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GC-MS, Phytochemical Analysis and In Silico Approaches of a Medicinal Plant *Acalypha indica*

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Abstract

The goal of this study is to look into the preliminary phytochemical and GC-MS analyses of a methanol extract of the leaves of *Acalypha indica*. Alkaloids, tannins, steroids, saponins, flavonoids, glycosides, and phenolic substances are found in leaf extract after phytochemical analysis. *A. indica* is a plant that grows in the southern Indian state of Tamil Nadu. It has medicinal characteristics and is used as a diuretic, anthelmintic, and respiratory disorders like bronchitis, asthma, and pneumonia. The present work is designed to investigate GC-MS. Thirty compounds are found in the leaf extract. The structure-based biological activities study supported the Pass online database findings from the current study support that the use of this plant compound responsible for the anticancer activity is based on structure-based drug development. Based on in-silico results, it was shown that the interactions of hydrogen bonds, binding affinity with JAK2 kinase inhibitors, tumor suppressor proteins pRB Retinoblastoma protein, and COX-2 target proteins were compared with known values. It can be considered an increase in the possibility of conniving potential anticancer drugs as an anti-inflammatory agent.

Keywords: *Acalypha indica*; Leaves; GC-MS; JAK2; Retinoblastoma protein; Anticancer activity.

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1. Introduction

Acalypha indica is a common annual herb found in the backyards of homes and squatter areas all over India's plains. Plants are used to treat bronchitis, pneumonia, asthma, and pulmonary tuberculosis as emetic, expectorant, purgative, and diuretic [1,2]. The herb is used in homeopathy to treat severe hacking symptoms such as lungs death, hemoptysis, and nascent phthisis. In recent years, auxiliary plant metabolites have been actively studied as a source of restorative operators. Currently, distinctive components derived from plants are being examined for their compatibility with contemporary pharmacological activity modes. Traditional medicinal approaches, particularly medicinal plants, continue to be important in providing the basic health needs of poor countries [3-12].

Acalypha indica has been shown to have post-coital antifertility effects, also known as 'Indian copperleaf' or 'Indian mercury,' a medicinal plant belonging to the Euphorbiaceae family. In folk medicine, the whole plant and leaves of *A. indica* have been used to treat skin injuries and anti-venom properties [13,14], Leaves of *A. indica* have been scientifically tested for their anti-bacterial [15], anti-inflammatory [16], and antioxidant [17] properties. Few studies isolated and identified the phytochemical constituents from the leaf extracts of *A. indica* [14,17-19]. However, photo components have been investigated using GC–MS. In this study, the methanolic extract of *Acalypha indica* was used for GC-MS analysis, and in silico atomic docking, Prediction of Action Spectra for Substances (PASS) examination of *Acalypha indica* so far. This inquiry aimed to survey the interaction of this distinguished phytochemical 2-methoxy-4-vinyl phenol with target proteins such as JAK2 kinase inhibitors, tumor silencer proteins pRB Retinoblastoma protein, and COX-2 target proteins to discover novel data for the improvement of successful anticancer drugs anti-inflammatory inhibitors.

2. Experimental

2.1. *Collection and processing of plant material*

The leaves were collected from the natural habitats of Cheyyar, Tamil Nadu, India. The plant *Acalypha indica*, Common name: Indian Copperleaf (Tamil: குப்பைமேனி) (family: Euphorbiaceae). The leaves were cleaned with water and distilled water thrice before being dried in the shade. A mechanical grinder was used to pulverize the leaves. For further investigation, the pulverized leaf was used.

2.2. *Sample extraction and phytochemical screening*

The 50 g of air-dried leaf powder was extracted extensively with 250 mL of methanol at a temperature below the boiling point using a soxhlet extractor. The methanol extract from the Soxhlet extractor was filtered before evaporating with a Rota vaporizer. Phytochemical testing was done on the pasty form extracts that resulted. The chemical

components of this methanol extract were identified by GC-MS analysis. Using the conventional procedure, the extracts were subjected to preliminary phytochemical screening to determine the presence of several chemical groups or compounds such as glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids, and phenolic compounds [20-22].

