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IN SILICO STUDIES OF *BYTTNERIA HERBACEA* Roxb. BIOACTIVE COMPOUNDS AGAINST ANTI-INFLAMMATORY (COX-1) PROTEIN

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ABSTRACT

The present study explored the potential of *Byttneria herbacea* Roxb. against inflammatory disease by conducting molecular docking studies. The SwissADME tool was utilized to perform a drug-likeness study, which was then followed by molecular docking using the AutoDock 4.2 software. *In silico*, GC-MS research identified 21 molecules, subsequently evaluated for drug-likeness properties. Based on the ADME analysis, six compounds were recognized as superior compounds. The docking analysis of these six molecules was performed with Autodock 4.2. Finally, two compounds were shown to be effective against Cyclooxygenase-2: 7-Methoxy-2,2-dimethyl-2H-1-benzothiopyran and 3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl) against the enzyme (COX-1). Excellent docking properties and the lowest binding energy (-6.94 and -6.90 kcal/mol) were also found. According to the data, *B. herbacea* aerial plant component showed a significant anti-inflammatory molecular docking effect.

Keywords: Anti-inflammatory activity; AutoDock; *Byttneria herbacea*; Molecular docking; Drug likeness

INTRODUCTION

Byttneria herbacea Roxb. (Family: Malvaceae) is a plant that is often found in peninsular India (Gujarat, Tamil Nadu, Odisha, and Bihar) and is known as a favorite odder (Khai) of deer (Sambar/Samar). Previous research has shown that indigenous societies employ the *B. herbacea* crude medication as a treatment for a variety of illnesses (**Sharma and Acharya, 2018**). It is effective in relieving bodily discomfort when taken orally (**Sreeramulu *et al*., 2012; Mairh *et al*., 2010; Sathish *et al*., 2021**). Diarrhea and gynecological issues are treated with the whole plant. The Odisha people have used the roots and leaves as vegetables (**Sharma *et al*., 2020**). In our earlier study, we reported the whole plant phytochemical profile of methanol extract bioactive compounds from *B. herbacea* plant and analyzed by GC-MS and revealed 24 compounds (**Sathish *et al*., 2021**). In addition, other researched also showed a significant antioxidant, antimicrobial activity (**Sharma and Acharya, 2018, 2020**). But very few studies are reported on in *silico* approaches.

Infection and injuries cause inflammation. It is linked to arthritis, cancer, stroke, and other neurological and cardiovascular diseases (**Nathan, 2002**). Due to their enhanced biological synthesis in inflamed tissue, prostaglandins (PGs) are the key competitors in forming the inflammatory response (**Stables, 2011**). COXs (prostaglandin G/H syntheses) are bifunctional enzymes that act as COX and a peroxidase. They exist in two different isoforms i.e. COX-1 and COX-2, and both are required for prostaglandin G/H synthesis (**Smith, 2000**). Despite their essential similarity, their expression profiles are substantially different. COX-2 is generally referred to as a "housekeeping enzyme" in the medical profession because it is primarily involved in physiological tasks such as maintaining and safeguarding renal function and controlling platelet aggregation via activation of the enzyme thromboxane A2 (TXA2). On the other hand, COX-1 is thought to be primarily essential for initiating and maintaining the inflammatory response, with minor physiological effects such as boosting prostacyclin (PGI2) production and decreasing platelet aggregation (**Oniga, 2017**).

In silico protein, analysis is a legitimate alternative research method at the molecular level. In drug design and discovery, molecular modeling and docking are most frequently used in this context. The molecular docking approach aids in the determination of the optimal binding orientation of single or multiple drugs to their target proteins, which are responsible for the development of illnesses and diseases. Sampling and scoring are two of the essential features of protein-ligand docking software. When a protein's binding site is sampled, it generates a variety of ligand-binding conformations. Scoring predicts the tightness of binding for various ligand conformations using a physical or empirical energy function (**Shoichet *et al*., 2002**).

