Research Paper

Antioxidant, Lipid Peroxidation and Molecular Docking Investigations of Solvent Extracts of *Argyreia imbricata*

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***Argyreia imbricata* belongs to the family Convolvulaceae and is commonly referred as imbricate wattle. The plant has been known to exhibit *in vitro* and *in vivo* anti-diabetic activities. In this study, we have prepared the leaves extract using three different solvents methanol, ethyl acetate, and chloroform separately by the Soxhlet extraction method. The extracts were analyzed for phytoconstituents by gas chromatography-mass spectrometry. The extracts were studied for total antioxidant activity, ferric reducing antioxidant power assay, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical cation decolorization assay, hydrogen peroxide scavenging assay and lipid peroxidation activities. Molecular docking was used to further study interaction of the phytoconstituents. L-ascorbic acid is used as a standard antioxidant in all the antioxidant assays. Among the three extracts, methanol extract showed the highest antioxidant activity, as measured by total antioxidant activity of 1.231µg/ml and ferric reducing antioxidant power of 14.9 µg/ml. Furthermore, methanol extract showed significant 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity with an half maximal inhibitory concentration value of 7.06 µg/ml, 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical cation decolorization assay with a value of 13.91 µg/ml, hydrogen peroxide scavenging assay with a value of 19.71 µg/ml and inhibited of lipid peroxidation with half maximal inhibitory concentration value of 128.4 µg/ml. The presence of different phytoconstituents was analyzed by gas chromatography-mass spectrometric analysis. The potential interactions between identified phytoconstituents and the antioxidant target protein were studied using docking study. The phytoconstituents of methanol extract scored the highest interaction with 2HE3. This study focuses on the pharmacological discovery process for preventing cell damage by oxidants.**

Key words: *Argyreia imbricata*, antioxidant activity, ferric reducing antioxidant power assay, 1,1-diphenyl-2-picrylhydrazyl, hydrogen peroxide scavenging assay, 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid, molecular docking

Studies from several years have evidenced that Reactive Oxygen Species (ROS) have a significant role in the pathophysiology of a wide range of illnesses. Enzymatic and non-enzymatic ROS defense systems are found in most living organisms. These processes can maintain a reactive equilibrium in healthy environments[1]. It is crucial to highlight that ROS plays an important role in the activation and development of inflammation. Inflammatory responses are triggered by an overabundance of the ROS produced by metabolic processes, including Superoxide Radical anion (SOR), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and nitric oxide (NO)[2].

Antioxidants shield the cellular damage from reactive oxygen species and the most common "antioxidants" include vitamins A (retinol), C (L-ascorbic acid), E (tocopherol), β-carotene,

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minerals like selenium, and naturally occurring polyphenols are all antioxidants. In foods such as fruits and vegetables, vitamins and β-carotene include conjugated double bonds and antioxidant functional groups. Antioxidants are available in the diet and in addition people also take supplements containing antioxidants[3]. Czernichow *et al.* study have shown that antioxidant supplements have adverse effect associated with metabolic syndrome. It is important to investigate plant-based sources with antioxidant and free radicle quenching properties without inducing metabolic syndrome[4].

Medicinal plants, on the other hand, have long been utilized in traditional medicine and have preventative properties, particularly in developing countries. Several medicinal plants' antioxidant capabilities have been investigated. Natural antioxidants including raw extracts or chemical components are extremely effective at preventing oxidative stress-induced damage[5]. A wide variety of herbal extracts and natural substances have been shown in preclinical studies to reduce oxidative stress. In macrophage cells, Thring *et al.* looked for anti-inflammatory potential in 30 different plant extracts. Interleukin (IL)-6 and tumor necrosis factor-α were found to be inhibited, while IL-10 production was increased, and the expression of Cyclooxygenase-2 and nitric oxide synthase was decreased. Their anti-inflammatory mechanisms were also identified[6].

With over 220 species, *Argyreia* is one of the major genera in the Convolvulaceae family, found across Asia, including India. *Argyreia imbricata* (*A. imbricata*) is prevalent in southern India at altitudes upto 300 m above mean sea level. The flowering and fruiting season is August-December for this dicotyledonous plant. A huge white woolly climber, asymmetrical, strigose leaves 8-12 cm long, with an obtusely sharp, rounded, or subcordate base, 3 cm petiole, 5 cm peduncle, small bracteoles and bracts. It has little flowers with a short pedicellate calyx lobe and a 2 cm long pink corolla and it has 5 mm berry, reddish, thickly hairy[7].

Earlier studies have shown that *A. imbricata* exhibited anti-diabetic effect in a streptozotocin-induced diabetes model in Wistar albino rats. This property was seen in different solvent extracts such as petroleum ether, chloroform, ethyl acetate and methanol extracts. The *in vitro* antidiabetic efficacy of all extracts was evaluated using α-amylase and β-glucosidase inhibition studies[8].

The *A. imbricata* has not been explored for its phytoconstituents and antioxidant, lipid peroxidation properties. In this study, we have prepared the different solvent extracts of the *A. imbricata* leaves. The extracts were analyzed for bioactive composition by Gas Chromatography-Mass Spectrometric (GC-MS) analysis, antioxidant and lipid peroxidation properties. Further, the molecular docking study was conducted. This is the first report to study the phytoconstituents analysis, antioxidant potentials of *A. imbricata* leaves solvent extracts and molecular docking studies for identified phytoconstituents.

MATERIALS AND METHODS

Materials:

Chemicals used in this study were gallic acid, sodium carbonate, folin-ciocalteau reagent, sodium phosphate, sulphuric acid, ammonium molybdate, sodium acetate, ferric chloride, Tripyridyl Triazine (TPTZ), hydrochloric acid, ascorbic acid, methanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), potassium persulfate, hydrogen peroxide, glacial acetic acid, sodium phosphate, monopotassium phosphate, potassium hydroxide, sodium dodecyl sulfate, thiobarbituric acid, trichloroacetic acid, monosodium phosphate, sodium chloride were purchased from Sisco Research Laboratories chemicals and SD Fine chemicals. All other chemicals and solvents used were of analytical grade.

Collection of plant material:

A. imbricata plant was collected from Tiptur local area, Tumkur district, Karnataka, India at a Latitude of 13° 15' 36.00" N Longitude of 76° 28' 48.00" E and during the period of 1st August 2021 to 30th November 2021. The plant was authenticated by Prof. Sharanappa, Department of Studies and Research in Biosciences, Hemagangotri, University of Mysore, Hassan. The plant herbarium was kept at Department of Studies and Research in Botany, Tumkur University, Tumakuru.

Extraction of plant material:

The *A. imbricata* leaves were collected washed

and shade dried. The dried leaves were powdered using a mixer and used for extraction. The leaves extract of *A. imbricata* was prepared by using three different solvents (methanol, ethyl acetate, and chloroform). The leaves were extracted with different solvents individually by using the Soxhlet extraction method. 20 g of leaves powder was suspended in a solvent and extracted for 9 to 10 h in the Soxhlet apparatus. The extracts were filtered and vacuum-condensed at 45°. The extracts were kept in the refrigerator until they were used.