2.3. *GC-MS analysis-identification of compounds*

GC-MS analysis of the seed extracts was performed using a Perkin-Elmer GC-Clarus 680 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite 5MS (5 % diphenyl/95 % dimethyl polysiloxane) fused capillary column (30.0 m, 0.25 mm ID, 250 μm df) at National College instrumentation facility (NCIF), Tiruchirapalli, Tamil Nadu. An electron ionization system was operated in electron impact mode with ionization energy of 70 eV for GC-MS detection. Helium gas (99.99 %) was used as a carrier gas at a constant flow rate of 1ml/min, and an injection volume of 1 μL was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C; the ion source temperature was 240 °C, the oven temperature was programmed as 60 °C (isothermal for 2 min), with an increase of 10 °C/min to 300 °C, ending with a 6 min isothermal at 280 °C. Mass spectra were taken at 70 eV, a scanning interval of 0.5 s, and 45 to 650 Da fragments. The solvent delay was 0 to 2 min, and the total GC/MS running time was 30 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.4.2. `Mass spectrogram of GC-MS was analyzed using the databases of about 1.75 lakhs of compounds. The spectrum of the unknown compounds was compared with the spectrum of the known compounds stored in the NIST-2015 libraries [12]. The name, molecular weight, and structure of the compounds of the tested materials were determined.

2.4. *In silico studies*

To study molecular docking, the commercial program Schrödinger was used [23]. Molecular docking studies are used to determine the bond between two molecules and the optimal direction of the ligand for structuring the complex with the least amount of energy. When a ligand atom is docked with a dynamic site of macromolecular targets, conformational changes can occur in the region of the dynamic site containing corrosive deposits of the amino group. Adaptability must be given not only to the ligand but also to the dynamic site building in order to find the most suitable dynamic site. On the other hand, a dynamic site is considered inflexible, and adaptability is provided solely to the ligand. According to these principles, filtration can be performed on a limited number of ligands if the information is associated with multiple ligands. The location of the dynamic

site will now provide adaptability after selecting a small number of ligands using rigid docking. This is known as induced docking (IFD).

Molecular mechanics force fields are used to assess the binding affinity of the docked receptor and ligand. Free binding energy increases in different areas. This can be written as

$$\text{“}\Delta G_{bind} = \Delta G_{solvent} + \Delta G_{conf} + \Delta_{Gint} + \Delta_{Grot} + \Delta_{Gtor} + \Delta G_{vib}\text{ ”}$$

Among the components are soluble effects, conformational changes in the protein and ligand, free energy due to protein-ligand interaction, internal rotations, and the energy of association of the ligand and receptor with the formation of a single complex as free viability due to shifts in the vibrational regime. The best compliance was determined depending on the docking rating and sliding energy. The docking score is determined by the sum of energies such as lipophilicity, hydrogen bonding, metal association, rotational bond tests, and rescue tests. The free binding energy, determined relative to the OPLS-AA force field, is slip energy. The best connection can be selected depending on the lowest sliding energy/docking assessment/both options [24-26].

2.4.1. *Target selection, preparation/ligand preparation*

Crystal structures of target receptors restricting localities of retinoblastoma protein (pRB) [PDB ID: 4CRI] [27], Janus kinase inhibitors (JAK2) [PDB ID: 5USY] [28] and cyclooxygenase (COX-2) [PDB ID: 5IKR] [29], the RCSB protein databank (http://www.rcsb.org/pdb/home/home.do) was used to obtain three types of the target protein [30]. Using the main module, proteins were constructed by eliminating water molecules, stabilizing charges, and correcting missing residues and side chains. The isolated chemical 2M4VP was identified by GC-MS and was obtained from the PubChem database of the National Centre for Biotechnology Information [31]. They have anti-inflammatory and anticancer properties. During the energy minimization process, the structure of the compound was minimized after integrating hydrogen atoms and changing bond placement using the steepest descent method with 1000 cycles and a conjugate gradient approach with 5000 cycles. Analysis of molecular docking results with graphic images was carried out using the visualization program/server: Pymol, PDB sum [32,33].

