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Free Radical Scavenging Activity and Oxidative Stress Inhibition of *Dalbergiella welwitschii* Essential Oils: Evidence from Experimental and Computational Studies

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Abstract

Free radicals are a growing public health concern because of their contribution to the development and progression of many diseases. Essential oils from medicinal plants have long displayed antioxidant properties that could be exploited in scavenging free radicals and preventing oxidative stress. This study examines the potential of *Dalbergiella welwitschii* essential oils to prevent oxidative stress by their ability to scavenge free radicals. Insilco studies on the potential of the major compounds present in the oils to inhibit the overexpression of free radical generating proteins (Human Xanthine Oxidase (XOR), Myeloperoxidase (MPO) and Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2)) was also conducted. The toxicity of the essential oils and its major compounds were ascertained using the Brine shrimp lethality test and ADMET studies. Aristolone (32.3%) and 13-isopimaradiene (88.13%) dominated the oils of the stems and leaves, respectively. The EC_{50} of the leaf oil (0.382 µg/mL) and the stem oil (0.369 µg/mL) indicated that they could effectively inhibit free radicals of DPPH even at minute concentrations. Molecular docking studies showed that 13-isopimaradiene had the highest binding affinity with XOR (-7.6 kcal/mol) and MPO (-7.8 kcal/mol) comparable to the standards Allopurinol (-6.0, -6.4 kcal/mol) Dextromethorphan (-6.2, 7.0 kcal/mol) and Ticlopidine (-7.7, -7.0 kcal/mol). Brine shrimp lethality test revealed that the oils were medium toxic in comparison to cyclophosphamide. The ADMET studies confirmed that the major compounds 13-isopimaradiene and aristolone are non-toxic, have good absorption and solubility hence, could be safely administered as drugs with no toxic side effects. These findings show that the essential oils of *Dalbergiella welwitschii* could be used as a natural alternative source of antioxidants. The major compounds could also serve as scaffolds in the development of drugs with antioxidant potential that could regulate free radical generation.

Keywords: *Dalbergiella welwitschii*, Essential oils, 13-isopimaradiene, Aristolone, Molecular docking

1 Introduction

Africa is blessed with abundant medicinal plants, and a large number of the population depend largely on these medicinal plants to meet their healthcare needs. Due to the underdeveloped nature of the pharmaceutical ecosystem, many public health challenges ravage countries within the African continent. Hence, there is a need to pilot research on random and targeted screening and computational approaches to identify bioactive molecules that could serve as scaffolds for drug discovery or as a direct source of phytopharmaceuticals.

One of the public health challenges is oxidative stress, which arises when there is an imbalance between free radicals generated in the body and antioxidants. Free radicals are generated in the body to help fight infections, but when produced in excess, they can be harmful the body (Lobo et al. 2010). In some cases, free radicals could be acquired externally due to environmental pollution, a high-fat and sugar diet, or smoking and alcohol consumption. They are also produced internally by reactive oxygen species-generating enzymes such as NADPH oxidase, myeloperoxidase, cytochrome P450, and xanthine oxidase (Martemucci et al. 2010). These enzymes catalyze free radical chemical reactions to help the body fight harmful pathogens, degrade drugs, support renal function, and promote tissue regeneration. However, an overexpression of these enzymes could lead to the development of various diseases related to oxidative stress (Kargapolova et al. 2021; Mohan et al. 2023).

A natural defense against oxidative stress exhibited by the body is the mechanism of antioxidants. These antioxidants exert their actions by either preventing free radical generation or blocking their access to important biochemical sites in the body. The body produces a few of these antioxidants, such as glutathione, ubiquinol, and uric acid. Most other antioxidants are supplied through diet and synthetic antioxidants sources (Sharifi-Rad et al. 2020). Many individuals in developing or underdeveloped countries due to poverty are unable to afford antioxidant-rich diets, hence limiting their options to synthetic antioxidants. Recent studies have shown that if used incorrectly, synthetic antioxidants can lead to carcinogenicity, induction of oxidative stress, and cytotoxicity (Xu et al. 2021). Given the rising exposure to free radicals, there is a need to explore natural and affordable alternative sources of antioxidants to combat the rising challenge of oxidative stress and its associated health risks. Most of the population in suburban and rural communities prefers the holistic use of herbal medicine arising from cultural attachment; hence it is necessary to provide

information on the antioxidant activity of local plants that could be easily and safely adopted as sources of natural antioxidants and to examine if the phytochemicals present in these plants could help inhibit the overexpression of free radical-generating enzymes that could mediate oxidative stress.

Essential oils obtained from medicinal plants are good sources of antioxidants. This may be due at times to the complex nature of their chemical composition, which may act synergistically to quench free radical reactions. The antioxidant activities of essential oils may also arise from the action of a few main compounds that dominate the oil (de Sousa et al. 2023).

In complementary alternative medicine, a major way of harnessing the antioxidant abilities of essential oils is through aromatherapy (Mot et al. 2022). In most cases, aromatherapy utilizes a blend of extracted essential oils for either oral or topical applications (Agarwal et al. 2022). In local herbal medicine, steam inhalation generates the antioxidant activities of plant essential oils (Gowrishankar et al. 2021). In steam inhalation, the patient inhales essential oils directly as medicinal herbs are being hydrodistilled. In Nigeria, this method of essential oil delivery is used to treat pulmonary issues, headaches, fevers, and flu, and relieve congestion.

This study examines the phytoconstituent, *in vitro* antioxidant, and cytotoxicity of the essential oils of *Dalbergiella welwitschii*; a medicinal shrub indigenous to tropical West Africa. The plant has been utilized as remedy for ailments associated with oxidative stress such as diabetes and inflammation (Olowokudejo et al. 2008; Adediwura and Akinla 2013; Boisa et al. 2021; Ajiboye et al. 2022; Diniso et al. 2022). *In silico* studies, of the effect of the major compounds present in the essential oils on free radical-generating enzymes was also conducted. This study was conducted to determine whether essential oils could be adopted as a natural antioxidant source to complement the body's defense against oxidative stress.

2.0 Materials and methods

Aerial parts of *Dalbergiella welwitschii* (Baker) Baker f. plant was collected in January 2022 from abandoned farmland in Jericho, southwest of Ibadan, Oyo State Nigeria. Latitude 7.3913215°N. Longitude 3.8572761°E and altitude of 180 meters. The plants were authenticated at the University of Ibadan Herbarium, where a voucher specimen was deposited (UIH-23199).

Dalbergiella welwitschii plants were rinsed with water separated into various organs and air dried. The air-dried leaves (74.11g) and stems (225.57g) were hydrodistilled using a Clevenger apparatus designed by the British pharmacopoeia specifications. The obtained essential oils were dried using anhydrous sodium sulphate (Na_2SO_4) and stored at 3°C before analysis and further studies.

The chemical constituents of the essential oils were determined using a gas chromatogram coupled to a mass spectrophotometer, fitted with a Rxi-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium gas was used as the mobile gas (1.00 mL/min). The temperature was maintained at 60°C for 3 minutes and set to increase at a rate of 20°C/min up to 120°C. after 3 minutes it was set to increase again to 180°C at a rate of 20°C/min. The sample injection mode was split (50:50), with the interface temperate set at a range of temperatures (260°C, 220°C, and 240°C). 0.5µl sample was injected using an autosampler. Electron impact ionization was utilized as an ionization source with an energy of 70 eV. A quantitative and qualitative analysis report for the chemical constituents present in the essential oils was generated using Shimadzu real-time analysis from the NIST17R library software. A positive identification of the chemical compounds was performed using the generated GC and mass spectral data. A comparison of retention indices with the reported retention index of compounds obtained using a closely related capillary column (DB5-MS) was also carried out (NIST 2023 accessed January 23, 2024).

