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Kavitha Rani Mari
Ph.D, Research Scholar, Periyar University, Department of Biochemistry, Salem, Tamil Nadu, India

Suriyavathana Muthukrishnan
Assistant Professor, Department of Biochemistry, Salem, Tamil Nadu, India

Correspondence
Suriyavathana Muthukrishnan
Assistant Professor, Department of Biochemistry, Salem, Tamil Nadu, India

Structural characterization and *insilico* study on *Pisonia alba* Leaves extract

Kavitha Rani Mari and Suriyavathana Muthukrishnan

Abstract
Urolithiasis is defined as the presence of one or more calculi in the kidney. ClC5 is located preliminary in the kidneys, particularly in proximal tubules. It helps to reabsorb nutrients, water and other materials from the bloodstream. *Pisonia alba* plant leaves were used for different maladies. The present research is focus to screen the secondary compounds from the P*isonia alba* leaves by GCMS and FTIR. The bioactive compounds were forwarded to *insilico* study with renal protein ClC5, followed by Lipinski's rule 5, molinspiration, bioactivity score and the toxicity by ADMET. The methanolic extract revealed 15 compounds in GCMS and 17 groups in FTIR, while in chloroform extract 11 bioactive compound in GCMS & 14 groups in FTIR analysis and in ethylacetate extract 9 phytocompounds in GCMS and 15 functional groups in IR spectral. The majorly repeated phytocompounds in GCMS were (Phytol, 3-O-Methyl glucose, Docosanoic acid, methyl ester, 3-Eicosene) docked with the renal target protein ClC5. Usually using standard drug for the urolithiasis tamsulosin hydrochloride and penicillamine were docked along with ClC5. *Insilico* study shows that the high affinity binding energy. The results showed the secondary metabolites has good affinity interaction. These create a platform to estimate the other contents.

Keywords: *Pisonia alba*, GC-MS, FTIR, *Insilico* study, ClC 5

1. Introduction
Natural products are the major source for maladies. Man researchers proved the various diseases have been cured by plant compounds which are mainly responsible for combating against vast diseases [1]. A long term, secondary metabolites was virtually ignored. At present status, the situation is different. The vast structural variability of these compounds has attracted the interest of chemists and the biological activity possessed by natural products. These have encouraged the pharmaceutical industry to search for new structures of new drugs [2]. Nowadays in the plant development program phytochemical compounds accumulate as an integral part were synthesized and widely consider as chemo protective agents and promoters for animal and human maladies [3]. Many researchers were focusing to explored the natural remedies to treat the countless disease condition. Though the chemically synthesized drugs are causing several side effect when comparing to the naturally derived bioactive compound or drug [4]. The isolation and identification of marker compounds in an natural drug is a prerequisite for quality control, since most of these compounds are not commercially available. For the analysis of volatile compounds in herbal medicines, Gas Chromatograph Mass spectrometry (GC/MS) have been extensively using. GC/MS has particularly served as a suitable and consistent method for the simultaneous determination of easily evaporate & normal temperature compounds in the multifaceted natural matrix of herbal extracts [5]. Fourier transform Infrared spectroscopy (FTIR) technique help to discover the medicine and the select the compound based on the functional group. This spectral analysis technique can perform on both purely separated compound or a mixture of complex without separation into individual components. This technique is highly sensitive and selective than the colorimetric method and it plays a vital role in pharmaceutical industries [6].
The kidney cells were consist lots of numerous specialized ion channels and transporters, which act a wall to control volume and ionic concentration by absorption or secretion of ions in urine. Each part of the kidney involved in filtration and ion concentration in ion channels. These ion channels mutually ensure suitable electrolyte homeostasis. Though a number of genetic mutation render and hereditary, these channels dys or non functional. Mutations to more than one ion channels are connected with a various symptom including proteinuria, progressive loss of renal function, and renal hypertension. The progressive loss of renal function, culminating in end-stage renal disease, is typically restorative by dialysis or

transplantation [7]. Zhihong Lin *et al*., 2011 provide significant information about the function of the intracellular voltage dependent cl/ p exchanger [8].

ClC-5 is a membrane protein in the kidney, its belongs to ClC family, ClC-5 is predominantly present in the early endosomes of proximal tubule cells and, to a lesser extent, in the thick ascending limb of Henle's loop and in the intercalated cells of the collecting duct [9]**.** ClC5 is essential for the renal reabsorption of low molecular weight proteins that can pass the glomerular filter into the primary urine. Mutations in *CLCN5*, the human gene encoding ClC5, cause Dent's disease, an X-linked disorder characterized by low molecular weight proteinuria and the urinary loss of phosphate and calcium that eventually lead to more variable, but clinical symptoms like kidney stones and nephrocalcinosis [10].

Protein and ligand docking is a molecular modeling technique. The goal of protein–ligand docking is to predict the position and orientation of a ligand (a small molecule) when it is bound to a protein receptor or enzyme. Pharmaceutical research employs docking techniques for a variety of purposes, most notably in the virtual screening of large databases of available chemicals in order to select likely drug candidates [11]. Significant progress has been made in computer-aided drug design by pharmaceutical companies at different stages of drug discovery, such as identifying new hits, enhancing molecule binding affinity in hit-to-lead, and lead optimization [12].

Pisonia alba is a tree which have used for traditionally to cure the various maladies in human beings. *Pisonia alba* belongs to *Nyctaginaceae* family. Elumalai *et al.,* 2012 [13], publicized in his review that the various parts of *Pisonia grandis* are extensively used in tribal folk medicines and also used as an Indian traditional medicine for an anti-diabetic [14], anti-inflammatory [15-17], wound healing [18], diuretic [19], analgesic [19], filariasis, dysentery [20] and rheumatic disorders, Antifungal [21]. Leaves of *Pisonia alba* consumed as vegetable and salad by human and fed to cattle [22]. This creates interests towards *Pisonia alba* leaves to screen and identify the bioactive compounds which are used for drugs discovery.

2. Materials and Methods

2.1 Collection and preparation of *Pisonia alba* leaves extract

The plant leaves were collected from the kolli hills, Namakkal District, Tamilnadu, India. The collected plant sample authorized by Prof. Jayaraman. P, Plant Anatomy Research Centre (PARC), Chennai-45. Registration no of the certificate: PARC/2015/3251.The collected leaves were cleaned repeatedly with tap water then followed by distilled water to remove the dust particles in the leaves and remove the spinal stem of the leaf were removed. The leaves were allowed to shadow dry and made fine powdered using electrical blender. 25 gms of fine powder of *Pisonia alba* leaves were extracted use of with 250 ml (1:10) RBFlask in order to polarity based solvents methanol, Ethyl acetate, chloroform solvent by soxhlets methods. The extracts were allowed to dry and stored for further analysis.

