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Research Article

Chemical Composition, Antioxidant, and Antimicrobial Activities of the Leaf and Fruit Essential Oils of the West African Plum, *Vitex doniana*

Jahnissi Frimpomah Odoom,[1] Caleb Impraim Aboagye,[1,2] Philipina Acheampong,[1,2] Isaac Asiamah,[3] Godfred Darko,[1] and Lawrence Sheringham Borquaye[1,2]

[1]*Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
[2]*Central Laboratory, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
[3]*Department of Chemistry, University of Cape Coast, Cape Coast, Ghana*

Correspondence should be addressed to Lawrence Sheringham Borquaye; lsborquaye.sci@knust.edu.gh

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Vitex doniana (West African plum or black plum) is a plant with varying phytoconstituents and biological activities across different countries. In this study, essential oils extracted from the leaves and fruits of *Vitex doniana* cultivated in Ghana were investigated for their antimicrobial and antioxidant activities. The antioxidant actions of the essential oils were determined using hydrogen peroxide (H_2O_2), phosphomolybdenum, thiobarbituric acid reactive substances (TBARS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. For both essential oils, the total antioxidant capacities ranged from 44 to 68 μg/g AAE, the IC_{50} values for H_2O_2 scavenging activity were between 87 and 242 μg/mL, whereas that for DPPH assay were between 322 and 599 μg/mL. The IC_{50} for the TBARS assay for both essential oils also ranged from 247 to 414 μg/mL. The antimicrobial activities of the essential oils were investigated using the broth dilution assay. The minimum inhibition concentration for the essential oils ranged from 12.5 to 50 mg/mL. Biofilm inhibitory activity was also evaluated for both essential oils, and the fruit essential oil showed a half-maximal inhibition of biofilm formation (BIC_{50}) at 44.40 ± 0.6 mg/mL, whereas the BIC_{50} value of the leaf essential oil was 109.1 ± 0.9 mg/mL. The fruit essential oil was superior to the leaf essential oil in inhibiting the secretion of pyoverdine. Molecular docking analyses suggested that methyl cinnamate, ethyl cinnamate, p-menth-4-en-3-one, trans-α-ionone, benzyl benzoate, isobutyl cinnamate, and folic acid likely interacted with LasR and algC proteins, and hence, contributed to the inhibition of biofilm formation and pyoverdine secretion. Essential oils from *Vitex doniana* could, therefore, be exploited as a natural source of radical scavenging and antimicrobial agents and could be useful in the pharmaceutical, food, and cosmetic industries.

1. Introduction

Essential oils are interesting natural plant products. Though they are obtained in minute quantities, they possess several biological properties such as antiviral, antioxidant, antibacterial, antifungal, and anti-inflammatory properties. Essential oils are usually identified by their strong fragrance which is peculiar characteristic of their source plants. These oils are mainly made up of mono- and sesquiterpenes, hydrocarbons, hydroxyl, and carbonyl derivatives, along with aliphatic aldehydes, ketones, alcohols, esters, and carboxylic acids. Essential oils have become an important part of everyday life. Their application is wide, extending from the pharmaceutical industries, food manufacturing and processing industries, perfumery and cosmetic industries, the medical sector to the agricultural domain [1]. Aromatic plants have an ancient legacy of application in traditional medicine due to their therapeutic properties. Interestingly, essential oils extracted from several plants have shown promise as potential therapeutic agents [2].

One area where essential oils have found profound application is in the mopping up of reactive chemical species. Uncontrolled reactive oxygen species (ROS) and reactive nitrogen species (RNS) production are known to cause redox imbalances and cellular oxidative stress. The effects of these ROS and RNS are harmful and have been reported to be critical in a variety of diseases such as neurodegenerative disorders, cancer, and autoimmune diseases. The oxidative degradation of lipids, described as lipid peroxidation, is associated with a marked decrease in the quality of food and loss of nutrients [3]. Antioxidants are powerful against oxidation effects and could massively help in the preventing and managing diseases caused by free radicals such as ROS. In the preservation of foods, antioxidants play a vital role in scavenging free radicals that induce lipid peroxidation. However, synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone, and propyl gallate, which are regularly used as preservants are frowned upon in the food industry due to their unsafe and unwanted side effects such as carcinogenicity [4]. Naturally derived antioxidants are generally preferred as they are considered safe and healthy. As a result, many studies have focused on unearthing natural antioxidants with low toxicity [5]. Many essential oils are known to have promising antioxidant effects and could be useful for various purposes. Essential oils obtained from various plant sources have good antioxidant activities and have, therefore, been touted as potential substitutes for artificial antioxidants [6]. These essential oils have been found to possess antioxidative compounds such as geraniol and carvacrol which elicit the antioxidant actions. The antioxidant ability of some of these compounds has been ascribed to the hydroxyl functionalities in their structure, which enables them to stabilize free radicals [7]. Rosemary and lavender essential oils have been used as a preservative in the food industry to control oxidation and retard food spoilage. They are used in the cosmetic industry to prevent oxidative stress and thus protect against skin damage and aging. They have also been shown to possess additional health benefits [8, 9].

Essential oils are also known to possess antimicrobial capabilities. Both foodborne and pathogenic microbes are susceptible to some essential oils. Bioactive volatile components such as terpenes, alcohol, esters, epoxides, aldehydes, ketones, and amines have been shown to play critical roles in the antibacterial actions of the essential oils [10]. These components can be present in major or minor quantities. The antimicrobial activities of volatile organic compounds have also been studied in modern medicine to uncover useful molecules for the treatment of human diseases, particularly those microbes that possess multidrug resistance capabilities. Different tactics are used by pathogenic bacteria to thwart the effects of antimicrobial substances. The emergence or acquisition of resistance genes, the growth of specialized persister cells, the modification of antimicrobial agents and/or antimicrobial target sites, the application of efflux methods, and the production of biofilms are a few examples of these [11, 12]. Some essential oils also possess antibiofilm and quorum sensing inhibitory capabilities [13–15]. Compounds such as carvacrol, linalool, and eugenol found in essential oils can inhibit biofilm formation and quorum sensing in the Gram-negative bacteria, *Pseudomonas aeruginosa* [16, 17].

P. aeruginosa uses a variety of virulence factors and processes, including biofilm formation, to successfully infect a wide range of hosts and defend itself against drugs and environmental stress [18]. Virulence factor production is mediated by quorum sensing (QS). The QS network in *P. aeruginosa* is organized in a multidimensional pyramid composed of about four interrelated signaling systems known as las, pqs, rhl, and iqs. As LasR sits at the apex of the QS circuit of *P. aeruginosa*, it has a vital role in infection formation and maintenance by the bacterium. *P aeruginosa* makes use of the LasR protein for the transcriptional regulation of quorum sensing signals that lead to the expression of virulence factors such as pyocyanin, pyoverdine, and proteases through a cascade of activities. LasR also regulates activities that support the formation of biofilm in *P. aeruginosa* [19]. Biofilms are structurally composed of three exopolysaccharides, namely, Psl, Pel, and alginate. Although the alginate polysaccharide is not required for the initiation of biofilm, it is a critical virulence agent in chronic infections, as it protects cells of *P. aeruginosa* from antimicrobial agents, and also suppresses the immune system of the host. The algC enzyme in *P. aeruginosa* is the only protein capable of catalyzing the conversion of mannose-6-phosphate to mannose-1-phosphate in alginate biosynthesis and the conversion of glucose-6-phosphate to glucose-1-phosphate in the biosynthetic pathway to the production of the LPS core [20, 21]. Inhibition of these 2 enzymes-LasR and algC-will interfere with biofilm formation in the bacteria. Thus, interfering with biofilm formation and quorum sensing processes are a potential route to dealing with the menace posed by *P. aeruginosa* and other related microorganisms.

Medicinal plants are known to possess useful compounds that have been shown to possess various biological activities [22, 23]. *Vitex doniana* (West African plum or black plum) is a flowering plant, of the family Lamiaceae, and is one such medicinal plant of importance. The *Vitex* genus has about 270 species of trees and shrubs in the tropical regions while a few species are also found in temperate zones [24]. *V. doniana* has a lot of useful applications—it serves as a source of food and medicine and provides timber for furniture [25]. Also, jam and syrup can be made from the fruit pulp. In folk medicine, the leaf, stem bark, root, and fruit pulp serve as ingredients in many medicinal preparations to treat ailments such as cancer, malaria, hypertension, headache, rash, fever, respiratory diseases, rheumatism, diarrhea, dysentery, high blood pressure, malnutrition, gonorrhea, and other inflammatory diseases [26, 27]. The essential oil of *V. doniana* has varying phytochemical constituents and biological activities. Sonibare and coworkers from Nigeria analyzed the leaf essential oil from *V. doniana* with β-phellandrene (31.13%), phytol (28.3%), and β-caryophyllene (12.6%) as the major constituents. The essential oil showed antimicrobial activity against *B. subtilis, P. mirabilis*, and *C. albicans* but not

against *S. aureus* and *P. aeruginosa* [28]. Another study on the constituents of the fruit essential oils in Nigeria identified eucalyptol (34.86%), 3-decenoic acid (16.91%), n-octanoic acid (14.86%), terpinene-4-ol (8.01%), and alpha terpineol acetate (4.29%) as the major constituents [29].

It is known that the chemical composition of essential oils is affected by cultivation conditions such as soil type and geographical location and environmental factors such as climate and habitat conditions [1]. As such, the chemical constituents of the fruit and leaf essential oils of *V. doniana* in Ghana were also analyzed in this study. The antioxidant and antibacterial capabilities of the essential oils were also assessed. This was followed by assessing the ability of the oils to interfere with biofilm formation and disrupt quorum sensing in *P. aeruginosa*. Finally, the potential role of the various oil constituents in biofilm formation and quorum sensing was evaluated by docking the compounds with the LasR and algC proteins. We report that the major compounds in the leaf and fruit essential oils of *V. doniana* from Ghana were long chain carboxylic acids, alkanes, and terpenes. The oils showed moderate antibacterial and antioxidant activities, with benzyl benzoate, isobutyl cinnamate, folic acid, trans-α-ionone, methyl cinnamate, and p-menth-4-en-3-one showing relatively strong binding affinities towards algC and LasR. Together, the results suggest that the oils could find use in pharmaceutical industries as antimicrobial agents and food processing industries as potential antioxidants against lipid peroxidation.

