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Constituents from *Psorospermum guineense* twigs, MDA-MB-231, lipoxygenase, antioxidant and Molecular Docking Studies

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Abstract

Cancer is one of the leading causes of mortality worldwide, characterized by the irregular proliferation of malignant cells and increasing resistance to known drugs. The importance of developing new chemotherapeutic agents is necessary. The present study focuses on *Psorospermum guineense*, used in traditional medicine to treat cancer, and associated carcinogenic processes such as tumour cell proliferation, differentiation, and apoptosis. A study of the chemical constituents of the twigs of *Psorospermum guineense* has resulted in the isolation and characterization of eight known compounds, namely: 3-geranyloxyemodine **1**, 3-β-fridelanol **2**, 3-geranyloxyemodine anthrone **3**, 2-geranylemodine **4**, Acetylvismione D **5**, Betulinic acid **6**, Ursolic acid **7** and Emodine **8** through LC-MS and selected 1D and 2D–NMR experiments. Single crystal x-ray diffraction analysis was used to confirm the relative configuration and conformation of compound **1**. Preliminary cytotoxicity assays against the MDA-MB-231 breast cancer cell indicated that Ursolic acid **7** was the most active, with an IC_{50} value of 9.88 μM and 95% CI of 9.03-10.5 μM. Acetylvismione D **5** exhibited better lipoxygenase inhibitory activity (19.8±0.16) and antioxidant activity (20.8±0.15) compared to the reference drugs baicalein (22.6 ± 0.08 μM) and Beta hydroxyl acid (BHA) (44.2 ± 0.07) respectively. Molecular docking studies were conducted to support lipoxygenase inhibitory activities of the test compounds.

Keywords: *Psorospermum guineense*, Breast Cancer, Antraquinones, single crystal x-ray, Lipoxygenase, Molecular Docking

1. INTRODUCTION

Cancer is one of the leading causes of mortality worldwide [1]. The irregular proliferation of malignant cells characterizes it in various stages with different biochemical, molecular and cellular events [2]. Cancer is caused by both internal factors (such as mutations, hormones and immune conditions) and external factors like chemicals, radiation and infectious microorganisms [3,4]. This is attributed to changes in lifestyle, such as smoking, unhealthy eating, lack of physical exercise and excessive consumption of alcohol [5].

In 2021, 18.1 million new cancer cases and 9.6 million deaths were reported globally [6]. The International Agency for Research on Cancer (IARC) reported that cancer's incidence, mortality and prevalence worldwide were attributed to 36 different types of cancer [6]. Lung cancer registered 11.6% of cancer incidences with 18.4% mortality. It was followed by breast cancer (11.6%), prostate cancer (7.1%), and colorectal cancer (6.1%) in mortality. Colorectal cancer (9.2%), stomach cancer (8.2%), and liver cancer (8.2%) were listed among the top four deadliest tumours. Lung, prostate, liver and stomach cancers represented the most deadly among males, while among females, breast, lung and colorectal cancers dominated [7]. In Africa, cancer recorded over 1 million cases yearly, and the most common include cervix, breast, liver and prostate cancers [5]. Cancer management is not a priority in developing countries, despite the increasing burden, largely due to limited resources and other pressing public health concerns such as HIV/AIDS, malaria and tuberculosis [1].

Cancer treatment is costly, and the resistance of tumour cells to the available antineoplastic drugs has exacerbated this [8]. Due to their lack of specificity, conventional cancer therapies, such as radiotherapies, present severe side effects and, in most developing countries, are inaccessible to cancer patients [9]. Thus, traditional medicine is gaining more attention in the chemoprotective management of cancer in Africa [1,10-11]. Over 3,000 plant species have been reported to have anticancer properties [12]. An example is *Taxus brevifolia*, from which taxol was isolated, a well-known cancer drug in the United States of America [13].

Psorospermum guineense Hochr. (sin. *P. glaucum* Hochr.) is a plant typically growing in French Guinea, Mali, Senegal, Tanzania, and Nigeria, where it is also known by the common name‘‘kari-diakouma" and the local population uses it to treat skin diseases (eczema, psoriasis, scabies, cold sores, and leprosy), syphilis, cancer and neuralgia [14]. Extracts of the leaves and root bark of *P. guineense* have shown anti-leishmanial and anticancer activity [14]. Previous phytochemical investigations of *Psorospermum* species reported the presence of bioactive xanthones, anthraquinones, vismiones and psorolactones [15].