Antioxidant activity was tested using the DPPH radical scavenging assay. The radical scavenging (%) power of the essential oils at 1.0, 0.5, and 0.25 mg/mL concentrations were measured and compared with standard dietary antioxidant BHA and ascorbic acid at the same concentrations. An initial stock solution of 1.0 mg/mL concentration was prepared by adding 0.5 mL of the essential oil, to 2 mL of methanol (Mbachu et al. 2023). Serial dilution was performed to obtain 0.5 mg/mL and 0.25 mg/mL concentrations. All concentrations were prepared in triplicate. A 0.1 mM concentration of the DPPH radical was prepared by dissolving 3.95 mg of DPPH in 100 mL of methanol. The radical reaction was initiated by adding 2 mL of DPPH solution to the test samples and incubating them in the dark for half an hour (Baliyan et al. 2022). A negative control sample was prepared using DPPH and methanol. The absorbance values (A_S) of all samples were read on a UV-VIS spectrophotometer at 517 nm. The absorbance of the control (A_C) containing DPPH solution and methanol was also measured. Before obtaining absorbance values, the spectrophotometer was blanked using methanol. The absorbance values obtained were used in

calculating the percentage inhibition (%I) of the oils, which in turn was used to determine the efficient concentration of antioxidants needed to reduce the DPPH concentration by 50% (EC_{50}) (Lagarto et al. 2001).

$$\%I = (A_C - A_S / A_C) \times 100 \quad (1)$$

Preliminary cytotoxicity screening was performed using the Brine Shrimp Lethality Assay (BSLA). Ten (T_N) Freshly hatched shrimp (*Artemia salina*) in seawater were treated with essential oils at concentrations of 1000, 100, and 10 ppm, respectively, in triplicate (Nguta et al. 2012). The total number of live shrimps (A_N) after 24 hours of exposure to the essential oils was noted and used to determine the percentage mortality (%M). The lethal concentration at 50% (LC_{50}) of the essential oils was extrapolated using the equation of regression generated from a plot of probit values transformed from percentage mortality against the logarithm of concentration. The LC_{50} values were categorized as either high or low in comparison to the cytotoxic drug cyclophosphamide (Baccouri and Rajhi 2001). A stock solution of 1000 ppm was prepared by adding 0.02 mL of essential oil and 0.2 mL of DMSO to 1.8 mL of seawater. Then, 0.5 mL of the 1000 ppm solution was further diluted with 2 mL of sea water to give a 100 ppm solution. The 10 ppm solution was obtained using the same dilution factor as the 100 ppm solution. The reference standard cyclophosphamide was prepared by dissolving 0.2 mg in a mixture of 0.2 mL DMSO and 1.8 mL of seawater.

$$\%M = (T_N - A_N / T_N) \times 100 \quad (2)$$

2.1 Molecular Docking

Of the essential oil constituents of *Dalbergiella welwitschii* identified, the two compounds (15-isopimaradiene and Aristolone) (Figure 1) and 3 FDA approved drugs (Allopurinol, Dextromethorphan, and Ticlopidine) used as standards were docked in each oxidative stress target to study their inhibitory or enzymatic actions at the atomistic level in the targets' receptor binding domain. The analysis and interpretation of binding interactions play a crucial role in potential drug design and elucidating the fundamentals of biochemical processes. The X-ray crystallographic deposited structural coordinates of human xanthine oxidase (XOR; PDB ID: 2E1Q, 2.6 Å resolution) (Yamaguchi et al. 2007), glycoforms of myeloperoxidase (MPO; PDB ID: 3F9P, 2.93 Å resolution) (Carpena et al. 2009), and nicotinamide adenine dinucleotide phosphate (NADPH)

oxidase (NOX2) (PDB ID: 7U8G, 3.20 Å resolution) (Noreng et al. 2022) were downloaded from the Protein Data Bank RCSB (2021 accessed December15, 2023). The protein was optimized by adding polar hydrogen bonds after deleting water molecules and other heteroatoms in the protein using the Biovia Discovery Studio. A molecular docking study of the compounds and targets was performed using AutoDock Vina incorporated in PyRx. The post-dock protein–ligand complex was analyzed using ChimeraX and Biovia Discovery Studio 2021.

2.2 Pharmacokinetic Analysis and Toxicity Prediction

The drug-likeness properties of the compounds were calculated using the SwissADME server (Daina et al. 2017), while the AdmetSAR2.0 online server (Yang et al. 2019) was mainly used for the analysis of the toxicity level of the compounds. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties are indicators for lead optimization that will scale through to become a drug candidate. The predicted result from SwissADME consists of physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and bioavailability score.

3.0 Results

Essential oils were obtained in a yield of 2.1% for leaves and 0.4% for stems. Seventeen compounds were identified in *Dalbergiella welwitschii* leaf and stem oils (Table 1), five in the leaves and 15 in the stems. The major compounds in the oils were 15-isopimaradiene (88.13%) in the leaves and Aristolone (32.93%) in the stems. In general, terpenes dominated the essential oils of *D. welwitschii* leaves and stems. The oils contained 4 monoterpene hydrocarbons, α-pinene in the stem oil only; while α-phellandrene, D-limonene and p-cymene were common to both the stem and leaf oils. Two oxygenated monoterpene alcohol were also present, eucalyptol in the stem and linalool in the leaves. An adamantane derivative in the stem oil was the only sesquiterpene hydrocarbon present in the essential oils. Five oxygenated sesquiterpenes were found in the stem oils, four of which were keto compounds and one hydroxy compound. The only diterpene observed was in the leaf oil. Terpenes are compounds with excellent antioxidant activity, which gives them the ability to ameliorate and protect against oxidative stress (Torequl-Islam et al. 2022). Diterpenes, especially the pimarane diterpenes, are classes of compounds with valuable bioactivities such as antioxidant, anti-inflammatory, and anti-rheumatoid properties.

Sesquiterpenes are compounds with the potential to cross the blood-brain barrier in addition to their many useful medicinal properties (Avila-Galvez et al. 2023). The presence of the diterpene 15-isopimaradiene and the sesquiterpene aristolone could impart good radical scavenging abilities to the oils and could help prevent diseases such as inflammation and neurodegenerative conditions.

Table 1 Essential oil constituents of *Dalbergiella welwitschii*

Identified compounds[a]	**RI(Exp)[b]**	**RI(Lit)[c]**	**Class of compound**	**LEO[d]**	**SEO[e]**
α-Pinene	948	940	MT	-	0.99
α-phellandrene	969	968	MT	1.66	2.83
D-limonene	1018	1010	MT	1.78	0.30
p-cymene	1042	1026	MT	2.17	1.60
β-methyl levulic acid	1046	-	NTD	-	1.78
Eucalyptol	1059	1033	MTO	-	0.60
Linalool	1082	1101	MTO	6.26	-
Thujopsenal	1407	-	STO	-	5.74
Ylangenal	1410	-	STO	-	1.47
Propyl-3-(propene-1-yl) adamantane	1433	-	ST	-	3.98
Aristolone	**1574**	**1723**	**STO**	**-**	**32.93**
Isolongifolene-5-one	1587	-	STO		4.54
5-hydroxy calamene	1757	-	STO	-	4.00
Nodecene	1900	-	HC	-	1.91
Falcarinol	1906	-	Fatty alcohol	-	2.79
15-isopimaradiene	**1926**	**1922**	**DT**	**88.13**	**-**
Heneicosane	2109	-	HC	-	30.54
Total identified				100.0	93.24
Class of compounds					
Monoterpene (MT)				5.61	5.72
Monoterpenoids (MTO)				6.26	0.60
Sesquiterpene (ST)				-	3.98
Sesquiterpenoids (STO)				-	48.68
Diterpene (DT)				88.13	-
Diterpenoids (DTO)				-	-
Total terpenes				100	58.98
Non terpenes				-	34.23

a-The constituents are listed in order of increasing retention index as eluted from an Rxi-5sil MS capillary column. Major compounds are in bold.
b- Retention index from this work, on a low-polar Rxi-5sil MS capillary column generated with NIST17R.lib.
c-Retention indices from literature on DB5-MS Capillary column with similar specifications curated using the NIST Chemistry webbook database. – indicates not available or not detected.
d – Leaf essential oils, e – Stem essential oils

The antioxidant activities of the essential oils, as expressed by their ability to scavenge free radicals of DPPH, were investigated, and compared with the standard known antioxidant butylated hydroxyl anisole (BHA) and ascorbic acid (Table 2). The percentage radical scavenging activity of the leaf oil was dose-dependent and increased with increasing concentration. For the stem oil, an increase in concentration did not significantly affect its scavenging ability. The effective inhibitory concentrations of the oils indicated that even at minute concentrations, the oils can still exert their antioxidant power and help to scavenge reactive oxygen species (ROS). The antioxidant activities of *Dalbergiella welwitschii* leaf and stem essential oil could be attributed to its high terpene content. Studies have shown that essential oils rich in terpenes content exhibit good antioxidant activities (Shaaban et al. 2012). Structure-activity relationship has shown that essential oils that contain monoterpenes with a strongly activated methylene group, oxygenated sesquiterpenes, or diterpenes of the abietane class usually exhibit high antioxidant activities (Gonzalez-Burgos and Gomez-Serranillos 2012). The major compound in the leaf, 15-isopimaradiene is an abietane-type diterpene and the major compound in the stem (-)-aristolone is an oxygenated sesquiterpene. It is possible that these two compounds are mainly responsible for the antioxidant activity of the oils. Monoterpenes with methylene groups, such as p-cymene and D-limonene, were also present in both oils. The increasing radical scavenging power of the oil with increasing concentration may suggest a synergistic contribution from the other compounds in the oils.