The binding mode is expected to be the top conformation. The three fundamental components of protein-ligand docking are system representation, conformational space search, and rating of candidate solutions. In docking, the scoring functions are solely responsible for the binding energy of the target proteins and the ligand. The docking score is derived based on the free energy required for binding (**Holt *et al*., 2008**).

MATERIAL AND METHODS

Target Protein Selection

The docking analysis focused on the anti-inflammatory COX-1 protein (PDB ID: 6Y3C). The docking configurations were collected from the PDB (https://www.rcsb.org/structure/6Y3C). COX-1 binding sites were ligand-free (Fig. 1). Protein heteroatoms were eliminated and replaced by polar hydrogen atoms. Additionally, the proteins were given partial atomic charges. The proteins were allocated molecular solvation parameters, and the data were converted to PDBQT format.

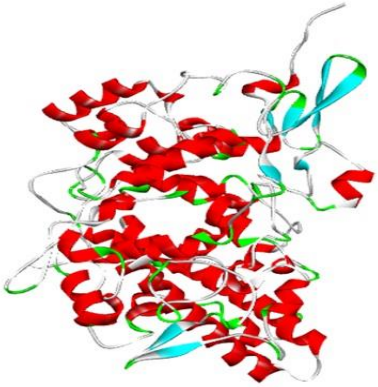


Figure 1 3D Structure of Human COX-1 Crystal Structure (PDB ID: 6Y3C)

SwissADME drug-likeness study of bioactive components

The chemical structure of *B. herbacea* compounds that had previously been reported in GC-MS analysis was downloaded in SDF (structure data format) using the PubChem data bank (http://pubchem.ncbi.nlm.nih.gov/). SwissADME external file option, files were imported and converted to molecular sketcher format using ChemAxon's Marvin JS (**Daina *et al.*, 2017**).

Ligand Selection

The ligand was produced according to Lipinski's rule (5–H bond donors, 500 Daltons Molecular Weight, 5 Log P for octanol-water partition coefficient, 10 H bond acceptors). The rule is critical when a pharmaceutically active leading structure is incrementally improved for higher activity, selectivity, and drug-likeness features throughout drug development.

Protein-Ligand Docking

In this study, docking of ligands towards COX-1 was performed with the help of AutoDock 4.2. AutoDock is a molecular docking software program that is freely available in the public domain (**Thomas *et al*., 2013**). To generate a collection of potential conformations, it comprises elements such as AutoGrid, AutoTors, and the Lamarckian genetic algorithm. There is a need for a program that can handle the flexible docking of ligands into identified protein structures on the fly. The proteins used in each docking experiment were kept rigid to allow for torsional flexibility in the ligands. AutoTors was used to define the rotatable bonds in the ligands, and a device called AutoGrid was used to generate the grid maps. The search for COX-1 was carried out in grid points of 80x80x80 with 0.675Å spacing between each point in the search grid (**Honmore *et al*., 2016; Zhang *et al*., 2019**). There are 30 docking runs with 150 participants in the docking experiment. Other than that, all other parameters were left in their usual defaults. The binding energy and bound conformations of docked structures are obtained from the AutoDock data. Following that, the results of the docking technique were analyzed with the help of BIOVIA Discovery Studio and Ligplot.

