CHARECTORIZATION AND MOLICULOR DOCKING STUDY OF CINNAMOMUM MALABATRUM ACTIVE CONSTITUENTS

***D Tirumala, Dr.V.Jayashree**

Department of Pharmacology, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai -600117

Corresponding author: tirumaladuguti@gmail.com Mobile: 9542735154

ABSTRACT

Cinnamomum malabatrum has been traditionally used in India. The extracts of the leaves after column chromatography the compounds were analyzed to the LCMS/MS has led to the identification of active principles, among the peaks, 6 peaks were selected as per reference standard which furnished in the chromatograms. The constituents of Cinnamomum malabatrum are Gallic acid, Trans – Aconitic acid, Chlorogenic acid, Resorcinol, Rhanmetin, and Quercetin. Molecular docking analysis revealed that compound 1, 4, 5 has better docking efficiency and forms hydrophobic interactions with human beta tubulin This is suggests that the compounds act as potential inhibitors of polymerization of beta tubulin and formation of spindle network apparatus in the rapidly dividing cells during metaphase configuration. This is the best evidence for cell cycle specific anti cancer activity.

Key words: Cinnamomum malabatrum, LCMS/MS, Molecular docking. cell cycle

INTRODUCTION

Cinnamomum malabatrum is one of the Ayurveda traditional plant for treating the diseases, as per review of literature and part of research work, this plant is subjected to extraction, isolation, characterization (analysis) by TLC, HPTLC, HPLC and by LCMS/MS method to knowing the active compounds in the extracts, commonly the ethanol extract had the potential compounds, as per LCMS/MS we were find Gallic acid, Trans – Aconitic acid, Chlorogenic acid, Resorcinol, Rhanmetin, and Quercetin these all are flavonoids and polyphones, hence we selected this plant extract for evaluation of anti cancer activity, MTT assay method was given satisfactory results, then we planned for molecular docking study for knowing the binding protein in the cell, molecular docking study revealed that plant active compounds which made a hydrophobic bonds with the beta tubulin protein which is present in the cytoplasm of the cell and the binding efficacy compared with the taxol natural anti

cancer drug finally I was concluded that Cinnomum malabatrum leaves methanolic extract shown cell cycle specific anti cancer activity.

MATERIALS AND METHODS

LC-MS/MS analysis

Dry filtrates were diluted to 1000 mg/L and filtered with a 0.2 µm microfiber filter prior to LC-MS/MS analysis LC-MS/MS analyses of compounds were performed using a Nexera model Shimadzu UHPLC coupled to a tandem MS instrument (Shimadzu, Kyoto, Japan). The liquid chromatography was equipped with LC-30AD binary pumps (Shimadzu, Kyoto, Japan), a DGU-20A3R degasser (Shimadzu, Kyoto, Japan), a CTO-10ASvp column oven (Shimadzu, Kyoto, Japan), and a SIL-30AC auto sampler (Shimadzu, Kyoto, Japan). The chromatographic separation was performed on a C18 reversed-phase Inertsil ODS-4 (150 mm × 4.6 mm, 3 µm, GL Sciences, Tokyo, Japan) analytical column. The column temperature was fixed at 40 °C. The elution gradient consisted of mobile phase A (water, 5 mM ammonium formate and 0.1% formic acid) and mobile phase B (methanol, 5 mM ammonium formate, and 0.1% formic acid). The gradient program with the following proportions of solvent B was applied t (min), B%: (0, 40), (20, 90), (23.99, 90), (24, 40), (29, 40). The solvent flow rate was maintained at 0.5 mL/min and injection volume was set as 4 µL.

Mass Spectroscopy (MS) Instrumentation

MS detection was performed using a Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source operating in both positive and negative ionization modes. LC-MS/MS data were collected and processed by Lab Solutions software (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) modes was used to quantify the analyses: the assay of investigated compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and/or the third one for confirmation. The optimum ESI conditions were determined as DL temperature; 250 °C, heat block temperature; 400 °C, nebulizing gas flow (nitrogen); 3 L/min and drying gas flow (nitrogen); 15 L/min

Protein preparation

The 3D crystal structure of human c-met kinase (PDB: 1JFF) was retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank website (https://www.rcsb.org/), and its original ligand and water were eliminated. The protein was

prepared using the protein preparation wizard Schrodinger 2020-3. The crystallographic inhibitor, ions (K+ and Mg+), and the unwanted water molecules were deleted and assigned bond orders, polar hydrogens added, created disulphide bonds, converted selenomethionine to methionines using Protein preparation wizard Schrodinger 2022-3. Checked and repaired missing atoms in the protein crystal structure, edited histidine hydrogens, assigned Raadii, added Kolman charges (-21.422), Gasteiger charges (-16.9996) were added and set Kollman charge field. The prepared proteins were validated through a Ramachandran plot (Fig. 2).Autodockvina was used to create the bioactive conformation. For autodockvina research, an enhanced PDB format known as PDBQT is used for the X, Y, and Z coordinates files grid box (center x = 2.2569, center y = -14.1235, center z = 13.3444), which contains atomic partial charges and atom types. Torsion angles were calculated to assign the flexible and non-bonded rotation of molecules. All of the chemicals in our data collection were docked in the protein's active site under investigation, and the findings were evaluated using Discovery Studio 2021.

Ligands selection

The Gallic acid, Quercetin, Resorcinol and Taxol were downloaded from pubchem in SDF (Structure Data File) 3D format. Marvin sketch was used to convert SDF files into PDB format. Using Auto Dock Partial charges (Kollmann and Giester) were added. Chosen torsion tree and set the torsions to 5, finally saved as PDBQT 3D format.

Molecular docking

The molecular docking investigation was carried out by genetic algorithm using the AutoDockVinaprogramme. We used Perl script to do virtual screening in AutodockVina (Command- perl vina_windows.pl). The Gallic acid, Quercetin, Resorcinol and Taxol were docked with human beta tubulin (PDB ID: 3JFF). Afterward, with the help of PYMOL software, the ligand docked-out files, were converted into PDB files for 2D-3D interactive visualization studies with the help of Biovia Discovery Studio 4.0. The docked complexes' shortening was done based on binding energy (kcal/mol) and dissociation constant (pM) as per AD Vina scoring, where more negative energy means stronger binding.