Table 2 Antioxidant activity of *Dalbergiella welwitschii* leaf and stem essential oils

Samples	Mean Percentage inhibition			EC_{50} (µg/mL)
	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	
Ascorbic acid	94.4±0.00	93.4±0.01	92.4±0.01	0.174±0.01
BHA	95.4±0.01	93.8±0.01	93.8±0.01	0.167±0.02
Leaf oil	76.9±0.02	71.7±0.01	66.0±0.00	0.382±0.01
Stem oil	72.6±0.02	72.1±0.01	71.3±0.01	0.369±0.01

Preliminary cytotoxic screening of the essential oils of *Dalbergiella welwitschii* was carried out using the brine shrimp lethality test (Table 3). The median lethal concentration (LC_{50}) of the essential oils was extrapolated from a plot of the percentage of dead shrimp converted to probit values against the logarithm of concentration. The leaf and stem essential oils were medium or low toxic. This was confirmed by a comparison of their LC_{50} values with that of the cytotoxic drug cyclophosphamide. This preliminary toxicity screening is essential to provide information on the potential safety and tolerability of the essential oils for adoption as pharmaceutical agents (McGaw et al. 2014). For potential drug agents, minimal to no toxicity is desirable, and the lethality test shows that the essential oils can be diffused, inhaled, or applied topically with no potential risk. The medium toxicity of *D. welwitschii* essential oils is ideal because it imparts to the oil's dual therapeutic activities. It will enable the plant not only to scavenge free radicals but also to prevent or halt any abnormal cell proliferation. Another important information that could be gained from the brine shrimp lethality test is the starting dose of essential oils that could be used for in vivo studies.

Table 3 Brine shrimp lethality assay of *Dalbergiella welwitschii* essential oils

Plant extract	Mean Percentage Mortality			LC_{50} (µg/mL)
	10 ppm	100 ppm	1000 ppm	
Leaf oil	20±0.82	47±0.54	73±0.72	153.69±0.36
Stem oil	43±0.98	60±0.00	73±0.54	474.79±0.28
Cyclophosphamide	13±0.72	80±0.47	100±0.00	52.84±0.17

3.1 Molecular Docking

The production of reactive oxygen species (ROS) is a fundamental process in various biological systems and is tightly regulated to maintain homeostasis. However, when this balance is disrupted, it can lead to oxidative stress, a state implicated in the pathogenesis of numerous diseases. This study evaluates three key enzymes involved in ROS production and regulation: xanthine oxidase (XO), myeloperoxidase (MPO), and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, specifically the NOX2 isoform. Xanthine oxidase (XO) catalyzes the oxidation of hypoxanthine to xanthine and further to uric acid, producing ROS as by-products. Excessive XO activity is associated with inflammatory responses and atherosclerotic plaque formation, which are

major cardiovascular risk factors. ROS generated by XO can damage cellular structures, contributing to various diseases. Under conditions of metabolic stress or inflammation, XO generates uric acid and free radical superoxide, exacerbating chronic inflammation and oxidative stress. Inhibiting XO activity is crucial for preventing oxidative stress-related pathological conditions. Second, myeloperoxidase (MPO), which is abundant in neutrophils, also contributes to ROS production. Under conditions of metabolic stress or inflammation, MPO generates uric acid and superoxide, leading to chronic inflammation and oxidative stress. Inhibiting MPO can attenuate oxidative damage and neuroinflammation, but it may compromise the body's defence against pathogens. Hence a delicate balance has to be achieved between the overexpression and over inhibition of this enzyme. Finally, NADPH oxidase, specifically the NOX2 isoform, produces superoxide essential for host defense and immunoregulation. However, excessive ROS production by NOX2 can lead to oxidative stress and tissue damage. Inhibition of NOX2 can mitigate oxidative stress-related diseases but may increase susceptibility to certain infections. Therefore, balanced regulation of NOX2 activity is crucial for maintaining health.

In this study, as presented in Table 4, we evaluated the binding affinities and type of intermolecular interactions of the most active molecules (15-isopimaradiene (T2) and aristolone (T3)) with the oxidative stress receptors encoded as XOR (2E1Q), MPO (3F9P), and NOX2 (7U8G) and compare them with three established FDA approved drugs. To study the binding efficacy of the selected compounds, molecular docking studies were conducted in the binding pockets of the receptors. The poses of the lowest conformation of each compound were examined on the basis of the binding scores (also known as Gibbs free binding energy,$\Delta G°$). The compounds exhibited good binding scores ($\Delta G°$) in complex with XOR, ranging from −5.4 to −7.7 kcal/mol. However, T2 performed better than most of the standard drugs and had a close score with Ticlopidine ($\Delta G°$ of -7.6 kcal/mol). The molecular interactions and the T2 and T3 positions in the XOR receptor are depicted in Figure 2. A 2D model representation of the molecular interaction between the standard drugs in the binding pocket of the XOR receptor is shown in Figure 3.

The control drug Allopurinol interact with at least one of the active site residues, such as VAL 803, ARG 881, and GLU 1262. For xanthine as a substrate, these active site residues can facilitate nucleophilic attack by forming hydrogen bonds with the inhibitor's carbonyl functional group[22]. Aristolone and ticlopidine have a single hydrogen bond interaction (distance) in complex with

GLN 768 (2.34 Å) and ALA 1084 (2.74 Å) residues, respectively. Allopurinol and dextromethorphan have two hydrogen bond interactions in complex with XOR residues (Table 4). Our results provide support at the molecular level for the hydroxylation activity of XOR towards allopurinol, a standard drug, (Yamaguchi et al. 2007). However, T2 exhibited a more stable conformation at the active site than allopurinol which showed specificity.

Furthermore, the free energy for the docked compounds with the MPO receptor ranged from -6.4 kcal/mol (Allopurinol) to -7.8 kcal/mol (T2), as shown in Table 4. T2 has the highest binding affinity within the binding pocket of the MPO receptor with ΔG^o = -7.8 kcal/mol. It sits in the receptor-binding domain and has an alkyl interaction with one of the side chains of hydrophobic residues, LEU 417, as shown in Figure 4. Meanwhile, T3 forms a hydrogen interaction with one of the hydrophobic residues ARG 333 with a distance of 2.04 Å, this is substantially different from the previous MD simulation (3.00 ± 0.19 Å) and the experimental structure (2.89 Å) (Carpena et al. 2009). As shown in Figure 5, the standard compounds also interact well with the receptor but may not be stable in the binding pocket of the receptor when subjected to thermodynamic and/or kinetic studies. There is no specific or well-documented information available regarding the standard compound's interaction with myeloperoxidase (MPO). The study emphasizes that T2 can be used as a lead compound for oxidative stress treatment because it has a good affinity for the target MPO.

As shown in Table 4, ticlopidine, one of the control drugs, exhibited the best binding affinity with NOX2, among the compounds used for the docking study with $\Delta G° = -7.8\ kcal/mol$. Next T2, and Allopurinol with the binding energy of -5.7, -5.4, and -5.2 kcal/mol, respectively. While others exhibited interactions with the amino acid residues in the receptor binding domain, they were well below the threshold. T2 has no hydrogen bond interaction with NOX2, but T3 exhibited one carbon hydrogen bond contact with LYS 102 with 2.82 Å. Figures 6 and 7 show the inhibitor-bound complex at the receptor-binding domain of NOX2. By this comparison, we can conclude that T2 can serve as the lead anti-oxidative stress candidate in XOR, MPO, and NOX2 receptors. However, T2 is more stable in XOR and MPO receptors and did not perform close to the standard drug in complex with NOX2. This indicates that the compounds showed specificity by having specific contacts with the key residues in the binding site of these receptors.