RESULTS AND DISCUSSION

Analysis of drug-likeness was performed to determine whether the bioactive compounds possessing favorable ADME characteristics were readily available. For drug-like compounds to be effective, they must have a high level of aqueous solubility, which can be predicted by three different methods: ESOL, logS (ALI), and (SILICOS-IT). Orally active medication should obey Lipinski five rules (Lipinski et al. 1997). Drug-likeness analysis of bioactive compounds listed (Tab 1). (1) Acetal; (2) 3-buten-2-one, 4-(5,5- dimethyl-1-oxaspiro[2.5]oct-4-yl); (3) 1, 3-diformyl -2- chloro-5-isopropylbenzene; (4) 7 -Methoxy-2, 2-dimethyl- 2 H - 1 - benzothiopyran; (5) (1Ar)-3-(Acetyloxy) (Acetyloxy) methyl-1a alpha, 1bbeta, 4, 4a, 5, 7a alpha, 7b, 8, 9, 9a-decahydro - 1, 1, 6, 8 alpha tetramethyl - 1H-cyclopropa[3,4]benz[1,2-e] azulene-4abeta, 5beta, 7b alpha, 9beta, 9a alpha - pentol 9, 9a-diacetate; (6) (2E,6E,10S,11S) (2E,6E,10S,11S) -7-Ethyl-10,11-dihydroxy-3,11- dimethyl-2,6-tridecadienoic acid methyl ester (Fig. 2). In accordance with the Lipinski RO5, bioactive compounds exhibited high drug-likeness parameters, such as good solubility and no excretion problems, because there is no pharmacokinetics P-gp (permeability glycoprotein) interference, with the exception of one compound (2E,6E,10S,11S) -7-Ethyl-10,11-dihydroxy-3,11-dimethyl-2,6-tridecadienoic acid methyl ester. Not an inhibitor of CYP enzymes and, in particular, CYP-inhibitory compounds (Zero Alerts for PAINS). It is also advantageous to use drug-likeness measures for *B. herbacea* bioactive chemicals because they adhere to the Lipinski RO5 rule, the Ghose ,Veber, Egan, Muegge rule, and the Bioavailability score of 0.55 for these bioactive chemicals.

Table 1 ADME prediction of *B. herbacea* bioactive compounds using Swiss ADME

Name of compounds	Drug Likeness – Lipinski Role of 5					Solubility			Pharmacokinetics		Lipinski Violations
	MW	HBD	HBA	HBR	log P	Log S (ESOL)	Log S (Ali)	Log S (SILICOS-IT)	GI absorption	CYP enzymes inhibitors	
Acetal	118.17	0	2	4	2.31	Very soluble	Very soluble	Soluble	High	No	0
3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl)	208.30	0	2	2	2.59	Soluble	Soluble	Soluble	High	No	0
Octadecanoic acid,(2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- (CAS)	446.66	0	4	20	6.33	Poorly soluble	Insoluble	Poorly soluble	Low	Yes	1
3-Ethyl-o-xylene	134.22	0	0	1	2.44	Soluble	Moderately soluble	Soluble	Low	No	1
1-Hexadecanol	242.44	1	1	14	4.41	Moderately soluble	Poorly soluble	Moderately soluble	High	Yes	1
11-Octadecenal	266.46	0	1	15	4.51	Moderately soluble	Poorly soluble	Moderately soluble	Low	Yes	1
2-Hexadecanol	242.44	1	1	13	4.45	Moderately soluble	Poorly soluble	Moderately soluble	High	No	1
7-Methoxy-2,2-dimethyl-2H-1-benzothiopyran	206.30	0	1	1	2.78	Soluble	Soluble	Soluble	High	Yes	0
1-Octadecene	252.48	0	0	15	5.05	Poorly soluble	Poorly soluble	Poorly soluble	Low	No	1
Lucenin 2	610.52	12	16	5	1.70	Very soluble	Soluble	Soluble	Low	No	3
1,3-diformyl-2-chloro-5-isopropylbenzene	210.66	0	2	3	1.99	Soluble	Soluble	Soluble	High	No	0
Petroselaidic acid	282.46	1	2	15	4.25	Moderately soluble	Poorly soluble	Moderately soluble	High	Yes	1
Methyl 14-methylpentadecanoate	270.45	0	2	14	4.59	Moderately soluble	Poorly soluble	Moderately soluble	High	Yes	1
Ethyl palmitate	284.48	0	2	16	4.65	Moderately soluble	Poorly soluble	Poorly soluble	High	Yes	1
Petroselaidic acid	282.46	1	2	15	4.25	Moderately soluble	Poorly soluble	Moderately soluble	High	Yes	1
9-Octadecenoic acid, ethyl ester	310.51	0	2	17	5.03	Moderately soluble	Poorly soluble	Poorly soluble	Low	Yes	1
Bacteriochlorophyll-c-stearyl	841.46	1	8	23	-83.24	Insoluble	Insoluble	Insoluble	Low	No	2
(1Ar)-3-(Acetyloxy)methyl-1aalpha,1bbeta,4,4a,5,7aalpha,7b,8,9,9a-decahydro-1,1,6,8alpha-tetramethyl-1H-cyclopropa[3,4]benz[1,2-e]azulene-4abeta,5beta,7balpha,9beta,9aalpha-pentol 9,9a-diacetate	492.56	3	9	7	3.12	Soluble	Soluble	Soluble	High	No	0
Stigmasterol	412.69	1	1	5	5.01	Poorly soluble	Poorly soluble	Moderately soluble	Low	No	1
(2E,6E,10S,11S)-7_Ethyl-10,11-dihydroxy-3,11-dimethyl-2,6-tridecadienoic acid methyl ester	312.44	2	4	11	3.56	Soluble	Moderately soluble	Soluble	High	No	0
Stigmast-5-en-3-ol, (3beta)-	414.71	1	1	6	4.79	Poorly soluble	Poorly soluble	Poorly soluble	Low	No	1

