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Anticancer Insights Of Phyla Nodiflora Against Cyclooxygenase-2 Through Conformational Molecular Dynamic Simulations

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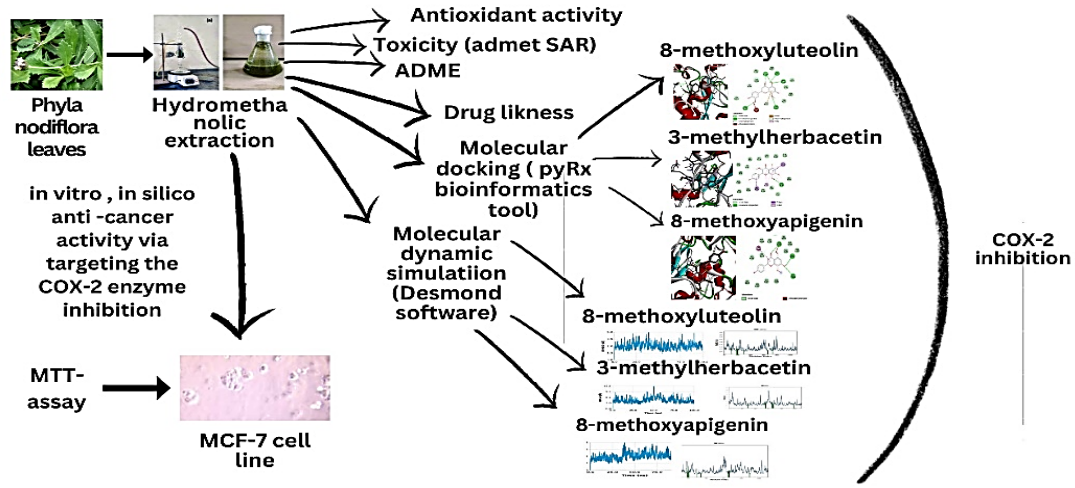
Abstract: *Cancer is one of the most fatal diseases throughout the world. Different types of approaches are used for the treatment of cancer and one of them is using medicinal plants to reduce side effects. For the treatment of cancer, there are many proteins and cytokines to be targeted like the cyclooxygenase (COX-2) enzyme. High levels of COX-2 enzyme and prostaglandin were found in patients suffering from cancer. This points towards the potential of targeting this enzyme to inhibit its enzymatic activity and thus reduce prostaglandin. Phyla nodiflora has been reported to induce apoptosis and cell cycle progression in MCF-7 breast carcinoma cells showing higher expression of transcription factors inducing tumorigenesis. Increased antioxidant activity in Ehrlich-Lettre ascites carcinoma (EAC-bearing) mice may be responsible for the anticancer effect. We decided to conduct in vitro experimental and Insilico study including antioxidant activity, molecular docking, molecular dynamic simulation, drug-likeness, ADME and toxicity to identify therapeutic agent of Phyla nodiflora to verify anticancer and antioxidant activity of the plant and to verify either these compounds are harmful or safe for human body. The results show that methanol extract of the plant has potential as antioxidant and molecular docking shows that compounds of the plant including 8-methoxyluteolin (-8.8kcal/mol), 8-methoxyapigenin (-8.3kcal/mol) and3-methylherbacetin (-9.1kcal/mol) to be best potential drug candidates as they have highest binding affinity, follow Lipinski guidelines of five as well and the RMSD and RMSF show that they have strong binding interaction with receptor and show stability. In this study we have reported three bioactive molecules 8-methoxyluteolin, 8-methoxyapigenin and 3-methylherbacetin from Phyla nodiflora which may act as drug candidates and therapeutic agents for treatment of cancer while targeting the COX-2 enzyme.*

Keywords: *Absorption; Metabolism; Excretion analysis; cyclooxygenase2; Lipinski guideline; Molecular docking; Phyla nodiflora; Toxicity*

Graphical abstract



INTRODUCTION

After cardiovascular disease, cancer is to be considered one the most common disease and reason of mortality. Antioxidants shield living things from harm brought on by the uncontrolled formation of reactive oxygen species (ROS). Chronic inflammation is caused due to the increased production of ROS resulting in cellular and tissue injury underlying many neurodegenerative, cardiovascular and metabolic syndromes. ROS induce the generation of macrophages in the tumor cells by the activation of distinct stimuli such as TNF-α causing a rapid burst of hydrogen peroxide and free radicals leading to the subsequent induction of tumor cell apoptosis. Mitochondrial dysfunctioning is worthwhile discussion in the progression of cancer suggesting its important contribution in the activation of caspase 1 transcription factors and mitochondrial uptake of Ca^{+2} (Joshi et al., 2017). Drug discovery and development of a novel drug using a multidrug target is an important approach to the treatment of cancer (Van & Botting 1995). Ligand-receptor docking studies can help to identify novel therapeutic and drug candidates (Bost et al., 2010). The most important enzyme cyclooxygenase-2 (COX-2) was found to be elevated in patients suffering from cancer (Rajakrishnan et al., 2008). A study conducted on Phyla nodiflora against breast and lung carcinoma showed that it has the potential to promote apoptosis and inhibit the growth of cancerous cells (Das et al., 2020). Natural drug compounds are potential candidates to combat different types of diseases (Rehman et al., 1999). Arachidonic acid is converted into prostaglandin by a rate-limiting enzyme cyclooxygenase (COX), found in the form of COX-1 and COX-2. COX-2 is associated with human cancer and its inhibition results in anti-inflammatory and anti-cancer activity (Picot et al., 2019). It has been reported that a large number of people use a non-steroidal anti-inflammatory drug for the treatment of inflammatory disease. But these drugs may adversely impact prostaglandin levels due to the inhibition of COX-1, not COX-2 which in turn causes hypermortality of the gastric cancer. COX-2 is phylogenetically more primitive that COX-1 and, while very similar, has critical differences, particularly the existence of a small pocket half way down the active enzyme site. A number of drugs achieve selectivity by binding to this pocket, including presumptively rofecoxib and celecoxib. Others, such as meloxicam, may inhibit COX-2 by different mechanisms. Truly selective COX-2 inhibitors have been shown to have no effect on gastric mucosal prostaglandin synthesis, to cause no acute injury, and no chronic ulceration compared to placebo (O Banion et al., 1991). At the site of infection, pro-inflammatory cytokines cause to activate COX-2 which in turn stimulates the proliferation of cancer cells, inhibits apoptosis and stimulates angiogenesis (Gunathilake et al., 2018). Both COX-2 and Prostaglandin E2(PGE2) have crucial roles in cancer development as COX-2 activate carcinogenesis while PGE2 has a negative role over natural killer cells to combat them which is one of the most important pathways to suppress the immune system (Ehrich et al., 1999). PGE2 is released by most of the cancer cells thus enhancing the growth of cancer and also suppressing the immunity of the system to combat carcinoma (Ballinger et al., 2001). Breast cancer is considered as 2^{nd} most highly cause of mortality in women. The level of COX-2 expression has been found to have a significant correlation with tumor invasiveness. The recent literature suggested measurement of COX-2 protein expression on western blot analysis and found a two-fold higher COX-2 expression in non-invasive MCF-7 breast cancer cells in comparison to benign MCF-10F breast cells and a more than 4-fold higher COX-2 protein levels ($p<0.01$) in the malignant tissue as compared to the normal tissue samples. In breast cancer including with invasive and ductal carcinoma in North American population, the COX-2 was found to elevate by 40% in breast cancer patients with invasive and ductal carcinoma (Rajkumar et al., 2013). For therapeutic purpose, the blockage of COX-2 enzyme has been found to play an effective role. So, it is one of the most important therapeutic target for drugs (Kokila et al., 2013). Inflammation is one the major part of cancer progression (Nivetha et al., 2017). This research work is conducted to evaluate potential bioactive constituents of Phyla nodiflora for treatment of cancers while targeting the COX-2 enzyme. The plant extract is active as antibacterial, antifungal, anti-inflammatory and anti-oxidant. No such study has been conducted using in silico approach to discover novel therapeutic drug candidates of Phyla nodiflora medicinal plant. The plant is active against inflammation so it will have an effective role against cancers as well.