2.2 GCMS analysis

After the preliminary screening 3 extracts were finally selected (methanol, chloroform, ethylacetate) to GC-MS analysis. Crude extracts of *Pisonia alba* were performed on a PerkinElmer Clarus 600(CT,USA) Gas Chromatography mass spectrum System, fitted with a Rtx-5MS capillary column (30 m ×0.25 µm inner diameter, × 0.25 µm film thickness; maximum temperature, 350 ˚C),coupled to a Perkin Elmer Clarus 600 ˚C Mass Spectrum. Ultra-high purity helium gas was used as carrier gas at a constant flow rate of 1.0 mL/ min. The injection, transfer line and ion source temperatures were all at 240 ˚C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The initial temperature of oven was programmed from 60 ˚C (60 ˚C hold for 2 min) ramp 10 ˚C/min to 300 ˚C hold for 6 minutes, solvent delay 2.50 min The crude samples were diluted in ratio of (1/100, v/v) with appropriate solvent and filtered. The particle-free diluted crude extracts (1 µL) were taken in a syringe and injected auto 260 ˚C and the sample injected into injector with a split ratio 10:1. All data were obtained by collecting the full-scan mass spectra within the scan range 40 to 600 Da. The percentage composition of the crude extract constituents was expressed as a percentage by peak area. The compound from chemical various was determined based on GC retention time and mass spectrum obtained is further compared with standard of NIST, wiley 08 library.

The concentration of such compound was calculated by the following formula:

Compound concentration percentage = [P1/P2] x 100

Where, P1-peak area of the compound and P2- whole peak areas in the fractionated extract [23].

2.3 FT-IR analysis

FT-IR spectral analysis was performed in the crude sample (methanol, ethylacetate, chloroform *Pisonia alba* leaves extract)along with a mixture of potassium bromide powder (KBr). The molecular functional vibrations of chemical groups which responsible for biological activity was recorded with Perkin-Elmer FT-IR spectrum e^1 spectrophotometer operated at a resolution of 2 cm^{-1} ranging from 4000 to 400 cm^{-1}.

2.4 *In silico* study in bioactive compound present in chromatogram technique

To have a better understanding about the inhibitory mechanism as well as the mode of interactions of the phytochemical compounds of the crude extract, docking analysis was accomplished using the Auto dock vina 1.1.2. The three dimensional crystallographic data file of ClCn5 protein were obtained from the Protein data bank (PDB) (http://www.pdb.rcbs.org), and the small molecules were retrieve from the pubchem compound database (http://www.ncbi.nlm.ni.gov/search). The screened compounds and the ClCn5 protein were docked (*Automated Docking of Flexible Ligands to Flexible Receptors*) with the help out of (i) autodock vina 1.1.2 (ii)MGL tool 1.5.6 (http://mgltools.scrips.edu). The other software (iii) discovery studio 3.5 is used to view the protein ligand interaction and to separate the crystallized ligand separation and (iv) PYMOL 1.3 to see the overlay of the ligand.

2.4.1 Protein and lignd preparation

Absolutely 5 bioactive compounds (from GCMS analysis) were used designed for docking studies. Ligand structure were obtain from different database like: Pubchem, chemdraw sketch or while analyzing GCMS the bioactive compounds structure (ligand)were obtained from the NIST library.

2.4.2 Molecular docking

Insilico study was carried out using autodock vina 1.1.2 software. A computational ligand target docking approach

was to analyze the structural complex of the Phytol, 3 O methyl glucose, Docosanoic acid, methyl ester, 3-Eicosene, 3,7,7,1,15,tetramethyl-2-hexadecene, Tamsulosin hydrochloride, Penicilamine (ligand) with the target (ClCn5-chloride gated voltage channel- PDB ID: 2J9L) in order to understand the specificity of the protein targets. During the selection of the ligand Lipinski's rule 5 was applied. Lipinski's rule 5 was essential to pharmacological industries to increase the activity and selection of ligand as well as drug like properties. Molinspiration were studied to calculate the molecular properties and drug likeness score; Grid: a computational principle for determining actively favorable binding site on molecules of identified structure. A scoring grid was computed using the ligand structure to reduce the computation time. ADMET helps us to know the toxicity bases [Computational Resource for Drug Discovery].

3. Results and Discussion

3.1 Volatile chemical composition determined by GCMS

GCMS analysis was used to identify the most prevailing volatile compounds present in *P.alba* leaves extracts. The results clearly depicts the presence of numerous constituents, which are responsible for the medicinal activity. The explored phytocompounds was confirmed based on the peak, molecular weight, molecular formula, retention time, structure and peak area percentage. In methanolic leaves extract of *P.alba* revealed 15 peaks in maximum run time 30 minutes. Table 1 shows the identified compounds occurs in methanolic extract of *P.alba* leaves, 8-Heptadecene is first compound read with a reduction of retention time (14.218min) and at last with highest retention time of (27.289min) Di-N-Decylsulfone compound was identified. 3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol constituents repeated 4 times in different retention time and sulfurous acid, Decyl 2-propyl Ester were also repeated two times at various retention time in methanolic leaves extract, following to *P.alba* ethylacetate leaves extract 11 peaks were observed during maximum run time 30 minutes. The biological active constituents present in ethylacetate extract showed at (Table 2), in less retention time (16.894min) 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-Ol compound were exhibited and in leading retention time (29.844min) Ergost-5,8(14)-Dien-3-Ol was identified. 3-O-methyl-D-glucose is repeated three times in distinct (RT). Interestingly, chloroform leaves extract *P.alba* exhibit a total no. of 9 peaks in maximum runtime 30 min. Table 3 states the phytocompounds present in the chloroform extract of *P.alba*, 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-Ol compound reveal at (16.914min) retention time and Trimethyl[4-(2-Methyl-4-Oxo-2-Pentyl)Phenoxy] Silane where as the last compound with longest retention time (30.395 min). 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-Ol and Trimethyl [4-(2-Methyl-4-Oxo-2-Pentyl) Phenoxy] Silane were the repeated in *P.alba* chloroform leaves extract, the position of peaks states the time of compound elution. Fig (1) exhibiting the highest peak range 16.89 and the percentage of peak area is 90.68, and the 19.645, peak area % is 100, Fig (2) display the ethylacetate extract of *P. alba %* of the peak area 100 the compound is 3-O-Methyl-D-Glucose, Fig (3) reveals the highest peak in16.91, the % of the peak area is 67.30 in chloroform extract. The peak indicates the abundance presence of phytocompounds in *P.alba* leaves extract. The chemical nature and biological significance of the explored compounds are presented in table (1), table (2) and table (3). In various retention time majorly repeated compounds are represented in table (4). The peaks indicates that the abundance of a constituents present in *P.alba* leaves extract.

