the glycation was inhibited by 50%, interpolated from the linear regression analysis. The result demonstrated that OA extract has anti-glycation properties at a concentration (47.6μg/mL) which is considered physiologically relevant and thus partially support its folkloric use in the treatment of diabetes.

Numerous research studies have demonstrated the biological activities of *Ocimum* species and their essential oils (Nakamura et al., 1999). However, the antibacterial activity of this botanical against our panel of bacteria is reported here for the first time. The results of anti-MRSA

susceptibility tests of OA extracts and norfloxacin (NOR) using the broth microdilution method are presented in Table 2. In this work, we considered the anti-MRSA activity of the extract significant (MIC<100 mg/L), moderate (100<MIC=512 mg/L) or weak (MIC>512 mg/L) against six MRSA strains based on the classification of Rios and Recio (2005). The MICs of the OA extract showed varied antibacterial activity between the range of 256 and ≥ 512 mg/L and norfloxacin between 0.5 and 64 mg/L. The plant extract exhibited a moderate activity towards MRSA-274869, ATCC-25923 and SA-1199B, having MIC values ranging from 256 to 512 mg/L but was inactive against NCTC-12981, NCTC-13373 and EMRSA-15 with MIC values > 512 mg/L. It is interesting to note that the OA extract inhibited the growth of SA-1199B that overexpresses the NorA efflux pump conferring a high level of resistance to certain fluoroquinolone. In this bacterium, where the mechanism of resistance has been confirmed to be the NorA efflux protein (Oluwatuyi et al., 2004; Gibbons, 2005) this suggests that the OA extract may contain an active principle that is not a substrate for the NorA efflux protein.

The use of combinatorial therapy has been suggested as a new approach to improve the efficacy of antimicrobial agents where monotherapy seems ineffective against drug resistant pathogens. Norfloxacin is a broad-spectrum antibacterial agent, commonly used for the treatment of a number of infectious diseases in clinical and veterinary medicine (Bjornsson et al., 2013; Beberok et al., 2015). In the recent times, the bacterial resistance to this agent has become an issue in antibiotic therapy. In this work, OA extract was tested for modulatory effect in the presence of norfloxacin towards NCTC-13373, SA-1199B, MRSA-274829 and NCTC-12981. The combination of the extract at one-half MIC with one-quarter MIC of norfloxacin showed no effect on the growth of NCTC-13373 and NCTC-12981 but displayed a reduction in the MIC of norfloxacin needed to attenuate the growth of MRSA-274829 and SA-1199B, respectively despite its weak antibacterial activity shown when tested alone. These results compared favourably well with reserpine a standard MDR inhibitor where the synergy interaction towards SA-1199B and MRSA-274829 is moderate. The observed activity indicated that OA extract could serve as a source of potential adjuvant of antibiotic or modulation of bacterial resistance mechanism (Oluwatuyi et al., 2004) considering the rich phytochemical constituents in the plant extract.

The synergistic interaction arising from the combination of the OA extract and norfloxacin was further explored by using a time kill assay against SA1199B and MRSA274829. This method provides qualitative information on the pharmacodynamics of antimicrobial agents (Basri et al., 2014). The results of the time-kill experiments are given in Fig 1 and 2. The data showed

that OA alone at one-half MIC induce cell death while norfloxacin at one-quarter MIC reduced the bacterial counts of SA-1199B and MRSA-274829 strains to 2.5 and 3.1 $\log_{10}$ CFU/mL respectively, as compared to the initial inoculum of 6 $\log_{10}$ CFU/mL after a 24h incubation period. However, the bacteriostatic activity of norfloxacin was enhanced upon the addition of the OA extract dramatically and increased its killing rate as compared to antibiotic alone. At 24 h of incubation, the bacterial growth of SA-1199B was completely attenuated to zero while MRSA-274829 was reduced to 1.8 $\log_{10}$ CFU/mL. This study provides a good concordance between the antibiotic potentiation activity and time-kill kinetics for the tested *S. aureus* strains. We found that the OA extract may perhaps have the potential to improve the effectiveness of norfloxacin activity by significantly reducing the growth of the effluxing bacteria SA-1199B and MRSA-274829. This result therefore confirms at least in part the use of OA extract in the treatment of microbial infections. Further studies are required to identify and characterized bioactive compounds responsible for the bacterial resistance modulation as well as probable mechanism of action.

High performance liquid chromatography coupled with diode-array detection (HPLC-DAD) is an indispensable tool for phenolic compound analysis due to its versatility, precision and relatively low cost (Zhao et al., 2008). Little or no scientific information is available for the polyphenolic composition of OA leaf extract. In this study, the chromatogram at 285 nm showed the presence of 32 peaks of phenolic compounds with retention times (RT) between 3 and 60 min. Here, none of the peaks identified based on their RT and UV/DAD absorption spectra corresponded with those of the standards (rutin, gallic acid, and quercetin) as shown in Fig 3. The HPLC fingerprints showed major peaks at the RTs (min) of 5.8, 10.2, 21.0, 24.7, 26.6 and 40.2. Zarlaha et al. (2014) identified rosmarinic acid (4.9 min) and caffeic acid (4.1 min) in *Ocimum basilicum* essential oil, a similar chemotype of *Ocimum americanum*, using HPLC coupled with DAD but these peaks are missing in this work, conceivably due to the difference in methodology and solvent system used. Nonetheless, the methanol crude extract from the OA leaves is rich in phenolic compounds and may justify the alcoholic decoction often employed during folkloric use for the treatment of diseases associated with oxidative stress. Further studies would be carried out to identify the major peaks of these compounds.

MTT is a rapid and high accuracy colorimetric approach currently in use to determine the cytotoxic nature of plant-derived products for new drug discovery (Mossman, 1983). The assay measured the integrity of the cell membrane via the enzymatic reduction of MTT to a blue formazan product. The number of surviving cells is directly proportional to the level of the

formazan product quantified using a simple colorimetric assay. Currently, there is a dearth of data on the toxicity of *Ocimum* spp. Nonetheless, *O. basilicum*, the most exploited species due to its robust therapeutic value and culinary uses has been reported to be somewhat toxic to certain tumour cells; HeLa (cervical cancer cells): 164.61±2.58 µg/mL; Femx (Human melanoma cells): 191.36±2.42 µg/mL; K502 (Erythroleukeamia cells): 157±2.25µg/mL and SKOV3 (Ovarian carcinoma cells: >200 µg/mL) (Zarlaha et al., 2014). The plant extract exhibited a weak cytotoxic effect on HepG2 hepatocarcinomal cells, having an IC_{50} value of 378 µg/mL, (Fig 4) which is significantly different as compared with the standard doxorubicin (0.5 µg/mL). This data therefore suggest that the OA extract did not impair mitochondrial viability and thus substantiates its 'safe' use as a complementary and alternative therapy for the treatment or prevention of diseases.

4. Conclusion

We have showed that *Ocimum americanum* had a strong antioxidative capacity which is evident from the data obtained from DPPH radical scavenging and ferric reducing antioxidant power due to its richness in phenolic compounds. The extract exhibited a synergistic interaction with norfloxacin in consonance with the time-kill kinetic data. Moreover, the OA extract was non-toxic towards human liver cells and hence, has some potential in the future design of safe combination anti-infective therapies against multidrug-resistant strains. Further studies are being carried out to isolate, characterize and understand the underlying mechanism of the extract-norfloxacin interaction.

Conflict of interest

The authors declare that there is no con+**********--++-*-*/

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flict of interest regarding the publication of this paper

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