RESULTS

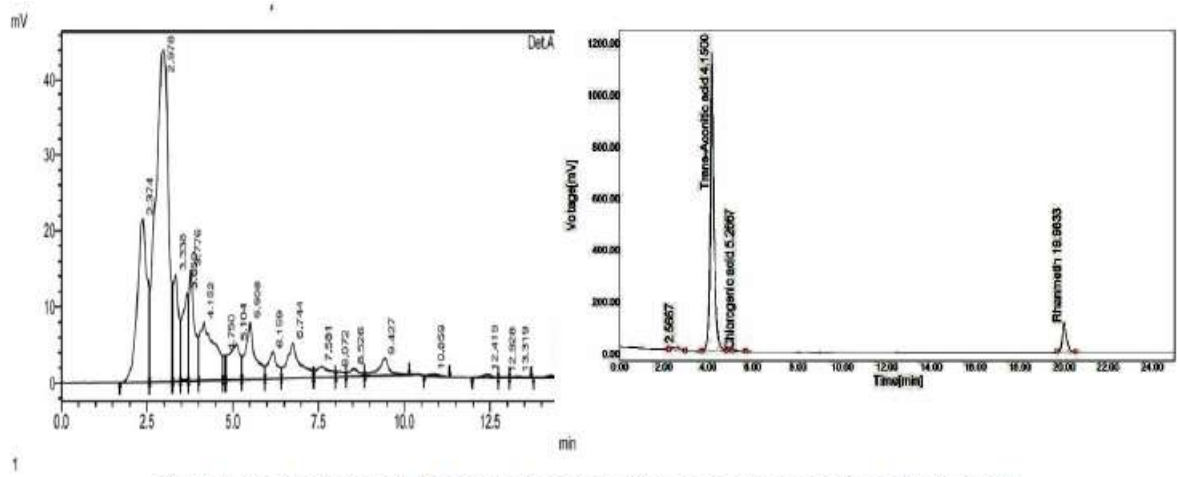


Fig : 1 LC-MS chromatogram of methanolic extract of Cinnamomum malabatrum {Peaks 1, 2,8}
Fig: 2 LC-MS chromatogram of methanolic extract of Cinnamomum malabatrum {Peak 16,17}

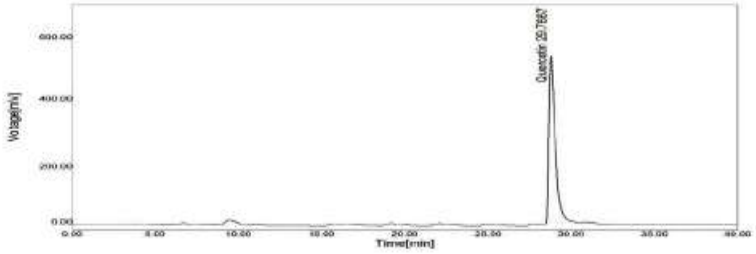


Fig : 3 LC-MS chromatogram of methanolic extract of Cinnamomum malabatrum [peak 18]

Important compound identified from the methanolic extract of Cinnamomum malabatrum by LC-MS/MS,

In the analysis of HPLC/MS analysis, peak 1 exhibited a negative molecular ion at [MS−H]+ at m/z of 170.12 corresponding to Gallic acid. Peak 2 had an m/z of 174.108 corresponding to Trans – Aconitic acid. Peak 8 showed an m/z of 354.31, which indicates chlorogenic acid. Peak 16 had a negative molecular ion at an m/z of 110.10 and was identified as Resorcinol. Peak 17 indicated an m/z of 316.26, which corresponds to Rhanmetin. Peak 18 exhibited an m/z at 302.23 corresponding to Quercetin

Table 1: **Protein Ligand Interactions Gallic acid**

Compound	**Distance**	**Category**	**Type**	**From**	**From Chemistry**	**To**	**To Chemistry**
Gallic acid	2.37389	Hydrogen Bond	Conventional Hydrogen Bond	SER236:HG	H-Donor	UNK0:O	H-Acceptor

	2.05935	Hydrogen Bond	Conventional Hydrogen Bond	GLY370:HN	H-Donor	UNK0:O	H-Acceptor
	2.94908	Hydrogen Bond	Conventional Hydrogen Bond	LEU371:HN	H-Donor	UNK0:O	H-Acceptor
	3.18047	Hydrogen Bond	Conventional Hydrogen Bond	UNK0:O	H-Donor	PRO360:O	H-Acceptor
	3.52935	Hydrogen Bond	Carbon Hydrogen Bond	ALA233:CA	H-Donor	UNK0:O	H-Acceptor
	4.70824	Hydrophobic	Pi-Alkyl	UNK0	Pi-Orbitals	PRO360	Alkyl