3.2 Analysis of Physicochemical descriptors and pharmacokinetic parameters

Assessment of crucial physicochemical, pharmacokinetic, and drug-like properties for single or multiple molecules is essential when dealing with new compounds. A significant number of drugs do not reach the clinical market because of suboptimal pharmacokinetics. SwissADME and AdmetSAR 2.0 web servers were used to predict the molecular physicochemical and pharmacokinetic characteristics of the top two potential candidates obtained from the experimental and docking studies, as shown in Table 5. The table displays the molecular weight (MW), the number of hydrogen bond acceptors (HBA) and donors (HBD), the topological polar surface area (TPSA), and the octanol-water partition coefficient (log P). Both compounds exhibit moderate molecular weight and relatively standard log P values, which indicates that they are comparatively large, lipophilic, and soluble molecules. These characteristics may influence their bioavailability, distribution, and elimination. The compounds adhere to Lipinski's rule of five (Lipinski et al. 2012) and Veber's rule (Verber et al. 2002), with only T2 having one violation, indicating that the compounds may possess good solubility, permeability, and oral bioavailability, and may also demonstrate high cell membrane penetration and target affinity.

Both compounds exhibit a relatively low topological polar surface area (TPSA). The polar surface area (PSA) or TPSA is a measure of a molecule's polarity and is defined as the surface sum over all polar atoms or molecules, primarily oxygen and nitrogen, including their attached hydrogen atoms. This reflects the molecule's ability to form hydrogen bonds with water molecules. This result elucidates why T2 does not have any hydrogen bonding interaction in the molecular docking study described in the previous section, as this compound does not contain any oxygen or nitrogen atoms. Therefore, the compound may require optimization to enhance TPSA and molecular complexity to boost its activity. A low PSA (≤ 90 Å^2) indicates high permeability through the cell membrane, especially across the blood-brain barrier (BBB). The results indeed show that both compounds can cross the BBB. Both T2 and T3 fall under category III in the acute oral toxicity test, indicating a moderate level of toxicity. Both T2 and T3 show positive results in human intestinal absorption, human oral bioavailability, and androgen receptor binding, indicating that they can be absorbed into the bloodstream when taken orally, be available in the body when taken orally, and play a role in male traits and reproductive activity.

Both compounds are not mutagenic because they show a 'negative' result in the Ames Mutagenesis test. Reproductive toxicity is a measure of a substance's potential to affect reproductive

capabilities, including chromosomal damage (mutations) and effects on fetuses (teratogenesis) (Wei et al. 2022). T2 is not reproductively toxic, but T3 is, as indicated by '+' for T3. Although there is no direct relationship between these parameters and ROS production, they can provide indirect indicators. For instance, oxidative stress causes hepatotoxicity, or liver damage (Xue et al. 2023). From Table 5, hepatotoxicity is present in T3 and not in T2. Therefore, it could be inferred that T3 is associated with increased oxidative stress in the liver. In addition, thyroid hormones can induce oxidative stress by increasing the metabolic rate and thus the production of ROS. Therefore, T2 is associated with oxidative stress by having positive thyroid receptor binding, but it depends on the tissue and metabolic state of the organism (Venditti and Di Meo 2006).

These predictions are important safety parameters for drug candidates because they can indicate the potential for adverse effects and regulatory hurdles. Therefore, further in vitro and in vivo studies are required to confirm the safety profile of these compounds.

4.0 Conclusion

The essential oils of *Dalbergiella welwitschii* being reported for the first time contain valuable terpene hydrocarbons and oxygenated terpenes, which may be responsible for its radical scavenging abilities. Aristolone (T3) and 15-isopimaradiene (T2) have good binding affinity with some of the targets of oxidative stress and can act as lead compounds for the treatment or inhibition of oxidative stress and its associated diseases. The compounds performed better than most of the standard drugs used in this study. The essential oils were medium toxic, which could be attributed to the presence of monoterpenes in the oils. The compounds have drug-like properties and good pharmacokinetic profiles with low expected toxicity potential and could act as lead compounds for the treatment of ROS.

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Availability of data and materials

Not applicable

Competing interest

The author reports that there are no competing interests to declare.

Ethics approval and consent to participate

Not applicable

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Table 4: Molecular interactions of the ligands with the selected targets

Ligands	Human Xanthine Oxidase (XOR) (PDB ID: 2E1Q)		Myeloperoxidase (MPO) (PDB ID: 3F9P)		Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2) (PDB ID: 7U8G)	
	Binding affinity kcal/mol	Molecular interaction residues (distance, Å)	Binding affinity kcal/mol	Molecular interaction residues (distance, Å)	Binding affinity kcal/mol	Molecular interaction residues (distance, Å)
15-isopimaradiene (T2)	**-7.6**	ARG 913 (5.09*), MET 1039 (5.33*), ALA 1084 (3.70*), ALA 1259 (5.09*)	**-7.8**	LEU 417 (5.18*) and LEU 420 (4.16*, 5.50*).	-5.4	ILE 67 (4.96*), HIS 101 (3.54*, 5.49*), LYS 102 (3.67*), ALA 105 (3.57*, 5.32*), TRP 106 (4.82*), ILE 189 (4.41*, 4.50*), ILE 190 (4.65*), and HIS 209 (5.23*).
Allopurinol	-6.0	ARG 881 (4.93) THR 1011 (2.11, 1.02^), and VAL 1012 (2.29^).	-6.4	MET 87 (2.55), ASP 94 (2.68), and PHE 332 (2.47, 2.62)	-5.2	HIS 101 (3.06), HIS 209 (2.24), and PHE 215 (3.04, Pi-donor HB).
Aristolone (T3)	**-5.4**	GLN 768 (2.34) and MET 1039 (4.96*)	**-6.8**	ARG 333(2.04)	-2.1	LYS 102 (2.82, Carbon HB), ALA 105 (4.03*), and ILE 190 (5.23*).
Dextromethorphan	-6.2	CYS 150 (4.94*), PHE 799 (2.24, 3.64#), ARG 913 (4.75), and MET 1039 (4.46**).	-7.0	HIS 95 (4.69*), ARG 239 (3.90"), HIS 336 (5.23*), PHE 407 (4.83*), and LEU 417 (5.39*).	0	LYS 102 (4.62*, 5.39*, 3.00 (Carbon HB)), TRP 106 (5.11*), and PHE 205 (5.28*).
Ticlopidine	**-7.7**	PHE 799 (4.82*), ARG (3.88*, 4.68*), ALA 1079 (5.08*), and ALA 1084 (2.74, 4.84*).	-7.0	PHE 332 (4.50*) and HIS 336 (4.19", 4.40")	-7.8	ILE 67 (4.77*), HIS 101 (3.59, Carbon HB), LYS 102 (5.04*), ALA 105 (4.34*, 4.94*), and PHE 212 (5.23*).

*Alkyl or Pi-alkyl, ** Carbon hydrogen bond, ^ unfavorable donor-donor, # pi-sigma, " Pi-cation

Table 5 Physicochemical descriptors and some pharmacokinetic parameters of major compounds

	Parameters	15-isopimaradiene (T2)	Aristolone (T3)
Physicochemical	Formula	$C_{20}H_{32}$	$C_{15}H_{22}O$
	Molecular weight (g/mol)	272.47	218.33
	Hydrogen bond acceptor	0	1
	Hydrogen bond donor	0	0
	TPSA ($Å^2$)	0.00	17.07
	MlogP	5.82	3.56
Pharmacokinetics and Toxicity	Androgen receptor binding	+	+
	Blood-brain barrier	+	+
	Caco-2	+	+
	Human intestinal absorption	+	+
	Human oral bioavailability	+	+
	Thyroid receptor binding	+	-
	Acute oral toxicity ©	III	III
	Ames mutagenesis test	-	-
	Reproductive toxicity	+	-
	Hepatotoxicity	-	+
	Carcinogenicity	-	-
	Respiratory toxicity	+	-

ii. Captions of Figures

Figure 1: Structures of the major compounds (T2 and T3) and standard compounds.

Figure 2 Structure of inhibitor-bound complex at the receptor binding domain of human XOR. (a) and (c) show the 3D molecular representation of XOR side chains of the amino acid residues interacting with T2 and T3, respectively. (b) and (c) show the 2D model of the interactions of XOR amino acid residues with T2 and T3, respectively.

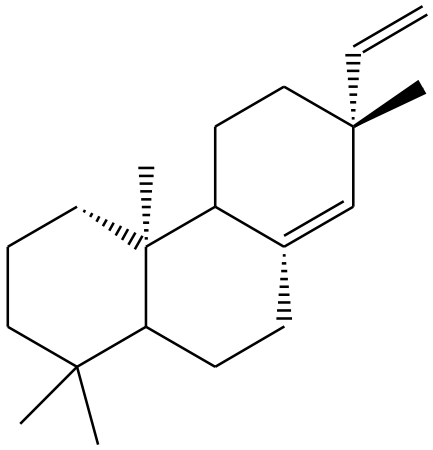
Figure 3: Molecular interaction between the XOR residues and FDA-approved drugs. (a) Allopurinol, (b) Ticlopidine, and (c) Dextromethorphan.