2. Methods

2.1. Plant Materials and Extraction of Essential Oil. Fresh leaves and matured fruits of *V. doniana* were obtained from Ekumfi Abor (5.2909°N, 0.94924°W) in the Central region of Ghana in September 2020. Samples were carried to the laboratory and stored at 4°C. Plant identity was authenticated at the Department of Herbal Medicine, KNUST. Voucher specimen identification codes for the fruit (KNUST/HMI/2017/F001) and the leaves (KNUST/HMI/2018/L058) of the plant were obtained, after which samples were deposited. The plant materials were cleared of any extraneous matter by washing. The fruit pulp was separated and the leaves were also cut into small pieces. The plant samples underwent steam distillation in a modified Clevenger-type apparatus for 3 hours. The pulp and leaves were weighed, and the masses were recorded before each extraction. Anhydrous sodium sulfate was used to dry the essential oils obtained after extraction. Oils were kept in the refrigerator at 4°C until needed for further analysis. Essential oil yields were determined and expressed as percentage w/w of the fresh materials.

2.2. Chemical Composition

2.2.1. GC-MS Analysis. The constituents of the oils were analyzed on a gas chromatograph-mass spectrometer (GC–MS–Perkin Elmer GC Clarus 580/Clarus SQ 8 S). The GC column used was ZB-5HTMS (5% diphenyl/95% dimethyl polysiloxane, DB-5 −30 × 0.25 μm ID × 0.25 μm DF). The GC program begun at 40°C and was kept for 2 min, followed by a gradual increase of 10°C every minute until the oven temperature got to 250°C. The rate was then changed to 20°C per minute to 280°C. An additional final hold at 280°C for 10 minutes completed the GC oven program. For the MS system, electron ionization was used. The system operated in the electron impact mode. Ionization energy of 70 eV was utilized. The injector and ion-source temperatures were maintained at 250°C and 220°C, respectively. The mobile phase consisted of ultrapure helium gas with a 1 mL/min flow rate that was kept constant. Sample injection volume was 1 μL. Mass spectra were taken with a scanning interval of 1 s and fragments from 50 to 4500 Da. The solvent delay was from 0 to 3 min, and the total run time was 34.5 min [13].

2.2.2. Compound Identification. To identify the oil constituents, the mass spectra obtained from GC-MS analyses were compared to those in the National Institute Standard and Technology (NIST) database and published literature. Similarity indices from the Wiley and NIST libraries for the GC-MS system and some published literature were used to assign compound names [28]. The peak areas were normalized, and the relative percentages of the various constituents were expressed as percentages.

2.3. Antioxidant Assays. Various assays were used to evaluate the antioxidant activities of the essential oils.

2.3.1. Phosphomolybdenum Assay (PM). Preparations were made with different concentrations of essential oils and ascorbic acid (as a standard drug). A test tube containing 1 mL of each test sample was mixed with 3 milliliters of the PM reagent, which consisted of 28 mM disodium hydrogen phosphate, 4 mM ammonium molybdate, and 0.6 M sulphuric acid. The mixture was then incubated at 95°C for 90 minutes. The same procedure was also carried out with the ascorbic acid, and the absorbance of the reaction mixtures was measured at 695 nm (using a Specord 200 Plus, Germany) against a blank solution after the mixtures had cooled to room temperature. The antioxidant capacity was expressed as μg of ascorbic acid equivalent (AAE) per gram of extract used [30].

2.3.2. DPPH Radical Scavenging Assay. Different concentrations (50 μL) of test samples were added to a 100 μL methanolic solution of DPPH, followed by incubation of the reaction mixture in the dark for some time (30 mins). Absorbances were taken at 517 nm (Analytik Jena Specord 200 Plus, Germany). Methanol was used as a negative control, with L-ascorbic acid as the positive control. The DPPH radical scavenging activity was calculated according to the following equation:

$$\%\ \text{DPPH radical scavenging activity} = \left(\frac{A_c - A_s}{A_c}\right) \times 100\%, \quad (1)$$

where A_c is the blank absorbance and A_s is the sample absorbance.

The % DPPH radical scavenging activity was plotted against the various concentrations to determine the IC_{50}.

2.3.3. Hydrogen Peroxide (H_2O_2) Scavenging Assay. To 0.065 mL of hydrogen peroxide (5 mM) was added 1.5 mL of variable amounts of the essential oil (made in 10% DMSO) and 0.25 mL of ferrous ammonium sulfate (1 mM), followed by sample incubation at room temperature in the dark for 5 mins. After this, 1.5 mL 1,10-phenanthroline (1 mM) was added and well shaken. They were again left to stand for 10 minutes. Absorbances were then taken at 510 nm. A blank solution made up of only ferrous ammonium sulfate and 1,10-phenanthroline was also made and carried through the analyses. The percentage amount of hydrogen peroxide scavenged by the oil was derived from the following equation:

$$\%\ \text{Hydrogen Peroxide Scavenged} = \frac{A_{\text{test}}}{A_{\text{control}}} \times 100, \quad (2)$$

where A_{test} is the sample absorbance and A_{control} is the absorbance of ferrous ammonium sulfate and 1,10-phenanthroline.

The IC_{50} values (extract concentration required to achieve 50% scavenging of hydrogen peroxide) were deduced from the graph of percentage hydrogen peroxide scavenged against concentration.

2.3.4. Inhibition of Lipid Peroxidation Assay (TBARS). To perform the assay, a 10% solution of egg homogenate in 1.5% KCl was used as the source of oxidizable substrate. A stock reagent was prepared using 1.5 mL of 20% acetic acid with pH 3.5 and 1.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) made in 1.1% (w/v) sodium dodecyl sulfate. Various amounts of essential oils made in methanol were added to 0.1 mL of egg yolk homogenate, followed by the addition of 0.4 mL of distilled water and 0.05 mL of $FeSO_4$ (0.07 M) to induce lipid peroxidation. The test tubes were allowed to stand at 37°C for 30 minutes, after which 1.5 mL of 20% acetic acid and 1.5 mL of the TBA stock reagent were added. The mixture was boiled for one hour at 95°C, cooled, and 5 mL of n-butanol was added. The mixture was then centrifuged and the absorbance of the butanol layer was measured at 535 nm. Methanol was used as a blank control, and BHT was used as a positive control [30]. The percentage inhibition of lipid peroxidation was derived from absorbance obtained using the following equation:

$$\%\ \text{Inhibition} = \left(\frac{A_c - A_s}{A_c}\right) \times 100\%, \quad (3)$$

where A_c is the blank absorbance and A_s is the sample absorbance.

The IC_{50} values were derived from the graph of % inhibition against concentration.

2.4. Antimicrobial Assay

2.4.1. Microbial Cultures. The microorganisms used for the study were obtained from the Microbiology lab of the Pharmaceutics Department KNUST. The strains of microorganisms used were Gram-positive bacteria (*S. aureus* ATCC 29213, *B. subtilis* NTCC 10073, *E. feacalis,* and *S. pneumoniae* ATCC 49619), Gram-negative bacteria (*P. aeruginosa* ATCC 27853 and *E.coli* ATCC 25922), and fungus (*C. albicans*).

2.4.2. Minimum Inhibitory Concentrations (MIC) Determination. Essential oil MIC was determined by transferring 100 μL of two-fold serial dilution of the oil into wells of a sterile 96-well microtiter plate containing 100 μL of double-strength nutrient broth with an inoculum of ~2.0×10^5 CFU/ml. Experiments for sterility control, growth control, and solvent control were also conducted. After incubation for 24 hours at 37°C, 20 μL of 1.25 mg/mL MTT solution was added to each well and further incubated for 30 min at 37°C. The MIC was determined as the lowest concentration of oil that inhibited microbial growth during the 24-hour incubation at 37°C. Each experiment was repeated 3 times [31].

2.5. Biofilm Inhibition (Crystal Violet Staining Assay). The crystal violet stain method was employed in assessing the antibiofilm activities of the essential oils [12]. Sterile 96-well microtiter plates were initially filled with 100 μL of various concentrations of the oils (MIC and sub-MIC). A 100 μL suspension of *P. aeruginosa* (PAO1) at 0.5 McFarland standard was pipetted into the wells. A control experiment was also set up, in which DMSO or gentamicin was utilized instead of essential oil. After incubating the plates for 24 hours at 37°C, the contents of the wells were emptied, followed by three washes with deionized water to eliminate loosely attached cells. Crystal violet (0.1%) was then added to the wells for staining, after which elution into a fresh microtiter plate was done. Absorbances were measured at 595 nm. The following equation was used to calculate biofilm inhibition:

$$\%\ \text{Inhibition} = \left(\frac{A_c - A_t}{A_c}\right) \times 100\%, \quad (4)$$

where A_c is the blank absorbance and A_t is the sample absorbance.

2.6. Pyoverdine Secretion Assay. Test tubes containing 4 mL broth cultures were inoculated with *P. aeruginosa* PA01 and incubated in the presence of sub-MIC doses of gentamicin and essential oils. After 48 hours, the mixture was centrifuged and the cell-free supernatant was collected and dispensed into a microtiter plate for pyoverdine measurement. The relative concentration of pyoverdine in treated supernatant compared to the control was measured using

a fluorescence spectrophotometer with excitation and emission wavelengths of 405 nm and 465 nm, respectively. Percentage inhibition of pyovedine secretion was evaluated relative to the control using equation (4) [12].