The phytocompounds derived from *P.alba* showed various fundamental medicinal properties. Such as antioxidant, antidiuretic, antifouling, anticancer, antiarthritic, antimicrobial. Phtytol: act in transplant rejection treatment [http://ncit.nci.nih.gov/ncitbrowser/conceptreport], gamma tocopherol: is naturally occurring fat soluble vit-E. It has the capable to scavenge free radical and protect from oxidative damages, similarly alpha tocopherol: is also a Vitamin E, it inhibits angiogenesis and tumor dormancy through suppressing vascular endothelial growth factor (VEGF) gene transcription (http://www.ncbi.nlm.nih.gv/mesh/68000975), Cyclotrisiloxane-Hexamethyl: used in intermediates, adherent and sealant chemicals (http://www.epa.gov/chemical-data-reporting.), dibutyl phthalate: larva trombiculidmite(http://ncit.nci.nih.gov/ncitbrowser/conceptreport), 3-O-Methyl-D-Gluose: it is located in extracellular and cytoplasm (http://hmdb.ca/metabolites/HMDB3422) At present, UPLCMS/MS and GCMS are two efficient and precise techniques to resolve the metabolomic profile of plant resources [24]. Gas chromatography of petroleum ether extracts of stems of *Pisonia grandis* showed that n-hexadecanoic acid and 6-octadecenoic acid are phytoconstituents. In root the chromatogram release the substantial phytoconstituents are 9-octadecenoic acid and n-hexadecanoic acid. Simple appearance in the chromatogram the major phytoconstituents observed in *Pisonia grandis* leaves extract that 9-coctadecenoic acid-1, 2,3-propanetriyl ester, phytol and n-hexadecanoic acid [25]. The similar results was report by Pradeesh *et al*., 2015 [26].

3.2 FTIR spectral analysis

IR spectrum analysis is basically a vibrational spectrum and it provides a wide platform to measure the wave length and intensity of the absorption. The basic principle of IR is to detect the bond in bioorganic sample. the presence of functional groups were differentiate based on the peak valve, it plays a vital role in physiochemical properties in the *Pisonia alba* extract, when run under IR region in the range of 400 - 4000 $cm^{-1.}$ This technique also provide molecular level information allowing investigation of molecular confirmation, functional groups and bonding types. These bands are clear, specific& sensitive to its structural confirmation. In table (5), (6) and (7) was explored the peak range, functional groups presence and the interpretation. In vast majorly esters, ethers, carboxylic acid, aromatic amines, saturated aliphatic are in strong, Alkanes, alkyl halides, primary amines are medium bonding, there is a week bonding phenol. The peak indicates that the abundance of a constituent present in *P.alba* leaves extract. Fig (4) delicate totally 17 peaks between 400 - 4000 cm^{-1} in *P.alba* methanolic leaves extract. Fig (5) depicts the 15 peak constituents in *P.alba* ethylacetate extract. The infrared spectra of protein are characterized by a set of absorption regions 600- 850 cm^{-1} known as the alkyl halide medium, aliphatic medium and aromatic strong. A total no of 14 peaks at Fig (6) was explored in *P.alba* chloroform leaves extract, the major functional groups are C-H stretching in range of 2800- 3000 cm^{-1}, C-Cl stretching in the range of 700 – 800 $cm^{-1.}$ C-N stretching in the range of 1026- 1074 $cm^{-1.}$ The functional group constituents are alkyl medium, aliphatic medium, alkanes medium. By analyzing the FTIR the functional groups were profiled they were helpful in drug discovery, pharmaceutical etc. In Hop extract the EI spectrum shows three absorption bands between 2850 and 3100 cm^{-1}

that correspond to the C–H stretching vibration in aliphatic and aromatic compounds. The aromatic structures are confirmed by C=C skeleton vibration bands between 1350 and 1600 cm^{-1} [27]. FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition [28]. FTIR analysis of *P.alba* exhibited a numerous range of functional groups, it order to identify the phytochemical compound and properties, OH bond established the presence of phenolic compound such as flavanoid and tannins [29]. Biological molecule plays a vital role in the formation of molecule like DNA, Carbohydrates, lipids and proteins. Carbon is a backbone of macromolecule which form chain or ring in the molecule and make role as substitution in element. Carboxyl group, amines, carboxyl are role in DNA, Protein, lipid and carbohydrates. Carbonyl group are in the form of amino acids while H bond involved in DNA complementary base paring in enzymes and substrate [30].

3.3 Molecular docking study

To validate the accuracy of the autodocking tool AUTODOCK Vina for the present purpose the ligand (ClCn5-chloride gated voltage channel- PDB ID: 2J9L) were docked with the five phytol compounds which were screened under by GCMS. The phytocompounds with their CID number were tabulated in table (8) the drug using for the urolithiasis disorders namely tamsulosin hydrochloride (CID: 129211) and penicillamine(CID: 5852) are chemically synthesized compound were also docked with the ClCn5 target.Table (8) describes the name of the compound, CID number, Canonical smile, Binding energy in Kcal/mole, number of hydrogen bond presence and the active site residues. Fig (7) shows the binding affinity between the target and ligand. Docosanoic acid, methyl ester, 3-Eicosene are identified with absence of the hydrogen bonds. The binding energy of the phytocompounds are 3-O-methyl D-glucose: -6.77, Phytol: -6.64, Docosanoic acid, methyl ester: -5.68, 3-Eicosene :-4.78, Tamsulosin hydrochloride: -7.29 and Penicillamine: -7.18. Active site residues: THR596, TYR617, LEU595, ILE722, SER618, ASP727, THR724, LYS726 were presented**.**

The canonical smile predominately used for molinspiration, ADMET. Table (9) shows the milog P, no of hydrogen atom acceptor and donor, mol.weight, of the ligand, polarity of surface. The level range of Kinase inhibitor, GPCR, Enzyme link, Nuclear receptor ligand, ion channel modulator and protease inhibitor were represented in table 9. Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulating and processing including smiles and sd.file conversion normalization of molecules, generation of tautomers, molecules fragmentation, calculation of various molecular properties, molecular modelings and drug discovery. Molinspiration supports internet chemistry community by offering free online services for calculation of important molecular properties (log p, hydrogen atom acceptor and donor, polar surface area) as well as the predicition of bioactive score for the most important drug targets. ADMET- absorption, distribution, metabolism, excretion and toxicity allow to eliminate compounds with unfavorable ADMET characteristics early on to avoid expensive reformulation later and to evaluate proposed structural refinements that are designed to improve ADMET properties, prior to resource expenditure on synthesis.