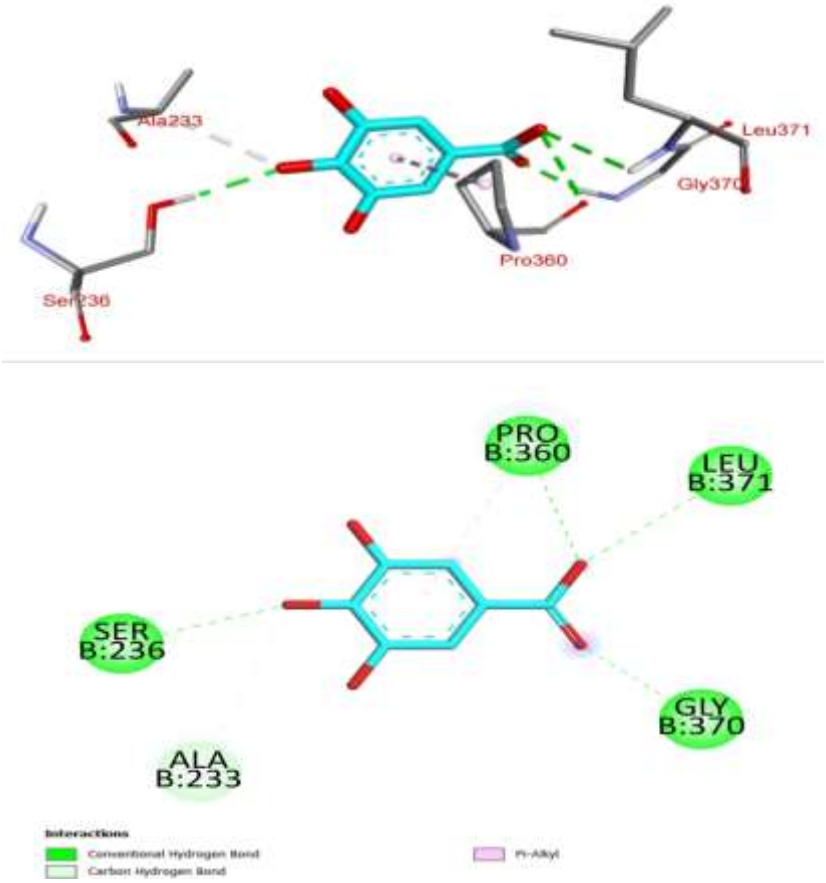


Fig: 4 Protein Ligand Interactions Gallic acid

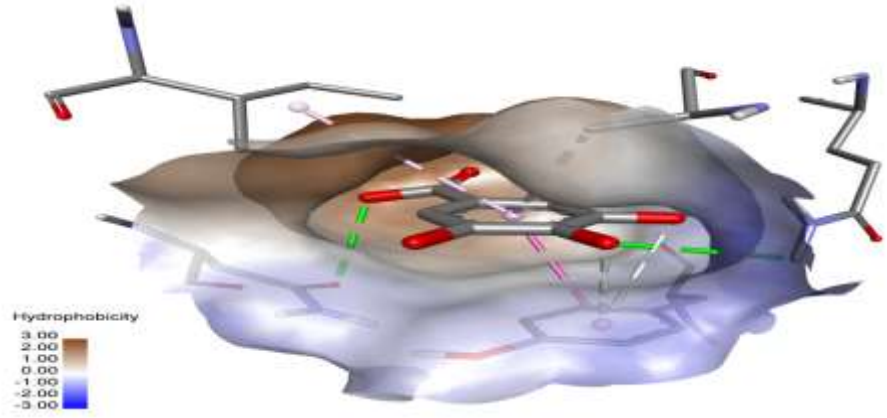


Figure 5: 1JFF protein Hydrophobic region around the Gallic Acid at the active site

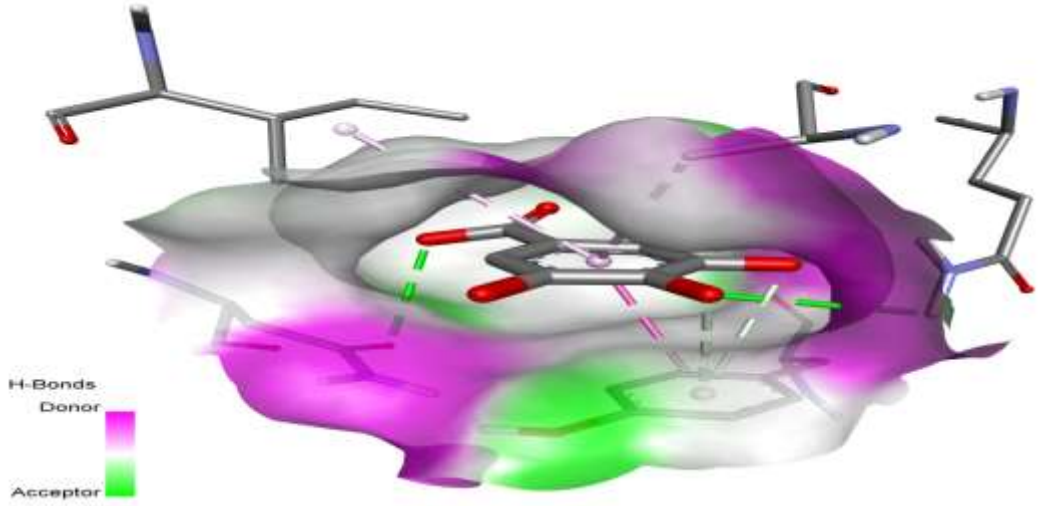


Figure 6: 1JFF protein Hydrophilic region around the Gallic Acid at the active site

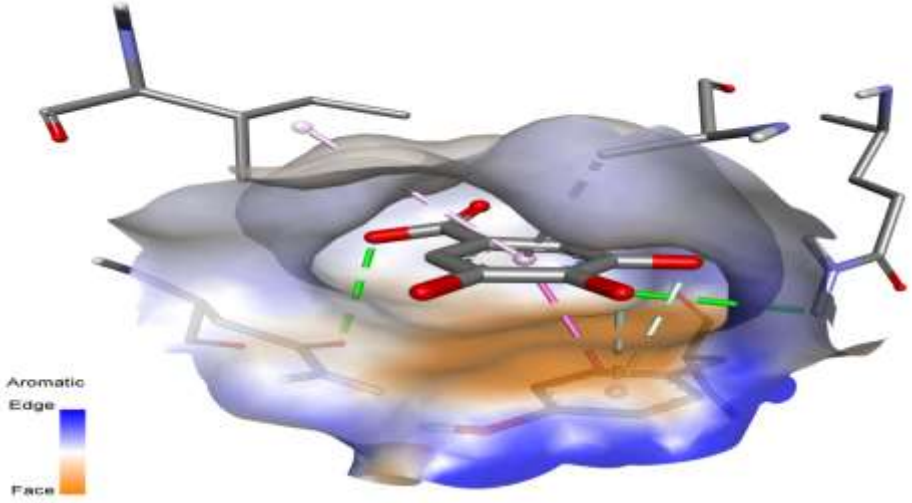


Figure 7: 1JFF protein aromaticity around the Gallic Acid at the active site

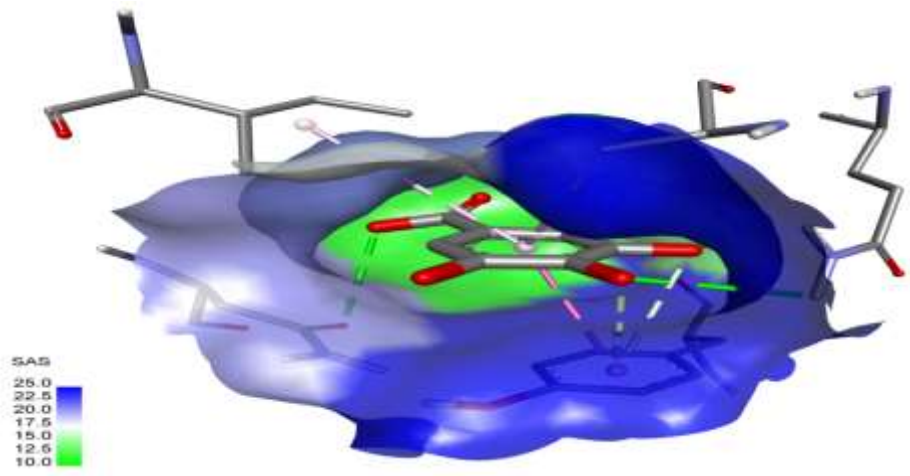


Figure 8: 1JFF protein SAS around the Gallic Acid at the active site

SAS= Solvent Accessible Surface

Among three compounds, Gallic acid showed binding affinity of -6.5 kcal/mol, as compared with reference drug Taxol binding affinity -9.9 kcal/mol for human Beta Tubulin (PDB: 1JFF).Gallic acid forms four conventional hydrogen bonds with amino acidsSer 236, Pro 360, Gly 370, Leu 371. One Carbon-hydrogen bond with Ala 233.