2.7. Computational Studies. The constituents of the oils were subjected to molecular docking to investigate how the compounds interact with the protein targets (LasR and algC) to identify compounds that potentially played a role in the inhibition of biofilm and quorum sensing by the essential oils.

2.7.1. Ligand Preparations. 3D models of the compounds were obtained using Spartan ′14 where all geometry optimizations were made with the Hartree Fock approximation on a 3–21 G basis. The ligands were prepared by the addition of polar hydrogens, merging of nonpolar hydrogens, and computing Gasteiger charges for all the atoms in the ligand [32, 33]. For virtual screening, the modeled compounds were converted to .sdf formats and prepared for docking by applying the mmff94 force fields and the steepest descent optimization algorithm. The minimized ligands were then converted to AutoDock compatible ligand formats (.pdbqt).

2.7.2. Protein Targets. The leading role of the transcriptional activator protein, LasR, in the activation of quorum-sensing-related virulence mechanisms among the other proteins involved in the quorum sensing circuit of *P. aeruginosa* made it an attractive protein to study. The X-ray structure of LasR was downloaded from the protein data bank, its PDB ID was 6V7X and it had a resolution of 2.90 Å [34]. The protein can cocrystallized with N-3-oxo-dodecanoyl-l-homoserine lactone as its native substrate. AQS1 (a QS antiactivator protein) was also crystallized with LasR.

Another protein target considered was the algC enzyme. AlgC plays a critical role in the alginate and lipopolysaccharide (LPS) biosynthetic pathways of *P. aeruginosa*. The crystal structure algC (phosphomannomutase/phosphoglucomutase) in *P. aeruginosa* was downloaded from the protein data bank with an ID of 2H5A cocrystallized with an inhibitor xylose-1-phosphate at a resolution of 1.72 Å [21]. In preparing the proteins for molecular docking, all crystallographic water molecules and cocrystallized ligands were deleted, polar hydrogens were added, and Gasteiger charges were computed for all protein atoms using AutoDockTools-1.5.7rc136.

2.7.3. Method Validation. A precision docking method was used in redocking the native ligands cocrystallized with the respective proteins–LasR and algC. The grid dimensions for docking N-3-oxododecanoyl-L-homoserine lactone to LasR were $X = 20.0068$, $Y = -3.27362$, $Z = -4.70686$, and size 19.2, whereas xylose-1-phosphate was docked with the dimensions, $X = -47.3532$, $Y = -14.3797$, $Z = 16.0256$, and size 10 for algC.

2.7.4. Molecular Docking. Molecular docking experiments were carried out with AutoDock Vina that has been extended into PyRx and UCSF Chimera [35]. The constituent compounds from the fruit and leaf essential oils were screened for preferential binding to the active sites of the LasR and algC enzymes in a global docking experiment. Compounds that bound in the active sites of the enzymes with binding affinities of −6.0 kcal/mol or better were further subjected to precision docking (using the same coordinates used for the method validation) to optimize the interactions made because of binding. The most stable conformations obtained from docking were considered the best poses based on their binding affinity and favorable interactions with critical amino acid residues present in the active site. These selected poses were then analyzed using Discovery Studio.

3. Results

Essential oil yields obtained from fruits and leaves of *V. doniana* were 0.04% and 0.12%, respectively. Yellow oil was obtained from the fruit while the leaf essential oil had a pale-yellow color. The total ion chromatograms (TIC) obtained for the fruit and leaf essential oils are shown in Figures 1 and 2, respectively. The compounds were identified through comparison of their mass spectra from the GC-MS run with NIST and Wiley spectra libraries. The fruit essential oil had 3-phenylpropanol (11.58%), hexyl acetate (10.46%), 3-decanoic acid, (E)- (9.70%), octanoic acid (5.16%), ethyl cinnamate (4.96%), and methyl cinnamate (4.91%) as the major constituents (Table 1). n-Hexadecanoic acid (15.90%), octacosane (8.45%), tetradecane (5.87%), hexadecane (5.56%), and oleic acid (4.74%) were the major compounds in the leaf essential oil (Table 2). Linalool was present in both oils. Some other interesting compounds identified in the leaf oil were benzyl benzoate, trans-α-ionone, trans-isoeugenol, folic acid, and γ-caprolactone and that of the fruit oil were linalool, benzene propanal, l-à-terpineol, citronellol, β-citral, α-citral, isobutyl cinnamate, hexadecanoic acid, and ethyl ester. The major compound classes of the leaf essential oil were alkanes (~24%), fatty acids (~16%), and terpenoids (~7%), whereas that for the fruit essential oil were fatty acids (~23%), esters (~13%), and alcohols (~11%), as shown in Table 3.

The antioxidant potential of the oils was determined using four assays—phosphomolybdenum, H_2O_2 scavenging, inhibition of lipid peroxidation, and DPPH assays. The results of the antioxidant assays are summarized in Table 4. The phosphomolybdenum assay was used to determine the total antioxidant capacity (TAC) of the essential oils. TAC was determined to be 44.0 ± 4.2 μg/g AAE and 67.5 ± 2.1 μg/g AAE for the fruit and leaf essential oils, respectively. The IC_{50} in the hydrogen peroxide assay was 87.9 ± 4.8 μg/mL and 241.2 ± 0.6 μg/mL, respectively, for fruit and leaf essential oils. Ascorbic acid and gallic acid were used as positive controls and both yielded IC_{50} values of 5.30 ± 0.2 μg/mL and 9.1 ± 1.6 μg/mL, respectively. The leaf essential oil was able to scavenge the DPPH radical with an IC_{50} value of 322.4 ± 0.7 μg/ml, whereas the IC_{50} of the fruit essential oil was 598.5 ± 0.8 μg/ml. Ascorbic acid showed

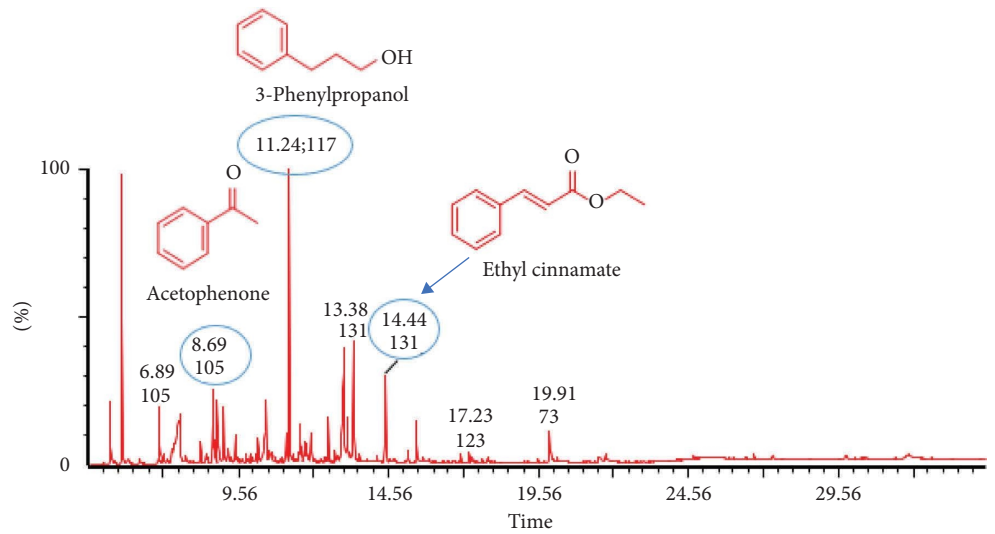

Figure 1: Total ion chromatogram (TIC) obtained from the GC-MS run of the fruit essential oil of *Vitex doniana*. Compounds were identified by comparison of MS spectra data with the NIST and Wiley libraries, as well as published literature. Chemical structures of some of the compounds are shown and were drawn with ChemDraw.

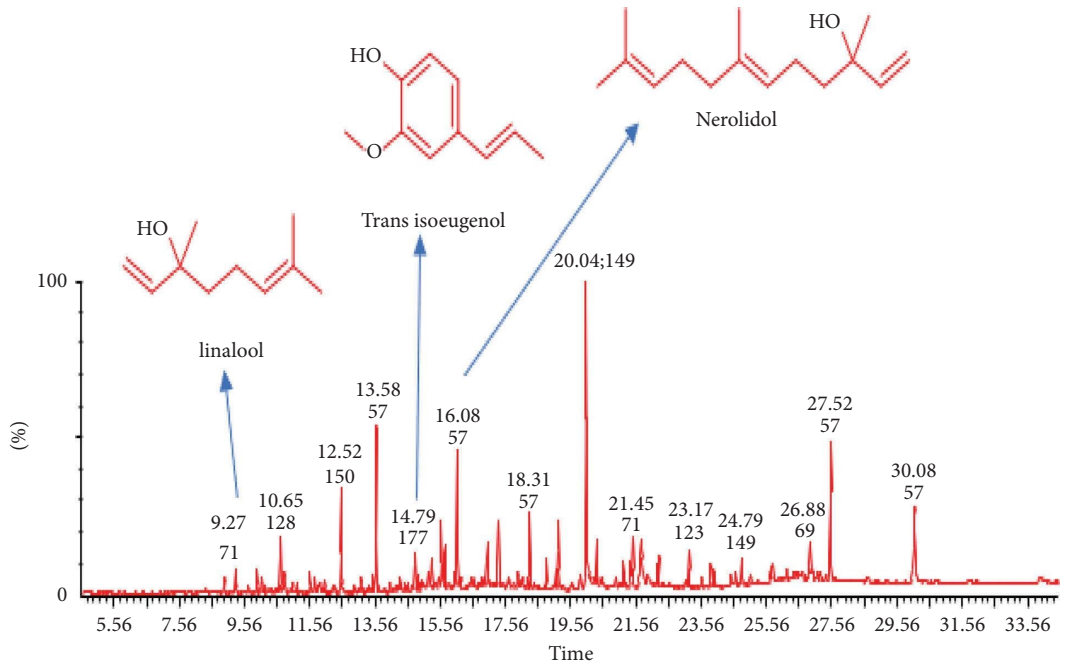

Figure 2: Total ion chromatogram (TIC) obtained from the GC-MS run of the leaf essential oil of *Vitex doniana*. Compounds were identified by comparison of MS spectra data with the NIST and Wiley libraries. Chemical structures of some of the compounds are shown and were drawn with ChemDraw.

better DPPH scavenging activity with an IC_{50} of $14.3 \pm 4.3\,\mu g/mL$. The IC_{50} for the leaf and fruit essential oils were $413.8 \pm 1.3\,\mu g/ml$ and $247.7 \pm 0.8\,\mu g/ml$, respectively, in the lipid peroxidation assay.