3. Results and Discussion

3.1. *Preliminary phytochemical analysis*

Qualitative preliminary screenings of extracts have been carried out at the beginning with exceptional chemical reagents to observe the phytochemical ingredients existing in methanolic extract. The extract indicates the presence of alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, and phenolic compounds

3.2. GC–MS studies

The methanolic extract of the leaf of *Acalypha indica* used to be subjected to GC–MS studies. Many plant phytochemical elements in the plant methanolic extract are listed in Table 1. Interpretation on mass spectrum GC-MS used to be carried out using the database of (NIST) [12]. Name, molecular weight, and shape of the aspects of substances have been ascertained in Table 1. Methanolic extract of *Acalypha indica* used to be subjected to GC-MS find out about for identification of medicinal properties and pass online biological activities are listed Table 1. According to the results, most of the medicinal properties of Phytocomponents are screened, Benzoic acid, Phytol, E-2-Hexenyl benzoate, 2-Methoxy-4-vinylphenol, beta-curcumin totally thirty compounds are observed in *Acalypha indica* leaf extracts.

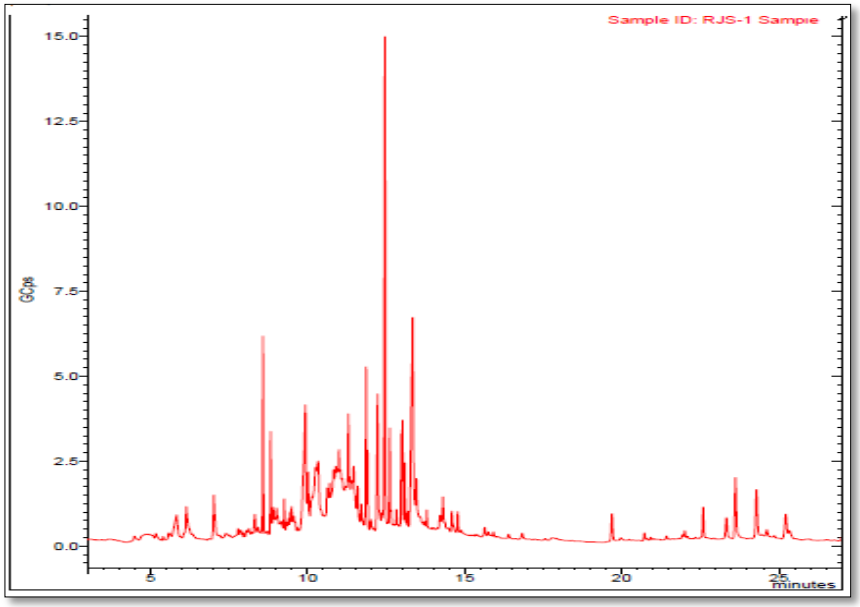

Fig. 1. The GC-MS Chromatogram spectrum of methanol leaf extract of *Acalypha indica*.

Table 1. GC-MS analysis revealed the presence of phytochemical components in methanol leaf extract of *Acalypha indica* and their Pass online biological activities.

SL. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Pass online activity [34]
1.	5.733	Benzoic acid	$C_7H_6O_2$	122	1.277	Testosterone 17beta-dehydrogenase (NADP+) inhibitor, Sugar-phosphatase inhibitor, Glutamyl endopeptidase II inhibitor, Prolyl aminopeptidase inhibitor
2.	6.102	Acetamide,N-(3-methyl-4-phenyl-5-pyrazolyl)-	$C_{12}H_{13}N_3O$	215	1.649	Acrocylindropepsin inhibitor, Chymosin inhibitor, Saccharopepsin inhibitor,