Quercetin

Table 2: **Protein Ligand Interactions Quercetin**

Compound	**Distance**	**Category**	**Type**	**From**	**From Chemistry**	**To**	**To Chemistry**
Quercetin	3.00901	Hydrogen Bond	Conventional Hydrogen Bond	THR276:HN	H-Donor	:UNK0:O	H-Acceptor
	3.1582	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	GLU27:OE2	H-Acceptor
	3.3464	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	ALA233:O	H-Acceptor

	3.39933	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	THR276:O	H-Acceptor
	4.746	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	PRO360	Alkyl

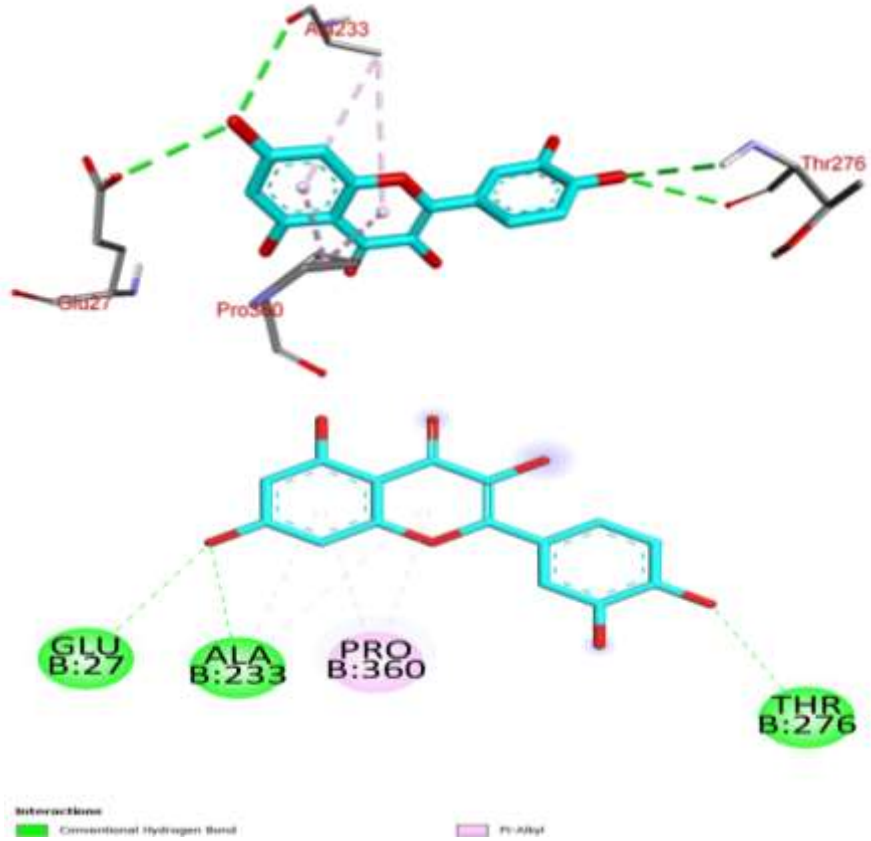


Fig : 9 **Protein Ligand Interactions Quercetin**

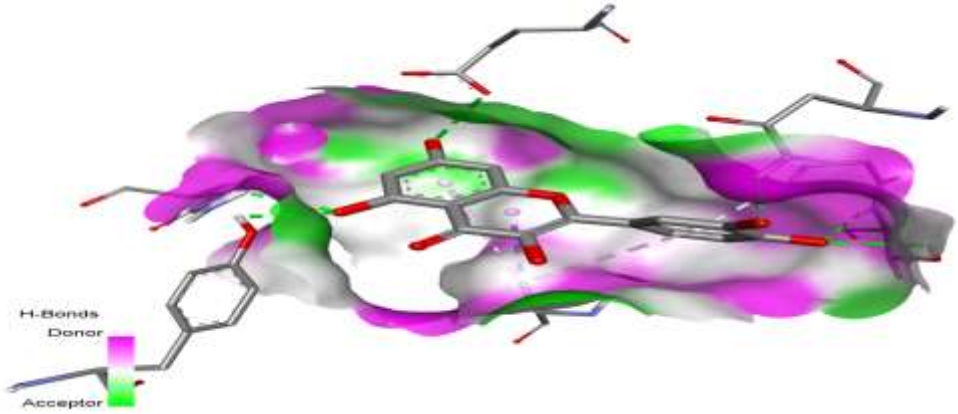


Figure 10 : 1JFF protein Hydrophilic region around the Quercetin at the active site

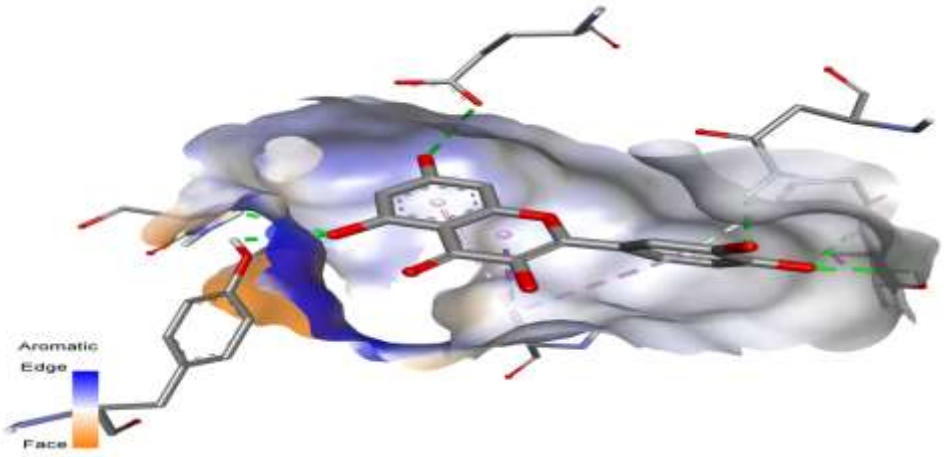


Figure 11: 1JFF protein aromaticity region around the Quercetin at the active site