In our attempts to isolate natural products for drug discovery and development from a rational approach as a part of a laboratory project on re-investigating Cameroon medicinal plants, we initiated a study to isolate constituents of *P. guineense*, with biological activity and search for potential targets or mode of action studies. In this paper, we have reported on the phytochemical studies of the twigs of *P. guineense*, single crystal x-ray diffraction analysis and

their bioactivity against MDA MB-231 breast cancer cell lines. The mechanism of action was determined by evaluating their antioxidant activity, lipoxygenase and urease enzyme inhibition activities. Moreover, molecular docking studies to support the better lipoxygenase inhibitory activities of the test compounds were also conducted.

2. MATERIALS AND METHODS

General Experimental Procedures. 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HSQC, HSQC-TOCSY, HMBC, TOCSY, NOESY, and ROESY) NMR spectra were recorded on a Bruker Ascend 600 (^{1}H 600 MHz and ^{13}C 150 MHz) NMR spectrometer equipped with a 5 mm cryoprobe. ^{1}H NMR chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (7.26 ppm,), coupling constants (J) are reported in Hertz (Hz), and diversity follows convention. Unless indicated otherwise, $CDCl_3$ served as an internal standard (77.2 ppm) for all ^{13}C spectra. The chemical shifts are quoted relative to TMS. X-ray data were recorded on a Bruker400 Machine. HRESIMS data were acquired on a Thermo LTQ Orbitrap mass spectrometer.

2.1. Sample Collection. *Psorospermum guineense* twigs were harvested from Limbo, a small locality between Wainamah and Ndop plane in the North West region of Cameroon (September, 2016) by a botanist Dr. Tacham Walters, of the University of Bamenda and a voucher specimen deposited at the Cameroon National Herbarium (HNC), (14432/SRFCam). The twigs collected were cut into small pieces, dried at room temperature and powdered.

2.2. Extraction and Isolation. At room temperature, powdered twigs (1.5 kilograms) were extracted by sonication in MeOH:CH2Cl2 (1:1 v/v). The filtrates were concentrated on a rotary evaporator under reduced pressure to yield 300 g of crude extract. A portion of the crude extract (150 g) was subjected to fractionation using hexanes, EtOAc and n-BuOH to afford hexane (30g), EtOAc (25g) and n-BuOH (20g) fractions. The LCMS analyses of the various fractions indicated that the hexane, EtOAc and n-BuOH fractions were rich in secondary metabolites. These fractions were each subjected to silica gel 60 (0.063 – 0.200 mm) column chromatography using hexanes, hexanes-EtOAc gradient systems and EtOAc. Fractions (150) of 150 mL each were collected and concentrated under a vacuum. The hexane fraction afforded four compounds namely; 3-geranyloxyemodine **1** (100 mg), 3-β-fridelanol **2** (10 mg), 3-geranyloxyemodine anthrone **3** (100 mg) and 2-geranylemodine **4** (75 mg). While the EtOAc fraction afforded; 3-geranyloxyemodine **1** (200 mg), 3-geranyloxyemodine anthrone **3** (200 mg), 2-geranylemodine **4** (150 mg) Acetylvismione D **5** (25 mg), Betulinic acid **6** (250mg), Ursolic acid **7** (250 mg). The n-butanol fraction afforded Emodine **8**(100mg)

3-geranyloxyemodine: Orange powder; 120^0C; Hexane-Ethyl acetate 4%; LR-MS *m/z*: 406.03 (calcd for $C_{25}H_{26}O_5$, 406.02). The 1D NMR spectra show complete agreement with those reported in the literature [15] (See supplementary information)

3-β-fridelanol: White powder; 60^0C; eluting using Hexane-Ethyl acetate 5%; LR-MS *m/z*: 428.40 (calcd for $C_{30}H_{52}O$, 428.38). The 1D NMR spectra show complete agreement with those reported in the literature [15] (See supplementary information)

3-geranyloxyemodine anthrone: Yellow powder; 118^0C; eluting using Hexane-Ethyl acetate 6%; LR-MS *m/z*: 392.07 (calcd for $C_{25}H_{28}O_4$, 392.05). The 1D NMR spectra show complete agreement with those reported in the literature [15] (See supplementary information)