						Acylcarnitine hydrolase inhibitor, Polyporopepsin inhibitor
3.	6.597	Pentadecanoic acid,14-methyl-,methyl ester	$C_{17}H_{34}O_2$	270	0.000	Acylcarnitine hydrolase inhibitor, Alkylacetylglycerophosphatase inhibitor, Alkenylglycerophosphocholine hydrolase inhibitor, CYP2J substrate
4.	6.972	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.296	Respiratory analeptic, Analeptic, Cardiotonic, Skeletal muscle relaxant
5.	7.734	7H-Furo[3,2-g][1]benzopyran-7-one,3,5-dimethyl-	$C_{13}H_{10}O_3$	214	0.924	Acrocylindropepsin inhibitor, Chymosin inhibitor, Saccharopepsin inhibitor
6.	8.157	Resibufogenin	$C_{24}H_{32}O_4$	384	1.431	Prenyl-diphosphatase inhibitor, Retinol dehydrogenase inhibitor, Ubiquinol-cytochrome-c reductase inhibitor
7.	8.509	Cyclopropaneoctanoic acid,2-[[2-[2-ethylcyclopropyl)methyl]cyclopropyl]methyl	$C_{22}H_{38}O_2$	334	3.137	CYP2J substrate, CYP2J2 substrate, Antieczematic, Linoleate diol synthase inhibitor, Lipid metabolism regulator
8.	8.792	Phytol	$C_{20}H_{40}O$	296	1.707	Acylcarnitine hydrolase inhibitor, Mucomembranous protector, Alkylacetylglycerophosphatase inhibitor, CYP2J substrate
9.	8.887	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	$C_{18}H_{30}O_2$	278	1.892	Phosphatidylcholine-retinol O-acyltransferase inhibitor, Sugar-phosphatase inhibitor, Acaricide
10.	9.114	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	1.314	Phobic disorders treatment, Membrane integrity agonist,
11.	9.473	2H-1-Benzopyran-2-one,7-methoxy-6-(3-methyl-2-oxobutyl)-	$C_{15}H_{16}O_4$	260	2.443	Eye irritation, inactive, Skin irritation, inactive, Cutinase inhibitor
12.	9.848	Benzofuran,2,3-dihydro-	C_8H_8O	120	6.448	Prenyl-diphosphatase inhibitor, Mucomembranous protector, BRAF expression inhibitor
13.	10.228	E-2-Hexenyl benzoate	$C_{13}H_{16}O_2$	204	7.376	Lipid peroxidase inhibitor, Peroxidase inhibitor, Antioxidant, TP53 expression enhancer, CYP2C12 substrate
14.	10.595	Benzamide,N-(1,3-dihydro-2-oxo-4-isobenzofuryl)-	$C_{15}H_{11}NO_3$	253	2.760	DELTA14-sterol reductase inhibitor, Prostaglandin-E2 9-reductase inhibitor,

						Antihypercholesterolemic
15.	10.890	1,2-Epoxycyclooct-3-ene,5,5-dimethyl-8-methylene-	$C_{11}H_{16}0$	164	9.836	Antihypercholesterolemic, DELTA14-sterol reductase inhibitor, Prostaglandin-E2 9-reductase inhibitor, CYP7 inhibitor
16.	11.226	4H-1-Benzopyran-4-one,2,3-dihydro-5,7-dihydroxy-2-phenyl-(s)-	$C_{15}H_{12}O_4$	256	5.195	Aspulvinone dimethylallyltransferase inhibitor, Feruloyl esterase inhibitor, Vanillyl-alcohol oxidase inhibitor
17.	11.424	BIS(2-Ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	5.403	Caspase 3 stimulant, Transcription factor NF kappa B stimulant, Transcription factor stimulant, Antineoplastic
18.	11.810	Squalene	$C_{30}H_5O$	410	4.539	Mucomembranous protector, Prenyl-diphosphatase inhibitor, Undecaprenyl-phosphate mannosyltransferase inhibitor
19.	12.043	dl-alpha-Tocopherol	$C_{29}H_{50}O_2$	430	2.658	Antieczematic, Glutamate-5-semialdehyde dehydrogenase inhibitor, Ubiquinol-cytochrome-c reductase inhibitor
20.	12.401	Campesterol	$C_{28}H_{48}O$	400	9.418	Aspulvinone dimethylallyltransferase inhibitor, Feruloyl esterase inhibitor, Membrane integrity agonist
21.	12.571	2-[4-methyl-6-(2,6,6-trimethyl-cyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-	$C_{23}H_{32}O$	324	2.593	Prenyl-diphosphatase inhibitor, Retinol dehydrogenase inhibitor, Ubiquinol-cytochrome-c reductase inhibitor
22.	12.922	gamma-Sitosterol	$C_{29}H_{50}O$	414	4.035	Antihypercholesterolemic, DELTA14-sterol reductase inhibitor, Prostaglandin-E2 9-reductase inhibitor, Cholesterol antagonist, CYP7 inhibitor,Alkenylglycerophosphocholine hydrolase inhibitor, Alkylacetylglycerophosphatase inhibitor
23.	**13.271**	**2-Methoxy-4-vinylphenol**	**$C_9H_{10}O_2$**	**150**	**10.307**	**Aspulvinone dimethylallyltransferase inhibitor, Feruloyl esterase inhibitor, Vanillyl-alcohol oxidase inhibitor, JAK2 expression**