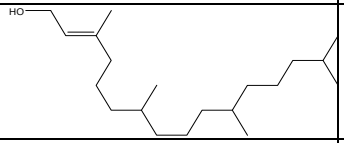
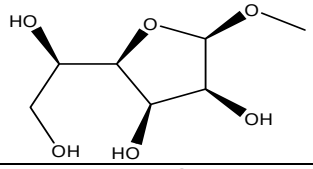
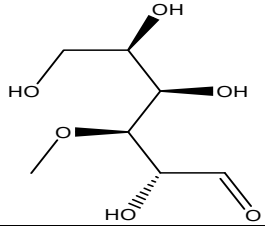
4. Conclusion

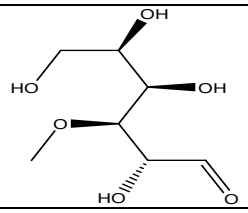
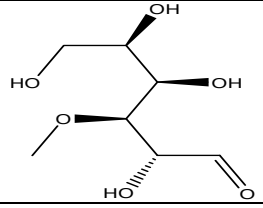
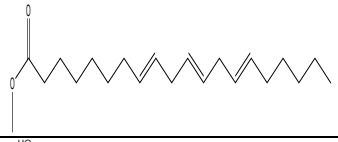
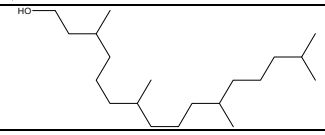
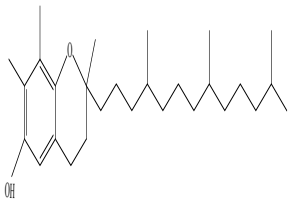
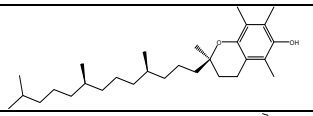
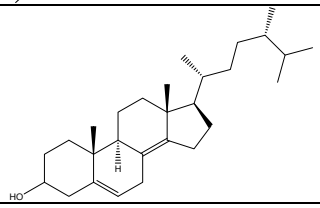
The phytocompounds detected by successive characterization using various solvents of *Pisonia alba* laves exhibits potential binding capability than the chemical synthesized drugs. The characterization study outlaid five bioactive compounds were further subjected to *insilico* study very effectively exhibited the binding affinity and drug (lead) compound optimization which is wanted by ADMET study. The phytocompounds are non toxic and more effective than chemically synthesized drugs. Our proposed research work confirm that the *Pisonia alba* plant leaves possess the bioactive phytochemical compounds.

5. Acknowledgement

The authors Kavitha Rani Mari and Suriyavathana Muthukrishnan are grateful to thank the UGC: Rajiv Gandhi National Fellowship (JRF) to carry out this part of work.

Table 1: GC-MS analysis in methanolic leaves extract *Pisonia alba*

S R	RT	Compound Name	M. Formula	Structure	Compoun d Nature	Biological Activity ***	Peak area %
1	16.894	3,7,11,15-Tetramethyl -2-Hexadecen-1-Ol [29,30]	$C_{20}H_{40}O$		Terpene Alcohol	Anti-microbial, Anti-Inflammatory activity	2.53
2	17.139	Beta.-D-Mannofuran oside, Methyl	$C_7H_{14}O_6$		-	-	2.02
3	17.334	3-O-Methyl-D-Glucose [31]	$C_7H_{14}O_6$		Sugar moiety	Preservative	8.18

4	17.739	3-O-Methyl-D-Glucose [31]	$C_7H_{14}O_6$		Sugar moiety	Preservative	21.18
5	17.794	Docosanoic Acid, Methyl Ester [32]	$C_{23}H_{46}O_2$		fatty acid	Therapeutic, Diagnostic.	9.89
6	18.270	3-O-Methyl-D-Gluose [31]	$C_7H_{14}O_6$		Sugar moiety	Preservative	100.00
7	19.475	8,11,14-Eicosatrienoic Acid, Methyl Ester	$C_{21}H_{36}O_2$		Long Chain Fatty Acid Methyl Ester	Anti-proliferation, Fatty acid studies	2.68
8	19.620	Phytol [30]	$C_{20}H_{40}O$		Diterpene	Hypocholesterolemic, Anti-microbial, Anticancer, Cancer Preventive, Diuretic Anti-inflammatory	12.05
9	27.013	Gamma.-Tocopherol [33]	$C_{28}H_{48}O_2$		Vitamin compound	Anti-ageing, Analgesic,Anti-diabatic, Anti-inflammatory, Anti-oxidant, Anti-dermatitic, Anti-leukemic, Antitumor, Anti-cancer, Hepatoprotective, Hypocholesterolemic, Anti-ulcerogenic, Vasodilator, Anti-spasmodic, Anti-bronchitic, Anti-coronary	8.32
10	27.673	Dl-.Alpha.-Tocopherol [34]	$C_{29}H_{50}O_2$		Alcoholic compound	Anti-inflammatory, antioxidant, anti-microbial, radical scavenging, anti-spasmodic	4.44
11	29.844	Ergost-5,8(14)-Dien-3-Ol	$C_{28}H_{46}O$		-	Antioxidant, Hypo-cholesterolemic	16.61

***- reveals the biological activities of the bioactive compounds in methanolic leves extract of *Pisonia alba* by Dr.Dukey method.

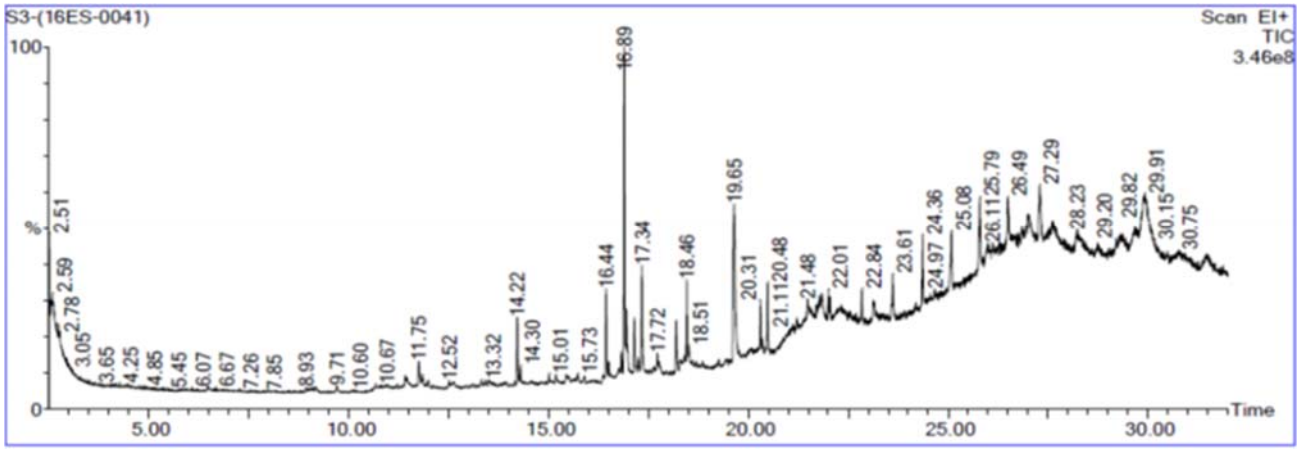
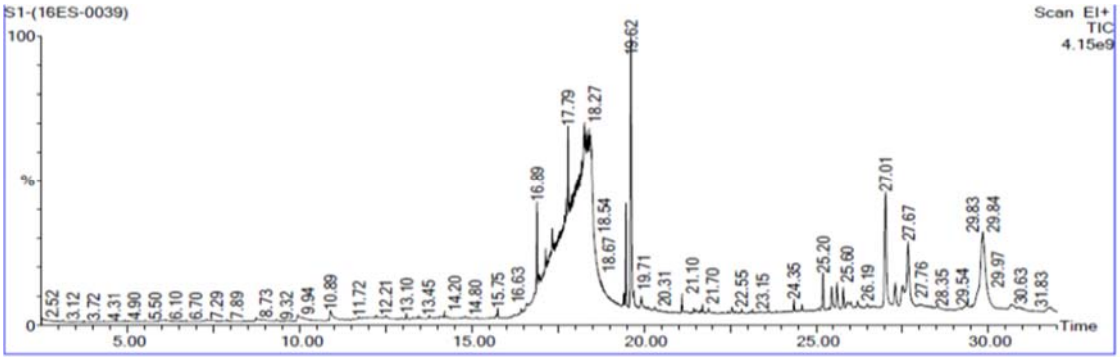
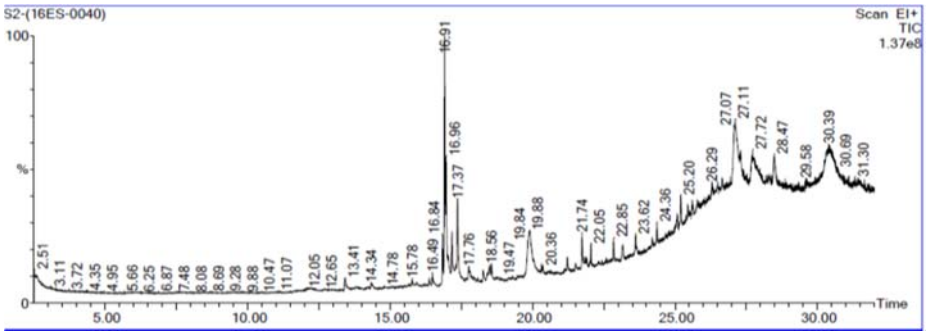