Determination of antioxidant activities:

Determination of Total Antioxidant Activity (TAA): TAA was done by the phosphomolybdenum technique according to the method of Prieto *et al.*[9]. In 1 ml of a standard reagent (0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate) different quantities of the extracts (100-500 µg/ml) were added. The tubes were capped and placed in a water bath at 95° for 90 min. The absorbance was measured at 695 nm against a blank after cooling to room temperature. The TAA per g of the extract was measured in mg of Gallic Acid Equivalence (GAE).

Ferric Reducing Antioxidant Power assay (FRAP): According to Benzi *et al.*[10], FRAP assay of extracts was determined. 1.0 ml FRAP reagent was mixed with 1.0 ml extracts (100-500 µg/ml). The FRAP reagent was made by combining 300 mM acetate buffer, 10 ml TPTZ dissolved in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio. The tubes were incubated for 30 min at 37°. At 593 nm, absorbance was measured against a blank. The FRAP values were measured in mg of GAE per g of extracts.

DPPH radical scavenging activity: According to Braca *et al.*[11], 50 µl of extracts (5-25 µg/ml) were added to 300 µl of an ethanolic solution containing 0.5 mM DPPH. The tubes were incubated for 5 min at 37° in a dark environment. At 517 nm, the absorbance was measured against a blank. The extract's radical scavenging activity, reported as percent inhibition, was estimated using the equation below.

Percentage inhibition of DPPH radical=[(absorbance control-absorbance test)/absorbance control]×100

ABTS radical cation decolorization assay: According to Seeram *et al.*[12], the ABTS radical cation decolorization experiment was done. To make an ABTS solution, water containing 7 mM ABTS was mixed with 2.45 mM potassium persulfate (1:1). For 16 to 20 h, this reaction mixture was kept in a container at room temperature. Using methanol as a diluting agent, we prepared a solution of ABTS that had an absorbance of 0.7 at 734 nm. After 30 min of incubation, various doses of extracts (10 to 50 µg/ml) were added to ABTS solution to make a total volume to 5 ml. The absorbance was read at 734 nm. Percent scavenging of extracts was used to measure the results, which were presented as percentages.

Hydrogen peroxide scavenging assay: Ruch *et al.* method's was used to determine the effectiveness of extracts in scavenging hydrogen peroxide[13]. Phosphate buffer was used to make a 40 mM hydrogen peroxide solution (50 mM pH 7.4). Hydrogen peroxide was added to extracts (10-50 µg/ml) in a total volume of 2 ml and the absorbance was read at 230 nm. To study the hydrogen peroxide scavenging activity in percentage, the above mentioned equation was used.

Lipid peroxidation: By measuring Thiobarbituric Acid Reactive Substances (TBARS), lipid peroxidation was determined as indicated by Ohkawa *et al.*[14]. The liver homogenate (10 % in cold phosphate buffered saline, pH 7.4) was treated with different concentrations of the extract (100-500 µg/ml) in water. The acetic acid (20 % v/v, pH 3.5), Sodium Dodecyl Sulfate (SDS) (8 % w/v, 0.2 ml), and thiobarbituric acid (0.8 % w/v, 1.5 ml) tubes were filled with the extracts. The mixture was kept in hot water bath for 45 min. The samples were read at 532 nm, L- ascorbic acid was used as the standard and the TBARS produced were extracted into 3 ml of 1-butanol. Malondialdehyde (MDA) equivalents were quantified in terms of nmol MDA produced per mg protein[15,16].

GC-MS analysis: GC-MS analysis was conducted for extracts (methanol, ethyl acetate, and chloroform) (model; QP 2010 plus, Shimadzu, Japan). At a rate of 2°/min, the oven temperature rose from 75 to 312°. Electron impact ionization was used to ionize the material (EI, 70eV). It was adjusted to 280° for the injector and 220° for the detector. Helium was used as a transportation gas. At 1.21 $ml.min^{-1}$, the carrier gas flow rate was established. 3 scans/s were used to scan compounds in the 40-60 m/z mass range. In a split injection approach, a Hamilton syringe was used

to inject each of the three extracts into the GC-MS. Phytoconstituents were identified by comparing mass spectra and retention time patterns to the program Chemstation.

Molecular docking:

Ligand Preparation: Phytocomponents identified from the GC-MS analysis of methanol, ethyl acetate and chloroform extract of *A. imbricata* leaves. The 3D structure of all the compounds was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih. gov/) and used in this study. All the compounds were subjected to minimization, the hydrogen atoms were added, followed by a minimization step with the Avogadro software. The ligands were built and energy-minimized using the MMFF94 force field.

Selection of target protein: As a receptor molecule for antioxidants, antioxidant target protein was used, the protein was retrieved from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) with PDB id: 2he3 was found from the literature. The 3D structure of this target protein was retrieved from PDB database (https://www.rcsb.org). The 3D structure of antioxidant target Protein was obtained from PDB database, and its PDB ID is 2HE3. For the protein structure, structural waters and ligands were removed.

Docking studies: Docking studies for the target protein, PDB ID: 2HE3 and phytoconstituents identified from the GC-MS analysis of methanol, ethyl acetate and chloroform leaves extract of *A. imbricata* were performed using Autodock vina software. The binding compounds were searched 25 times using AutoDock Vina's scoring tool with default settings. Visually analyzing categories with RMSD<2 Å. Docking models regarded receptors rigid while ligands flexible. The lowest-binding energy conformations that replicated key interactions were chosen. The results were also analyzed using Discovery Studio 2021.

Statistical analysis:

The data are presented as the mean±Standard Deviation (SD). Antioxidant experiments were conducted using one-way ANOVA tests to compare the half-maximal Inhibitory Concentration (IC_{50}) values of different solvent extracts. To figure out the IC_{50} values, Graph Pad Prism 5 was used.

RESULTS AND DISCUSSION

Majority of the medicinal plants are known to be associated with antioxidant property[17]. The exhibited property depends on the phytoconstituent composition and concentration of the different phytoconstituents present in the extract. As a result, the antioxidant activity of plant extracts cannot be assessed using a single approach[18]. To explore the various processes responsible for antioxidant activities, solvent extracts of *A. imbricata* leaves (methanol, ethyl acetate, and chloroform) were tested for antioxidant activities such as TAA, FRAP, DPPH, ABTS and hydrogen peroxide scavenging activity.