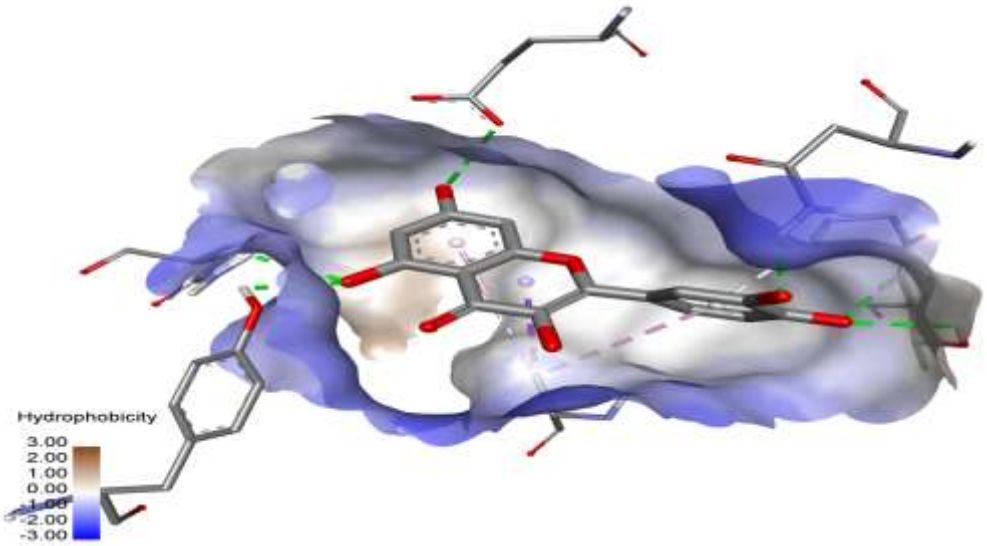


Figure 12: 1JFF protein Hydrophobic region around the Quercetin at the active site

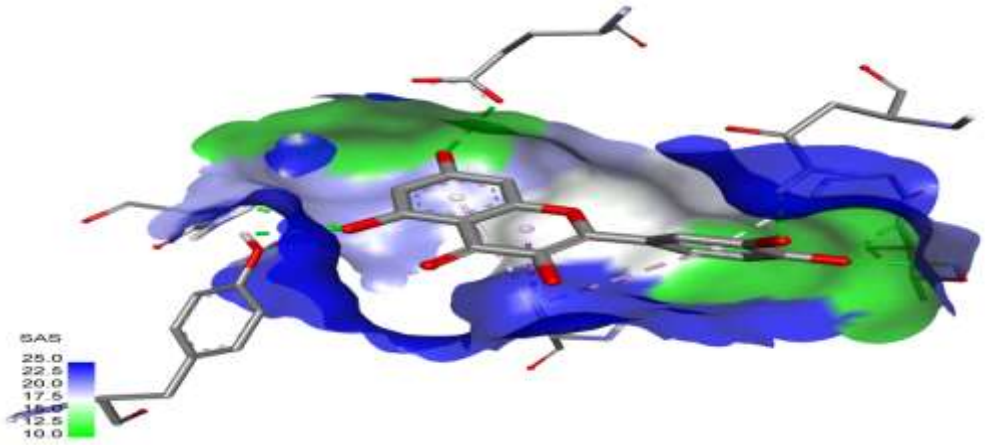


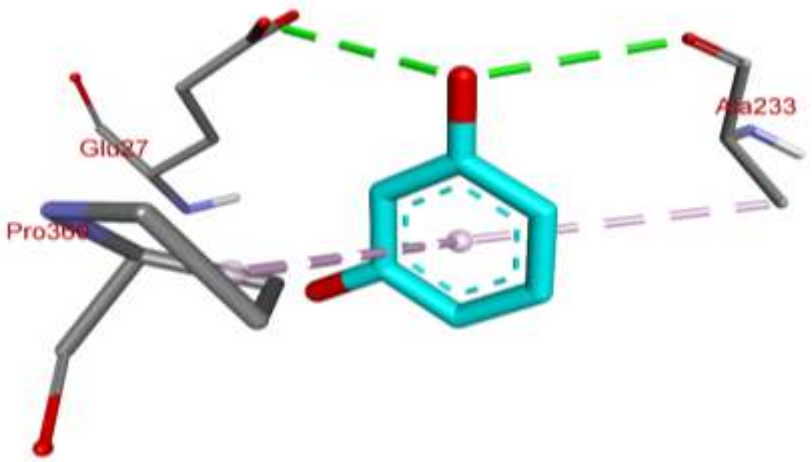
Figure 13 : 1JFF protein SAS around the Quercetinat the active site

Among three compounds, Quercetin showed binding affinity of -7.6 kcal/mol, as compared with reference drug Taxol binding affinity -9.9 kcal/mol for human Beta Tubulin (PDB: 1JFF). Gallic acid forms three conventional hydrogen bonds with amino acids Glu 27, Ala 233 and Thr 276. One Pi-Alkyl bond with Pro 360.

Resorcinol

Table 3: **Protein Ligand Interactions Resorcinol**

Compound	**Distance**	**Category**	**Type**	**From**	**From Chemistry**	**To**	**To Chemistry**
Resorcinol	3.32848	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	GLU27:OE2	H-Acceptor
	3.31564	Hydrogen Bond	Conventional Hydrogen Bond	:UNK0:O	H-Donor	ALA233:O	H-Acceptor
	4.87432	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	ALA233	Alkyl
	4.96068	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	PRO360	Alkyl