Figure 4: The structure of inhibitor-bound complex at the receptor binding domain of MPO (PDB ID: 3F9F). (a) and (c) show the 3D molecular representation of MPO side chains of the amino acid residues interacting with T2 and T3, respectively. (b) and (c) show the 2D model of the interactions of MPO amino acid residues with T2 and T3, respectively.

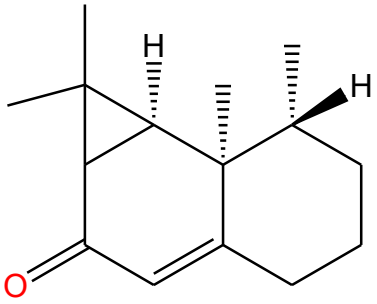
Figure 5: Molecular interaction between the residues of MPO and FDA-approved drugs ((a) Allopurinol, (b) Ticlopidine, and (c) Dextromethorphan.

Figure 6: Structure of inhibitor-bound complex at the receptor binding domain of NOX2 (PDB ID: 7U8G). (a) and (c) show the 3D molecular representation of NOX2 side chains of the amino acid residues interacting with T2 and T3, respectively. (b) and (c) show the 2D model of the interactions of NOX2 amino acid residues with T2 and T3, respectively.

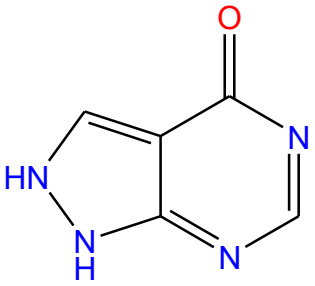
Figure 7: Molecular interaction between the residues of NOX2 and FDA-approved drugs ((a) Allopurinol, (b) Ticlopidine, and (c) Dextromethorphan



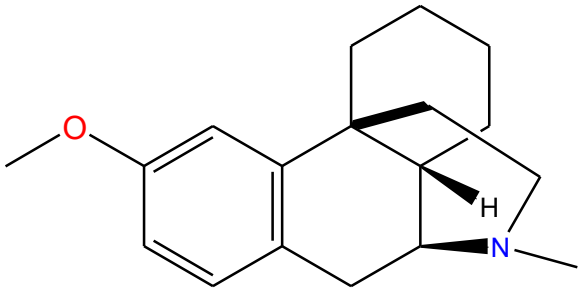
15-isopimaradiene
(T2)



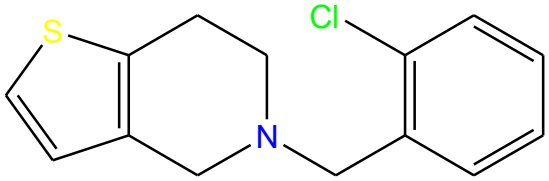
Aristolone
(T3)



Allopurinol



Dextromethorphan



Ticlopidine

Figure 1

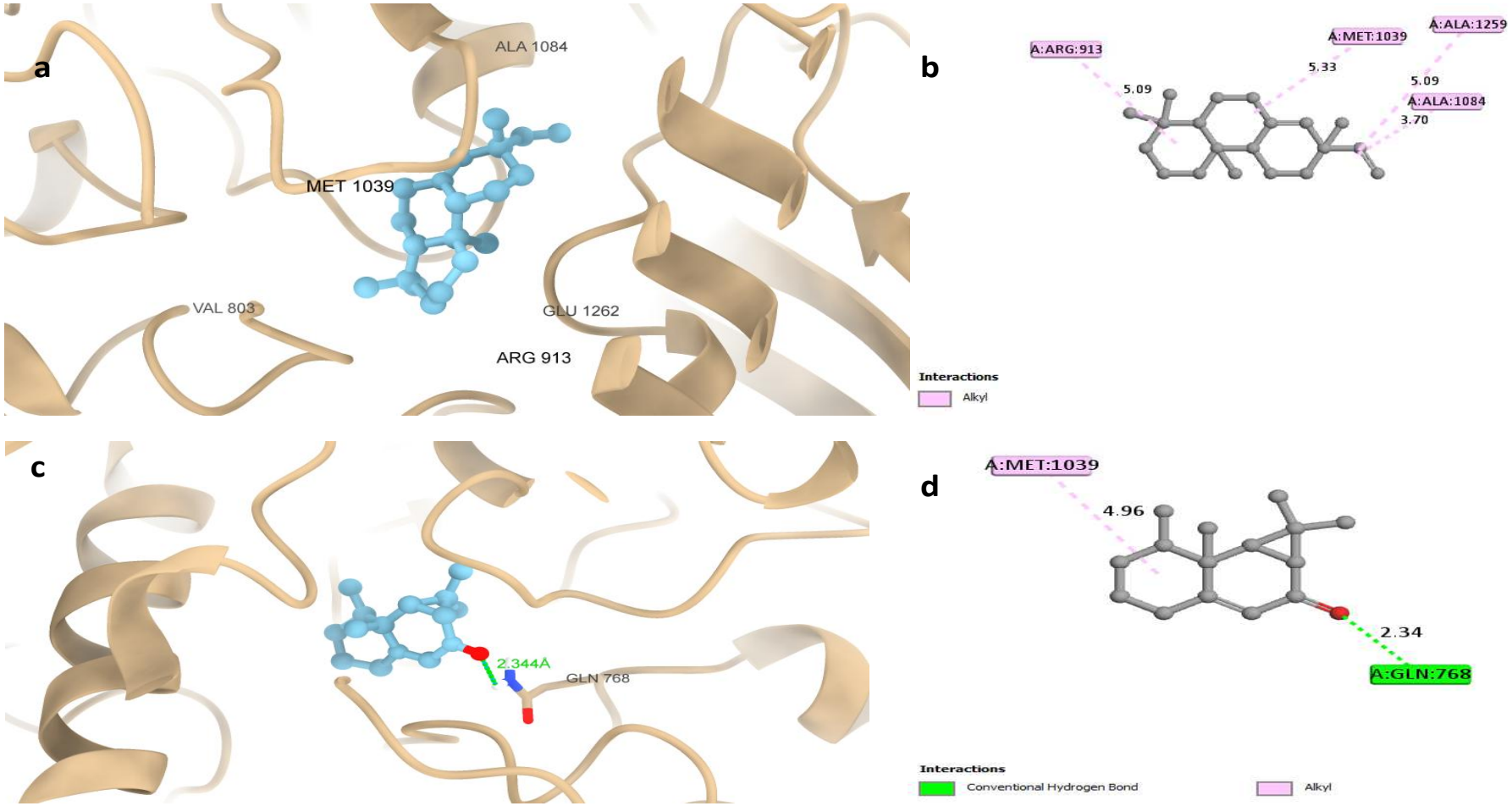

a
ALA 1084
MET 1039
VAL 803
GLU 1262
ARG 913
b
A:ARG:913
A:MET:1039
A:ALA:1259
A:ALA:1084
5.09
5.33
5.09
3.70
Interactions
Alkyl
c
2.344Å
GLN 768
d
A:MET:1039
4.96
2.34
A:GLN:768
Interactions
Conventional Hydrogen Bond
Alkyl