An acidic pH resulted in the development of a green phosphate/Mo (V) complex that had a TAA. It measures the overall antioxidant capacity of both water- and fat-soluble antioxidants[19]. TAA is a quantitative measure of the antioxidant capacity of the extracts and it is reported in GAE. The total antioxidant capacity of extracts were shown to be in the following order; methanol extract>ethyl acetate extract>chloroform extract (Table 1). Methanol extracts had the highest overall TAA among the three extracts. The TAA of methanol extract increases steadily as extract concentration increases from 100 to 500 µg/ml. The GAE value was found to be 1.231±0.02, 1.216±0.02 and 1.182±0.01 µg/ml GAE at 500 µg/ml of methanol extract, ethyl acetate extract and chloroform extract respectively. All the three extracts exhibited significant TAA. Earlier study using *Garcinia cambogia* seed extracts (methanol, ethyl acetate and acetone), the methanol extract showed highest TAA at a concentration of 92.63 µg/ml[20].

In FRAP assay, antioxidant capacity is measured by the reduction of the ferric ion complex TPTZ. In the presence of antioxidants, the Fe^{3+}/ferricyanide combination can be reduced to Fe^{2+}/ ferrocyanide. As a result, the binding of the ligand to Fe^{2+} produces a highly vivid navy-blue hue. As a result, the quantity of reduced iron may be measured and associated with the number of antioxidants by detecting the production of Perl's Prussian blue at 700 nm[21]. The results revealed a considerable antioxidant capacity in terms of FRAP measured as GAE of all the extracts, which enhanced steadily with increasing concentrations from 100 to 500 µg/ml of samples. The antioxidant activity of the extracts were observed to decrease in the following order: methanol extract>chloroform extract>ethyl acetate extract (Table 2). Among the extracts, methanol extract showed the highest antioxidant power activity of the three extracts. The GAE value was found to be

14.9±0.03, 17.1±0.04 and 10.1±0.04 µg/ml GAE at 500 µg/ml of methanol extract, ethyl acetate extract, chloroform extract respectively. Likewise, Uddin *et al.*[22] study have shown the FRAP activity of methanol extract of *A. argentea* (Roxb) was 150.83±4.26 µmol ascorbic acid/g. The capacity of plant extracts to donate hydrogen atoms was tested by decolorizing a methanol solution of DPPH. DPPH in methanol creates a violet/purple color that fades to yellow in the presence of antioxidants[23]. We investigated the extract's radical scavenging properties by measuring changes in the absorbance of the DPPH at 517 nm. The DPPH radicals were scavenged by all three extracts in a dose-dependent manner. The methanol extract outperformed the other two extracts in terms of radical scavenging activity. The percentage inhibition of free radical (DPPH) scavenging activity at 25 µg/ml of concentration, the methanol extract displayed radical scavenging activity with an IC_{50} of 7.06 µg/ml, whereas the ethyl acetate and chloroform extracts showed IC_{50} value of 9.836 µg/ml and 10.03 µg/ml, respectively (fig. 1). Similarly, Mahendra *et al.*[24] have shown DPPH radical scavenging activity in *Argyreia osyrensis* Roth plant extract.

In ABTS method, antioxidants that are capable of donating electrons can convert the blue-green ABTS radical solution into a colorless form. All extracts scavenged the ABTS radical in a concentration-dependent manner (10-50 µg/ml) (fig. 2). The methanol extract exhibited more activity as a radical scavenger than the other two extracts. The methanol extract have shown to scavenge ABTS with an IC_{50} value of 13.91 µg/ml, while the ethyl acetate and chloroform extracts showed IC_{50} of 16.52 µg/ml and 18.60 µg/ml, respectively. Similarly, Kekuda *et al.*[25], showed the maximum ABTS scavenging activity in methanol extract of *Argyreia cuneata* (willd.) Ker Gawl plant. The leaves methanol extract was more effective in scavenging ABTS radicals with IC_{50} value of 9.34 µg/ml followed by flower methanol extract (IC_{50} value 14.98 µg/ml) and stem extract (IC_{50} value 22.47 µg/ml). The plant extracts ability to scavenge H_2O_2 is measured spectrophotometrically by the disappearance of H_2O_2 at 230 nm. H_2O_2 is found in low concentrations in the natural environment. It decomposes fast into oxygen (O_2) and water (H_2O), producing hydroxyl radicals (OH), which can cause lipid peroxidation and DNA damage[26]. Fig. 3 depicts the hydrogen peroxide radical scavenging capabilities of the extracts. The methanol extract showed highest hydrogen peroxide radical scavenging activity (percent) with IC_{50} 19.71 µg/ml value compared to the other two extracts, ethyl acetate having IC_{50} value 25.10 µg/ml and chloroform having IC_{50} value of 22.36 µg/ml. Likewise, Dintakurthi *et al.*[27] exhibited H_2O_2 scavenging activity of *Argyreia pilosa* Wight and Arn. (Whole Plant). In this study, the methanol and ethyl acetate extracts were found to have the highest percentage of radical scavenging activity.

Lipid peroxidation is a chain reaction that causes membrane damage by peroxidizing lipid molecules, particularly polyunsaturated fatty acids. The initial contact produces a second radical, which may react with another macromolecule, resulting in cell malfunction[28]. Lipid peroxidation has the potential to be damaging due to its uncontrolled and self-enhancing nature. This results in the disruption of biomembranes, lipids and other essential cell components. When cells are subjected to oxidative stress, Malonaldehyde (MDA), an end product of lipid peroxidation, increases. Fig. 4 depicts the inhibitory action of extracts in methanol, ethyl acetate and chloroform was determined at concentrations of 100-500 µg/ml. The IC_{50} values for methanol, ethyl acetate and chloroform extracts were found to be 128.4 µg/ml, 189.4 µg/ml, and 182.6 µg/ml, respectively. The methanol extract significantly showed inhibition of lipid perioxidation when compared to other two extracts. Similar study by Shreedhara *et al.*[29] exhibited the lipid peroxidation inhibition activity of *Argyreia nervosa* chloroform extract.

TABLE 1: TAA OF DIFFERENT SOLVENT EXTRACTS OF *A. imbricata*

	TAA in GAE µg/ml of extract		
Concentration of extract (µg)	TAA (GAE)		
	Methanol	Ethyl acetate	Chloroform
100	1.13±0.01	1.14±0.01	1.13±0.01
200	1.16±0.01	1.17±0.01	1.14±0.01
300	1.18±0.01	1.18±0.01	1.16±0.01
400	1.21±0.02	1.20±0.01	1.18±0.01
500	1.22±0.02	1.21±0.02	1.19±0.01

TABLE 2: FRAP ASSAY OF DIFFERENT SOLVENT EXTRACTS OF *A. imbricata*

FRAP activity in GAE µg/ml of extract			
Concentration of extract (µg)	FRAP (GAE)		
	Methanol	Ethyl acetate	Chloroform
100	2.1±0.02	1.12±0.03	1.0±0.02
200	5.4±0.02	3.1±0.03	1.9±0.02
300	8.4±0.03	4.1±0.03	5.2±0.02
400	10.1±0.03	5.1±0.04	8.5±0.04
500	14.9±0.03	7.1±0.04	10.1±0.04