To evaluate antimicrobial capabilities, the essential oils from the leaves and fruits of *V. doniana* were tested against 7 microorganisms, 6 bacteria strains and 1 fungus. Table 5 summarizes the results of the antimicrobial susceptibility tests. The MICs of the fruit essential oil ranged from 6.25 mg/mL to 25.00 mg/mL, with that of the leaf essential oils ranging between 19.75 mg/mL and 79.00 mg/mL. Ciprofloxacin was used as a positive control with its MIC ranging from $0.31\,\mu g/mL$ to $5.00\,\mu g/mL$. In all cases, the fruit essential oils displayed a superior antimicrobial activity in comparison to the leaf essential oil. The activity of both essential oils against Gram-positive bacteria was also better than the activities displayed in Gram-negative bacteria. *E. faecalis* was most susceptible to the action of both essential oils, with an MIC of 25.00 mg/mL against the leaf essential oil and 6.25 mg/mL against the fruit essential oil.

Table 1: Chemical composition of the essential oil obtained from the fruit of *Vitex doniana*.

S/N	Compound name	% C	RT	SI
1	1-Hexanol	2.51	5.29	99.47
2	Benzaldehyde	2.55	6.89	97.66
3	1-Heptanol	0.41	7.07	97.89
4	3-Octanone	0.80	7.33	84.60
5	Hexanoic acid	10.46	7.60	97.65
6	Acetic acid and hexyl ester	0.59	7.78	96.30
7	Benzeneacetaldehyde	1.00	8.30	95.48
8	γ-Caprolactone	0.63	8.46	94.61
9	Acetophenone	4.33	8.68	99.57
10	Cis, cis-4,6-octadienol	3.28	8.87	99.05
11	2-Nonanone	2.57	9.07	98.09
12	Linalool	0.77	9.21	98.57
13	Phenylethyl alcohol	1.39	9.45	97.40
14	3-Nonen-2-one	0.34	9.82	98.99
15	Benzenepropanal	1.25	10.20	91.06
16	Octanoic acid	5.16	10.46	90.56
17	1-Methylene indene	0.81	10.57	84.25
18	L-à-terpineol	0.63	10.64	97.80
19	Citronellol	1.14	11.16	96.72
20	3-Phenylpropanol	11.58	11.23	99.28
21	β-Citral	0.56	11.36	99.27
22	Cyclooctylidene-(2-phenylaziridin-1-yl) amine	2.85	11.58	99.60
23	α-Citral	1.17	11.78	93.83
24	2-Propenal, 3-phenyl-	0.82	11.84	97.62
25	1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl	1.15	11.98	100.00
26	Cyclohexa-2,4-dienylmethanol	0.50	12.79	94.43
27	3-Decenoic acid, (E)	9.70	13.08	97.40
28	3-Phenyl-1-propanol acetate	1.36	13.17	98.05
29	Methyl cinnamate	4.91	13.38	100.00
30	1-Cyclohexen-1-ol, 2,6-dimethylacetate	0.52	14.16	79.73
31	Ethyl cinnamate	4.96	14.44	95.60
32	1,8(2H,5H)-Naphthalenedione, hexahydro-8a-methyl-, cis-	0.53	15.19	97.67
33	Mellein	2.29	15.50	98.47
34	Isobutyl cinnamate	0.49	16.95	97.75
35	Spirio-10-(2,11-dioxabicyclo[4.4.1]undeca-3,5-diene)-2′-(oxirane), 1,3,7,7-tetramethyl-	0.48	17.23	99.14
36	Cyclohexane, 1,2,4,5-tetraethyl-	0.43	17.32	96.80
37	n-Hexadecanoic acid	2.88	19.90	98.82
38	Hexadecanoic acid, ethyl ester	0.80	20.20	97.43
39	cis-vaccenic acid	0.71	21.59	97.61
40	n-Propyl 9,12-octadecadienoate	0.52	21.79	94.31
41	1-Monolinoleoylglycerol trimethylsilyl ether	1.98	24.71	96.56
42	Octadecane, 1,1′-[1,3-propanediyl bis(oxy)]bis-	4.05	25.66	96.89
43	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1.40	26.06	64.41
44	Squalene	1.34	26.76	87.39
45	Hentriacontane	1.39	27.37	80.91

S/N: compound number in order of elution; % C: percentage composition of compound in essential oil; SI: similarity index (library search of purity value of a compound); RT: retention time in minutes.

In the antibiofilm assay (Table 6), both fruit and leaf essential oils were screened against *P. aeruginosa* PAO1 strain. The fruit and leaf essential oils showed strong biofilm inhibition, with inhibition rates of over 50% at sub-MICs. At MIC/32, both the fruit and leaf essential oils were able to inhibit biofilm formation by >50%. At MIC, the fruit and leaf essential oil showed maximum inhibitions of $71.5 \pm 5.5\%$ and $65.5 \pm 2.6\%$, respectively, while gentamycin showed its maximum inhibition of $89.1 \pm 1.6\%$ at its MIC. Generally, there was a dose-dependent relationship for all treatment groups in biofilm inhibition. The concentrations of the fruit and leaf essential oil required for half-maximal inhibition of biofilm formation (BIC_{50}) were 44.40 ± 0.6 and 109.1 ± 0.9 mg/mL, respectively. The BIC_{50} of gentamycin was 25 ± 0.3 μg/ml. The pyoverdine quantification was used to evaluate the ability of the fruit and leaf essential oil to interfere with the production of pyoverdine, and the results are given in Table 7. At MIC, the percentage inhibition of fruit and leaf was 44.07% and 18.67%, respectively. Overall, the fruit essential oil was a better inhibitor of pyoverdine secretion in comparison to the leaf essential oil.

Table 2: Chemical composition of the essential oil obtained from the leaves of *Vitex doniana*.

S/N	Compound name	%C	RT	SI
1	Linalool	1.07	9.27	99.78
2	Menth-2-en-1-ol	0.84	9.93	97.71
3	2,6-Nonadienal, (E,Z)-	0.79	10.09	92.22
4	Naphthalene	2.43	10.64	97.48
5	Folic acid	1.31	10.77	98.07
6	p-Menth-4-en-3-one	0.88	11.58	99.38
7	5-Methylene-1,3a,4,5,6,6a-hexahydropentalen-1-ol	0.70	11.85	95.69
8	2-Methoxy-4-vinylphenol	3.83	12.51	99.12
9	4-Cyclooctene-1-methanol	0.84	13.12	83.48
10	Chloroacetic acid, 2-tetradecyl ester	0.65	13.47	88.37
11	Tetradecane	5.87	13.58	93.70
12	Trans-isoeugenol	1.04	14.33	98.18
13	Trans-á-ionone	1.74	14.79	97.78
14	1,3-Cyclohexanedione, 5,5-dimethyl-2-propyl-	0.84	15.17	87.02
15	2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone	1.35	15.27	98.99
16	Mellein	2.40	15.60	95.89
17	α-Nerolidol	2.09	15.71	95.18
18	Pentafluoropropionic acid, 4-hexadecyl ester	0.73	15.98	91.14
19	Hexadecane	5.56	16.07	95.92
20	Calarene epoxide	0.77	16.57	92.19
21	Selina-4(15),7(11)-diene	0.69	16.92	91.13
22	Patchouli alcohol	2.55	16.99	97.39
23	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	2.96	17.34	99.32
24	1-Heptatriacotanol	0.73	17.63	99.71
25	Tetradecanoic acid	0.70	17.92	89.52
26	Benzyl benzoate	0.62	18.09	71.83
27	Octadecane	2.62	18.31	97.24
28	2-Pentadecanone, 6,10,14-trimethyl-	1.16	18.80	97.52
29	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.90	19.08	99.46
30	Hexadecen-1-ol, trans-9-	3.72	19.17	92.28
31	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	0.67	19.59	98.24
32	Palmitoleic acid	1.11	19.87	92.82
33	n-Hexadecanoic acid	15.90	20.03	97.05
34	Eicosane	1.87	20.34	99.68
35	1-Octadecanol	1.14	21.15	97.71
36	Phytol	3.06	21.44	97.52
37	Oleic acid	4.74	21.70	97.55
38	Octadecanoic acid	0.98	21.90	96.69
39	Heneicosane	0.91	22.20	96.76
40	Benzoic acid, tetradecyl ester	1.07	22.27	99.65
41	Benzoic acid, tridecyl ester	1.54	23.17	99.22
42	Heptacosane	0.94	23.83	87.12
43	Benzoic acid, tetradecyl ester	0.95	23.94	98.90
44	Pentacosane	0.79	25.75	96.78
45	Squalene	2.82	26.87	96.17
46	1,2-Benzenedicarboxylic acid, dinonyl ester	0.67	27.11	93.54
47	Octacosane	8.45	27.51	98.72

S/N: compound number in order of elution; % C: percentage composition of compound in essential oil; SI: similarity index (library search of purity value of a compound); RT: retention time in minutes.