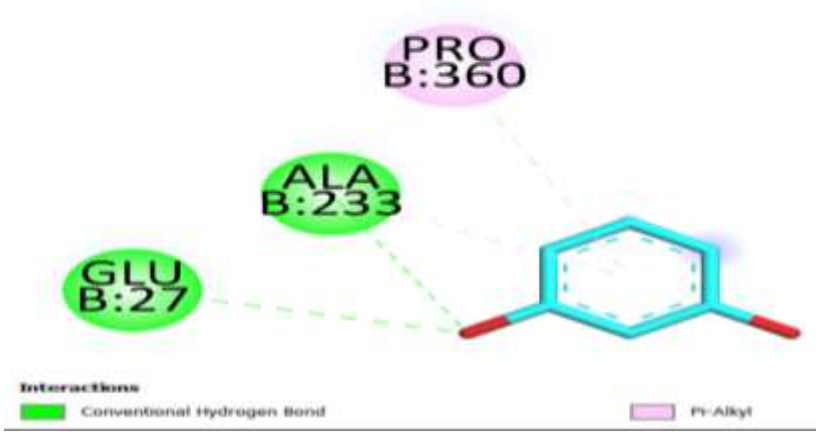


Fig :14 **Protein Ligand Interactions Resorcinol**

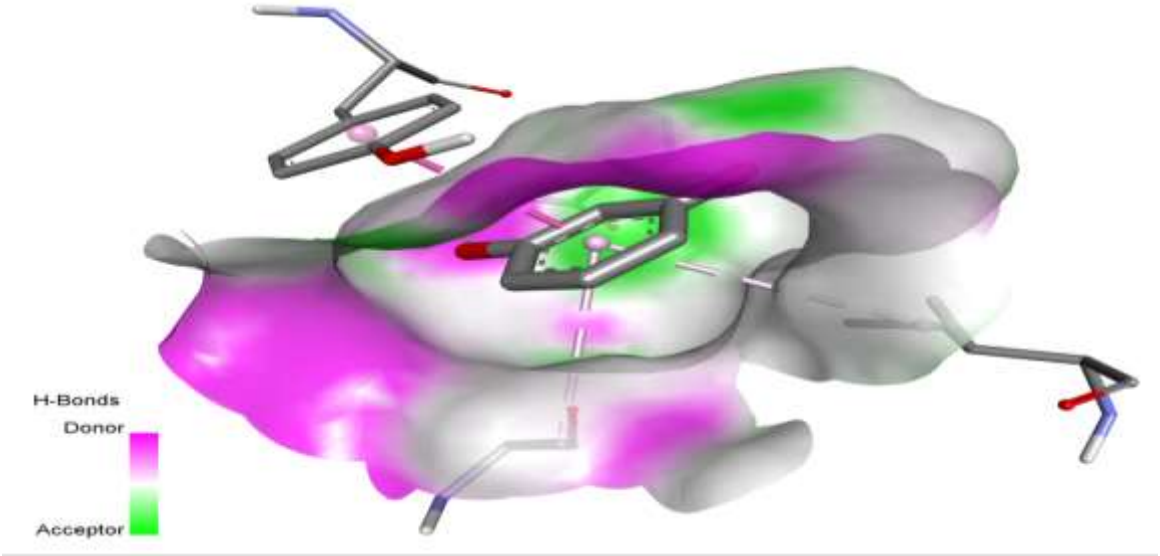


Figure 15 : 1JFF protein Hydrophilic region around the resorcinol at the active site

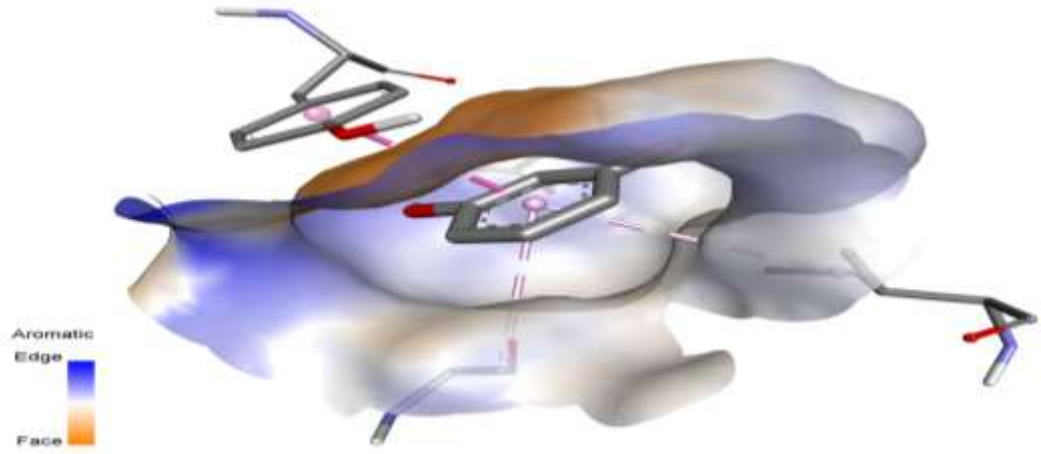


Figure 16: 1JFF protein aromaticity region around the resorcinol at the active site

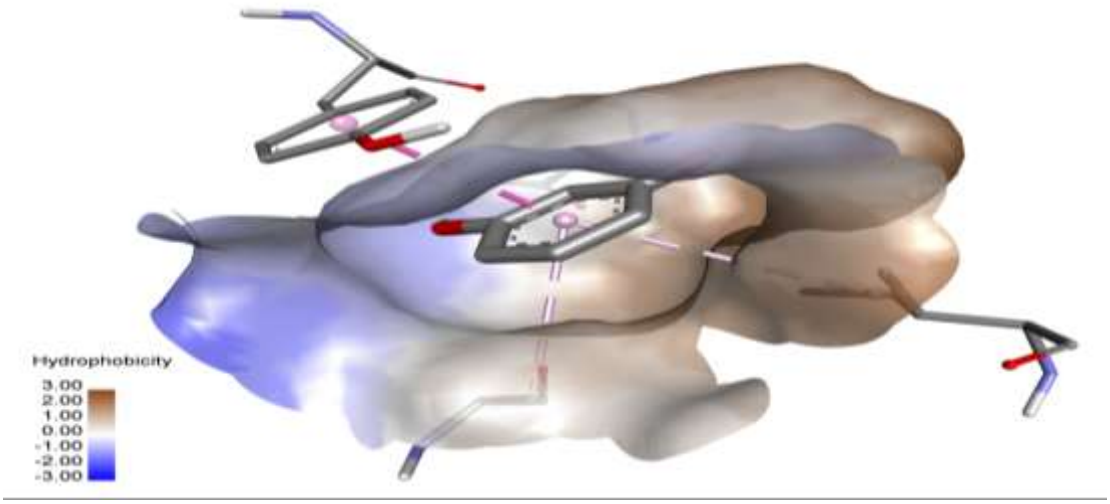


Figure 17: 1JFF protein Hydrophobic region around the resorcinol at the active site