2-geranylemodine: Yellow powder; 78-80^0C; eluting using Hexane-Ethyl acetate 7%; LR-MS *m/z*: 406.02 (calcd for $C_{25}H_{26}O_5$, 406.01). The 1D NMR spectra show complete agreement with those reported in the literature [15] (See supplementary information)

Acetylvismione D: Yellow powder; 216^0C; eluting using Hexane-Ethyl acetate 15%; LR-MS *m/z*: 452.07 (calcd for $C_{27}H_{32}O_6$, 452.05). The 1D NMR spectra show complete agreement with those reported in the literature [15] (See supplementary information)

Betulinic acid: White powder; 288^0C; eluting using Hexane-Ethyl acetate 25-27%; LR-MS *m/z*: 456.20 (calcd for $C_{30}H_{47}O_3$, 256.20). The 1D NMR spectra show complete agreement with those reported in the literature [15] (See supplementary information)

Ursolic acid: White powder, 270^0C; eluting using Hexane-Ethyl acetate 40-60%; LR-MS *m/z*: 456.35 (calcd for $C_{30}H_{48}O_3$, 456.33). The 1D NMR spectra show complete agreement with those reported in the literature [15] (See supplementary information)

Emodine: Yellow powder; 240^0C; eluting using Hexane-Ethyl acetate 20%; HR TOF-MS ESI *m/z*: 270.045 corresponding to $C_{15}H_{10}O_5$. The 1D NMR spectra show complete agreement with those reported in the literature [15] (See supplementary information)

2.3. Biological screening.

2.3.1 Cytotoxicity

Cytotoxicity was determined through cells plated in 96-well plates (Nest Scientific) at a density of 2000 cells per well in 100 μL of media per well. On the following day, the media was aspirated, and 100 μL of media in which a serial dilution of DMSO-dissolved compound (or DMSO as a control) was added to wells in triplicate. Three days later, the relative number of metabolically active cells was determined by the addition of the CellTiter-Glo reagent (Promega)

and measurement of chemiluminescence according to the manufacturer's protocol using a Fluoroskan Ascent FL (Thermo Scientific). To calculate the percent viability, the signal from background wells (media only) was subtracted from each well, and then the remaining chemiluminescence value was normalized to the signal from wells with the equivalent amounts of DMSO. Graphing was done using GraphPad Prism 6 (GraphPad Software). IC_{50} values were calculated by fitting a non-linear curve using the "log-inhibitor vs normalized response" function given by the equation Y=100/{1+10[(X-LogIC50)]}.

2.3.2 Lipoxygenase inhibitory activity

Lipoxygenase (LOX) inhibiting activity was measured by modifying the spectrophotometric method developed by Tappel [16]. Lipoxygenase solution was prepared so that the reaction mixture's enzyme concentration was adjusted to a 0.05 absorbance/min rate. The reaction mixture comprised 160 µL of 100mM sodium phosphate buffer at pH 8, 10 µL of the test solution and 20 µL of LOX solution. The contents were mixed and incubated for 10 min at 25 °C. It was then initiated by adding 10 µL substrate solution (linoleic acid, 0.5mM, 0.12 %w/v tween 20 in a ratio of 1:2) and the change in absorbance at 234 nm was followed for 6 min. The concentration of the test compound that inhibited lipoxygenase activity by 50 % (IC_{50}) was determined by monitoring the effect of increasing concentrations of these compounds in the assays on the degree of inhibition. The IC_{50} values were calculated using EZ-Fit, Enzyme kinetics Program (Perrella Scientific In., Amhherset, USA).

2.3.3 Evaluation of urease inhibition activity

Urease (Jack bean) solution (25 µl) was mixed with the 5 µl compound (500 µg) and incubated at 30°C for 15 min. Aliquots were taken and immediately transferred to assay mixtures containing urea (100mM) in buffer (40 µl) and re-incubated for 30 min in 96 well plates. The indophenol method determined urease activity based on measuring ammonia produced [17]. 50 µl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to wells. An increase in absorbance was measured after 50 min at 630 nm on a microtitre plate reader (Spectramax Plus 384 Molecular Device, USA). The final volume of the reaction is 200 µl at pH 8.2 (0.01 M K_2HPO_4. $3H_2O$, 1mM EDTA and 0.01 M $LiCl_2$). All reactions were performed in triplicates. The standard used in this assay was Thiourea, and percentage inhibitions were calculated by the formula:

$$100 - \left[\frac{\text{Optical Density of test cpd}}{\text{Optical Density of control(with no cpd)}} \right]$$

2.3.4 Determination of DPPH radical scavenging activity

1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) is a quick method most researchers use to determine antioxidant activities.