						inhibitor, Gluconate 2-dehydrogenase (acceptor) inhibitor
24.	13.605	Lupeol	$C_{30}H_{50}O$	426	1.803	Caspase 3 stimulant, Transcription factor NF kappa B stimulant, Transcription factor stimulant
25.	14.213	1,6,10-Dodeca-triene,7,11-dimethyl-3-methylene	$C_{15}H_{24}$	204	1.961	All-trans-retinyl-palmitate hydrolase inhibitor, CYP2J substrate
26.	14.569	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	$C_{15}H_{22}$	202	0.751	Membrane integrity agonist, Aspulvinone dimethylallyltransferase inhibitor
27.	14.733	beta-curcumene	$C_{15}H_{24}$	204	0.834	Antieczematic, Glutamate-5-semialdehyde dehydrogenase inhibitor, Ubiquinol-cytochrome-c reductase inhibitor
28.	19.631	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180	0.539	Anti-inflammatory, Aspulvinone dimethylallyltransferase inhibitor
29.	22.536	2Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one	$C_{10}H_{10}O_3$	184	0.568	Mucomembranous protector , Sugar-phosphatase inhibitor

3.3. *Binding mode analysis by molecular docking*

Molecular docking efficiency was achieved using Glide to study the careful intermolecular contact between the ligand and the tumor suppressor target proteins. An induced docking simulation was performed to perceive the approach of ligand binding to the tumor suppressor protein retinoblastoma (pRB), JAK2 kinase inhibitors, and cyclooxygenase (COX-2), among others, to find information for advanced structural development, the docking results are shown in Table 2.

3.3.1. *Target- I: Retinoblastoma protein (pRB)*

The molecular docking study showed that the compound 2-Methoxy-4-vinylphenol (2M4VP) was deeply embedded in the active site of the retinoblastoma protein (pRB), two interactions with hydrogen bonds and hydrophobic interactions, reflecting the greater stability, as well as the affinity of the studied molecule for binding through the receptor. The hydrogen bond developed between the amino acids PRO-808 and MLY-810 of the pRB protein with the ligand is 2.8 and 3.05 Å, the slip index is -5.79, and the slip energy is -21.486 kcal/mol in the active site. The residues of hydrophobic interactions were 4 Å

long SER-807, LEU-809, ILE-804, and TYR-805. The compound designated as a docked IFD ligand forms a strong complex through the tumor suppressor protein retinoblastoma, as shown in Fig. 2A. shows the interaction of hydrogen bonds and hydrophobic bonds (Table 2) in the active site. Interaction of co-crystals Fig. 2B.

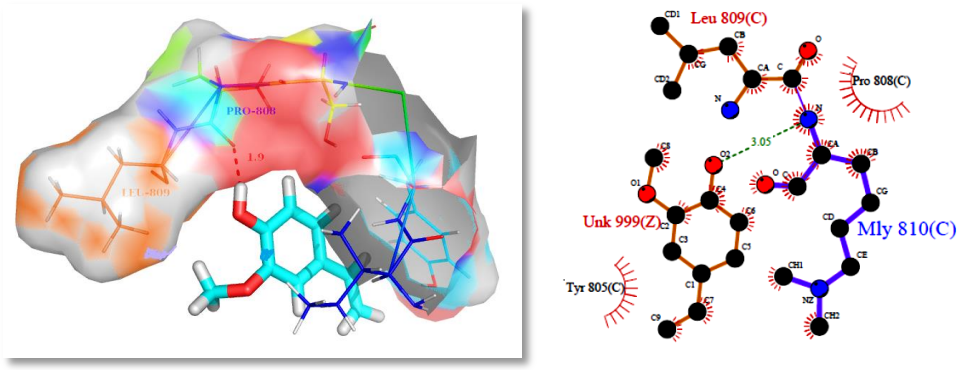

Fig. 2A. Validation of molecular docking: Pymol 3D diagram and LigPlot diagram above represent the interaction between the Retinoblastoma (pRB), active site residues, and the ligand 2M4VP.