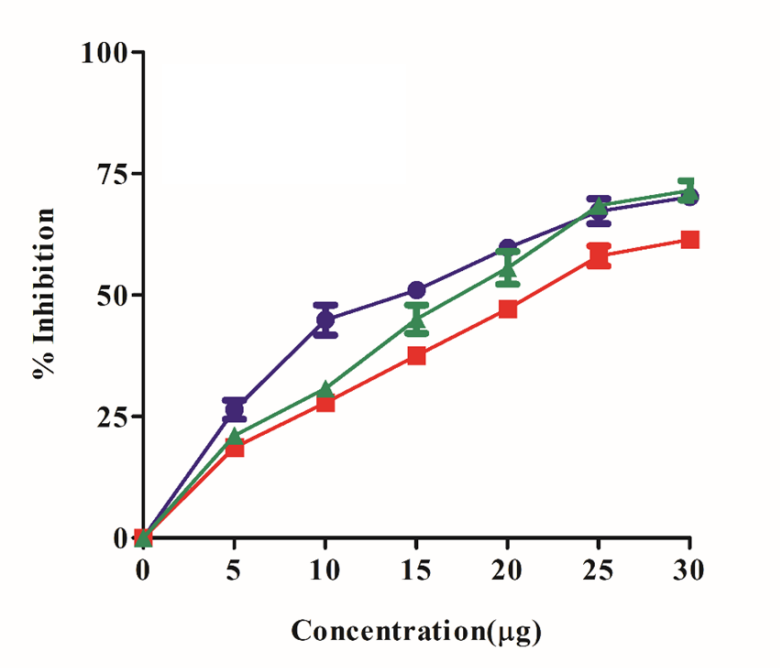

Fig. 1: 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity
Note: ( ): Methanol; ( ): Ethyl acetate and ( ): Chloroform

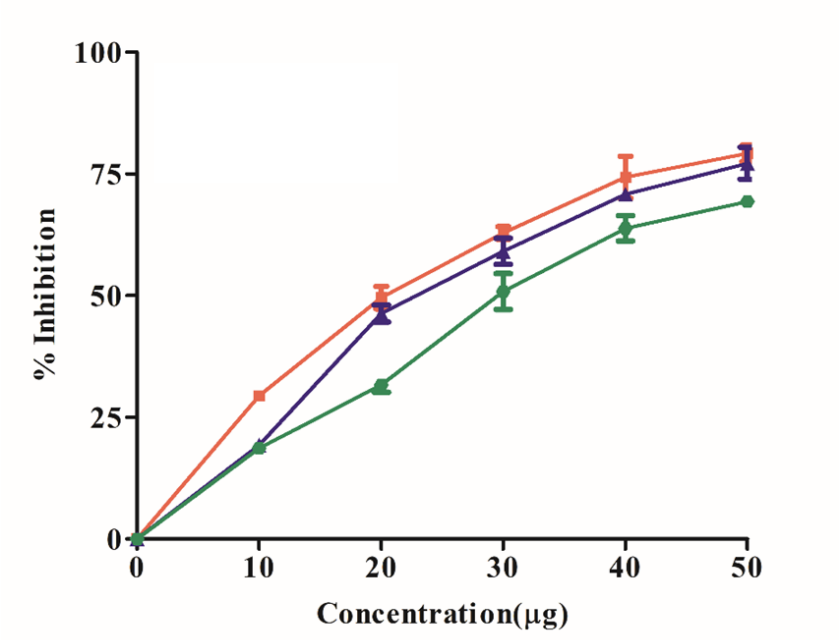

Fig. 2: ABTS radical cation decolorization activity
Note: ( ): Methanol; ( ): Ethyl acetate and ( ): Chloroform

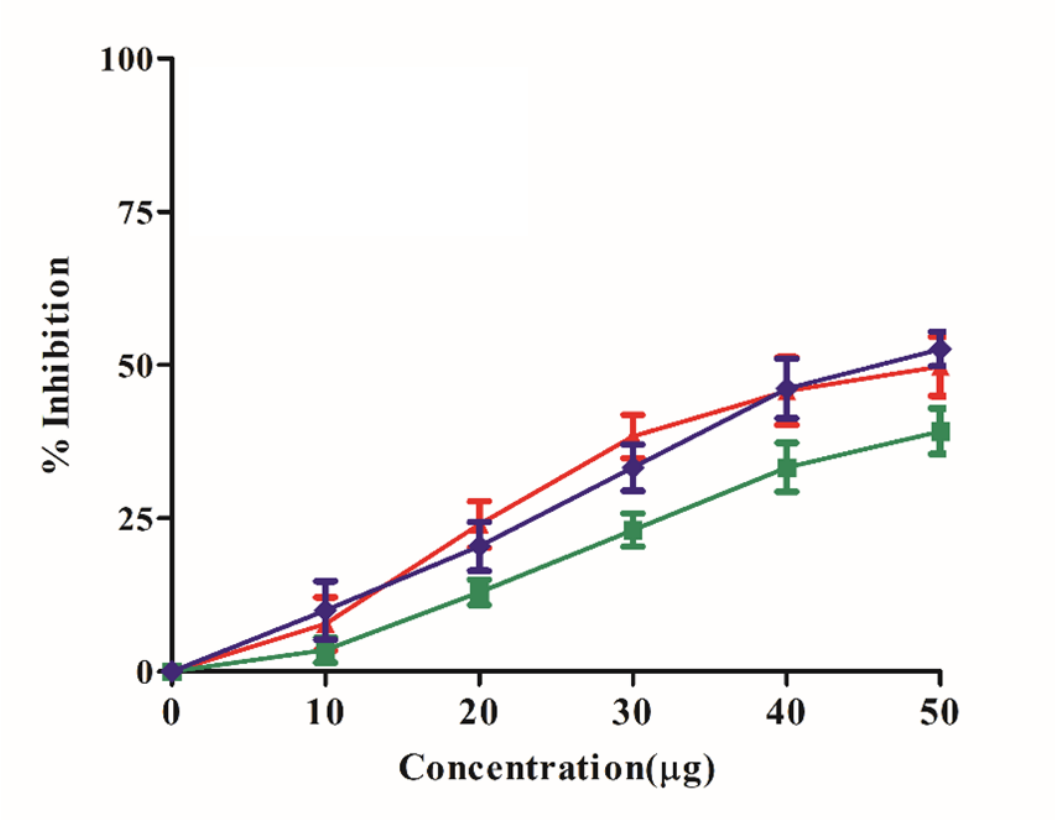

Fig. 3: Hydrogen peroxide scavenging activity
Note: ( ): Methanol; ( ): Ethyl acetate and ( ): Chloroform