Virtual screening was used to identify the most probable inhibitors of the selected protein targets from the various constituents of the fruit and leaf essential oils. A global docking of the compounds was initially carried out to identify compounds that bind in the active site of the proteins. Precision docking was then used to evaluate the binding affinities and nature of the interactions of these compounds in the active site. Against LasR, 18 of the compounds were preferentially bound to the protein in the active site pocket (Figure 3). These compounds were 2-methoxy-4-vinyl phenol, 3-phenylpropanol, α-citral, acetophenone, benzene acetaldehyde, benzene propanal, folic acid, isobutyl cinnamate, menth-2-en-1-ol, methyl cinnamate, naphthalene, -menth-4-en-3-one, phenol-2,6-dimethoxy-4,2-propenyl, trans-α-ionone, trans-isoeugenol, benzyl benzoate, citronellol, and L-α-terpineol. In docking of these compounds against LasR using the grid dimensions $X = 20.0068$, $Y = -3.27362$, $Z = -4.70686$, and size 19.2, it was observed that acetophenone (−7.2 kcal/mol), p-menth-4-en-2one (−7.2 kcal/mol), methyl cinnamate (−7.3 kcal/mol,

Table 3: Classes of compounds identified in *Vitex doniana* leaf and fruit essential oils.

Compound class	Composition of leaves essential oil (%)	Composition of fruits essential oil (%)
Terpenoids	6.45	6.91
Aldehydes	0.57	4.36
Alkanes	23.04	6.09
Vitamin	0.93	—
Alcohol	5.11	10.79
Phenol	4.57	1.78
Ester	4.92	12.58
Ketone	0.96	6.25
Epoxide	0.55	—
Fatty acid	16.01	22.46
Carboxylic acid	1.95	—
Amine	—	2.21

— Class of compounds absent in given essential oil.

Table 4: Summary of antioxidant activities of *Vitex doniana* fruit and leaf essential oils.

Sample	Total antioxidant capacity (TAC) (μg/g AAE)	H_2O_2 scavenging activity IC_{50} (μg/mL)	DPPH radical scavenging activity IC_{50} (μg/mL)	TBARS IC_{50} (μg/mL)
Fruit essential oil	44.0 ± 4.2	87.9 ± 4.8	598.5 ± 0.8	247.7 ± 0.8
Leaf essential oil	67.5 ± 2.1	241.2 ± 0.6	322.4 ± 0.7	413.8 ± 1.3
Ascorbic acid	NA	5.3 ± 0.2	14.3 ± 4.3	NA
BHT	NA	NA	NA	9.2 ± 1.7

Data represented as mean ± standard deviation, $n = 3$; TBARS: thiobarbituric acid reactive substance assay; nd: not applicable.

Table 5: Antimicrobial activity of the leaf and fruit essential oils of *Vitex doniana*.

	Minimum inhibition concentration (mg/ml)		
Microorganisms (gram status)	Leaf (mg/mL)	Fruits (mg/mL)	Ciprofloxacin (ug/mL)
[#]*Candida albicans*	79.00	25.00	5.00
Pseudomonas aeruginosa (−)	79.00	25.00	0.08
Escherichia coli (−)	>79.00	25.00	0.31
Staphylococcus pneumoniae (−)	79.00	25.00	0.63
Staphylococcus aureus (+)	39.50	12.50	5.00
Enterococcus feacalis (+)	19.75	6.25	1.25
Bacillus subtilis (+)	39.50	25.00	1.25

[*]MICs of ciprofloxacin are in μg/ml; +, Gram-positive bacterial; −, Gram-negative bacterial; [#]*Candida albicans* is a fungus.

Table 6: Inhibition of biofilm formation in *P. aeruginosa* by leaf and fruit essential oils of *Vitex doniana*.

Concentrations	% biofilm inhibition		Gentamycin
	Fruit	Leaf	
MIC	71.52 ± 5.5	65.53 ± 2.6	89.06 ± 1.6
MIC/2	69.01 ± 4.9	64.06 ± 2.3	87.82 ± 2.9
MIC/4	65.65 ± 3.3	61.12 ± 1.6	77.42 ± 7.2
MIC/8	60.05 ± 6.9	59.33 ± 1.9	63.63 ± 2.4
MIC/16	58.82 ± 7.1	56.19 ± 2.3	57.68 ± 3.8
MIC/32	54.98 ± 3.4	52.89 ± 1.4	56.84 ± 4.0

Data represented as mean ± standard deviation, $n = 2$; concentrations of essential oils or gentamycin used were based on their minimum inhibitory concentration (MIC) against *P. aeruginosa*; gentamycin was used for comparison purposes. BIC_{50} values computed from dose-response curve.

Table 7: Inhibition of pyoverdine secretion in *P. aeruginosa* by leaf and fruit essential oils of *Vitex doniana*.

Concentrations	% Pyoverdine inhibition		Gentamycin
	Fruit	Leaf	
MIC	44.07	18.67	44.44
MIC/2	41.03	18.26	41.67
MIC/4	35.56	12.86	38.27
MIC/8	31.61	7.88	37.65
MIC/16	28.27	5.81	34.26
MIC/32	9.42	0.83	13.58

isobutyl cinnamate (−8.4 kcal/mol), benzyl benzoate (−10.1 kcal/mol), and folic acid (−10.3 kcal/mol) were bound to the protein with the best binding affinities (Table 8). Benzoyl benzoate, folic acid, methyl cinnamate, acetophenone, and p-menth-4-en-2-one all interacted with Ser 182 via hydrogen bonding. Hydrophobic interactions with Tyr117 and Leu 89 were also prevalent for the compounds (Figure 4).

Against algC, methyl cinnamate, ethyl cinnamate, p-menth-4-en-3-one, and trans-α-ionone were the only compounds that were bound to the protein at its active

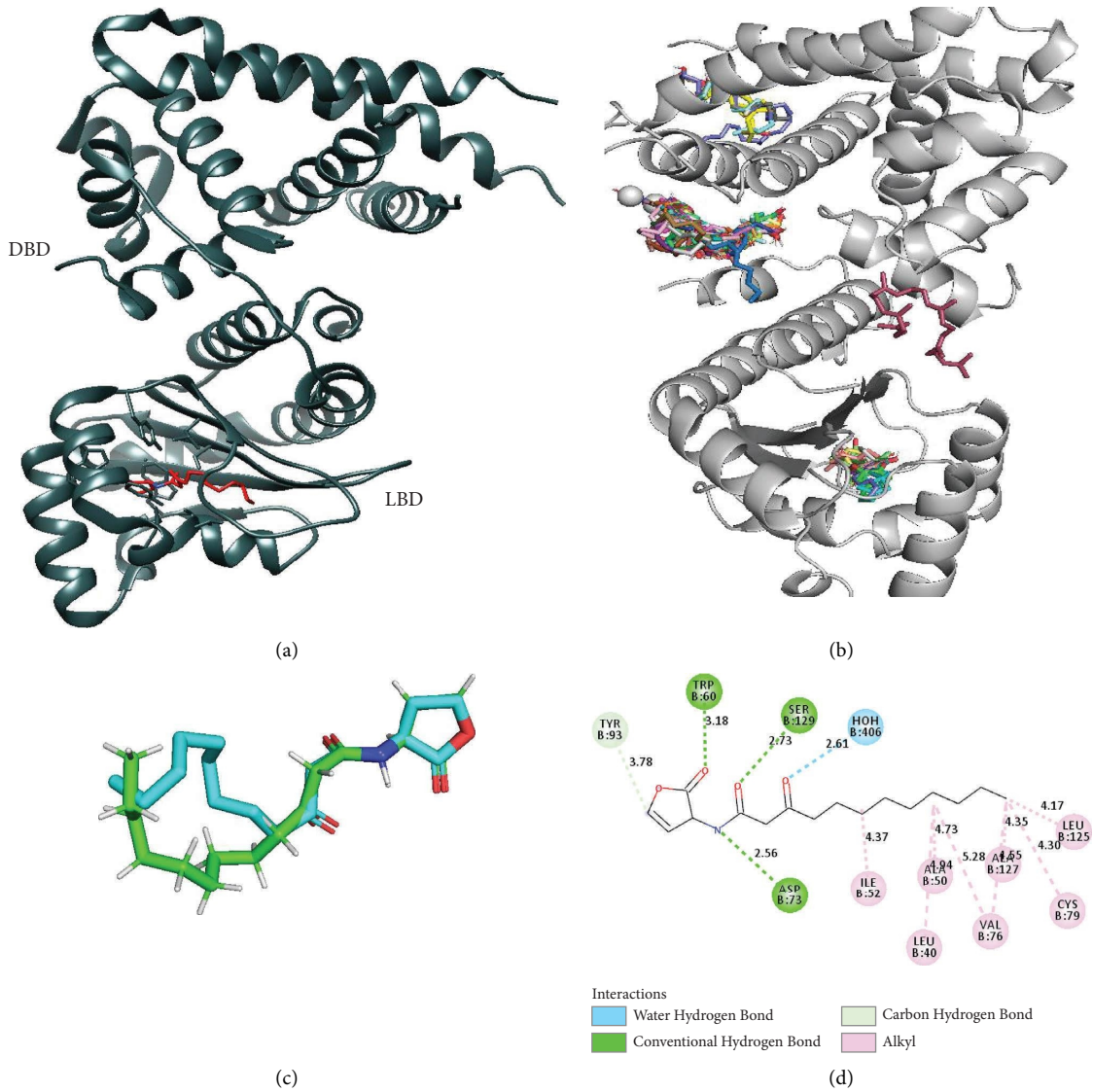

FIGURE 3: The transcriptional activator, **LasR:** (a) crystal structure of Las R enzyme of *P. aeruginosa* in complex with AHL. In red is the AHL molecule (DBD: DNA binding domain, LBD: ligand/AHL binding domain); (b) global docking of compounds from the essential oils of *V. doniana* showing the compounds which preferred to bind in the ligand binding domain of LasR; (c) superimposition of redocked and crystallized poses of AHL against Las R of *P. aeruginosa*. Crystallized pose in green, and redocked pose in cyan. An RMSD of 0.231 Å was observed. Redocking of AHL produced a binding affinity of −8.4 kcal/mol; (d) 2D interactions of AHL at the Las R binding site.

site (Figure 5). These compounds had binding energies between −4.9 and −5.7 kcal/mol and interacted with key active site residues as well (Table 9). Methyl cinnamate formed hydrogen interactions with Asn 415, Tyr 9 and Arg 412, and hydrophobic interactions with Val 421 and Ser 414, with a binding affinity of −5.6 kcal/mol. Ethyl cinnamate had a binding affinity of −5.7 kcal/mol with hydrogen bond interactions with Asn 424, Tyr 17, and Arg 421, and hydrophobic interactions with Val 430 and Ser 423. P-menth-4-en-3-one had no hydrogen interactions but formed hydrophobic interactions with His 320, His 299, and Tyr 9, with a binding affinity of −4.9 kcal/mol. Trans-a-ionone formed hydrogen bond interactions with His 320, with a binding affinity of −5.0 kcal/mol (Figure 6).