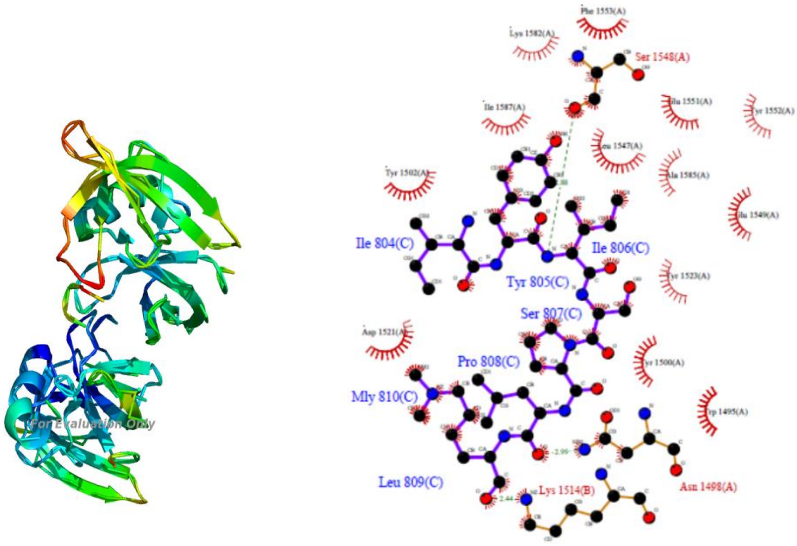

Fig. 2B. Validation of molecular structure: The Pymol 3D diagram and LigPlot diagram above represent the interaction between the Retinoblastoma (pRB), active site residues, and the co-crystal interaction.

3.3.2. *Target- II: Janus kinase inhibitors (JAK2)*

The result of the docking of Janus kinase inhibitors with the 2M4VP molecule consumes an excellent binding affinity with the target protein JAK2 due to the given frequency of two interactions of hydrogen bonds between the ligands. The active site of the LEU-932

amino acids and the GLU-930 protein with the ligand are 3.1 and 3.0 Å. (Fig. 2b) with a slip estimate of -8.989 and slip energy -48.093 kcal/mol. The upper interaction of the ligand with the JAK2 protein describes the reputation of the hydroxyl group in the chemical structure about the binding affinity to the center and residues of the hydrophobic region: VAL-911, LEU-902, LEU-983, ALA-880, MET-929, TYR-931, LEU- 855, TYR-934, MET-865, PRO-933, VAL-863, VAL-984 of the JAK2 protein kinase region. An additional negative Glide value indicates a better ligand interaction with the target protein associated with the co-crystal value shown in Table 2.