Fig 1: GC-MS analysis in methanol leaves extract of *Pisonia alba*



This graph exhibit the secondary metabolite compound at different peak line. The highest peak 19.62 is a phytol it is a diterpene compounds, compound eluted at 20 min

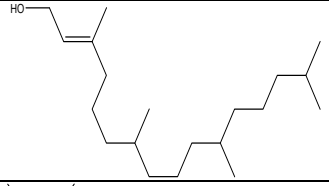
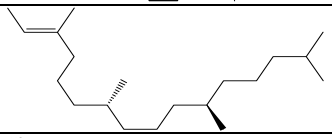
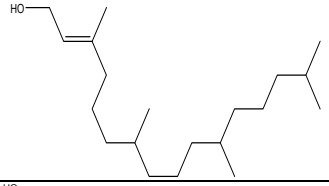
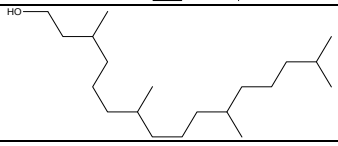
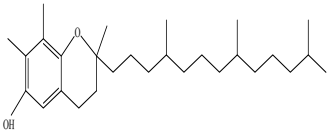
Fig 2: GC-MS analysis in ethylacetate leaves extract *Pisonia alba*

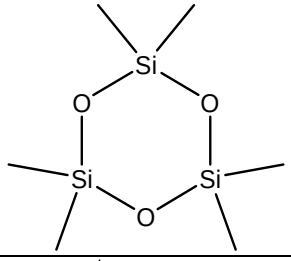
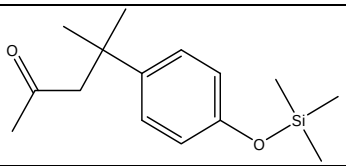
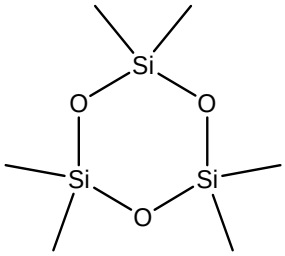
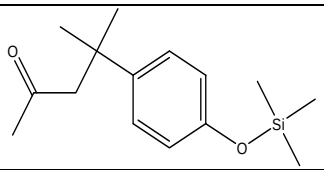


Graph express the presence of secondary compounds at different peak line. The highest peak 16.91 shows the presence of 2-Hexadecene, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]- The peak area % is 31.64

Fig 3: G-MS analysis in chloroform leaves extract

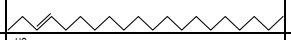
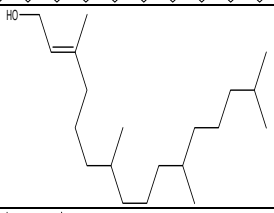
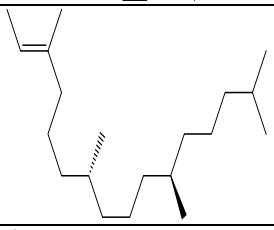
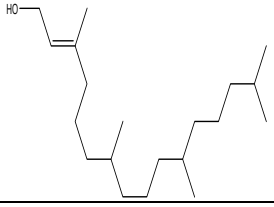
Table 2: GC-MS analysis in chloroform leaves extract *Pisonia alba*

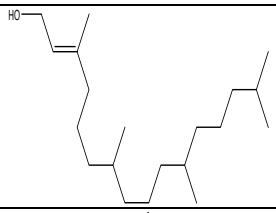
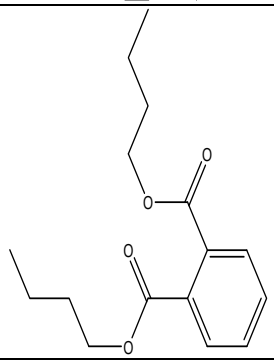
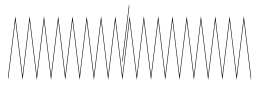
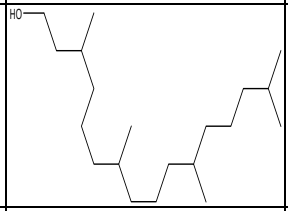
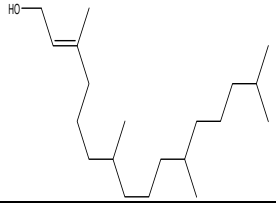
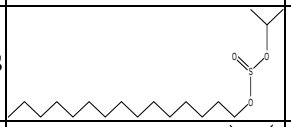
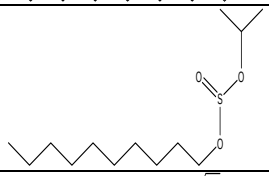
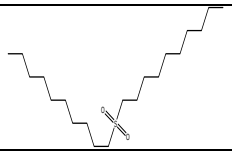
S. No	RT	Compound Name	M. Formula	Structure	Compound Nature	Biological Activity ***	Peak area %
1	16.914	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol [29], [30]	$C_{20}H_{40}O$		Terpene Alcohol	Anti Microbial, Anti-Inflammatory Activity	67.30
2	16.964	2-Hexadecene, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]- [35]	$C_{20}H_{40}O$		-	Fuel, Transplant rejection treatment,metabolic disease treatment, Gaucher disease treatment.	31.61
3	17.369	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol [29],[30]	$C_{20}H_{40}O$		Terpene Alcohol	Anti Microbial, Anti-Inflammatory Activity	22.53
4	19.900	Phytol [29]	$C_{20}H_{40}O$		Diterpene	Hypocholesterolemic, Antimicrobial, Anticancer, Cancer Preventive, Diuretic Anti Inflammatory	71.47
5	27.108	.Gamma.-Tocopherol [33]	$C_{28}H_{48}O_2$		Vitamin compound	Anti-ageing, Analgesic,Anti-diabatic, Anti-inflammatory, Anti-oxidant, Anti-dermatitic, Anti-leukemic, Anti-tumor, Anti-cancer, Hepatoprotective, Hypocholesterolemic, Anti-ulcerogenic, Vasodilator, Anti-spasmodic, Anti-	100.00