The free radical scavenging activity was measured by 1, 1-diphenyl-2-picryl-hydrazine (DPPH) [18]. DPPH solution (0.3mM) was prepared in ethanol. The activity was measured in different concentrations of each compound ranging 62.5 µg- 500 µg. 5 µl of different concentrations ranging (62.5 µg - 500 µg) of each sample was mixed with 95 µl of DPPH solution in ethanol. The prepared dilutions were dispersed in 96 well plate and incubated at 37°C for 30 min. The absorbance was measured at 515 nm in microtitre plate reader (Spectramax Plus 384 Molecular Device, USA). The percent radical scavenging activity of root extracts was determined against methanol treated control.

$$\text{DPPH Scavenging effect (\%)} = \frac{Ac - As}{Ac} \times 100$$

Where:

Ac = Absorbance of Control (DMSO treated)

As = Absorbance of Sample

Molecular docking studies

Molecular docking studies were performed using AutoDock 4.2 software [19, 20] to study the molecular interaction and binding mode of the purified compounds. For this purpose, the crystal structure of human lipoxygenase (PDB code: 3V99) was obtained from the protein data bank (http://www.rcsb.org). All water molecules of 3V99 were removed. The polar hydrogen atoms, Kollman and Gasteiger charges, were added to the protein structure's amino acid residues using AutoDock Tools (ADT, version 1.5.6) [21]. The 3D molecular structures of the compounds were optimized using ChemDraw 3D. Then, the required AutoDock format (pdbqt) of the receptor and ligands were obtained using AutoDock Tools 1.5.6. A total of 200 docking calculations of the purified compounds were carried out using the Lamarckian genetic algorithm method (LGA). The population size, the maximum number of evaluations (medium), and the maximum generation were set at 150; 2,500,000 and 27,000, respectively. The grid box was centered on the enzyme's active site with x, y, and z coordinates of 17.778 -80.36, -30.62 Å. The number of points in

the x, y and z dimensions was 40 × 50 × 50. The spacing between grid points was set at 0.375 Å. The molecular visualizations were executed in Python3.4.

2.4. X-ray Crystallographic Analysis. Red crystals of compound **1** were obtained from AcOEt-hexanes. Data for complex **1** were collected at 150 K on a Bruker D8 Quest with IμS microfocus source using Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods and refined by full-matrix least-squares refinement on F2 after multiscan absorption correction of the data using SADABS [22,23]. Data was processed using the Bruker AXS SHELXTL software, version 6.14 [24]. Crystallographic data of **1**: C25 H26 O5 (M = 406.46); Triclinic crystal (0.358 x 0.101 x 0.040 mm^3); space group P-1; unit cell dimensions a = 4.5662(6) Å, b = 12.7014(19) Å, c = 18.1090(2) Å, α= 100.464(7)°, β = 93.846(5)°, γ=95.467(4)°, V = 1024.3(2) $Å^3$; Z = 2; ρ_{calcd} = 1.318Mg/m^3; μ = 0.091 mm^{-1}; 15 280 reflections measured (2.557° ≤ θ ≤ 28.354°), 4925 unique (Rint = 0.0590), which were used for all calculations; the final refinement produced R1 = 0.0852, wR2 = 0.1932 (all data); and R indices (all data) R1 = 0.1604, wR2 = 0.2299. The crystallographic data of **1** were deposited at the Cambridge Crystallographic Data Centre under deposition number CCDC 2022971. Copies 18 of the crystallographic data can be obtained for freefrom the Cambridge Crystallographic Data Centre, 12 Union Road,Cambridge CB2 1EZ, UK e-mail: deposit@ccdc.cam.ac.uk or fax:(+44) 1223-336-033] or by www.ccdc.cam.ac.uk/conts/retrieving.html