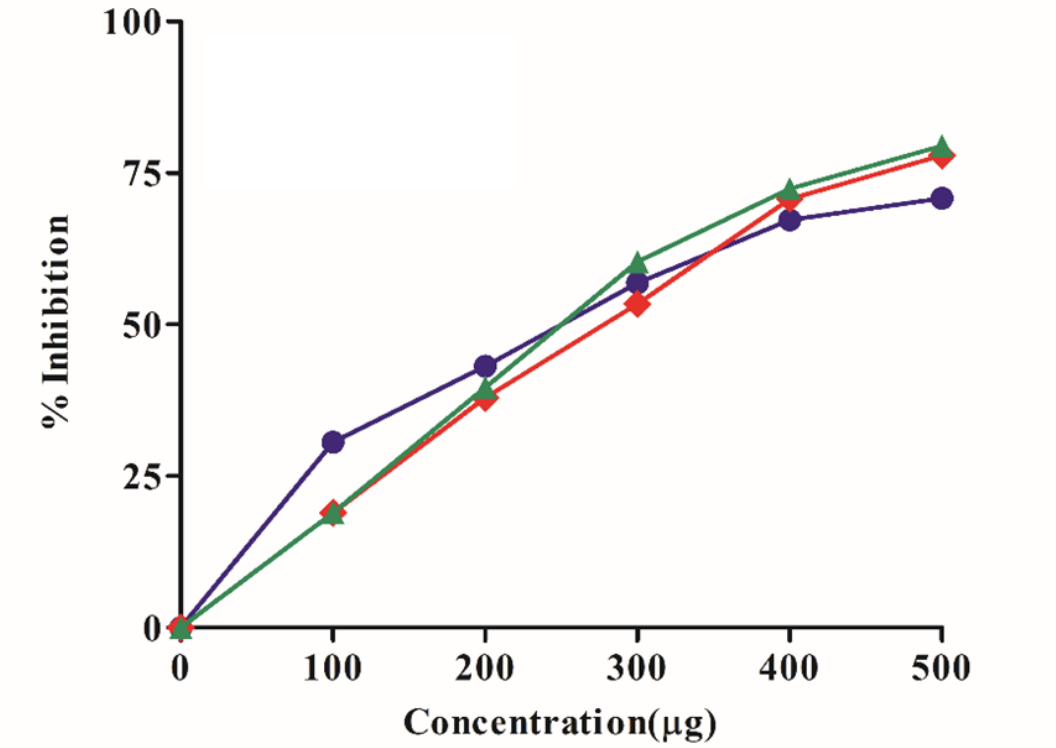

Fig. 4: Lipid peroxidation
Note: ( ): Methanol; ( ): Ethyl acetate and ( ): Chloroform

The analysis of the phytoconstituents in the extracts was analyzed by GC-MS method[30]. The potential active compounds responsible for antioxidant activity in *A. imbricata* leaves extracts was identified using GC-MS analysis. Table 3-Table 5 shows the retention period and peak area percentage of several bioactive principles (fig. 5). The GC-MS analysis of the extracts revealed 26, 46 and 23 different molecules in methanol, ethyl acetate, and chloroform extracts respectively. The identified phytoconstituents belong to alkane, alcohols and ketones group of secondary metabolites. Likewise, Bharati *et al.*[31] studied the GC-MS analysis of *Argyreia Nervosa* Burm. F plant. They obtained 26 phytoconstituents belonging to different group of secondary metabolites.

Molecular docking is a very important tool in structural molecular biology, computer-aided drug designing and to analyze the interaction of phytoconstituents with target molecule. The goal of ligand-protein docking is to figure out how a ligand and a protein that has a known 3D structure will interact with each other with mode of binding with the active site of the molecule[15]. Docking analysis was performed using specific pharmacological targets such as antioxidant target protein (PDB id: 2HE3) in an attempt to justify the antioxidant activity for the phytoconstituents of all three extracts from GC-MS data. The docking results of antioxidant target protein revealed that the compounds Cholest-5-en-3-ol and 13,14-Epoxyursan-3-ol of methanol extract have a significant binding mode with docking scores of -6.3 and -8.6 Kcal/mol. The compound Cholest-5-en-3-ol formed hydrogen bonding with THR:98 amino acid. 13,14-Epoxyursan-3-ol formed hydrogen bonding with THR:100 amino acid. Further, the interaction was stabilized by alkyl bonds (fig. 6). The 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 9,10-Dihydrodeoxynivalenol of ethyl acetate extract has substantial binding modes with docking scores of -5 and -5.8 Kcal/mol, respectively (fig. 7). The 3,7,11,15-Tetramethyl-2-hexadecen-1-ol formed hydrogen bonding with LYS:87 amino acid. The 9,10-Dihydrodeoxynivalenol formed hydrogen bonding with LEU:101 and ASN: 12. Further, the interaction was stabilized by alkyl bonds. In chloroform extract D:B-Friedo-B':A'-neogammacer-5-en-3-ol and 2-Propenoic acid indicate substantial binding mechanisms showing -7.7 and -5.3 kcal/mol docking scores. The D:B-Friedo-B':A'-neogammacer-5-en-3-ol formed hydrogen bonding with ARG:29 and 2-Propenoic acid formed hydrogen bonding with TYR:98 (fig. 8). Similar study from Kulkarni *et al.*[32] showed molecular docking of LC-MS analysis phytoconstituents of petroleum ether, chloroform, ethyl acetate, acetone, methanol and water extracts of *Argyreia nervosa* (Burm. f) Bojer (Table 6-Table 8).

In conclusion, the three different extracts of *A. imbricata* leaves were analyzed for antioxidant activities by different assay methods, in which all the extracts exhibited significant antioxidant capacities. In addition, all the three extracts inhibited lipid peroxidation activity. GC-MS was used to identify a variety of secondary metabolites from methanol extract, Cholest-5-en-3-ol and 13,14-epoxyursan-3-ol showed highest binding energy with antioxidant target protein by in silico. THR: 98, THR:100, LYS:87, LEU:101 ASN:15, ARG:29 and TYR:127 were the essential amino acid residue involved in the intermolecular interactions. This study focused on the ethnopharmacological phytoconstituents in preventing cell damage by oxidants. Further *in vivo* studies will give a better approach towards the antioxidant capacities of the molecules identified in the extracts.