MATERIALS AND METHODS

3.1. Plant collection and leaf Extraction

Plant Phyla nodiflora was collected from district Bannu, KPK, Pakistan and after drying the leaves of the plant were converted into powder form. Two different types of solvent, methanol and water were used for leaf extraction.

3.2. Antioxidant Activity

For antioxidant activity methanol and water extract of leaf was used at different concentrations i.e. 1mg, 3mg, 5mg, and 7mg.

3.3. Data Collection

Active constituents of Nodiflora Halleridone, 8-methoxyluteolin, 3-methylherbacetin, 8-methoxyapigenin and Halleron were downloaded from the PubChem database in sdf format and receptor COX-2 (PDB: 5IKR) was downloaded from Protein Data bank in pdb format (www.pdb.org/pdb).

3.4. Protein Preparation

Receptor protein (PDB: 5IKR) was opened in discovery studio software, 2021(https://discover.3ds.com/discovery-studio-visualizer-download) to remove undesired substances. All the Hetaatom, water molecules and bounded ligands were removed from the protein, Hydrogen atoms were added and were saved as in pdb format (Jabeen et al., 2021).

3.5. Ligand Preparation

Ligands downloaded from PubChem database(https://pubchem.ncbi.nlm.nih.gov/)are in sfd format so they were uploaded in Open Babel window of PyRx and were converted into pdb format (Indravathi et al., 2016).

3.6. Molecular Docking

To evaluate the ligand-protein interaction of five selected active components of Nodiflora against COX-2, molecular docking approach was applied using PyRx bioinformatics tool (Bhadra, 2020). COX-2 enzyme as a receptor protein was uploaded in PyRx and was converted into a macromolecule. Using Open Babel window, all ligand molecules were uploaded; the energy was minimized and were then converted into pdbqt format. Docking was run after creating grad box using vina wizard. Discover studio 2021 was used for results visualization including 2D structure, hydrogen bonding and bond length (Sharma et al, 2013).

3.7. Drug-Likeness and ADME

SwissADME online software (http://www.swissadme.ch/) was used for checking drug-likeness and also for ADME analysis. ADME is absorption, distribution, metabolism and excretion (Gomha et al., 2021). Canonical smiles of ligand compounds were retrieved from PubChem and were pasted in swissADME to analyze drug-likeness and ADME parameters.

3.8. Toxicity

Canonical smiles of all the ligands were retrieved from PubChem database and were pasted in Protox-ii online web server (http://tox.charite.de/protox_II) (Khadera et al., 2024) and admetSAR (http://lmmd.ecust.edu.cn/admetsar2) for toxicity determination. Protox-ii online and admetSAR was run to check hepatotoxicity, mutagenicity and cytotoxicity, AMES toxicity, carcinogenicity and acute oral toxicity of the compounds.

3.9. Bioactivities

Orally administrated drugs need to be compatible with the drug-likeness properties to be finalized as pharmaceutically consistent with their bioactivity score and that's why we used Molinspiration Toolkit for prediction of selected compounds (https://www.molinspiration.com/cgi-bin/properties).

3.10. Molecular Dynamics Simulation:

Molecular dynamics (MD) simulation is a powerful computational technique used to study the behavior and properties of molecules and materials at the atomic level. Desmond is a software package developed by Schrödinger for MD simulations of biological systems, including proteins, nucleic acids, and membranes (Tamilselvi et al., 2022).

The stability of protein-ligand interactions was investigated through molecular dynamics (MD) simulations. Specifically, MD simulations were carried out on the most stable complexes with high binding energies. To determine the dynamic binding behavior and binding stability of protein-inhibitor

complexes in their docked pose, MD simulations were performed using the Desmond module of Schrödinger. A detailed description of the methodology can be found elsewhere (Hanwell et al., 2012; Van Gunsteren et al., 1996; Trott et al., 2010; Tian et al., 2018).

RESULTS AND DISCUSSION

2.1. Antioxidant Activity

Sample	mg	Abs. 1	Abs. 2	Abs.3	avarage abs	%Average-Absorbance
Methanol Extract	1	0.314	0.319	0.316	0.316333	42.69324
	3	0.295	0.297	0.297	0.296333	46.31643
	5	0.224	0.27	0.267	0.253667	54.04589
	7	0.194	0.196	0.198	0.196	64.49275
Water Extract	1	0.4822	0.4844	0.4876	0.484733	12.18599
	3	0.479	0.4812	0.478	0.4794	13.15217
	5	0.4957	0.4227	0.4934	0.4706	14.74638
	7	0.4768	0.475	0.4689	0.473567	14.20894
Ascorbic Acid		0.025	0.027	0.029	0.027	95.1087

Abs*-Absorbance**, A-O*-** Antioxidant.

Table .1 Antioxidant activity of leaf extract of Phyla nodiflora plant at different concentrations at OD=517 nm