3. RESULTS AND DISCUSSION

The air-dried twigs of *Psorospermum guineense* used in traditional medicine to treat cancer (1.5 Kg) were extracted using a mixture of CH_2Cl_2-MeOH (1:1) in a sonicator bath to afford 300 g of extract. 150 g of the extract was partitioned using hexanes, EtOAc and n-BuOH to afford hexane (30 g), EtOAc (25 g) and n-BuOH (20 g) fractions. Analysis of the high-resolution LC-MS of the various fractions revealed the presence of different compounds. The major compounds were isolated from these fractions, characterized based on the interpretation of their 1D and 2D NMR values, and compared with those described in the literature. The hexane extract was subjected to repeated column chromatography to yield 3-geranyloxyemodine **1**, 3-β-fridelanol **2**, 3-geranyloxyemodine anthrone **3** and 2-geranylemodine **4**, the EtOAc fraction afforded; 3-geranyloxyemodine **1**; 3-geranyloxyemodine anthrone **3**, 2-geranylemodine **4**, Acetylvismione D **5**, Betulinic acid **6**, and Ursolic acid **7** while the n-butanol fraction afforded 3-geranyloxyemodine **1**, 3-β-fridelanol **2**, 3-geranyloxyemodine anthrone **3** and 2-geranylemodine **4**, Acetylvismione D **5** and Emodine **8**. See Figure 1[15]. The stereochemistry of the double bonds and conformation of the geranyl moiety of compound **1** was attributed based on single crystal x-ray analyses (Figure 2).

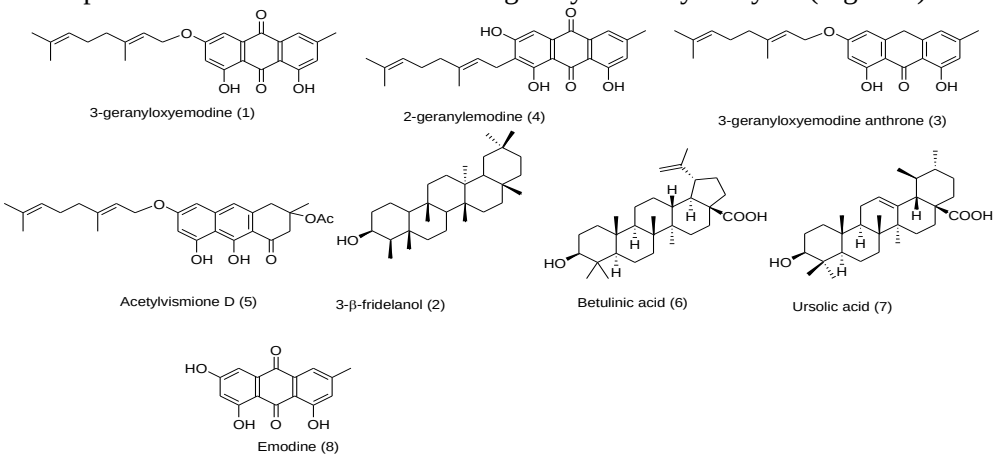

Figure 1: Isolates from the Hexane, EtOAc and nBuOH extracts of the twigs of *Psorospermum guineense* [15].

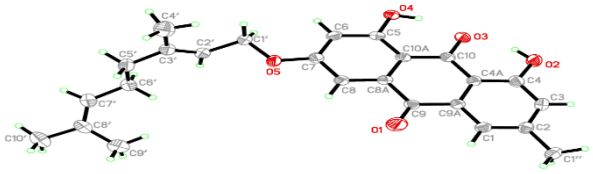

Figure 2: Single crystal X-ray structure of 3-geranyloxy Emodine **1**

The relative viability of the MDA-MB-231 breast cancer cell line exposed to these compounds at multiple concentrations was analyzed to ascertain if any of the isolates exhibited cytotoxic activity. MDA MB 231 cells were plated in a 96-well format. The indicated compounds, dissolved in DMSO, were serially diluted in culture media prior to addition to the culture. After a 3-day incubation time, relative viability was indirectly determined by measuring

total ATP in each well using CellTiter-Glo (Promega, Figure 3). Following this assay, the IC_{50} value was determined with 95% confidence interval. Several compounds showed moderate cytotoxic activity against MDA-MB-231 breast cancer cells. Compound **7** (ursolic acid) gave the best activity with an IC_{50} of 9.88 µM and 95% CI of 9.03- 10.5 µM.