TABLE 3: PHYTOCOMPOUNDS IDENTIFIED BY GC-MS ANALYSIS IN METHANOL EXTRACT OF A. imbricate

S No.	Compound Name	Molecular weight	Molecular formula	Retention time	Peak area %
1	5-O-Methyl-d-gluconic acid dimethyl amide	237.25	$C_9H_{19}NO_6$	8.363	3.08
2	1-Octadecyne	250.5	$C_{18}H_{34}$	14.936	0.65
3	1-Heptadecanol	256.5	$C_{17}H_{36}O$	17.354	0.81
4	Phytol	296.5	$C_{20}H_{40}O$	17.641	1.52
5	Octasiloxane	336.68	O_7Si_8	18.825	1.53
6	Bis[di(trimethylsiloxy)phenylsiloxy] trimethylsiloxyphenylsiloxane	793.5	$C_{33}H_{60}O_7Si_8$	19.626	2.13

7	Heptasiloxane	292.59	O_6Si_7	19.979	2.09
8	Cyclononasiloxane	414.9	$H_{18}O_9Si_9$	21.053	2.77
9	Cyclodecasiloxane	741.5	$C_{20}H_{60}O_{10}Si_{10}$	23	3.14
10	2,6,10,14,18,22-Tetracosahexaene	326.6	$C_{24}H_{38}$	23.24	0.99
11	1-Tetracosanol	354.7	$C_{24}H_{50}O$	23.693	1.77
12	Heptasiloxane	292.59	O_6Si_7	23.864	2.66
13	Silicic acid	192.23	$H_8O_8Si_2$	24.527	1.33
14	Tetracosamethyl-cyclododecasiloxane	889.8	$C_{24}H_{72}O_{12}Si_{12}$	24.719	2.17
15	1-Triacontanol	438.8	$C_{30}H_{62}O$	25.083	2.29
16	Stigmasterol	412.7	C29H48O	27.035	3.08
17	Cholest-5-en-3-ol	386.7	$C_{27}H_{46}O$	27.664	1.4
18	Alpha -Amyrin	426.7	$C_{30}H_{50}O$	28.271	2.46
19	13,14-Epoxyursan-3-ol	470.7	C31H50O3	28.619	5.37
20	Lupeol	426.7	$C_{30}H_{50}O$	28.856	2.3
21	D:B-Friedo-B':A'-neogammacer-5-en-3-ol	426.7	$C_{30}H_{50}O$	29.006	1.57
22	18,19-Secolupan-3-ol	430.7	$C_{30}H_{54}O$	29.544	5.33
23	Tetracosamethyl-cyclododecasiloxane	889.8	$C_{24}H_{72}O_{12}Si_{12}$	29.917	4.15
24	D:A-Friedooleanan-3-ol,	428.7	$C_{30}H_{52}O$	30.467	1.97
25	Friedelan-3-one	426.7	$C_{30}H_{50}O$	30.861	17.06
26	Tetrapentacontane	759.4	$C_{54}H_{11}O$	32.052	5.39

TABLE 4: PHYTOCOMPOUNDS IDENTIFIED BY GC-MS ANALYSIS IN ETHYL ACETATE EXTRACT OF *A. imbricata*

S No.	Compound name	Molecular weight	Molecular formula	Retention time	Peak area %
1	Isoamyl acetate	130.18	$C_7H_{14}O_2$	3.186	0.47
2	5-O-Methyl-D-Gluconic Acid Dimethylamide	237.25	$C_9H_{19}NO_6$	8.318	2.58
3	Heneicosane	296.6	$C_{21}H_{44}$	11.094	0.5
4	Heneicosane	296.6	$C_{21}H_{44}$	13.561	0.62
5	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	238.32	$C_{14}H_{22}O_3$	14.63	0.38
6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296.5	$C_{20}H_{40}O$	14.947	1.62
7	Tetrapentacontane	759.4	$C_{54}H_{11}0$	15.41	0.76
8	Eicosane	282.5	$C_{20}H_{42}$	17.366	0.9
9	1-Nonadecene	266.5	$C_{19}H_{38}$	17.649	1.53
10	Phytol	296.5	$C_{20}H_{40}O$	18.959	2.97
11	Oxirane	44.05	C2H4O	19.583	0.41
12	Dotriacontane	450.9	$C_{32}H_{66}$	20.745	0.48
13	Dotriacontane	450.9	$C_{32}H_{66}$	21.244	0.74
14	Dotriacontane	450.9	C32H66	21.498	0.65
15	Tetrapentacontane	759.4	$C_{54}H_{11}0$	22.223	0.54
16	Dotriacontane	450.9	$C_{32}H_{66}$	23.247	0.58
17	Dotriacontane	450.9	$C_{32}H_{66}$	23.596	0.71
18	Squalene	410.7	$C_{30}H_{50}$	23.693	1.02
19	Tetrapentacontane	759.4	$C_{54}H_{11}0$	23.994	1.55

20	1-Pentacosanol	368.7	$C_{25}H_{52}O$	24.13	1.08
21	1,6,10,14,18,22-Tetracosahexaen-3-ol	426.7	$C_{30}H_{50}O$	24.265	3.63
22	Triacontane	422.8	$C_{30}H_{62}$	24.399	2.03
23	Tetrapentacontane	759.4	$C_{54}H_{11}0$	24.62	1.65
24	Tetrapentacontane	759.4	$C_{54}H_{11}0$	24.951	1.66
25	2-Octadecyl-propane-1,3-diol	328.6	$C_{21}H_{44}O_2$	25.088	1.72
26	Tetrapentacontane	759.4	$C_{54}H_{110}$	25.599	2.03
27	1-Triacontanol	438.8	$C_{30}H_{62}O$	25.918	1.01
28	Vitamin E	430.7	$C_{29}H_{50}O_2$	26.24	0.68
29	1-Pentacosanol	368.7	$C_{25}H_{52}O$	26.623	0.85
30	Eicosanoic acid	312.5	$C_{20}H_{40}O_2$	26.743	1.55
31	Tetrapentacontane	759.4	$C_{54}H_{110}$	26.845	2.24
32	Ergost-5-en-3-ol	400.7	$C_{28}H_{48}O$	27.046	0.55
33	1-Pentacosanol	368.7	$C_{25}H_{52}O$	27.681	0.44
34	Stigmasterol	412.7	$C_{29}H_{48}O$	27.933	1.74
35	Gamma-Sitosterol	432.7	$C_{29}H_{52}O_2$	28.291	4.68
36	13-Methyl-Z-14-nonacosene	420.8	$C_{30}H_{60}$	28.637	0.49
37	4,4,6a,6b,8a,11,12,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	424.7	$C_{30}H_{48}O$	28.877	3.61
38	9,19-Cyclo-9.beta.-lanostane-3.beta.,25-diol	444.7	$C_{30}H_{52}O_2$	29.029	1.74
39	Lup-20(29)-en-3-ol	426.7	$C_{30}H_{50}O$	29.229	16.77
40	B-Friedo-B':A'-neogammacer-5-en-3-ol	426.7	$C_{30}H_{50}O$	29.565	6.81
41	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol	222.37	$C_{15}H_{26}O$	29.848	0.3
42	1,4-Dimethyl-8-isopropylidenetricyclo[5.3.0.0(4,10)] decane	204.35	$C_{15}H_{24}$	30.09	0.4
43	Naphthalene	128.169	$C_{10}H_8$	30.884	2.3
44	9,10-Dihydrodeoxynivalenol	298.33	$C_{15}H_{22}O_6$	31.159	0.38
45	D:A-Friedooleanan-3-ol	428.7	$C_{30}H_{52}O$	32.061	10
46	Friedelan-3-one	426.7	$C_{30}H_{50}O$	33.434	7.09