4. Discussion

Essential oils are complex combinations of volatile substances produced by living organisms and extracted from a whole plant or plant. The essential oils from the leaves and fruits of *V. doniana* were obtained through steam distillation with yields of 0.04% and 0.12% for the fruit and leaf essential oils, respectively. These yields are typical of essential oils extracted from plants using similar methods [14, 36]. Sonibare and coworkers reported the yield of leaf essential oil from *V. doniana* to be 0.04% using hydrodistillation [28]. The difference in essential oil yield from these 2 studies could be influenced by the extraction techniques employed. It has been reported that plant variety and geographical location may also affect the quantity of essential oils obtained from plants [1].

Table 8: Binding free energies and molecular interactions of some selected compounds docked against the LasR protein.

Compound	Binding affinity (kcal/mol)	Hydrogen bonding	Hydrophobic interactions
Folic acid	−10.3	Leu 178, Leu 92, Ser 182, Asp 126, Leu 163	Ala 103, Leu 93, Tyr 100, Ala 123, Val 129, Leu 89, Tyr 117, Trp 141
Benzyl benzoate	−10.1	Ser 182, asp 126	Tyr 117, Leu 89, Leu 163, pha 154, Ala 158, Trp 141
Isobutyl cinnamate	−8.4	Tyr 109	Trp 141, Asp 126, Leu 89, Ile 105, Tyr 117
Methyl cinnamate	−7.3	Ser 182	Ala 158, Leu 163, Phe 154, Trp 141, Tyr 117, Leu 89, Tyr 109
Acetophenone	−7.2	Ser 182	Trp 141, Leu 163, Ala 158, Phe 154
P-menth-4-en-3-one	−7.2	Ser 182	Trp 141, Tyr 117, Tyr 109, Trp 113, Leu 89, Trp 141
3-Phenylpropanol	−6.9	Asp 126	Ala 158, Trp 141, Leu 163, Phe 154
Benzene propanal	−6.9	Tyr 117	Leu 163, Pha 154, Trp 141. Ala 158
Naphthalene	−6.9	Ser 182	Tyr 109, Leu 89, Tyr 117, Trp 113, Asp 126
L-α-terpineol	−6.9		Phe 154, Ala 158, Leu 163, Trp 141, Tyr 101, Leu 89, Tyr 117
Phenol-2,6-dimethoxy-4,2-propenyl	−6.7	Tyr 109, thr 128	Ala 180, Val 129, Trp 113, Phe 154, Leu 163, Ala 158, Leu 89, Asp 126, Tyr 117, Ile 105
Trans-α-ionone	−6.7		Ile 105, Leu 89, Tyr 117, Tyr 109, Val 129, Ala 180
Benzene acetaldehyde	−6.6	Ser 182	Leu 163, Phe 154, Trp 141, Ala 158
Menth-2-en-1-ol	−6.6	Tyr 117, Asp 126	Leu 89, Tyr 109, Trp 141, Ala 158, pha 154
Trans-isoeugenol	−6.6	Ser 182	Ala 180, Thr 128, Leu 89, Asp 126, Tyr 117, Tyr 109, Trp 141, Leu 163, Ala 158
2-Methoxy-4-vinyl phenol	−6.5	Ser 182	Tyr 109, Trp 141, Ala158, Pha 154, Leu 89, Ala 180, Asp 126
α-citral	−6.4		Tyr 117, Leu 89, Tyr 109, Trp 141, Leu 163, Phe 154, Ala 158
Citronellol	−6.3	Tyr 117	Leu 89, Tyr 109, Ala 158, Leu 163, Trp 141, Phe 154

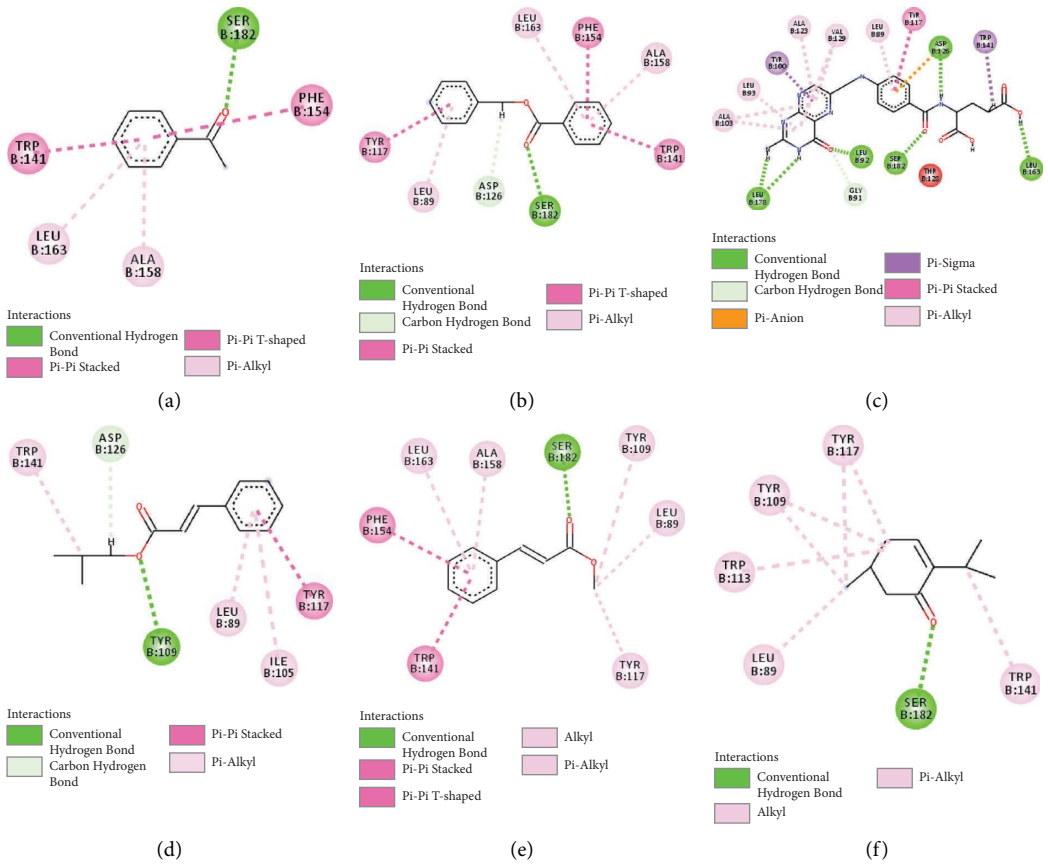

Figure 4: Molecular docking results: 2D view of interactions between top compounds and the LasR protein. (a) Acetophenone, (b) benzyl benzoate, (c) folic acid (d) isobutyl cinnamate, (e) methyl cinnamate, and (f) p-menth-4-en-3-one.

Table 1 shows the compounds identified in the fruit essential oil. Forty-five components were identified. These compounds were mainly fatty acids (~23%), esters (~13%), alcohols (~11%), and terpenoids (~7%), as shown in Table 3. The main constituents of the fruit essential oil were methyl cinnamate (4.91%), ethyl cinnamate (4.96%), octanoic acid (5.16%), 3-decanoic acid, (E)- (9.70%), and hexanoic acid (10.46%), with 3-phenylpropanol (11.58%) as the major constituent. Chinyere and coworkers also determined aroma constituents in the fruit syrup of *V. doniana* from Nigeria and identified terpenes to be the most abundant volatile constituents as they accounted for the largest proportion of the total aroma (43.99%) [29]. Environmental factors such as temperature and light, as well as cultivation conditions such as soil type and properties, can impact the composition of essential oils. These factors may have contributed to the observed differences in the fruit oils from Ghana and Nigeria. Linalool, methyl cinnamate, octanoic acid, and decanoic acid were also found to be present in both studies though their fractions in both works differed. Derivatives of cinnamates and linalool have been reported to possess a various bioactivities such as antibacterial, antifungal, insecticidal, and larvicidal properties [37]. Methyl cinnamate and linalool are widely used in the pharmaceutical, cosmetic, and food industries; and their repellent, insecticidal, and larvicidal properties have been reported as well [38].

The constituents of the leaf essential oil are shown in Table 2. In total, 47 compounds were identified. These compounds were mainly alkanes (23.04%), fatty acid (16.01%), terpenoids (6.45%), alcohol (5.11%), ester (4.92%), and phenols (4.57%). Minor classes of compounds present were carboxylic acid (1.95%), ketone (0.96%), aldehydes (0.57%), and epoxides (0.55%), as shown in Table 3. Phytol (3.06%), oleic acid (4.74%), tetradecane (5.87%), and n-hexadecanoic acid (15.90%) were the major compounds present in the leaf essential oil. In 2009, the essential oil components of *V. doniana* leaves from Nigeria were reported [28]. A comparison of the constituents of the oils shows marked differences. Only phytol was identified in both Nigerian and Ghanaian cultivars of *V. doniana*. Terpenoids were the most prevalent class of compounds in the Nigerian cultivar. Differences in essential oil composition of the same plant from different locations have been reported in the literature. Environmental conditions such as light and temperature, and cultivation conditions such as soil properties and type have been identified to be influential factors that contribute to differences in the chemical constitution of essential oils [39, 40]. N-hexadecanoic acid which was present in this oil in a high amount has been documented to have anti-inflammatory, antibacterial, antioxidant, and hypocholesterolemic properties [41].