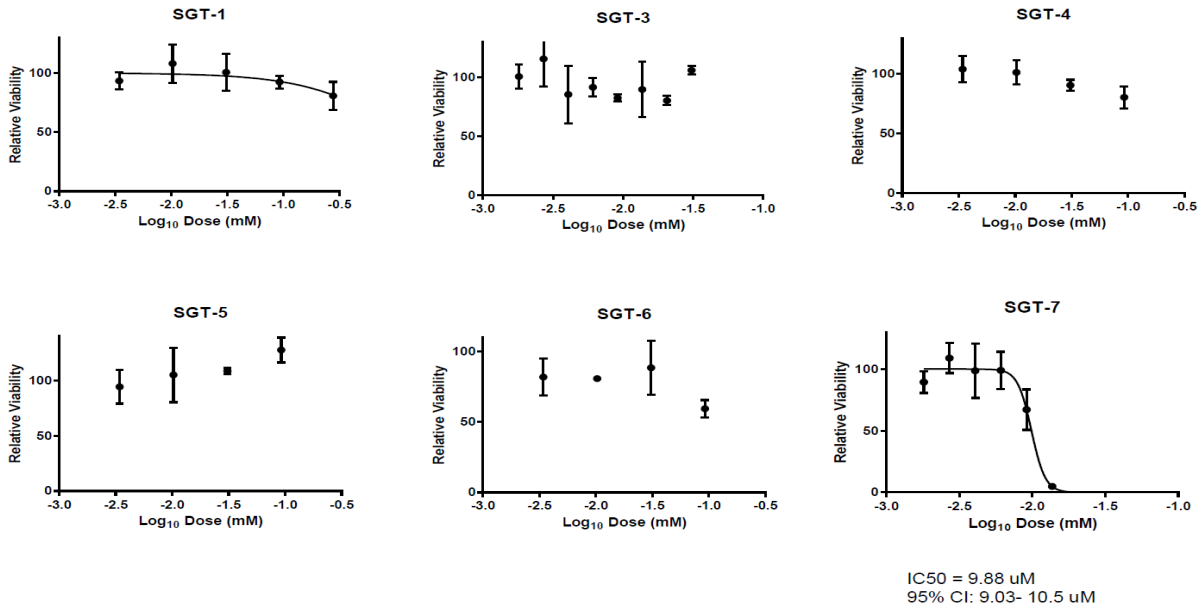

Figure 3: Cytotoxic activity of compounds **1** and **3-7**

To get a deeper inside on the possible mechanism of action, these compounds were tested on some known cancer targets [16].

The isolated compounds were equally tested for their LOX inhibitory activity, antioxidant activity and urease inhibition activity. The quinone, Acetylvismione D **5** exhibited better lipoxygenase inhibitory activity (19.8±0.16 µM) and antioxidant activity (20.8±0.15 µM) compared to the reference drugs baicalein (22.6 ± 0.08 µM) and Beta hydroxyl acid (BHA) (44.2 ± 0.07 µM) respectively. None of these compounds were more active than Thiourea for urease inhibition activity. The rest of the isolated compounds showed moderate activity, as shown in Table 1. All of the isolates exhibited their inhibitory activity in a concentration-dependent manner. Baicalein (IC_{50} = 22.6 ± 0.08 µM). Beta hydroxyl acid (BHA) (44.2 ± 0.07 µM) and Thiourea (24.2± 0.09 µM) were used as a positive control.

Table 1 Antioxidant activity, lipoxygenase inhibitory activity and urease inhibition activity of compounds from the twigs of *Psorospermum guineense*[a]

Sr. #	Compound	Antioxidant Activity IC_{50} value (µM)	Lipoxygenase Inhibition Activity IC_{50} value (µM)	Urease Inhibition Activity IC_{50} value (µM)
1.	SGT 1 (1)	85.6 ± 0.05	98.6 ± 0.36	65.6± 0.17
2.	SGT 2 (2)	25.7± 0.11	28.7± 0.81	55.1± 0.82
3.	SGT 3 (3)	53.4± 0.29	76.6± 0.93	52.1± 0.71
4.	SGT 4 (4)	35.6± 0.86	38.9± 0.25	44.8± 0.16
5.	SGT 5 (5)	20.8± 0.15	19.8± 0.16	38.9± 0.18
6.	SGT 6 (6)	78.8± 0.79	91.1± 0.77	66.5± 0.22
7.	SGT 7 (7)	79.7± 0.57	87.7± 0.10	81.6± 0.30
8.	BHA	44.2 ± 0.07	-	
9.	Baicalein[b]	-	22.6 ± 0.08	
10.	Thiourea			24.2± 0.09

[a] Values are the mean ± SEM of three experiments ($p < 0.05$).