Figure 2

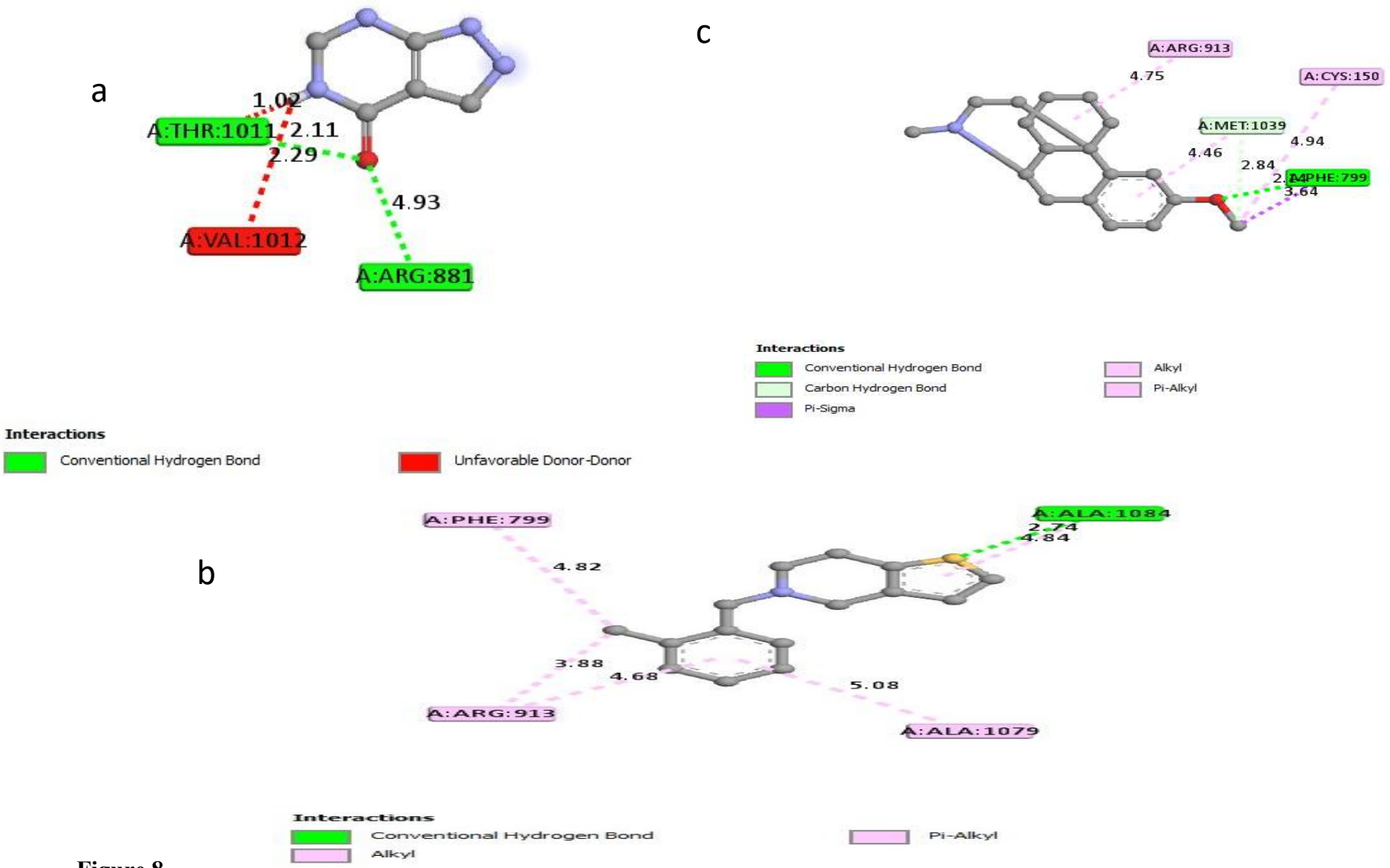

a
1.02
A:THR:1011
2.11
2.29
4.93
A:VAL:1012
A:ARG:881
Interactions
Conventional Hydrogen Bond
Unfavorable Donor-Donor
c
A:ARG:913
4.75
A:CYS:150
A:MET:1039
4.94
4.46
2.84
A:PHE:799
3.64
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Alkyl
Pi-Alkyl
b
A:PHE:799
A:ALA:1084
2.74
4.84
4.82
3.88
4.68
5.08
A:ARG:913
A:ALA:1079
Interactions
Conventional Hydrogen Bond
Alkyl
Pi-Alkyl

Figure 8.

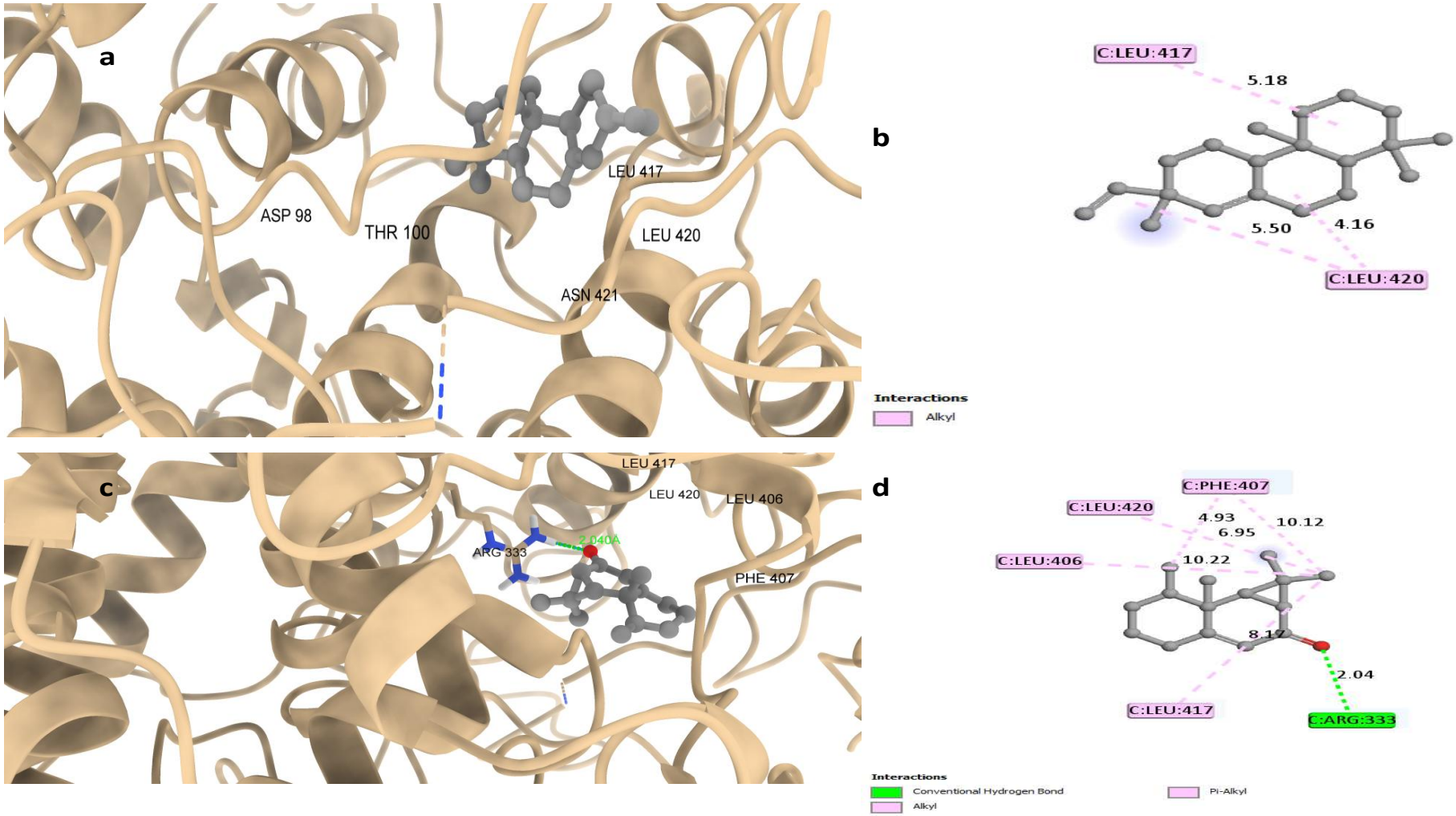

a
LEU 417
ASP 98
THR 100
LEU 420
ASN 421
b
C:LEU:417
5.18
5.50
4.16
C:LEU:420
Interactions
Alkyl
c
LEU 417
LEU 420
LEU 406
2.040A
ARG 333
PHE 407
d
C:PHE:407
C:LEU:420
4.93
10.12
6.95
C:LEU:406
10.22
8.17
2.04
C:LEU:417
C:ARG:333
Interactions
Conventional Hydrogen Bond
Pi-Alkyl
Alkyl

Figure 9:

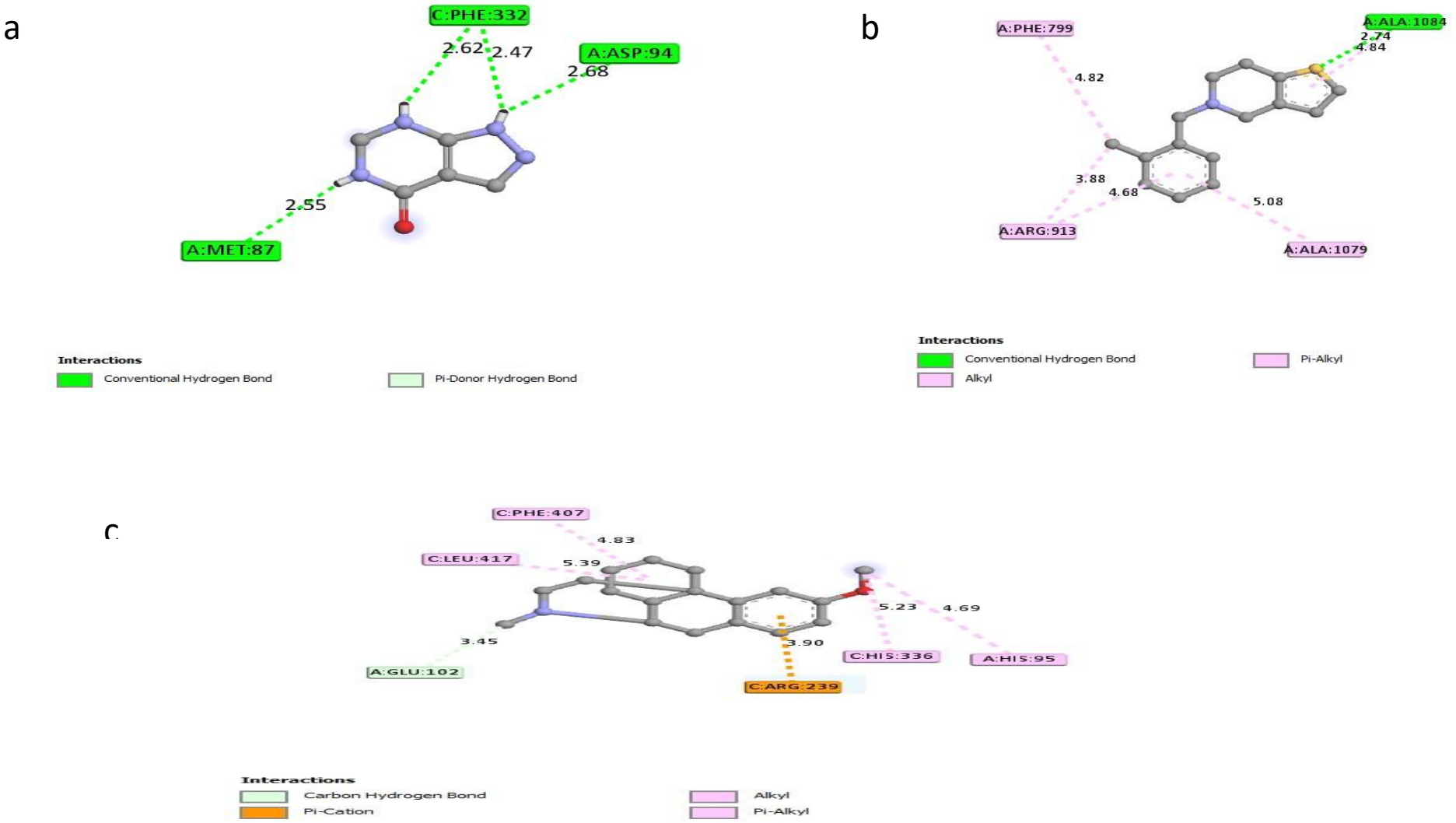

a
C:PHE:332
2.62
2.47
A:ASP:94
2.68
2.55
A:MET:87
Interactions
Conventional Hydrogen Bond
Pi-Donor Hydrogen Bond
b
A:PHE:799
A:ALA:1084
2.74
4.84
4.82
3.88
4.68
5.08
A:ARG:913
A:ALA:1079
Interactions
Conventional Hydrogen Bond
Pi-Alkyl
Alkyl
c
C:PHE:407
4.83
C:LEU:417
5.39
5.23
4.69
3.45
3.90
C:HIS:336
A:HIS:95
A:GLU:102
C:ARG:239
Interactions
Carbon Hydrogen Bond
Alkyl
Pi-Cation
Pi-Alkyl

Figure 10

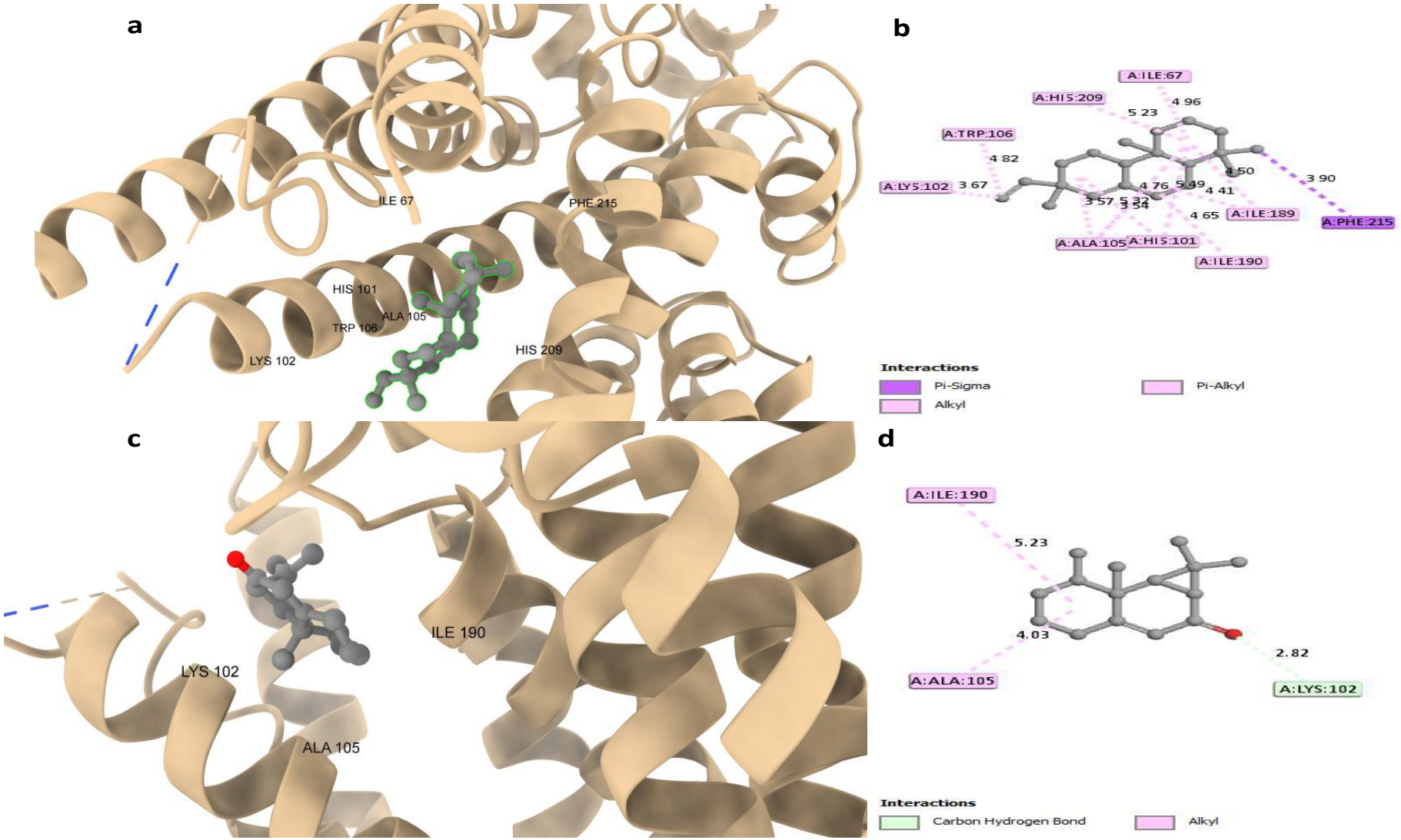

a
ILE 67
PHE 215
HIS 101
ALA 105
TRP 106
LYS 102
HIS 209
b
A:ILE:67
A:HIS:209
A:TRP:106
A:LYS:102
A:ILE:189
A:PHE:215
A:ALA:105
A:HIS:101
A:ILE:190
Interactions
Pi-Sigma
Pi-Alkyl
Alkyl
c
LYS 102
ILE 190
ALA 105
d
A:ILE:190
A:ALA:105
A:LYS:102
Interactions
Carbon Hydrogen Bond
Alkyl