TABLE 5: PHYTOCOMPOUNDS IDENTIFIED BY GC-MS ANALYSIS IN CHLOROFORM EXTRACT OF *A. imbricate*

S No.	Compound Name	Molecular weight	Molecular formula	Retention time	Peak area %
1	Bacteriochlorophyll-c-stearyl	841.5	$C_{52}H_{72}MgN_4O_{42}$	18.024	1.21
2	Tetrapentacontane,1,54-dibromo-	917.2	$C_{54}H_{10}8Br_2$	20.67	4.06
3	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	410.7	$C_{30}H_{50}$	24.016	8.6
4	Tetrapentacontane	759.4	$C_{54}H_{110}$	24.432	1.59
5	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	412.7	$C_{29}H_{48}O$	24.816	2.77
6	Tetrapentacontane	759.4	$C_{54}H_{110}$	26.065	3.4
7	1-Triacontanol	438.8	$C_{30}H_{62}O$	26.181	2.16
8	Vitamin E	430.7	$C_{29}H_{50}O_2$	26.78	1.01
9	Tetrapentacontane	759.4	$C_{54}H_{110}$	27.073	0.44

10	Tetracontane-1,40-diol	595.1	$C_{40}H_{82}O_2$	27.687	0.52
11	Tetrapentacontane	759.4	$C_{54}H_{110}$	28.261	9.2
12	1-Triacontanol	438.8	$C_{30}H_{62}O$	28.464	1.43
13	Stigmasterol	412.7	$C_{29}H_{48}O$	28.566	3.1
14	Gamma -Sitosterol	414.7	$C_{29}H_{50}O$	29.374	5.25
15	Hexacontane	843.6	$C_{60}H_{122}$	30.116	0.66
16	3.alpha.,7.beta.-Dihydroxy-5.beta.,6.beta.-epoxycholestane	418.7	$C_{27}H_{46}O_3$	30.842	2.72
17	Lup-20(29)-en-3-ol	468.8	$C_{32}H_{52}O_2$	31.053	12.63
18	D:B-Friedo-B':A'-neogammacer-5-en-3-ol	426.7	$C_{30}H_{50}O$	31.334	5.58
19	Tetrapentacontane	759.4	$C_{54}H_{110}$	31.694	9.41
20	Tetracontane-1,40-diol	595.1	$C_{40}H_{82}O_2$	32.011	1.58
21	2-Propenoic acid	164.16	$C_9H_8O_3$	32.9	1.35
22	D:A-Friedooleanan-3-ol, (3.alpha.)-	428.7	$C_{30}H_{52}O$	33.25	11.53
23	Friedelan-3-one	426.7	$C_{30}H_{50}O$	33.415	7.21

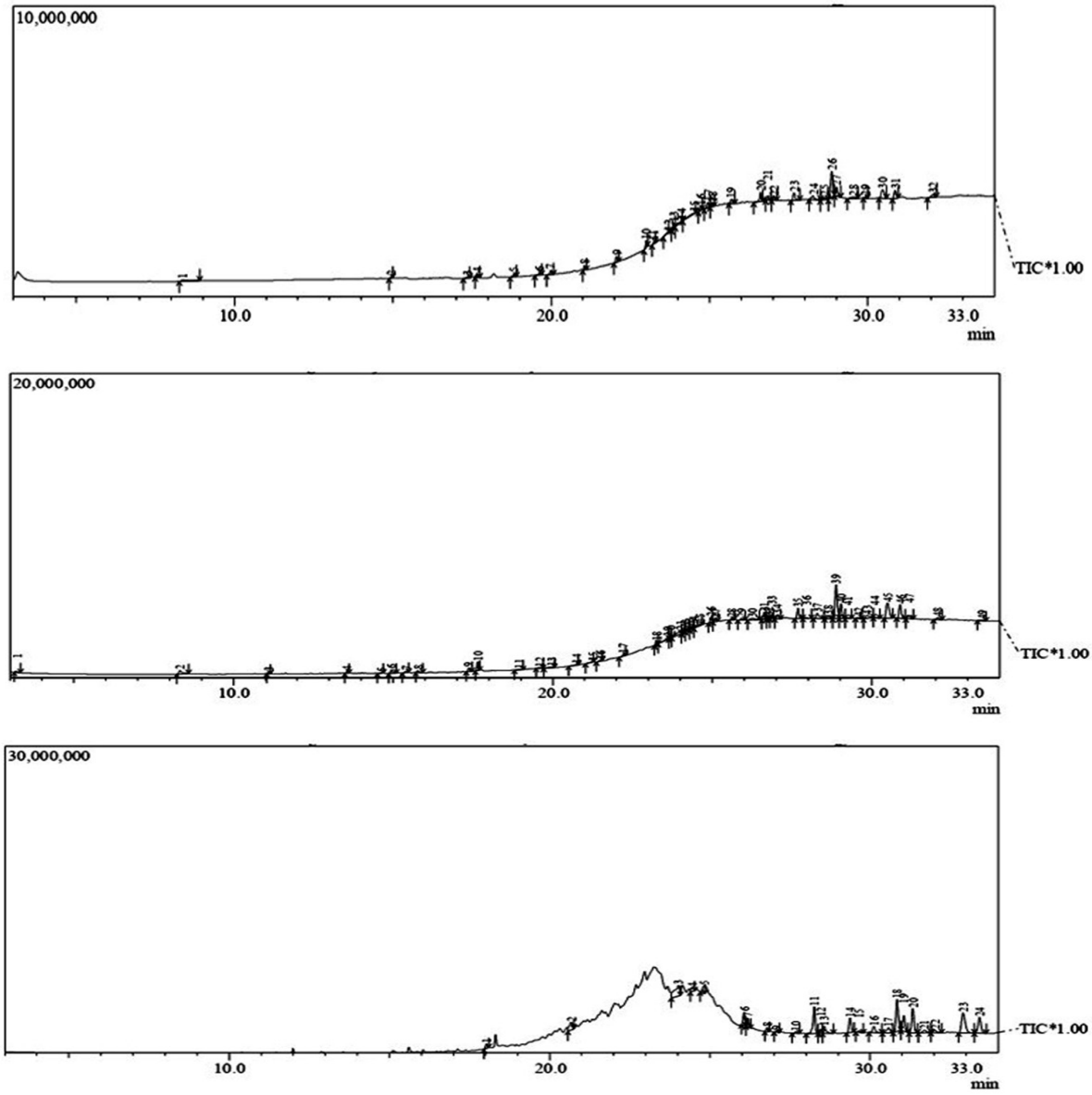

Fig. 5: Chromotograph of the GC-MS analysis of different solvent extract of *A. imbricata* leaves