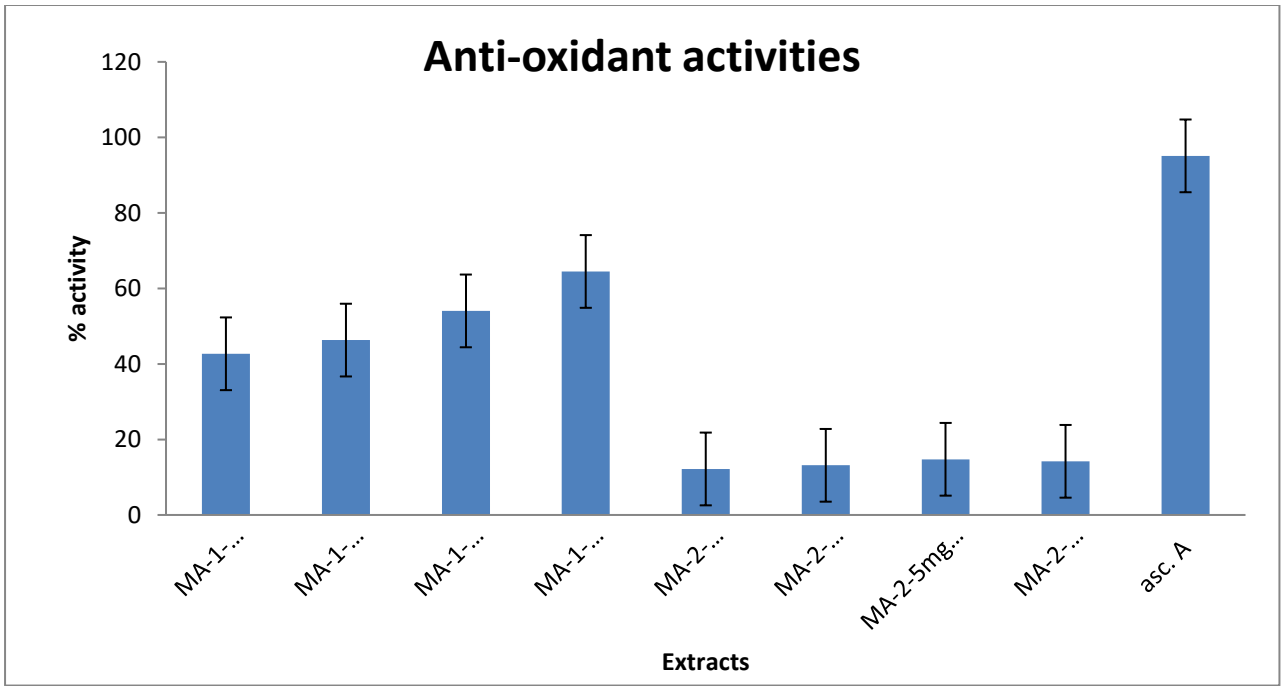


Figure .1 Graphical presentation of Antioxidant activity of methanol and water extract of Phyla nodiflora leaf.

***MA-1**-Methanol extract, ***MA-2**- Water extract, ***asc.A**- Ascorbic acid

The antioxidant activity of leaf extract of Phyla nodiflora was analyzed at different concentrations and in three independent experiments. The results show that when the average absorbance at 517 nm decreases the scavenging effect of the leaf extract increases. Table 1 and Figure1 shows that the methanol extract of leaf is more effective for antioxidant activity and when the dose of methanol extract is increased the scavenging effect of the plant extract is noted to be increased. The antioxidant activity of methanol extract is 42.69% when 1mg dose of the leaf extract is used while it increases to 64.49% when dose of leaf extract is increased to 7mg. Antioxidant activity, when ascorbic acid is used was noted 95.10%.

2.2. Molecular Docking

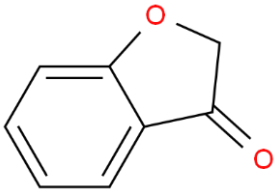
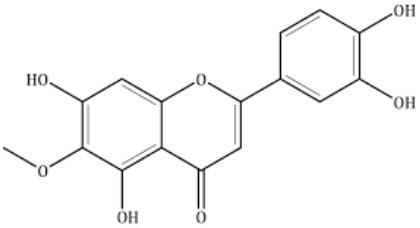
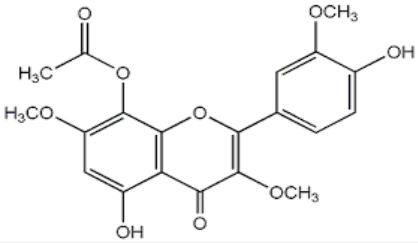
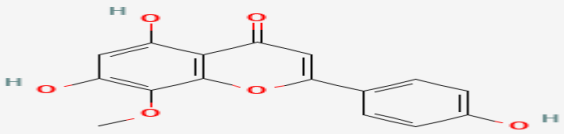
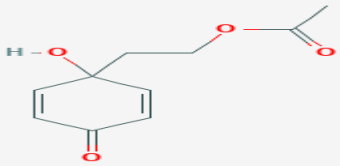
S.NO	Compound names	Structure Depiction
1	Halleridone	
2	8-methoxyluteolin	
3	3-methylherbacetin	
4	8-methoxyapigenin	
5	Halleron	

Table .2 List of five selected bioactive constituents with compound name and structure depiction of Nodiflora.

S.No	Compounds	Binding Affinity (Kcal/mol)	Hydrogen Bond	Bond Distance (Å)
1	Halleridone	-6.1	GLN A:461	2.16
2	8-methoxyluteolin	-8.8	TRP B:139, ASP A:229, ARG A:333, LEU A:238, ASN B:144	2.51, 2.70, 2.42, 2.68, 2.22
3	8-methoxyapigenin	-8.3	ARG A:333, GLU B:140, LEU A:238	2.61, 2.20, 2.97
4	Halleron	-5.6	ARG B:376, ASN A:375, ARG A:376	2.29, 1.83, 2.15
5	3-methylherbacetin	-9.1	CYS B:36	2.38

Table .3 Molecular docking results of selected bioactive compounds of Nodiflora medicinal plant.

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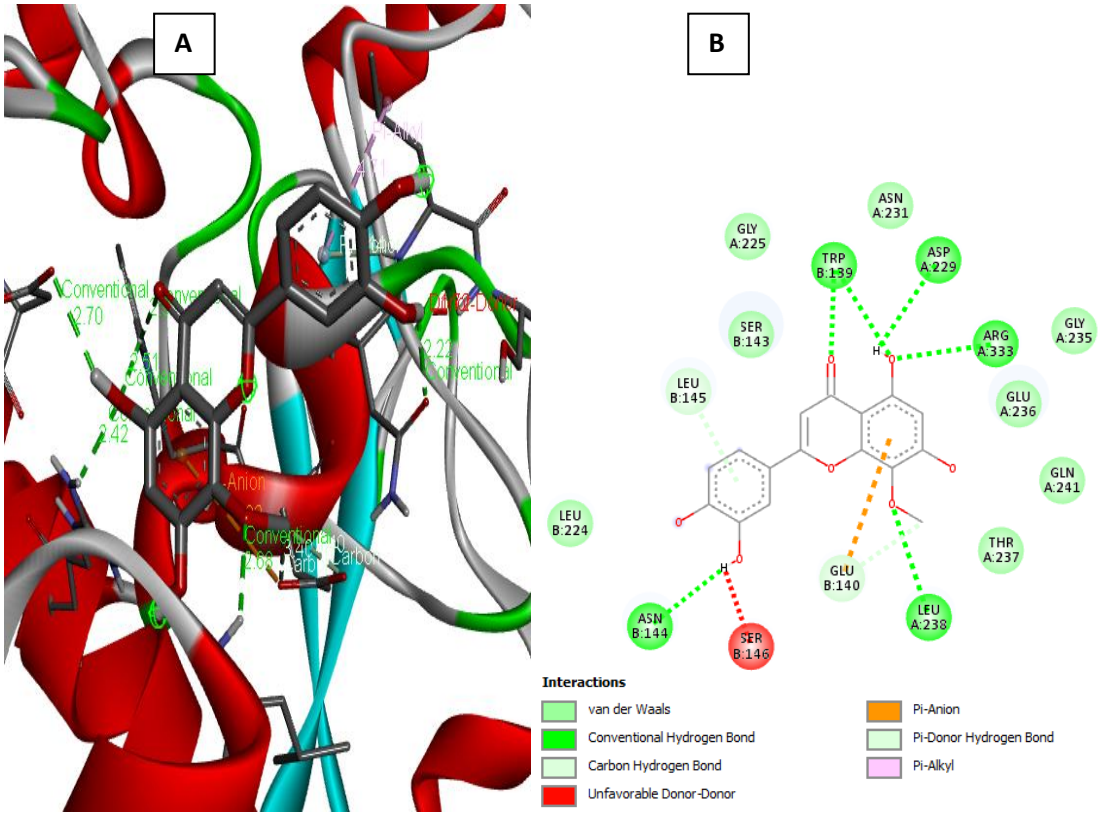

Figure .2 "A" shows the interaction of 8-methoxyluteolin with receptor protein COX-2 while "B" shows the 2D interaction and bonding present in them.