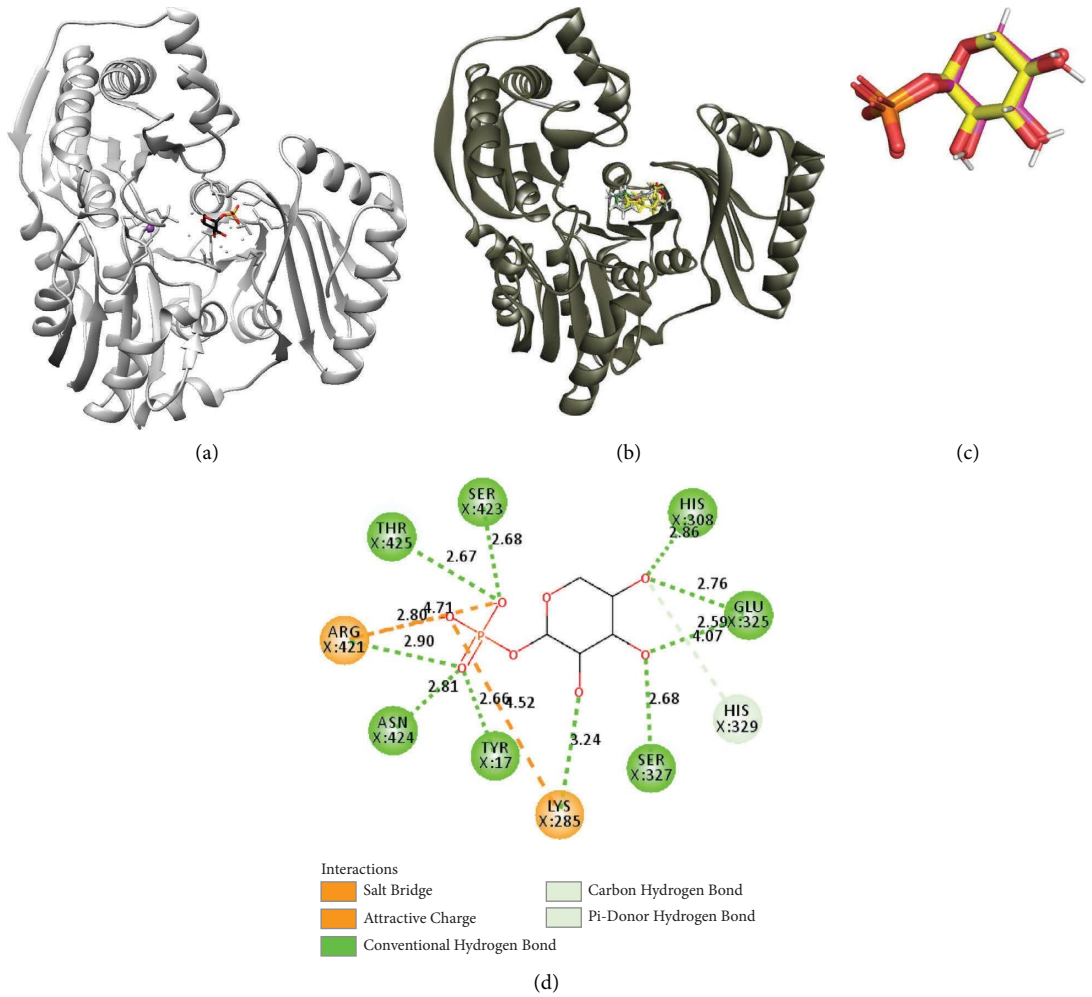

FIGURE 5: The phosphomannomutase/phosphoglucomutase enzyme in *Pseudomonas aeruginosa*, **algC:** —(a) crystal structure of algC enzyme in complex with xylose-1-phosphate (in black is xylose-1-phosphate and Mg metal in purple); (b) global docking of compounds from the essential oils of *V. doniana* showing the compounds which preferred to bind in the xylose-1-phosphate binding pocket of the enzyme; (c) superimposition of redocked and crystallized poses of xylose-1-phosphate against algC. Crystallized pose is in yellow and redocked pose is in pink. An RMSD of 0.082 Å was observed. Redocking of xylose-1-phosphate produced a binding affinity of −7.0 kcal/mol; (d) 2D interactions of xylose-1-phosphate at the algC active site.

TABLE 9: Binding free energies and molecular interactions of some selected compounds docked against the algC protein.

Compound	Binding affinity	Hydrogen bonding	Hydrophobic interactions
Methyl cinnamate	−5.6	Asn 415, Tyr 9, Arg 412	Val 421, Ser 414
Ethyl cinnamate	−5.7	Asn 424, Tyr 17, Arg 421	Val 430, Ser 423
P-menth-4-en-3-one	−4.9		His 320, His 299, Tyr 9
Trans-α-ionone	−5.0	His 320	Ser 318, His 100

Since essential oils are known to be antimicrobial, the essential oils of *V. doniana* were evaluated against some microorganism, Gram-positive and negative bacteria, and a fungus, and the data are summarized in Table 5. The antibacterial activity of the fruit essential oil was significantly better, with MIC values between 6.25 mg/mL and 25.00 mg/mL. The Gram-positive bacteria, *E. faecalis* and *S. aureus* were the organisms most susceptible to the antimicrobial action of the fruit essential oils. The MIC of the leaf essential oil against all of the test species ranged from 19.75 to 79 mg/mL. The leaf essential oil was also particularly effective against Gram-positive bacteria, *S. aureus*, *B. subtilis*, and *E. faecalis*. In general, the Gram-negative bacteria were least susceptible to the action of the fruit and leaf essential oils. This is probably because a protective capsule encapsulates Gram-negative bacteria. Gram-negative bacteria also have an exterior membrane beneath the capsule that shields them from antimicrobial agents. Endotoxins are released when this membrane is damaged, and this adds to the resilience of Gram-negative bacteria and increases their resistance to

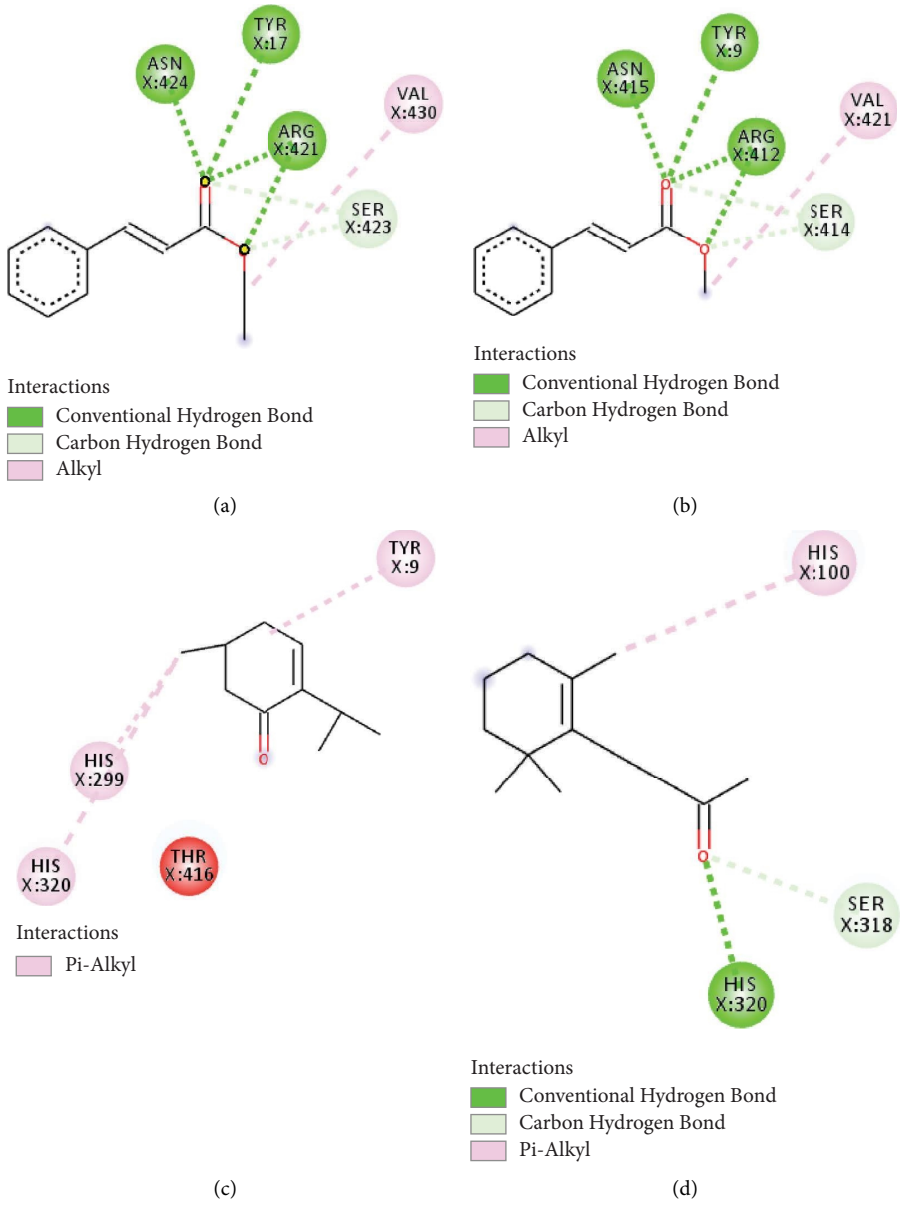

Figure 6: Molecular docking results: 2D view of interactions between compounds and the algC enzyme, (a) ethyl cinnamate, (b) methyl cinnamate, (c) p-menth-4-en-3-one, and (d) trans-α-ionone.

antimicrobial agents [42, 43]. Essential oils usually exert their antimicrobial action via a coordinated synergistic action between the components of the oil. The activity is usually a function of the components present, as well as the amounts of each component present in the mixture [1, 40].