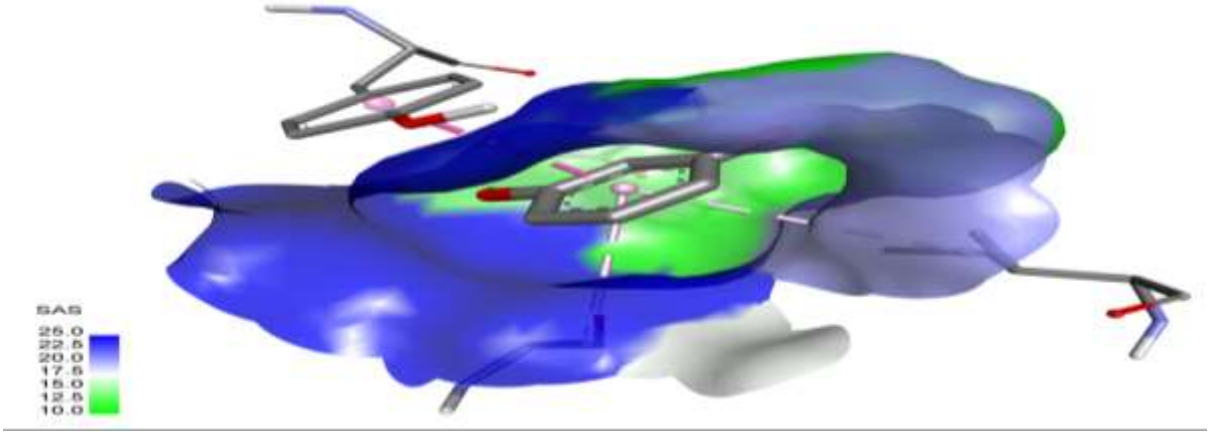


Figure 18: 1JFF protein SAS region around the resorcinol at the active site

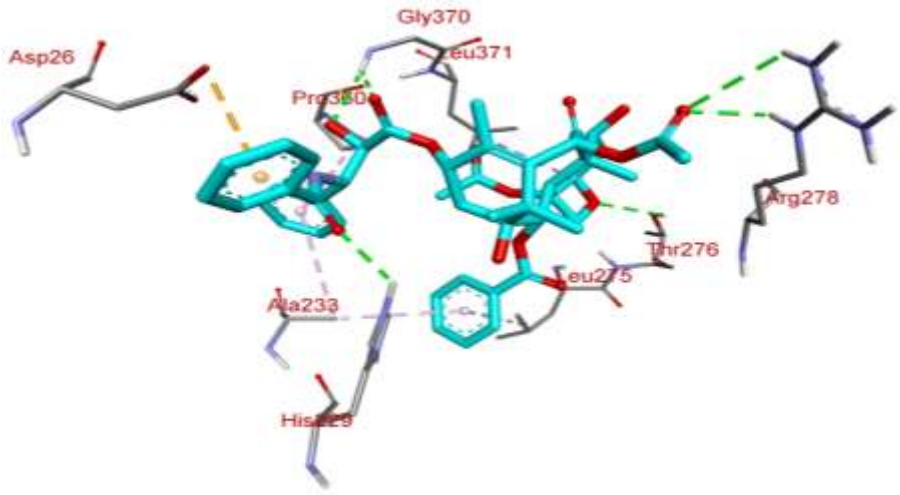
Among 3 compounds, Resorcinol showed binding affinity of -6.5 kcal/mol, as compared with reference drug Taxol binding affinity -9.9 kcal/mol for human Beta Tubulin (PDB: 1JFF). Resorcinol forms two conventional hydrogen bonds with amino acidsGlu 27, Ala 233. One Pi-Alkyl bond with Pro 360.

Taxol

Table 4: **Protein Ligand Interactions Taxol**

Compound	**Distance**	**Category**	**Type**	**From**	**From Chemistry**	**To**	**To Chemistry**
Taxol	2.64454	Hydrogen Bond	Conventional Hydrogen Bond	HIS229:HE2	H-Donor	:UNK0:O	H-Acceptor

	2.8691	Hydrogen Bond	Conventional Hydrogen Bond	THR276:HG1	H-Donor	:UNK0:O	H-Acceptor
	2.46389	Hydrogen Bond	Conventional Hydrogen Bond	ARG278:HE	H-Donor	:UNK0:O	H-Acceptor
	3.01934	Hydrogen Bond	Conventional Hydrogen Bond	ARG278:HH21	H-Donor	:UNK0:O	H-Acceptor
	2.35988	Hydrogen Bond	Conventional Hydrogen Bond	GLY370:HN	H-Donor	:UNK0:O	H-Acceptor
	2.36284	Hydrogen Bond	Conventional Hydrogen Bond	GLY370:HN	H-Donor	:UNK0:O	H-Acceptor
	3.63564	Electrostatic	Pi-Anion	ASP26:OD2	Negative	:UNK0	Pi-Orbitals
	4.80864	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	ALA233	Alkyl
	4.95297	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	PRO360	Alkyl
	4.52962	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	ALA233	Alkyl
	4.78964	Hydrophobic	Pi-Alkyl	:UNK0	Pi-Orbitals	LEU275	Alkyl