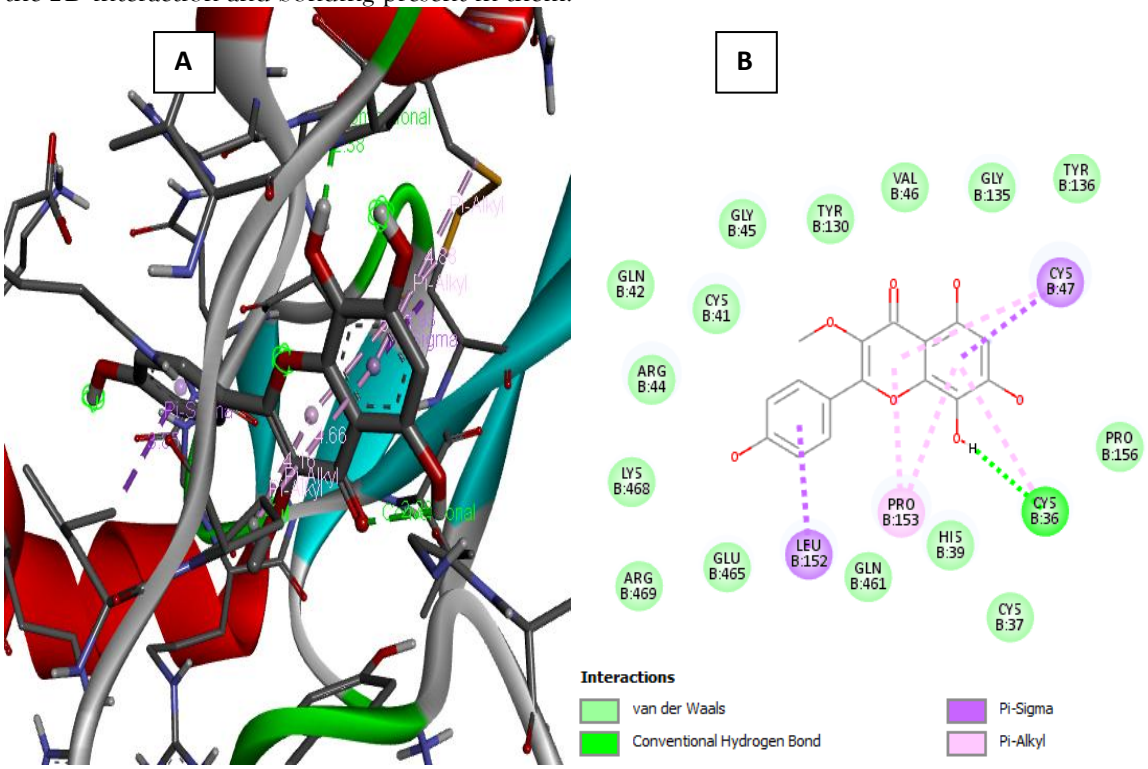

Figure .3 "A" shows the interaction of 3-methylherbacetin with receptor protein COX-2 while "B" shows the 2D interaction and bonding present in them.

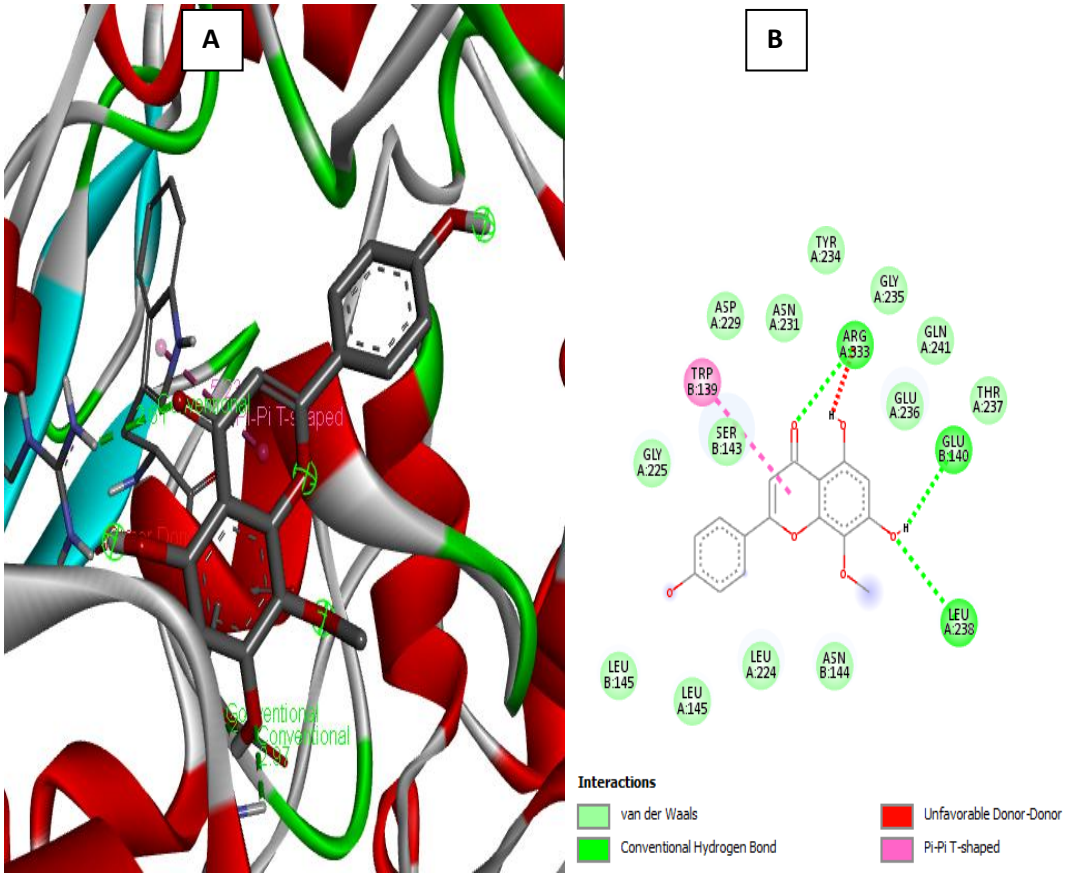

Figure .4 "A" shows the interaction of 8-methoxyapigenin with receptor protein COX-2 while "B" shows the 2D interaction and bonding present in them.