Table 6 shows the biofilm inhibitory assay for both fruit and leaf essential oils. Biofilms play a critical part in infectious diseases. It is estimated that biofilm accounts for 80% of microbial infections in humans [44]. *P. aeruginosa* is a commonly used model in investigating antibiofilm activity and was, therefore, employed in this work. Both fruit and leaf essential oils were good inhibitors of biofilm formation. Even at the least sub-MIC tested (MIC/32), both essential oils inhibited biofilm formation by over 50%. At the MIC, biofilm inhibitions were $71.52 \pm 5.5\%$ and $65.53 \pm 2.6\%$ for the fruit and leaf essential oils, respectively. The fruit essential oil exhibited a better biofilm activity with a BIC_{50} value of 44.40 ± 0.6 mg/mL as compared to 109.1 ± 0.9 mg/ml for the leaf essential oil. Biofilms form when individual, free-floating bacteria attach to a surface and begin to create a microcolony that grows into a community enclosed in a protective extracellular matrix [45]. Inhibition of biofilm formation may be due to the disruption of the initial bacterial adhesion necessary for biofilm formation [12]. Some of the constituents of the oils have been reported to inhibit biofilm formation, and these compounds may have played a role in the biofilm inhibitory action of the *V. doniana* essential oils. Linalool inhibits biofilm formation in both *C. albicans* and *P. aeruginosa* [17, 46]. Since biofilm formation in *P. aeruginosa* is controlled by the QS machinery,

the ability of the oils to disturb QS was also investigated in the pyoverdine quantification assay. The fruit essential oil was observed to inhibit pyoverdine formation much better than the leaf essential oil. Pyoverdine, which is a primary *P. aeruginosa* siderophore sequesters iron from the host organism and also functions as a signaling molecule for QS. Pyoverdine that has been coupled to iron binds to the *Pseudomonas* cell receptor FpvA, and this complex then interacts with the antisigma factor FpvR to upregulate the exotoxin A and pyoverdine itself [47]. This is one of the main virulence strategies utilized by *P. aeuruginosa*.

To further probe the molecular basis of the antibiofilm and antiquorum sensing activities of the essential oils, molecular docking was used to evaluate the binding of the constituents of the essential oils to 2 proteins that play a key role in biofilm formation and quorum sensing regulation in *P. aeruginosa*. These proteins are LasR and algC. The LasR protein, when activated, plays the pivotal role of multimerizing and activating transcription of RhlR and other virulence proteins as part of its regulon. The RhlR protein in turn facilitates the production of rhamnolipids which provide architectural integrity to biofilm structure [48]. Inhibition of the LasR protein not only result in a marked decline in the production of virulence factors such as pyocyanin and pyoverdine but could also result in a cascade of effects that involves inhibiting biofilm formation. Validation of the docking method used involved redocking the cocrystallized ligand found in the crystal structure to the LasR protein and investigating the reproduction of critical interactions. Redocking replicated conventional hydrogen bonding interactions between the native AHL with Ser129 and Trp60 and pi-alkyl interactions with Leu125 and Val76 of the LasR protein (Figure 3). The redocked native ligand conformation matched the original pose from the protein data bank with a root mean squared deviation (RMSD) of 0.231 Å (Figure 3). The AHL was bound to the active site with a binding energy of −8.4 kcal/mol. The results suggest that the docking protocol is valid and could be utilized for studying the modeled compounds.

From the initial global docking, 18 of the compounds bound preferentially to the active site of the LasR protein (Figure 3). Of these 18 compounds, the top 4 compounds with binding affinities comparable to or better than the native AHL were benzyl benzoate, isobutyl cinnamate, folic acid, and methyl cinnamate (Table 8). Benzyl benzoate which accounts for 0.62% of leaf essential oil and isobutyl cinnamate which accounts for 0.49% of the fruit essential oil had binding energies as low as −10.1 kcal/mol and −8.4 kcal/mol, respectively. These results are indicative of the spontaneity associated with the binding event observed, as well as the strength of the binding conformations. As a result, benzyl benzoate and isobutyl cinnamate could be regarded as possible inhibitors of the LasR protein since they bind effectively to LasR and prevent the homoserine head group of the autoinducer from binding. It has been reported that benzyl benzoate possesses *in vitro* antiquorum sensing activity which results in the inhibition of pyocyanin, elastase, and proteolytic activities, as well as biofilm formation [49]. Similarly, cinnamic acids have been shown to attenuate quorum-sensing-related virulence factors and biofilm formation in *P. aeruginosa* [50]. The study further revealed that cinnamic acids can act as competitive inhibitors of the transcriptional activator, LasR. This outcome is similar to what is observed in the case of isobutyl cinnamate, a cinnamic acid ester derivative. Though present in minute quantities, the tendency of benzyl benzoate and isobutyl cinnamate to competitively bind to the active site and make key interactions that drive the transcriptional activity of the receptor provides a molecular basis for the observed antibiofilm activity seen in both fruit and leaf essential oils. Additionally, the remaining 16 compounds from the virtual screening had relatively high to moderate binding affinities which could synergistically act to further inhibit the activity of the receptor.

Biofilms are composed primarily of microbial cells and an extracellular polymeric substance (EPS). The enzyme, algC is involved in the biosynthesis of alginate and lipopolysaccharide (LPS), and these are key components of the EPS of *P. aeruginosa* [51]. The dual roles of algC in alginate and LPS biosynthesis of *P. aeruginosa* makes it a good target for inhibitor design. Results from docking of the library of compounds against this enzyme revealed 4 ligands that could likely inhibit the activity of the protein. These are methyl cinnamate, ethyl cinnamate, p-menth-4-en-3-one, and trans-α-ionone. Interestingly, both methyl cinnamate and ethyl cinnamate made conventional hydrogen bonding interactions with Arg421 (Figure 6), a key amino acid residue involved in the activity of the enzyme [20]. The similarity in binding energy (−4.9 and −5.7 kcal/mol), as well as interactions made by the compounds, suggest a combined effort from the individual compounds in inhibiting the algC enzyme. The constituents of the leaf and fruit essential oils of *V. doniana* are, therefore, pivotal in the antibiofilm and antiquorum sensing activities observed against *P. aeruginosa*.

Antioxidants decrease oxidative damage directly by quenching free radicals or indirectly by inhibiting the activity of these free radicals [52]. The antioxidant assays performed on the essential oils revealed promising results, as shown in Table 4. In all the assays, antioxidant activities were dose-dependent as expected. The leaf essential oil exhibited better performance than the fruit essential oil in the hydrogen peroxide scavenging assay. However, the activity of both oils could be considered moderate since the percent inhibition of hydrogen peroxide was below 50% for both oils. In the DPPH scavenging radical assay, the IC_{50} values obtained for both essential oils showed promising inhibition of the DPPH radical. The antioxidant activity of the leaf essential oil was higher than that of the fruit essential oil, as it demonstrated substantial inhibition of lipid peroxidation in the TBARS assay, while the fruit essential oil only exhibited moderate inhibition. Many compounds disrupt the development of the radical needed in the initiation step, quench radicals that are generated during chain propagation, or modulate the action of enzymes that mop up reactive species in exerting their antioxidant action. Due to their multicompound composition, the antioxidant activity of essential oils has been attributed to the synergistic action of the

individual constituents [6]. The promising antioxidant activity of the essential oil of the leaf of *V. doniana* could be ascribed to the presence of phenolic compounds, which were relatively higher in the leaf essential oil than in the fruit essential oil. Phenolic compounds are usually antioxidative by quenching peroxyl radicals through a formal hydrogen atom [6]. Antioxidant activity is, however, not attributed to only phenols but maybe also due to other secondary metabolites such as terpenoids, carotenoids, and vitamins [53]. Linalool, phytol, citronellol, and folic acid are some constituents of *V. doniana* fruit essential oil that have been reported in the literature to possess antioxidant activity [7]. ROSs can damage lipids, nucleic acids, and proteins. The impressive antioxidant activity of the *V. doniana* essential oils means they can be utilized as a source of natural antioxidants in food additives and also as preservatives, especially since the use of synthetic antioxidants like BHA and BHT are frowned upon by consumers. The essential oils of *V. doniana* could also find use as preservatives in animal feed formulation.

5. Conclusion

The leaf and fruit essential oils of *V. doniana* were mainly made up of terpenoids, phenols, fatty acids, and esters. The oils showed very good antioxidant and antimicrobial properties. The oils were also able to inhibit biofilm formation and pyoverdine secretion in *P. aeruginosa*. Methyl cinnamate, ethyl cinnamate, p--menth-4-en-3-one, and trans-α-ionone were shown to bind well to the algC protein and hence may have interfered with biofilm formation. On the other hand, benzyl benzoate, isobutyl cinnamate, folic acid, trans-α-ionone, and methyl cinnamate likely interacted with LasR and hence, interfered with biofilm formation and pyoverdine secretion. Together, the results obtained from this study indicate that the essential oils obtained from *Vitex doniana* fruits and leaves could be exploited as a natural source of antimicrobial and antioxidant agent, and could find use in the pharmaceutical, cosmetic, and food industries.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

LSB, IA, and GD conceived the study. All experiments were designed by LSB, GD, IA, JFO, PA, and CIA. JFO, CIA, and PA performed all experimental works. Molecular docking work was done by IA, CIA, and PA. JFO, CIA, PA, and LSB analyzed the data. Initial manuscript was drafted by JFO, CIA, and PA. All authors read and approved the final manuscript.

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