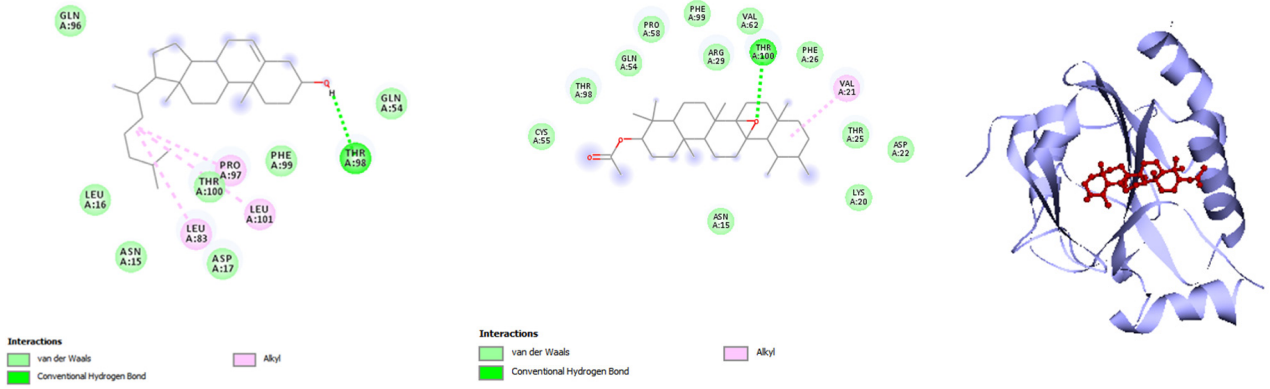

Fig. 6: Molecular docking conformation of different phytoconstituents of methanol extract of *A. imbricata* leaves

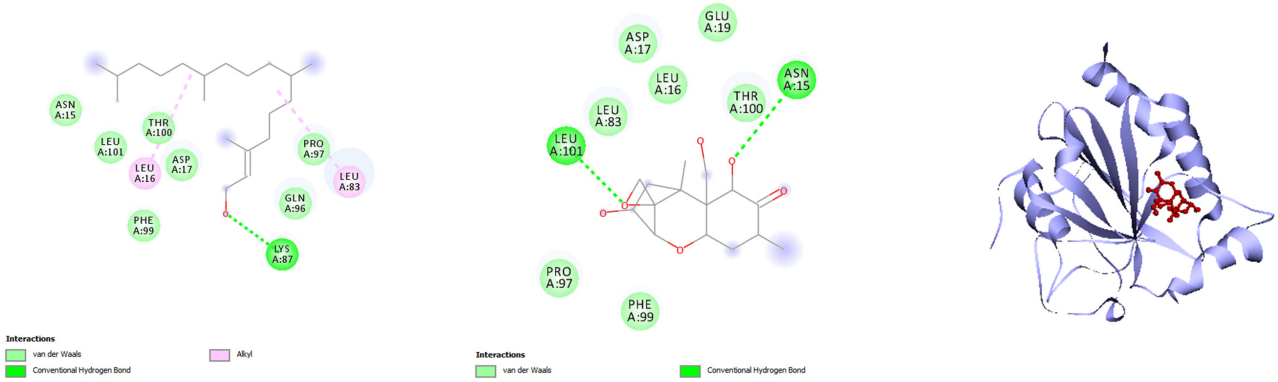

Fig. 7: Molecular docking conformation of different phytoconstituents of ethyl acetate extract of *A. imbricata* leaves

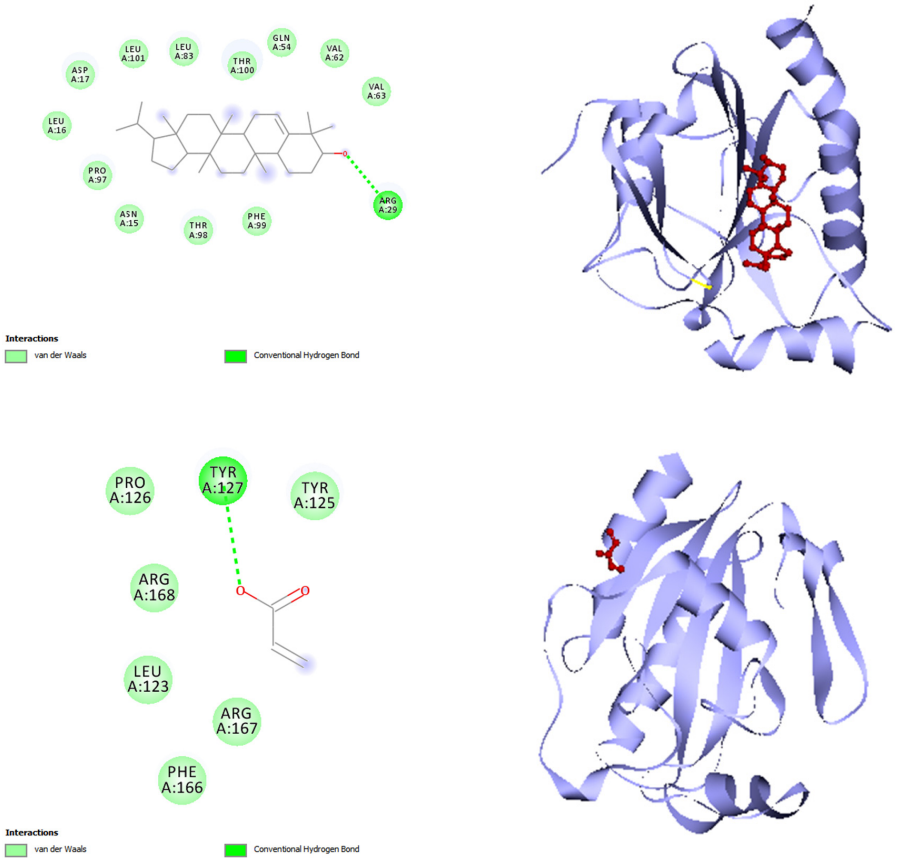

Fig. 8: Molecular docking conformation of different phytoconstituents of chloroform extract of *A. imbricata* leaves

TABLE 6: DOCKING SCORE (Kcal/mol) OF THE PHYTOCONSTITUENTS FROM THE METHANOL EXTRACT OF *A. imbricata* LEAVES EXTRACTS

Ligand	PubChem id	Energy scores (Kcal/mol)
Cholest-5-en-3-ol	304	-6.5
13,14-Epoxyursan-3-ol	605176	-7.9

TABLE 7: DOCKING SCORE (Kcal/mol) OF THE PHYTOCONSTITUENTS FROM THE ETHYL ACETATE EXTRACT OF *A. imbricata* LEAVES

Ligand	PubChem id	Energy scores (Kcal/mol)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5366244	-5
9,10-Dihydrodeoxynivalenol	573016	-5.9

TABLE 8: DOCKING SCORE (Kcal/mol) OF THE PHYTOCONSTITUENTS FROM THE CHLOROFORM EXTRACT OF *A. imbricata* LEAVES

Ligand	PubChem id	Energy Scores (Kcal/mol)
D:B-Friedo-B':A'-neogammacer-5-en-3-ol	623604	-7.8
2-Propenoic acid	637542	-5.5

Conflict of interests:

The authors declared no conflict of interests.

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