						bronchitic, Anti-coronary	
6	27.293	Cyclotrisiloxane, Hexamethyl [36]	$C_6H_{18}O_3SI_3$		Phenolic compounds	Anti-microbial potential, Anti-oxidant activity	24.02
7	27.719	Trimethyl[4-(2-Methyl-4-Oxo-2-Pentyl)Phenoxy]Silane	$C_{15}H_{24}O_2SI$		-	-	39.82
8	28.474	Cyclotrisiloxane, Hexamethyl-	$C_6H_{18}O_3SI_3$		Phenolic compounds	Anti-microbial potential, Anti- oxidant	22.43
9	30.395	Trimethyl[4-(2-Methyl-4-Oxo-2-Pentyl)Phenoxy]Silane	$C_{15}H_{24}O_2SI$			-	98.66

*** -Indicates the biological activities of the bioactive compounds in chloroform leaves extract of *Pisonia alba* by Dr.Dukey's method

Table 3: GC-MS analysis in ethylacetae leaves extract of *Pisonia alba*

S. No	RT	Compound Name	M. Formula	Structure	Compound Nature	Biological Activity**	Peak are%a
1	14.218	8-Heptadecene	$C_{17}H_{34}$				19.73
2	16.444	3-Eicosene, (E)- [38]	$C_{20}H_{40}$		Alkane	-	22.84
3	16.894	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol [29], [30]	$C_{20}H_{40}O$		Terpene Alcohol	Anti-microbial, Anti-inflammatory activity	90.68
4	16.949	2-Hexadecene, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]- [35]	$C_{20}H_{40}O$		-	Transplant rejection treatment, metabolic disease treatment, Gaucher disease treatment.	23.64
5	17.144	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol [29], [30]	$C_{20}H_{40}O$		Terpene Alcohol	Anti-microbial, Anti-inflammatory activity,Resistant gonorrhea	17.91

6	17.339	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol [29], [30]	$C_{20}H_{40}O$		Terpene Alcohol	Anti-microbial, Anti-inflammatory activity	33.52
7	18.195	Dibutyl Phthalate [37]	$C_{16}H_{22}O_4$		Ester	Anti-microbial and Anti-fouling,Anti-malarial	18.45
8	18.460	17-Pentatriacontene [38]	$C_{35}H_70$			Anti-inflammatory Anti-cancer, Anti-bacterial, anti-arthritic	18.84
9	19.645	Phytol [29]	$C_{20}H_{40}O$		Diterpene	Hypocholesterolemic, Anti-microbial, Anti-cancer, Cancer Preventive, Diuretic Anti-inflammatory	100.00
10	20.481	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol [29], [30]	$C_{20}H_{40}O$		Terpene Alcohol	Anti-microbial, Anti-inflammatory activity	17.56
11	23.607	Hexatriacontane [39]	$C_{36}H_{74}$		Higher alkane	Anti-fungal against fungal spores germination, Antioxidant, Anti-tumour, Antibacterial	21.02
12	24.357	Heptacosane, 1-Chloro	$C_{27}H_{55}CL$				22.51
13	25.083	Sulfurous Acid, Pentadecyl 2-Propyl Ester [36]	$C_{18}H_{38}O_3S$		Alkyl sulphonic ester	-	30.05
14	26.488	Sulfurous Acid, Decyl 2-Propyl Ester [36]	$C_{20}H_{42}O_2S$		Alkyl sulphonic ester	Not reported	27.95
15	27.289	Di-N-Decylsulfone	$C_{13}H_{28}O_3S$		Alkyl sulphonic ester	Not reported	35.84

*** - helps to know the biological activities of the bioactive compounds in ethylacetate leaves extract of *Pisonia alba* by Dr. Dukey method

Table 4: Majorly exhibited compounds in three crude leaves extracts of *Pisonia alba* (methanol, ethylacetate, chloroform)

S. No	Name of compound identified
1.	3-O-Methyl-D-Glucose(3)*
2.	Phytol(3)*
3.	Gamma tocopherol(2)*
4.	Trimethyl[4-(2-Methyl-4-Oxo-2-Pentyl)Phenoxy] 2)* Silane(
5.	Cyclotrisiloxane, Hexamethyl (2)*
6.	Sulfurous Acid, Decyl 2-Propyl Ester(2)*

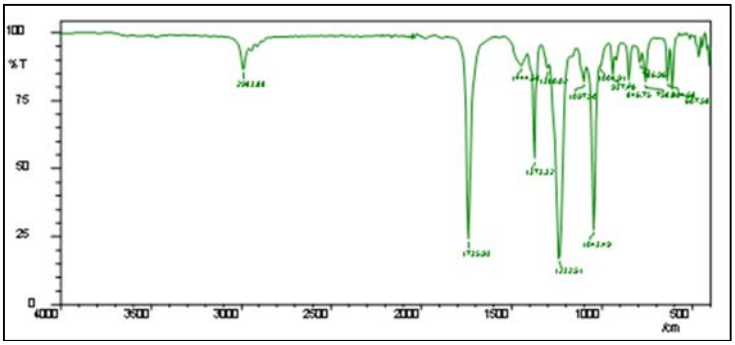
* indicates the bioactive compounds present in the methanol, chloroform and ethyl acetate leaves extract of *Pisonia alba*. These are the secondary metabolities repeated in the three crude leaves extract. Numbers at bracket denotes the number of times repeated in GCMS techniques