[b] Positive control

Some significant results of the *in silico* studies are summarized in Table 2. The experimental IC_{50} values of isolates revealed that the better LOX result was obtained for compound **5** (Table 1). This is in agreement with the estimated free energies of binding of compound **5** and Baicalein (Table 2). Docking analysis showed that, the compound **5** created conventional H-bonding, carbon-H bond, Pi-Pi T-shape, alkyl and Pi-alkyl interactions with amino acids in the active site of LOX (Fig. 5) and had lowest binding affinity (ΔG Binding = -8.14 kcal/mol). Also, docking studies indicated that Baicalein had no carbon-H bond but Van der Waals, conventional H-bonding, Pi-donor H-bond, Pi-Lone Pair, Pi-Pi T-shaped, Amide Pi-stacked and Pi-alkyl interactions with amino acids of the active site residues of LOX (Fig. 4) and had higher binding affinity (ΔG Binding = - 7.81 kcal/mol),

Table 2 Summarizing the important docking results. The H-bond distances have been measured between related atoms

Compound	**Estimated $\Delta G_{Binding}$ (kcal/mol)**	**Hydrogen bonding**		
		Interacted ligand functional group	**Interacted amino acid**	**Distance (Å)**
3-geranyloxyemodine	- 7.81	C=O	ALA 672	2.76
2-geranylemodine	- 8.76	OH C=O	ALA 672 GLN 557	2.03 2.74
3-geranyloxyemodine anthrone		C=O	PHE 177	2.68
Acetylvismione D	- 8.14	O	PHE 177	2.67
3-β-fridelanol	- 9.02	OH	ASN 554	1.81
Betulinic acid	- 9.17	OH	ASN 554	2.27
Ursolic acid	- 9.79	COOH OH	PHE 177 ASN 554	2.63 1.89
Baicalein	- 7.81	OH	VAL 671 ALA 672	1.95 2.00