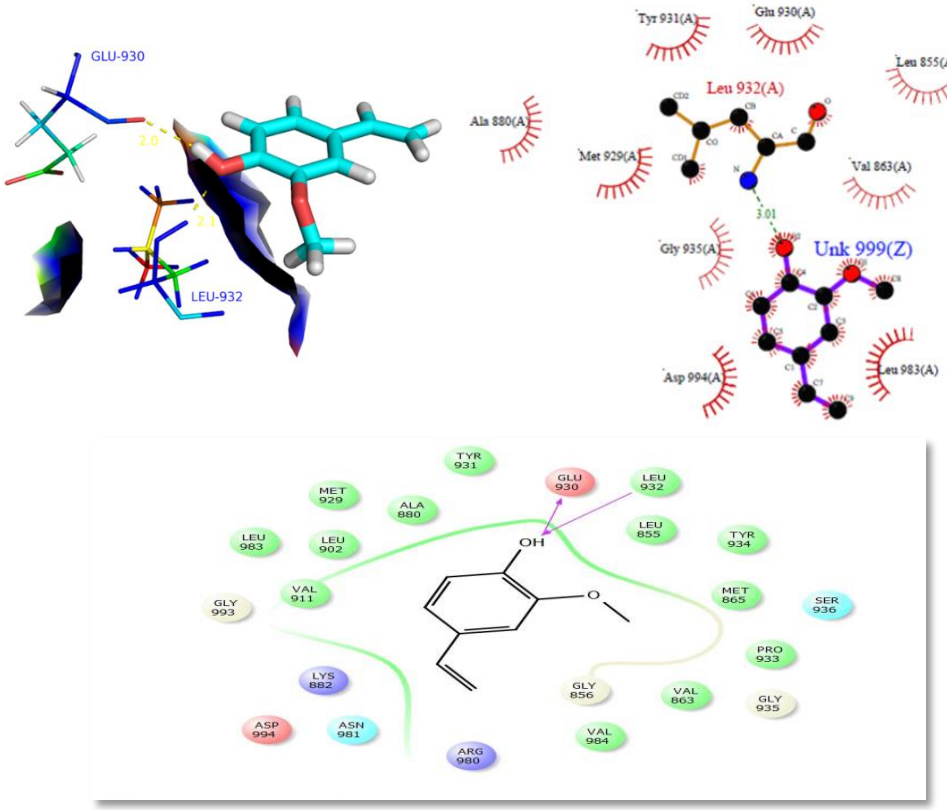

Fig. 3. Validation of molecular docking: Pymol 3D diagram, 2D diagram, and LigPlot diagram above represent the interaction between the Janus kinase inhibitors (JAK2) - (PDB ID: 5USY) active site residues and the ligand 2M4VP.

3.3.3. *Target- III: Cyclooxygenase (COX-2)*

The structure of the co-crystal line ligand is in the complex of cyclooxygenase (COX-2) with (PDB ID: 5IKR) in the active center. The docked complex (2M4VP) had a docking rating of -7.518 and slipped energy of -26.195 kcal/mol. The docked complex had two interactions at the hydrogen bond with the remainder of the active site TYR-385, SER-530, and TRP-387 Pi-Pi by stacking interactions at a distance of 2.88 Å and 2.89 Å. The

IFD result and (Fig. 4) show the interactions of hydrogen bonds in the active site. The interaction of the ligand upstream through the COX-2 protein describes the reputation of the hydroxyl group in the chemical structure concerning the attraction of binding to the hinge and hydrophobic residues of the region: PHE-518, MET-522, VAL-523, TRP-387, LEU-518, LEU-352, PHE-381, GLY-526 cyclooxygenase protein. An additional negative Glide value indicates a good interaction of the ligand with the target protein compared to the co-crystal value shown in Table 2.

Among the well-matched conformations of the title molecule, in-depth intermolecular interaction between the ligand and target protein of retinoblastoma (pRB), Janus kinase inhibitors (JAK2), and cyclooxygenase proteins (COX-2) are studied. An exciting development is the type of protein-ligand interaction that finds application in tumor suppressive medicine.