Figure 11

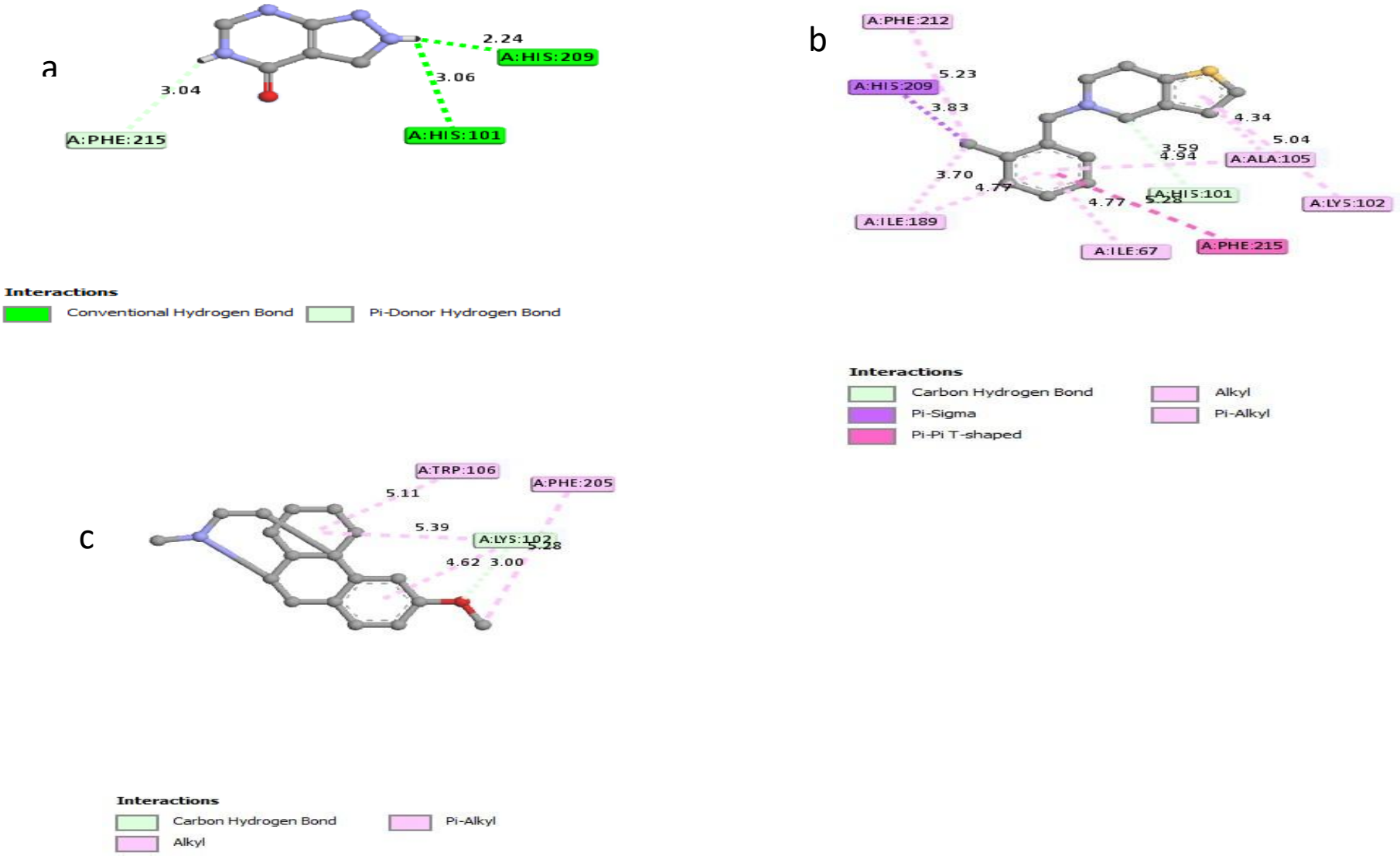
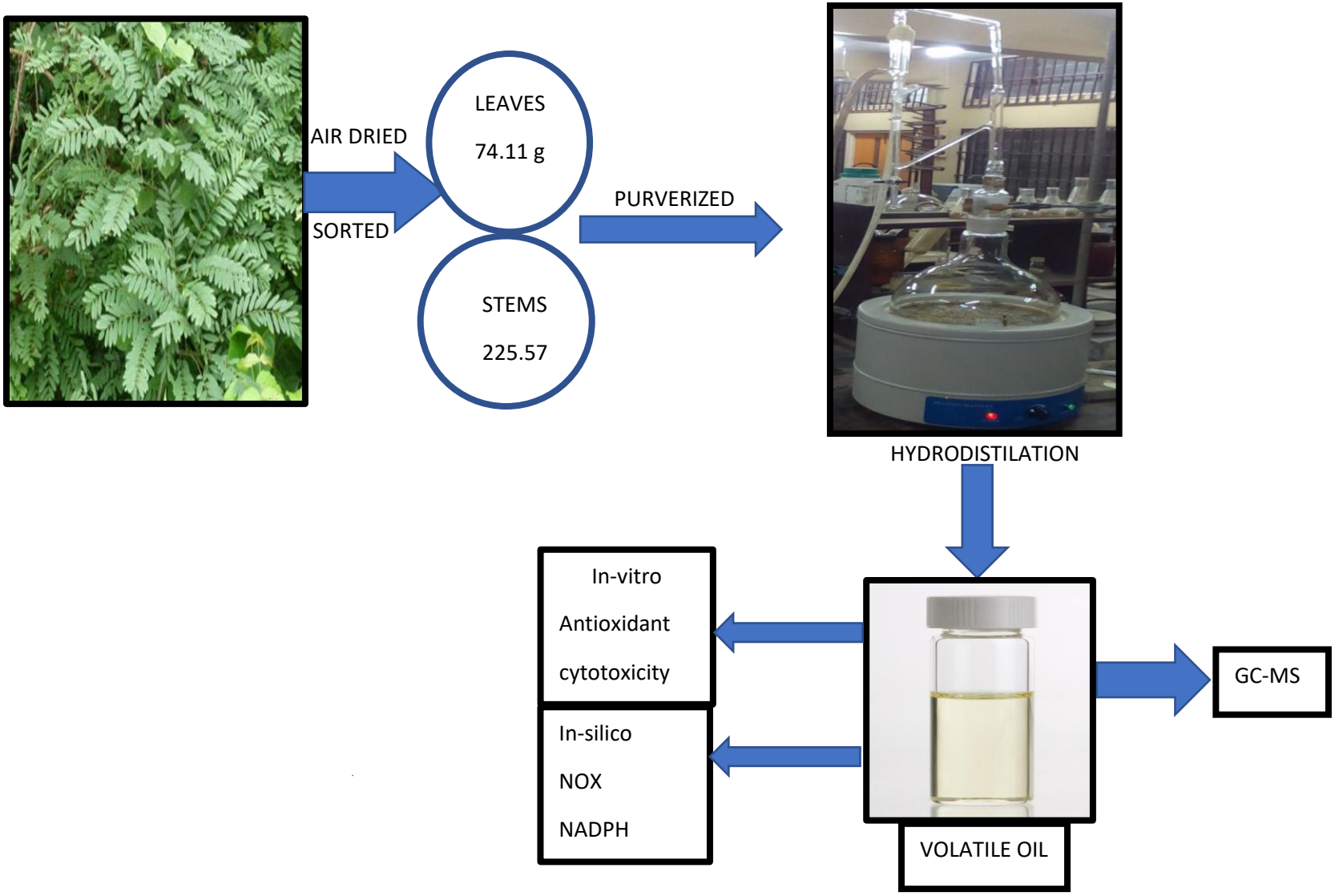

a
2.24
A:HIS:209
3.06
3.04
A:PHE:215
A:HIS:101
Interactions
Conventional Hydrogen Bond
Pi-Donor Hydrogen Bond
b
A:PHE:212
5.23
A:HIS:209
3.83
4.34
5.04
3.59
4.94
A:ALA:105
3.70
4.77
A:HIS:101
4.77
5.28
A:LYS:102
A:ILE:189
A:ILE:67
A:PHE:215
Interactions
Carbon Hydrogen Bond
Alkyl
Pi-Sigma
Pi-Alkyl
Pi-Pi T-shaped
c
A:TRP:106
A:PHE:205
5.11
5.39
A:LYS:102
5.28
4.62
3.00
Interactions
Carbon Hydrogen Bond
Pi-Alkyl
Alkyl

Figure 12


AIR DRIED
SORTED
LEAVES
74.11 g
STEMS
225.57
PURVERIZED
HYDRODISTILATION
In-vitro
Antioxidant
cytotoxicity
In-silico
NOX
NADPH
VOLATILE OIL
GC-MS