Molecular docking for five bioactive constituents was carried out against COX-2 enzyme to evaluate their role as anti-cancer agents. Molecular docking has a key role in ligand-protein and protein-protein interaction and binding sites of receptor to which a ligand molecule binds which is extensively used in modern drug discovery (Silentin et al., 2015). Table 3 shows that four out of five selected compounds have a high binding affinity and low binding energy which shows their strong interaction with receptor protein COX-2. The results show that 3-methylherbacetin, 8-methoxyluteolin, 8-methoxyapigenin and Halleridone have binding affinity -9.1, -8.8, -8.3 and -6.1 respectively. Generally for active drug compounds -6.00 kcal/mol is considered as potential binding energy (Laskowski et al., 2011). Hydrogen bond and bond distance were also analyzed for all the ligand interacting with receptor protein COX-2. Molecular docking study of COX-2 reported that sesame oil is considered as a traditional medicine having -6.0 to -8.4kcal/mol binding affinity (Pagadala et al., 2017). Another study conducted to explore potential inhibitors of COX-2 show that xanthoxyletin having -8.9kcal/mol binding affinity is a potential agent to inhibit COX-2 expression (Hashem et al., 2022). Curcumin was found to interact with Gln192, Asp347, Arg513, Leu351,Leu383, Leu357, Arg120 and Glu524 residues of the COX-2 enzyme (Afroz et al., 2019). It is evident from Table 3 and figures(2B,3B and 4B) that Gln461, Trp139 (2), Asp229, Arg333 (2), Leu238 (2), Asn144, Cys36, Glu140, Arg376 (2) and Asn375 residues of COX-2 enzyme having bond length 2.16Å, 2.92 Å and 2.51 Å, 2.70 Å, 2.42 Å and 2.61 Å, 2.68 Å and 2.97 Å, 2.22 Å, 2.38 Å, 2.20 Å, 2.29 Å and 2.15 Å and Asn375 having 1.83Å respectively interacting with our selected bioactive constituents of Phyla nodiflora. The bioactive molecule 8-methoxyluteolin makes the most stable complex with receptor protein due to a high number of hydrogen bonds. The hydrophobic interaction in the binding site of COX-2 is driven by Tyr385 and Tyr387 (Bathinin et al., 2016).

2.3. ADME ANALYSIS and Drug-Likeness

Properties	Features	Halleridone	8-methoxyluteolin	3-methylherbacetin	8-methoxyapigenin	Halleron
Physiochemical Properties	MW(g/mol)	154.16	316.26	316.26	300.26	196.2
	H.B Donor	1	4	4	3	1

	H.B Acceptor	3	7	7	6	4
	MR	38.35	82.5	82.5	80.48	49.81
	Arom. Heavy Atom	0	16	16	16	0
	Rotatable Bonds	0	2	2	2	4
	TPSA	46.53	120.36	120.36	100.13	63.6
Lipophilicity	logP(SILICOS-IT)	0.95	2.06	2.06	2.55	0.88
Water Solubility	Logs(SILICOS-IT)	-0.43	-3.94	-3.94	-4.52	-0.8
Pharmacokinetics	GI Absorption	High	High	High	High	High
Drug-likeness	Lipinski	Yes: 0 Violation	Yes: 0 Violation	Yes: 0 Violation	Yes: 0 Violation	Yes: 0 violation
Lead likeness		Yes: 1 Violation	Yes: 0 Violation	Yes: 0 Violation	Yes: 0 Violation	Yes:1 violation

Table .4 ADME analysis and drug-likeness results of selected bioactive constituents of Phyla Nodiflora.

ADME study was carried out using swiss ADME to identify different parameters like physical properties, lipophilicity, Lipinski and solubility of bioactive constituents (Zhang et al., 2017). For any drug development, ADME has a key role as many drug candidates fail a clinical trial. No more than two violations are acceptable for orally drug candidate (Banerjee et al., 2018). Lipinski rule of five (≤5 H.bond donor, ≤10 H.bond acceptor, m.weight ≤500Da, Molar Refractivity 40-130 and ≤logP) (Schrodinger, 2019) was followed for selected compounds to analyze whether these may act as drugs or not. The results in Table 4 show that all selected bioactive molecules pass the Lipinski rule of five and have no single violation. Lead likeness shows that Halleridone and Halleron have just one violation and all other molecules have zero violation. Gastrointestinal absorption and solubility class shows that all molecules are highly soluble. For water solubility Logs values ranges from -10 to 0 which indicates different category of solubility i.e. -10, -6, -4, -2 and 0 for insoluble, poorly soluble, soluble, very soluble and highly soluble respectively (Jorgensen et al., 1999). Table 4 shows that all compounds are in the range of solubility and no one is insoluble in the selected candidates. The five compounds were solely selected on the basis of wet analysis provided the literature. Although the aqueous extract were prepared but the DPPH assay demonstrated that the antioxidant activity is more pronounced in ethanolic extract as compared to the aqueous.

Compound Name	Hepatotoxicity	Mutagenicity	Cytotoxicity
Halleridone	Inactive	Inactive	Inactive
8-methoxyluteolin	Inactive	Inactive	Inactive
3-methylherbacetin	Inactive	Inactive	Inactive
8-methoxyapigenin	Inactive	Inactive	Inactive

Table .5 Toxicity results of five selected bioactive constituents of Phyla nodiflora medicinal plant.

Compound	hERG inhibition	Rat(LD50,mol/kg)	AMES	Carcinogen	Actute oral toxicity	Carcinogenesity (class 3)
Halleridone	Weak	1.7272	Non	Non	III	Non-required
8-methoxyluteolin	Weak	2.6388	Non	Non	III	Non-required
3-methylherbacetin	Weak	2.6388	Non	Non	III	Non-required
8-methoxyapigenin	Weak	2.7192	Non	Non	III	Non-required
Cisplatin	Weak	2.2490	Yes	Yes	II	Non-required

Table .6 Toxicity profiling of four selected compounds (with high binding affinity) using admetSAR server.

Online Protox-II server was used to analyze all constituents which may behave like a drug candidate to check their toxicity like hepatotoxicity, mutagenicity and cytotoxicity. Table 5 shows that Halleridone, 8-methoxyluteolin, 3-methylherbacetin, and 8-methoxyapigenin are non-toxic and can be used as a drug candidate as they obey Lipinski and have no toxicity. To validate and cross check the toxicity of selected compounds, they were analyzed using admetSAR which shows that all our studied candidates are AMES non-toxic, non-carcinogenic (Table 6). Further all compounds were found to be weak inhibitors for human ether-a-go-go-related gene (hERG) and showed rat acute toxicity with a median lethal dose (LD50) of 2.63 mol/kg. The results can be compared with cisplatin which is carcinogen, AMES toxic, hERG weak inhibitor and has Rat acute toxicity of 2.24 (Vijayakumar et al., 2018). As per the predicted acute

oral toxicity, all compounds lie in 'class III'. Compounds of this class have LD50 values <5000 mg/kg and were generally considered suitable from a druggable point of view. Study based on clinical trials is directed for the reported compound (Zarghi & Arfaei 2011).