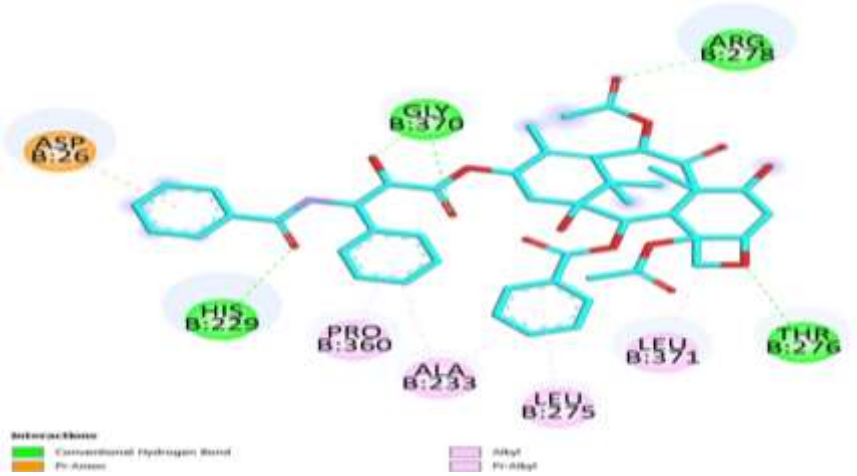


Fig :19 **Protein Ligand Interactions Taxol**

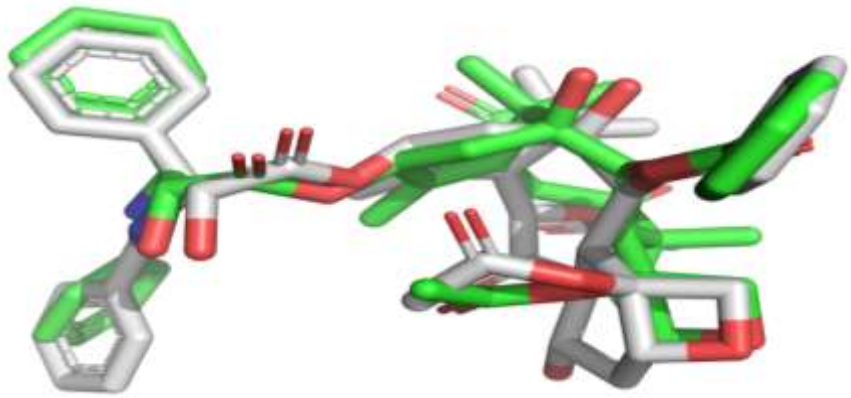


Fig:20 Re docked with Cocrystal pose

RMSD- 2.248

Reference drug Taxol showed binding affinity -9.9 kcal/mol for human Beta Tubulin (PDB: 1JFF). Taxol forms five conventional hydrogen bonds with amino acids His 229, Thr 276, Arg 278, Gly 370, Gly 370. Five Pi-Alkyl bonds with Ala 233, Ala 233, Leu 275, Pro 360, and Leu 371. One Pi-Anion bond with Asp 26.

Table :5 **Protein Ligand beta tubulin (** Beta Tubulin (PDB ID: 1JFF)

Compound	**Distance**	**Category**	**Type**	**From**	**From Chemistry**	**To**	**To Chemistry**
beta tubulin	2.4309	Hydrogen Bond	Conventional Hydrogen Bond	GLY370:HN	H-Donor	UNK0:O	H-Acceptor
	2.51542	Hydrogen Bond	Conventional Hydrogen Bond	GLY370:HN	H-Donor	:UNK0:O	H-Acceptor
	2.73067	Hydrogen Bond	Conventional Hydrogen Bond	LEU371:HN	H-Donor	:UNK0:O	H-Acceptor
	3.25298	Hydrogen Bond	Carbon Hydrogen Bond	HIS229:CE1	H-Donor	:UNK0:O	H-Acceptor
	3.42285	Hydrogen Bond	Carbon Hydrogen Bond	HIS229:CE1	H-Donor	:UNK0:O	H-Acceptor
	2.95578	Hydrogen Bond	Pi-Donor Hydrogen	ARG278:HN	H-Donor	:UNK0	Pi-Orbitals

			Bond				
	4.58477	Hydrophobic	Amide-Pi Stacked	SER277:C,O; ARG278:N	H-Donor	:UNK0	Pi-Orbitals
	4.64197	Hydrophobic	Pi-Alkyl	:UNK0	H-Donor	LEU219	Alkyl
	4.71746	Hydrophobic	Pi-Alkyl	:UNK0	H-Donor	ALA233	Alkyl

Table :6 Molecular Docking Results

S. No	Compound	Binding Energy (Kcal/Mol)
1	Gallic acid.pdbqt	-6.5
2	Quercetin.pdbqt	-7.6
3	Resorcinol.pdbqt	-5.1
Standard	Taxol.pdbqt	-9.9
	Co-Crystal	-9.7

CONCLUSION

The present study was concluded that the methanolic leaves extract Cinnamomum malabatrum having the potential compounds which are flavonoids and polyphenols

Flavonoids and polyphenols were characterized by the LCMS/MS method to know the compounds which are Gallic acid, Trans – Aconitic acid, Chlorogenic acid, Resorcinol, Rhanmetin, and Quercetin, these compounds were having anti cancer activity it was proved by the MTT assay method, and also we were subjected to molecular docking study for molecular level interaction (Pharmacodynamics) in the cell, we find all these compounds were interacted with beta tubulin protein which is present in the cytoplasm and inhibit the spindle apparatus functioning during the cell division process especially in metaphase configuration, hence these plant extract was confirmed as cell cycle specific anti cancer activity.

REFERENCES

1. N. Savithramma, M. Ling Rao and S. Ankanna: Preliminary Phytochemical Screening of Some Important Medicinal Plants. Middle-East Journal of Scientific Research 2012; 2-1:139-145.
2. N. Md. Azmathullah, M. Asrar Sheriff and A.K. Sultan Maiden: Phytochemical Screening of Calotropis procera Flower Extracts and their Bio-Control Potential on Culex sp. Mosquito Larvae and Pupae. International Journal of Pharmaceutical & Biological Archives 2011; 26:1718-1721.
3. Solanki R.: Some medicinal plants with antibacterial activity. International Journal of Comprehensive Pharmacy 2010 1(4):1-4.

4. Dahiru, D, William, E.T. and Nadroi M.S: Protective effects of Ziziphus mauritiana leaf extract on carbon tetrachloride-induced liver injury. African Journal Biotechnology 2005; 4(10):1177-1179
5. Majumdar, S.H, Chakraborthy G.S, Kulkarni K.S: Medicinal Potentials of Semecarpus anacardium Nut- A review. Journal of Herbal Medicine & Toxicology 2008; 2(2):9-13.
6. Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi and Mohammadreza Verdian-rizi: Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai Journal of Pharmaceutical Sciences 2008; 32:17-20.
7. Mohamad Asif, S.H.Ansari, Md.Rafiul Haque and Nazia Kalam: Estimation of Phenolic, Flavonoid contents and Antioxidant activity in nut galls of Quercus infectoria Olivier. Journal of Pharmacy Research 2012; 5(7):3855-3857.
8. Rabe T and Van Staden J: Isolation of an Antibacterial Sesquiterpenoid from Warburgia salutary. Journal of Ethanopharmocology 1997; 73:171-174.
9. P.B.Mallikharjuna, L.N.Rajanna, Y.N. Seetharam and G.K.Sharanabasappa: Phytochemical Studies of Strychnos potatorum L.f. A Medicinal Plant. Journal of Chemistry 2007; 24:510-518.
10. Silverstein RM, Webster FX, Kiemle DJ: Proton NMR spectrometry and Spectrometric identification of organic compounds. John Wiley and Sons, Seventh edition 2005, 127-204.
11. 1Nagesh Simhadri VSDNA,1, Muthuchamy Muniappan,2 Iyanar Kannan,3 and Subramanyam Viswanathan, Phytochemical analysis and docking study of compounds present in a polyherbal preparation used in the treatment of dermatophytosis, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6101157/
12. Burhan Durhan, Emine Yalçın, Kültiğin Çavuşoğlu, Molecular docking assisted biological functions and phytochemical screening of Amaranthus lividus L. extract, Scientific Reports volume 12, Article number: 4308 (2022
13. Kero Jemal, Molecular Docking Studies of Phytochemicals of Allophylus serratus Against Cyclooxygenase-2 Enzyme, BioRxiv journal December 5, 2019