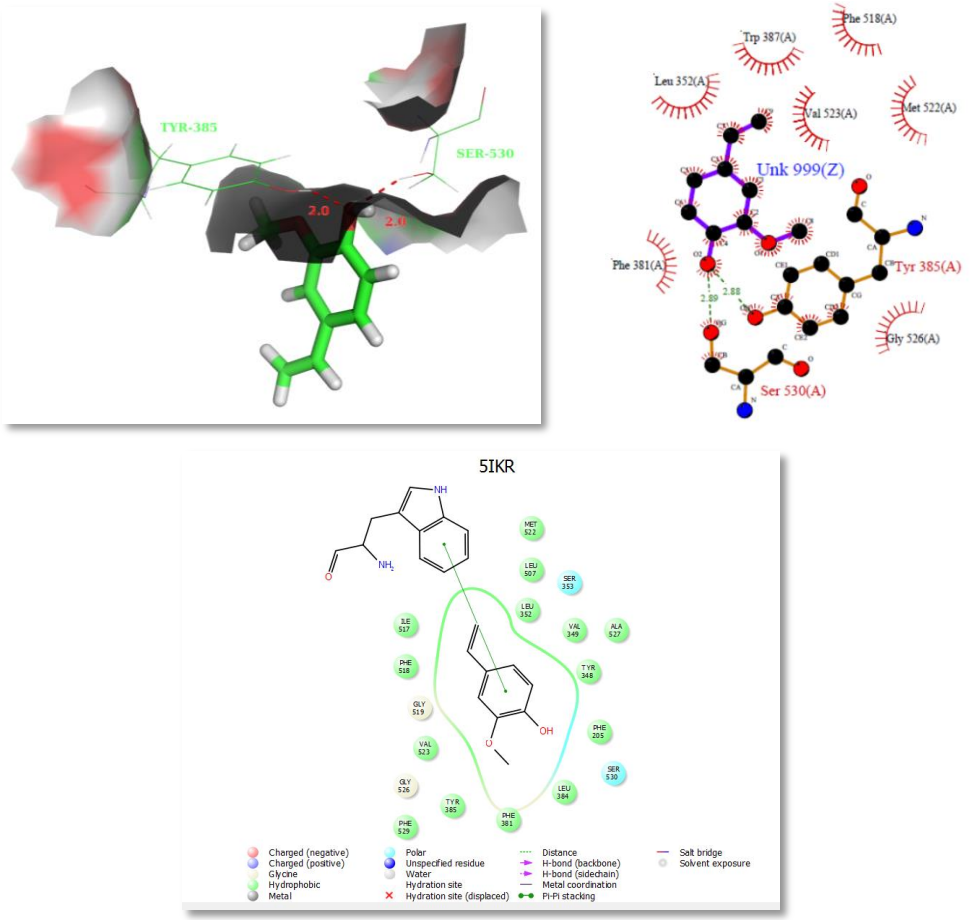

Fig. 4. Validation of molecular docking: Pymol 3D diagram, 2D diagram, and LigPlot diagram above represent the interaction between the cyclooxygenase (COX-2) - (PDB ID: 5IKR) active site residues and the ligand 2M4VP.

Table. 2. Molecular docking studies results of the compound 2M4VP against Tumor suppressor protein Retinoblastoma (pRB), Janus kinase inhibitors (JAK2), and Cyclooxygenase (COX-2).

Targets (PDB ID)	Comps.	Hydrogen Bond (D-H…A)	Distance (Å)	Hydrophobic interactions	Docking Score	Glide Energy (kcal/mol)
pRB PDB ID: 4CRI	2M4VP	MLY-810 (N-H.....O) (O-H....O) PRO-808	3.05 2.8	SER-807 LEU-809, ILE-804, TYR-805	-5.799	-21.486
JAK2 PDB ID: 5USY	Co-crystal-*SKE	LEU-932 (N-H.....N) LEU-932 (N-H.....O) (N-H....O) GLU-930 (N-H.....O) LEU-855 (N-H.....O) LYS-943	3.09 2.78 2.89 3.14 2.87	VAL-911,LEU-902, LEU-983, ALA-880, MET-929, TYR-931, LEU-855, TYR-934, MET-865, PRO-933, VAL-863, VAL-984	-6.732	-35.148
	2M4VP	LEU-932 (N-H.....O) (O-H....O) GLU-930	3.0 3.1	VAL-911,LEU-902, LEU-983, ALA-880, MET-929, TYR-931, LEU-855, TYR-934, MET-865, PRO-933, VAL-863, VAL-984	-8.989	48.093
COX-2 PDB ID: 5IKR	*ID-8	(O-H...O) SER-530 (O-H...O) TYR-385	2.77 2.82	VAL-349, LEU-531, TRP-387, LEU-352, TYP-387, ALA-527, GLY-526, MET-522, VAL-523	-6.930	-28.122
	2M4VP	(O-H...O) SER-530 (O-H...O) TYR-385 TRP-387	2.89 2.88 Pi-Pi stacking	PHE-518, MET-522, VAL-523, TRP-387, LEU-518, LEU-352, PHE-381, GLY-526	-7.518	-26.195

***** SKE - 4-({5-Amino-1-[(2,6-Difluorophenyl)carbonyl]-1h-1,2,4- Triazol-3-Yl}amino) benzenesulfonamide
*ID8 - 2-[(2,3-Dimethylphenyl)amino]benzoic acid [Mefenamic acid]

4. Conclusion

In summary, the GC-MS evaluation of the *Acalypha indica* leaf methanolic extract plant is an awesome supply of phytoconstituents, particularly 2M4VP with a magnificent binding appeal to tumor suppressor proteins (pRB, JAK-2, and COX-2) in docking studies and the PASS evaluation approach of phytoconstituents. This suggested many sizeable things for the phytochemicals studied. The molecular docking results showed that 2M4VP exhibits significant binding affinity for tumor suppressor proteins through hydrogen bonding and hydrophobic interactions. In the future, 2M4VP will be used by many researchers as an anticancer and tumor suppressor drug.

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