2.5. Bioactivity Prediction

Bioactivities	Halleridone	8-methoxyluteolin	3-Methylherbacetin	8-methoxyapigenin
GPCR ligand	-0.77	-0.10	-0.11	-0.09
Ion channel modulator	-0.52	-0.01	-0.17	0.01
Kinase inhibitor	-1.62	0.17	0.21	0.17
Nuclear receptor ligand	-0.52	0.19	0.21	0.22
Protease inhibitor	-0.73	-0.25	-0.28	-0.26
Enzyme inhibitor	0.04	0.22	0.25	0.23

Table .7 Bioactivity score of selected bioactive compounds according to Molinspiration cheminformatics software.

One of the greatest tools for assessing the bioactivity scores of natural chemicals for therapeutic targets is Molinspiration as demonstrated in (Table 7). The likelihood that a compound has notable biological activity depends on its bioactivity score. Compounds with bioactivity values above 0.00 are most likely to be noteworthy, while those with scores between -0.50 and 0.00 are moderately dynamic and those with scores below -0.50 are inactive. Our analysis demonstrates that many routes may be implicated in the chemical compounds' physiological actions. It might also be brought on by interactions with nuclear receptor ligands, GPCR ligands, ion channel modulator ligands, enzyme inhibitors, and protease inhibitors. The results of bioactivity scores show that all selected compounds are highly active to act as a ligand of enzyme inhibitor, 8-methoxyapigenin, 8-methoxyluteolin and 3-methylherbacetin are highly to act as ligand of nuclear receptor and kinase inhibition while 8-methoxyapigenin can bind as a ligand of ion channel modulator. The result shows that 8-methoxyapigenin, 8-methoxyluteolin and 3-methylherbacetin are moderately dynamic toward GPCR and protease inhibitor. The overall finding reveals that our selected ligand molecules are associated with all pharmacological targets.

2.6. Molecular Dynamic Simulation

Molecular dynamics simulations were performed on the top hits containing high binding energies. Over the simulation period, the projected conformational changes from the initial structure were presented in terms of root mean square deviation (RMSD). Moreover, structural stability, atomic mobility, and residue flexibility at times of interaction of protein-hit were expressed with root mean square fluctuation (RMSF) values. The peaks of RMSF graph represent the fluctuation portion of the protein through the simulation. The N- and C-terminal show more changes than any other portion of the protein. Alpha helices and beta strands show less fluctuation, as they are stiffer than the unstructured part of protein, than loop portion. The RMSD of the 8-methoxyapigenin-complex showed small deviation from 2.4 Å to 4.2 Åat almost 30 ns and 45 ns after 75 ns the system was equilibrated. It indicates of the stability of the protein-protein complex (Figure 5). The RMSD of 8-methoxyluteolin-complex showed small deviation initially and after 50 ns the system was equilibrated and stabilized throughout the simulation (Figure 6). For the complex of 3-methylherbacetin there was deviation of almost 2.5 Å from almost 40 ns to 65 ns and after that the system was stabilized (Figure 7).

Similarly, the Root Mean Square Fluctuation (RMSF) is useful for characterizing local changes along the protein chain. Peaks reflect sections of the proteins that fluctuate the most during the simulation on the RMSF. Protein tails (both N- and C-terminal) are more likely to change than other regions of the protein. Alpha helices and beta strands, for example, are usually stiffer and less fluctuating than the unstructured component of the protein. The residues with higher peaks, according to MD trajectories, belong to loop areas or the N and C-terminal zones. For RMSF there was no fluctuation where the ligands made contacts (green lines) with the receptor indicating the strong binding of ligands with receptor proteins (Figure 8, 9 and 10).

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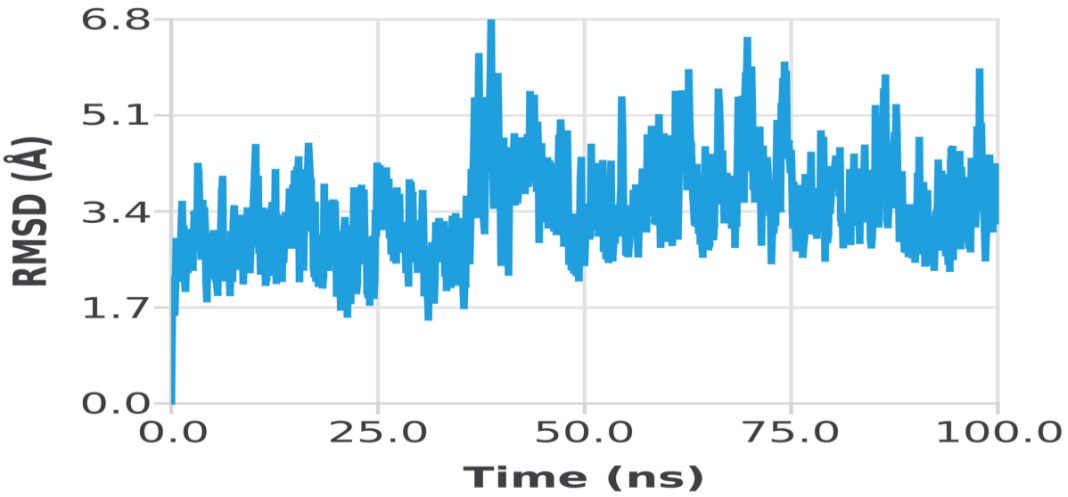