Table 5: FT-IR analysis in methanolic leaves extract of *Pisonia alba*

S. No	Peak	Group	Consituents
1.	3371.57	N–H stretching,	1º 2º amines, amides,medium
2.	2922.16	C-H Stretching	Alkanes medium
3.	2850.79	C-H Stretching	Alkanes medium
4.	1641.42	-c=c Stretching	Alkenes medium
5.	1379.10	C-H bending	Alkanes
6.	1265.30	C-O-H bending	Alcohol and phenol, broad and weak,
7.	1197.79	C–H wag (–CH2X)	Alkyl halides,medium
8.	1172.72	C–H wag (–CH2X),	Alkyl halides,medium
9.	1118.71	C-O Stretching	Alcohols,carboxylic acids, esters, ethers, strong
10.	1099.43	C-O Stretching	Alcohols,carboxylic acids, esters, ethers, strong
11.	1072.42	C-O Stretching	Alcohols,carboxylic acids, esters, ethers, strong
12.	1014.56	C-O Stretching	Alcohols,carboxylic acids, esters, ethers, strong
13.	896.90	C-H"oop"	Aromatics strong
14.	827.46	C-Cl Stretching	Alkyl halides medium
15.	736.81	C-H"oop"	Aromatics strong
16.	532.35	C-Br Stretching	Alkyl halides medium
17.	511.14	C-I Stretching	Iodide compounds

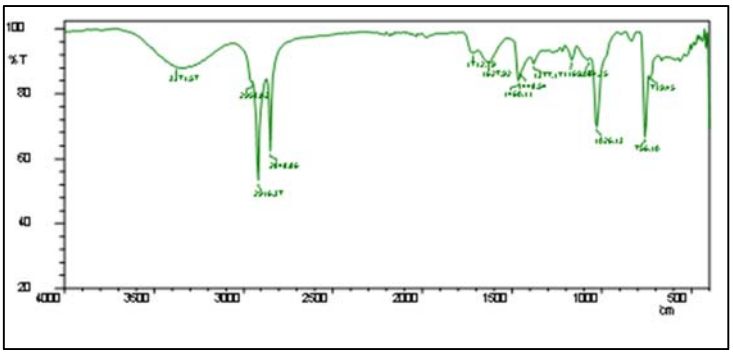
Table 6: FT-IR analysis in chloroform leaves extract of *Pisonia alba*

S. No	Peak	Group	Consituents
1.	3371.57	N–H stretching,	1º 2º amines, amides,medium
2.	2922.16	C-H Stretching	Alkanes medium
3.	2850.79	C-H Stretching	Alkanes medium
4.	1641.42	-c=c Stretching	Alkenes medium
5.	1379.10	C-H bending	Alkanes
6.	1265.30	C-O-H bending	Alcohol and phenol, broad and weak,
7.	1197.79	C–H wag (–CH2X)	Alkyl halides,medium
8.	1172.72	C–H wag (–CH2X),	Alkyl halides,medium
9.	1118.71	C-O Stretching	Alcohols,carboxylic acids, esters, ethers, strong
10.	1099.43	C-O Stretching	Alcohols,carboxylic acids, esters, ethers, strong
11.	1072.42	C-O Stretching	Alcohols,carboxylic acids, esters, ethers, strong
12.	1014.56	C-O Stretching	Alcohols,carboxylic acids, esters, ethers, strong
13.	896.90	C-H"oop"	Aromatics strong
14.	827.46	C-Cl Stretching	Alkyl halides medium
15.	736.81	C-H"oop"	Aromatics strong
16.	532.35	C-Br Stretching	Alkyl halides medium
17.	511.14	C-I Stretching	Iodide compounds



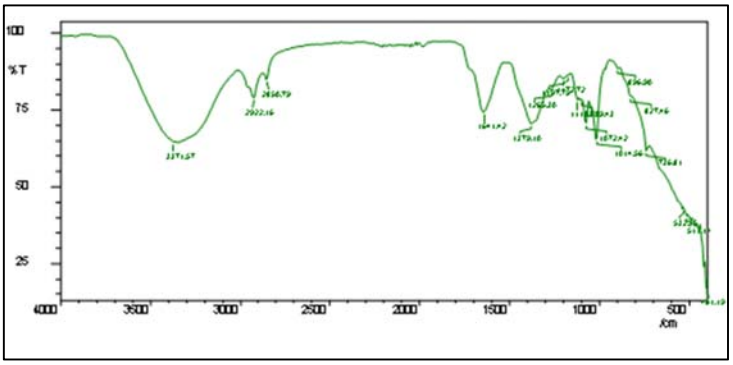
The graph represent the (3) Deep band (DB) at 1736.93- ester, saturated aliphatic band, 1232.51- aliphatic medium,1043.49- aliphatic

Fig 4: FT-IR analysis in ethylacetate leaves extract *Pisonia alba*



This graph express the presence of 1 Broad band (BB), 1 Deep band. 3371.-1˚,2˚ amines medium, 2916.37- alkanes

Fig 5: FT-IR analysis in chloroform leaves extract of *Pisonia alba*



FTIR analysis in methanolic extract exhibit the presence of Broad band at 3371.57-1˚,2˚ amined medium

Fig 6: FT-IR analysis in methanolic leaves extract of *Pisonia alba*

Table 7: FT-IR analysis in ethylacetate leaves extract of *Pisonia alba*

S.no	Peak	Group	Constituents
1.	2983.88	C-H Stretching	Alkanes medium
2.	1735.93	C=O Stretching	Ester, saturated aliphatic strong
3.	1444.68	C–C stretch	Aromatic medium
4.	1373.32	CH3 bendinging	Alkanes
5.	1300.02	C-N Stretching	Aromatic amines,strong
6.	1232.51	C–N stretching	Aliphatic amines,medium
7.	1097.50	C–N stretching	Aliphatic, medium
8.	1043.49	C–N stretching	Aliphatic, medium
9.	1004.91	C-OStretching,	Alcohol, carboxylic groups,esters
10.	937.40	O–H bending	Carboxylic acid,medium
11.	846.75	C–Cl stretching	Alkyl halides,medium
12.	786.96	C–Cl stretching	Alkyl halides,medium
13.	758.96	C–Cl stretching	Alkyl halides,medium
14.	634.58	-C≡C:C-H bending	Alkynes, strong
15.	607.58	C-Br Stretching	Alkyl halide,medium

It show the peak range and functional groups presence and its constituent in ethylacetate crude extract of *Pisonia alba*