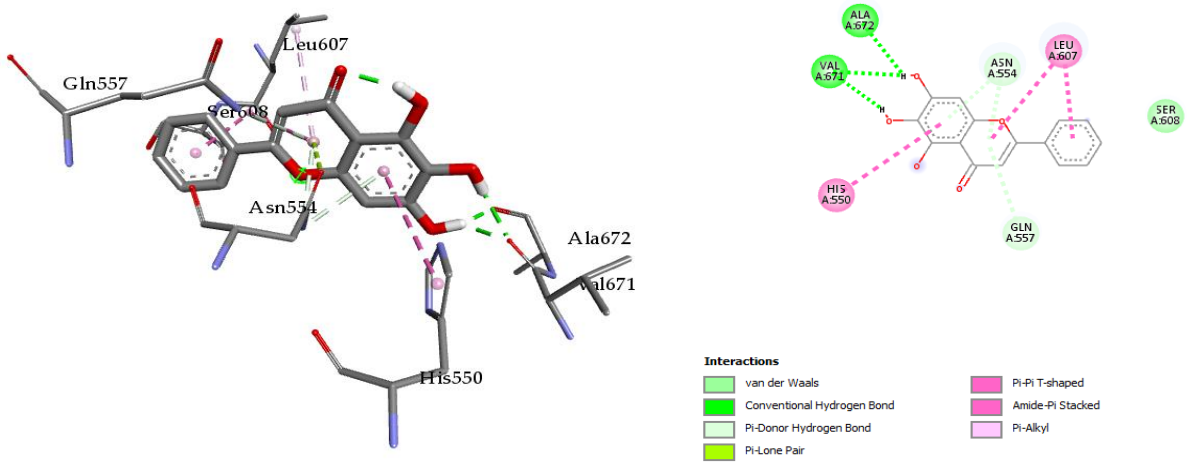

Figure 4 Interactions between Baicalein and the active site of the 3V99

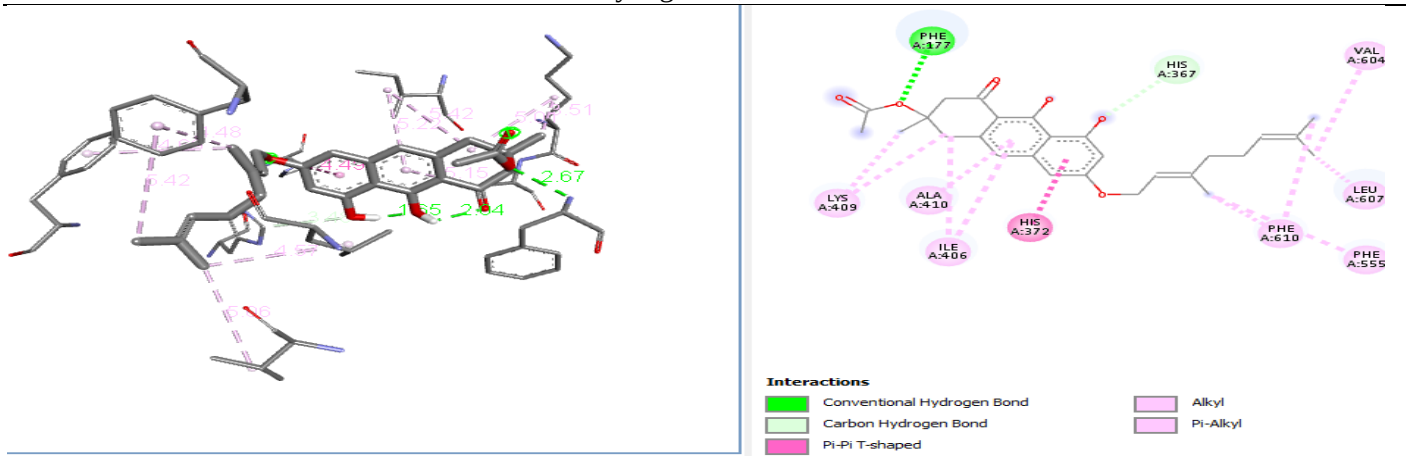

Figure 5 Interactions between Acetylvismione D and the active site of the 3V99

4. DISCUSSION

Antioxidants can track down and neutralize free radicals preventing chain reactions which can cause cancer. More body cells are kept healthy and less susceptible to cancer. Lipoxygenases or ureases could cause oxidative stress or inflammation.

LOX-mediated products elicit diverse biological activities needed for neoplastic cell growth, influencing growth factor and transcription factor activation, oncogene induction, tumour cell adhesion stimulation, and apoptotic cell death regulation [25]. Agents that block LO-catalyzed activity may effectively prevent cancer by interfering with signalling events needed for tumour growth. Enzyme activity inhibitors may be better candidates for chemopreventive intervention, because these enzymes' inhibition directly reduces fatty acid metabolite production, with concomitant damping of the associated inflammatory, proliferative, and metastatic activities that contribute to carcinogenesis. So, the discovery of new lipoxygenase inhibitors with more inhibitory activity is needed. In fact, in a few studies, LOX inhibitors have prevented carcinogen-induced lung adenomas and rat mammary gland cancers [25]. Bacteria can produce urease, which is a major virulence factor that may also play a role in carcinoma development.

To explore the activities of the present compounds and binding interactions between ligands and lipoxygenase, the structures of isolated compounds (**1-8**) were docked into the active site of LOX (PDB code: 3V99). The structure and conformation of compound **1** was unambiguously confirmed from single crystal analysis. The analysis revealed that the geranyl group was not a linear chain. This conformation was a very important factor which was considered for the docking.

5. CONCLUSION

In our attempts to isolate natural products for drug discovery and development from a rational viewpoint, the cancer disease, its mode of inhibition (antioxidant) or possible targets (lipoxygenase or urease) was evaluated. Bioactive constituents have been isolated from the twigs of *Psorospermum guineense*, the stereochemistry and conformation of the geranyl sub unit of compound **1** were established from single crystal x-ray analysis, and the isolates evaluated for their biological activities. Ursolic acid **7** was the most active compound tested for triple-negative breast cancer. All the quinolic derivatives (1-4) tested showed better antioxidant activity than BHA, which was used as a reference drug. The lipoxygenase (15-LOX) inhibitory activity of the isolated compounds was investigated for the first time with Acetylvismione D showing the best activity with IC_{50} value in the micromolar range. *In silico* study of isolated compounds showed possible binding modes on the enzyme target lipoxygenase (15-LOX) (Observed were conventional H-bonding, carbon-H bond, Pi-Pi stacked, alkyl and Pi-alkyl interactions between enzyme-substrate.) and confirmed the experimental results. Also, the molecular docking analysis confirmed the potential of the studied compounds for future drug discovery investigations. Further QSAR and kinetic studies are needed to understand the inhibitory mechanism of corresponding compounds and relative derivatives.

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