Figure 5. Root Mean Square Deviation plot of 8-methoxyapigenin-Complex

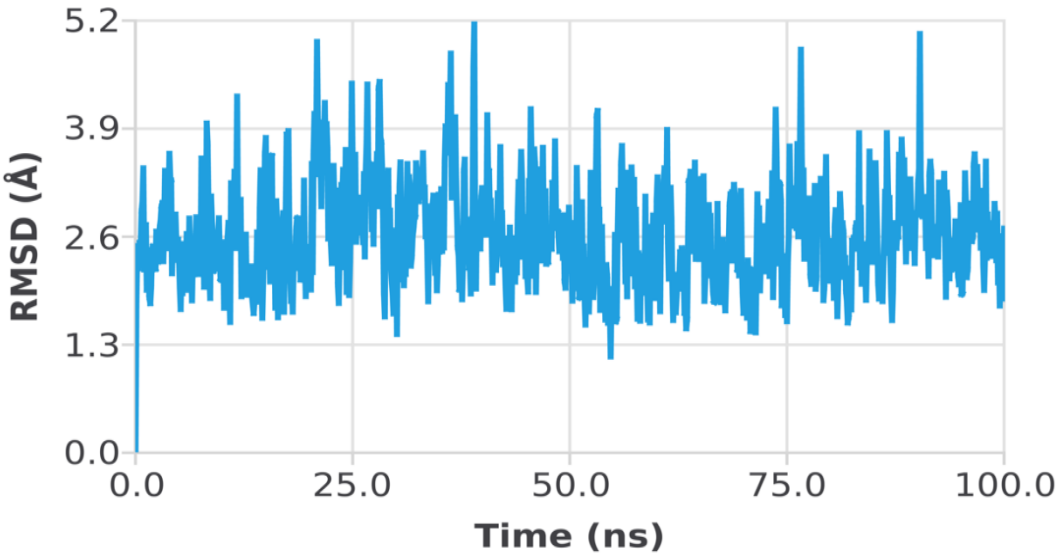

Figure 6. Root Mean Square Deviation plot of 8-methoxyluteolin-Complex

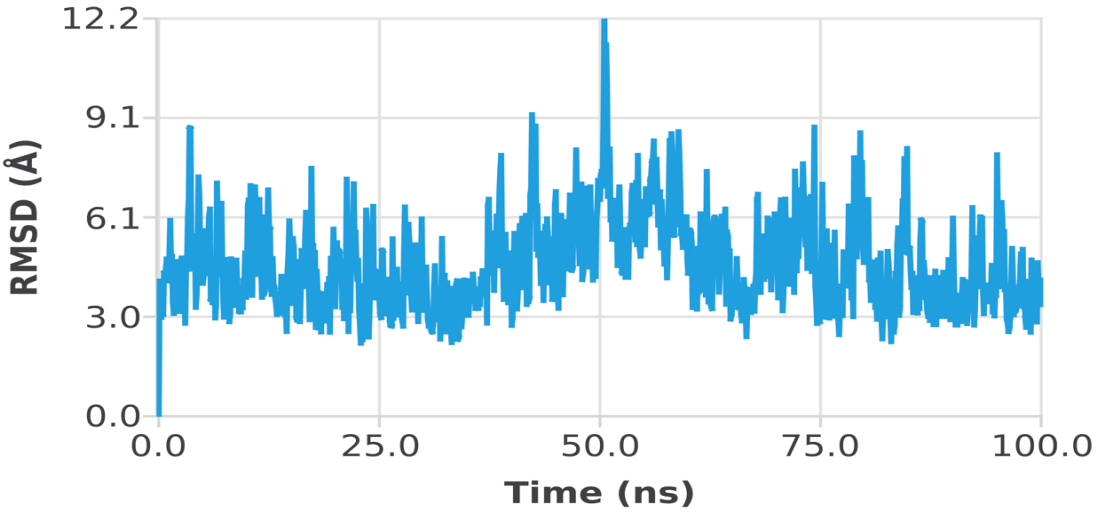

Figure 7. Root Mean Square Deviation plot of 3-methylherbacetin-Complex

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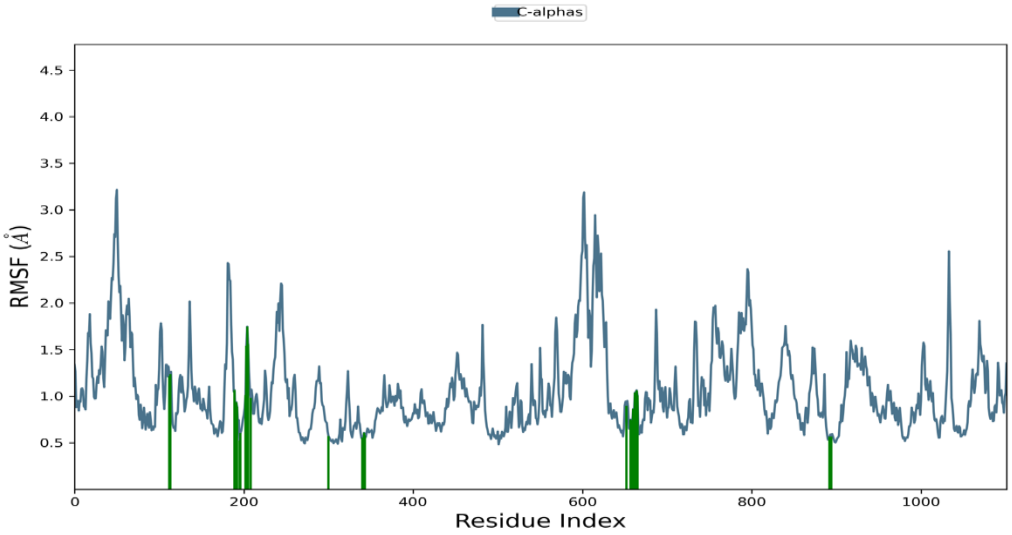