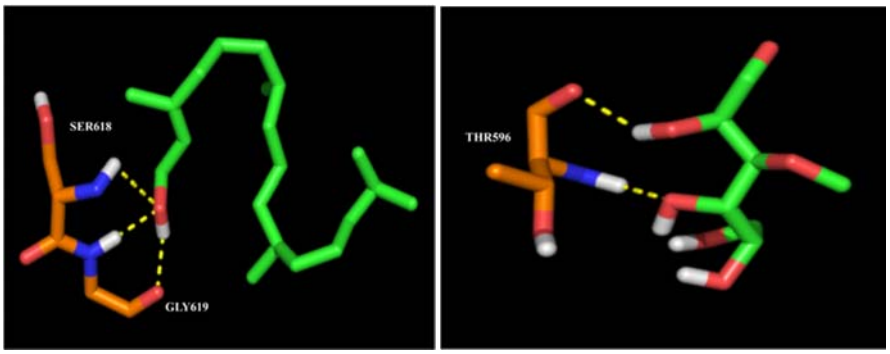


Fig 7(A): Phytol **Fig 7(B):** 3-O-methyl D glucose

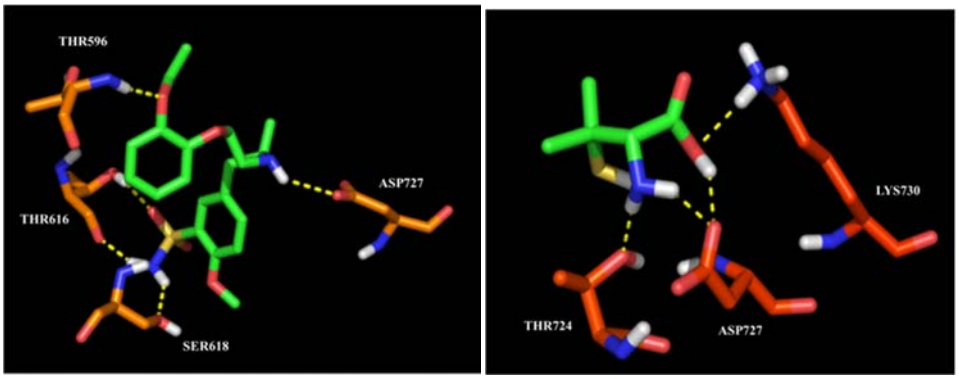


Fig 7(C): Tamslosin Hydrochloride **Fig 7(D):** Penicillamine

Fig 7: Insilico study in phytocompounds with ClC5

Table 8: Binding affinity energy of docked compounds

S. No:	Compound Name	CID	Canonical smile	Binding energy (kcal/mol)	No. of H-bonds
1.	Tamsulosin hydrochloride	129211	CCOC1=CC=CC=C1OCCNC(C)CC2=CC(=C(C=C2)OC)S(=O)(=O)N.Cl	-7.29	5
2.	Penicillamine	5852	CC(C)(C(C(=O)O)N)S	-7.18	4
3.	Phytol	5280435	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C	-6.64	3
4.	3-O-methyl D-glucose	298225	COC(C(C=O)O)C(C(CO)O)O	-6.77	2
5.	Docosanoic acid, methyl ester	13584	CCCCCCCCCCCCCCCCCCCCCC(=O)OC	-5.68	0
6.	3-Eicosene	5365051	CCCCCCCCCCCCCCCCC=CCC	-4.78	0
7.	3,7,11,15-tetramethyl-2-hexadecene	5366161	CC=C(C)CCCC(C)CCCC(C)CCCC(C)C	-3.12	0

Table 9: Molinspiration properties (Drug Likeness Score)

Properties	Ligands (From GCMS)					Ligands (Standard chemical drugs)	
	Phytol	3-O-methyl Glucose	Docosanoic acid, methyl ester	3-Eicosene	3,7,11,15-tetramethyl-2-hexadecene	Penicillamine	Tamusolin Hydrochloride
miLogP	6.76	-2.25	9.22	9.08	7.77	-1.90	2.58
TPSA	20.23	207.22	26.30	0.00	0.00	63.32	99.89
N atoms	21	13	25	20	20	9	28
Molecular weight	296.54	194.18	354.62	280.54	280.54	149.22	408.52
nONO(acceptor)	1	6	2	0	0	3	7
nOHNH	1	4	1	0	0	3	3
N violation	1	0		1	1	0	0
N rotb	13	6	21	16	12	2	11

Table 10: Molinspiration bioactivity score of the targets

Bioactives	Ligands (From GCMS)					Ligands (Standard chemical drugs)	
	Phytol	3-O- methyl Glucose	Docosanoic acid, methyl ester	3-Eicosene	3,7,11,15-tetramethyl-2-hexadecene	Penicillamine	Tamusolin hydrochloride
GPCR ligand	0.11	-0.63	0.02	0.08	-0.04	-1.47	-0.04
Ion Channel Modulator	0.16	-0.15	-0.04	0.08	0.01	-1.16	-0.23
Kinase inhibitor	-0.32	-0.85	-0.16	-0.20	-0.45	-2.52	-0.23
Nuclear receptor ligand	0.35	-0.66	0.05	0.06	0.22	-1.93	-0.35
Protease inhibitor	0.00	-0.35	0.06	-0.08	-0.16	0.96	0.06
Enzyme inhibitor	0.31	0.20	0.04	0.15	0.22	-0.71	-0.04

Table 11: Properties analysis and ADMET profile

S. No	ADMET Profile	Tamsulosin hydrochloride	Penicillamine	Phytol	3-O-methyl glucose	Docosanoic acid, methyl ester	3-Eicosene	3,7,11,15-tetramethyl-2-hexadecebe
1.	Blood-Brain Barrier	+;P:0.375	+;P:0.5821	+;P: 0.9375	+;P: 0.5966	+;P:0.9848	+;P:0.9982	+;P:0.9442
2.	Human Intestinal Absorption	+;P:0.9846	+;P:0.9278	+;P: 0.9846	+;P: 0.8547	+;P:0.9881	+;P:0.9961	+;P:0.9895
3.	CaCo2 permeability	+;P:0.6445	+;P:0.7778	+; P: 0.6445	+;P: 0.8090	+;P: 0.8141	+;P: 0.8304	+;P: 0.6999
4.	AMES toxicity	Non AMES toxic;P:0.9132	AMES toxic; P: 0.6358	Non AMES toxic; P: 0.9132	Non AMES toxic; P: 0.6021	Non AMES toxic;P0.9765:	Non AMES toxic;P:0.9901	Non AMES toxic;P:0.9518
5.	Carcinigenisi	Non-	Non-	Noncarcinogens;	Non-	carcinogens;	carcinogens;	carcinogens;

		carcinogens; P:0.6507	carcinogens; P:0.6491	P: 0.5055	carcinogens; P:0.8133	P:0.5347	P:0:6346	P:0.5632
6.	Biodegradation	Ready biodegradable P:0.8931	Not Ready biodegradable P:0.8739	Ready biodegradable; P: 0.8931	Ready biodegradable; P: 0.9415	Ready biodegradable; P: 0.8747	Ready biodegradable; P: 0.5971	Ready biodegradable; P: 0.7909
7.	Acute oral toxicity	III;P: 0.8552	III;P:0.7879	III;P:0.8552	IV;P: 0.5774	III;P: 0.8589	III;P: 0.8244	III;P: 0.8971
8.	Rat acute oral toxicity	P:1.6146	P:2.0294	P:1.6146	P:1.0530	P:1.4915	P:1.6166	P:1.5057

P: Probability in QSAR analysis. ADMET profile by Jie Shen data.

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