Figure 8. Root Mean Square Fluctuation plot of 8-methoxyapigenin-Complex

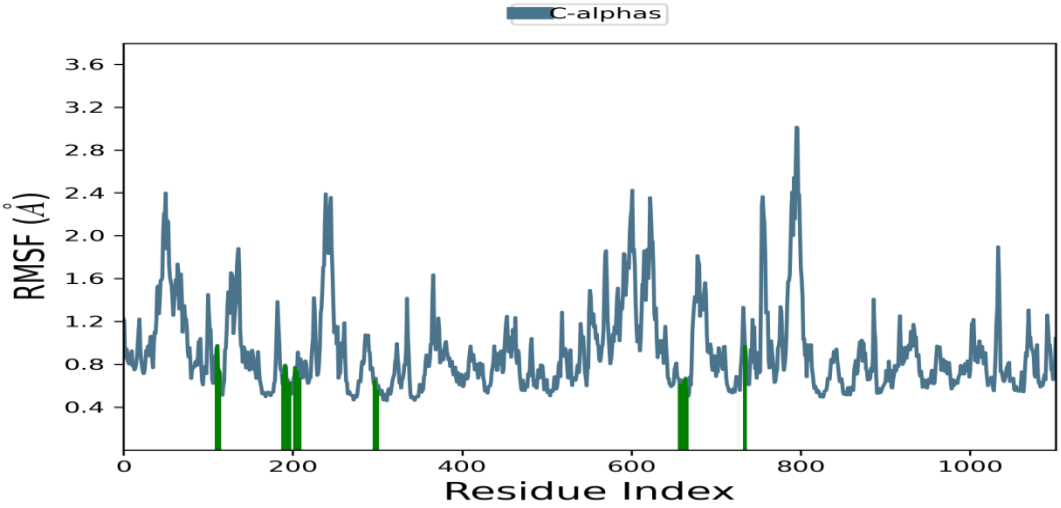

Figure 9. Root Mean Square Fluctuation plot of methoxyluteolin-Complex

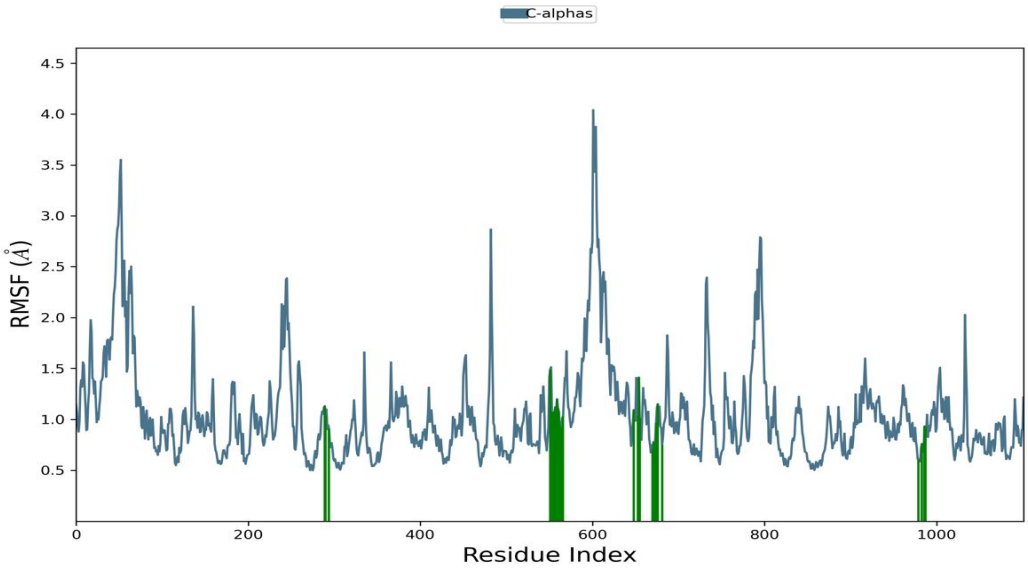

Figure 10. Root Mean Square Fluctuation plot of methylherbacetin-Complex

Herbal remedies known as flavonoids have a variety of medicinal activities, including antioxidant, anti-inflammatory, analgesic, anti-carcinogenic, anti-bacterial infection, anti-fungal, and antiviral effects (Mustarichie et al., 2014).

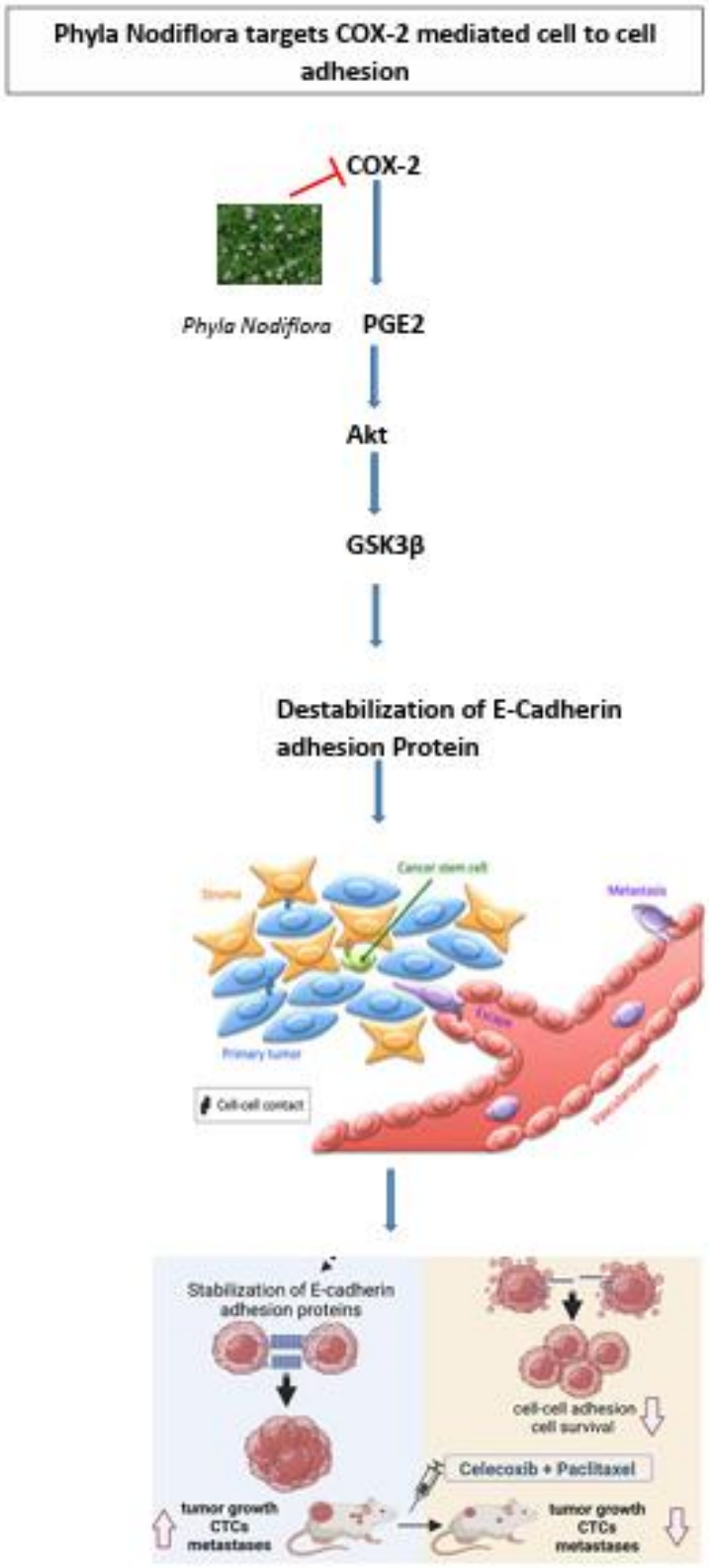

Figure: 11 Pathophysiology of COX-2 targeted by Phyla Nodiflora

The leaves and stems of Phyla nodiflora have anti-proliferative and apoptotic effects on human breast cancer cell lines when they are extracted in methanol and ethyl acetate. Through apoptosis, the extracts at 90–120 µg/ml might stop the proliferation of cancer cells (Prabhavathi 2020). The anticancer effect in

breast cancer for methanol extract of Phyla nodiflora was evaluated using MCF7 cell line and MTT assay which shows that the plant extract has potential for inhibition of MCF7 cells with IC_{50} ranges from 90 to 120µg/ml. Another in vitro anticancer study of plant extracts using NCI-H450 cell lines for human lung cancer show the potential effect as anti-proliferative for tested cell lines with IC_{50} value of 10µg/ml. Increased antioxidant activity in EAC-bearing mice may be responsible for the anticancer effect (Daze & Hof 2016). Halleridone and Hallerone isolated from nodiflora show anticancer, anti-tumor and antifungal. 8-methoxyluteolin and 8-methoxyapigenin, methanol extract of A.Judaica medicinal plant, exhibits significant bioactivities including anti-inflammatory, hepatoprotective, anti-diabetic and antioxidant activities (Huang et al., 2009). Herbacetin demonstrated anti-carcinogenic and anticancer effects in vitro against SK-MEL-5 melanoma and A431 cutaneous squamous cell carcinoma at 10 and 20 M doses (Pandey et al., 2017). According to three different assays, i.e. the AKT kinase assay, In silico assay and ex vivo pull-down assay, Herbacetin suppress the activity of AKT and associated signaling pathways like GSK3 and RSK2 by directly binding to AKT (Shukla et al., 2018).

CONCLUSIONS

It is concluded hereby that Phyla nodiflora bioactive compounds play a great role as antioxidant and anti-cancer agents. The study shows that three compounds that are docked against the COX-2 enzyme, have a high binding affinity score and Molecular dynamic simulation, RMSD and RMSF value, also support the strong binding of the compounds with COX2, obey all guidelines of Lipinski, and are biologically active hence showing that these compounds 8-methoxyluteolin, 3-methylherbacetin and 8-methoxyapigenin may act as novel therapeutic agents in the future for the treatment of cancers.

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Conflict of Interest: Authors declare no conflict of interest

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