Table 2 Target protein and selected ligands interaction

Compound name	Binding Energy (kcal/mol)	H-Bond Interactions	Distance (Å)
Acetal	-3.73	HIS383 HIS386	2.93 2.96
3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl)	-6.90	ARG469	2.76
1,3-diformyl-2-chloro-5-isopropylbenzene	-6.45	CYS47 TYR130 TYR130	2.96 2.77 2.94
7-Methoxy-2,2-dimethyl-2H-1-benzothiopyran	-6.94	-	-
(1Ar)-3-(Acetyloxy)methyl-1aalpha,1bbeta,4,4a,5,7aalpha,7b,8,9,9a-decahydro-1,1,6,8alpha-tetramethyl-1H-cyclopropa[3,4]benz[1,2-e]azulene-4abeta,5beta,7balpha,9beta,9aalpha-pentol 9,9a-diacetate	-5.46	ARG49 ASP135 ASP135 ILE 137	2.97 2.78 2.97 3.09
(2E,6E,10S,11S)-7-Ethyl-10,11-dihydroxy-3,11-dimethyl-2,6-tridecadienoic acid methyl ester	-5.24	CYS41A CYS41A	2.76 2.80

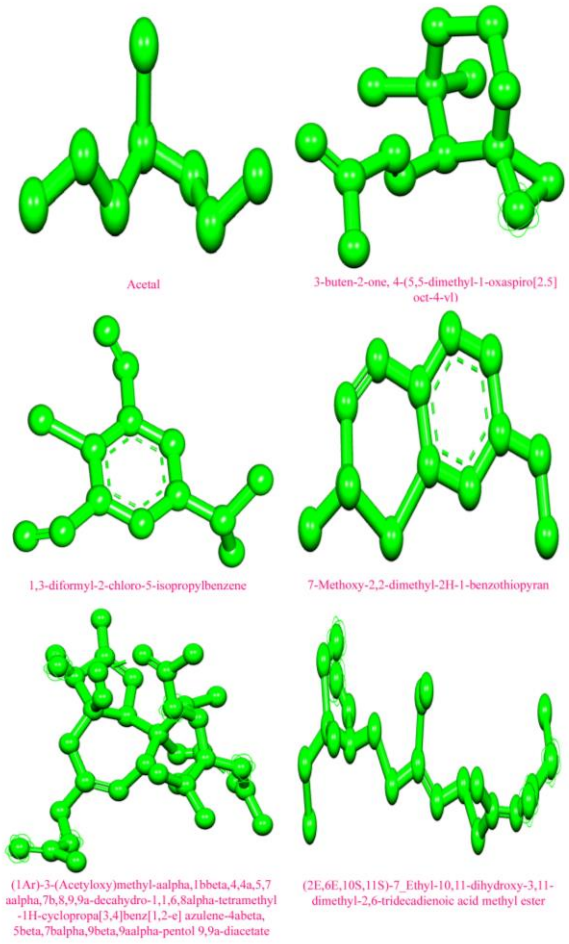
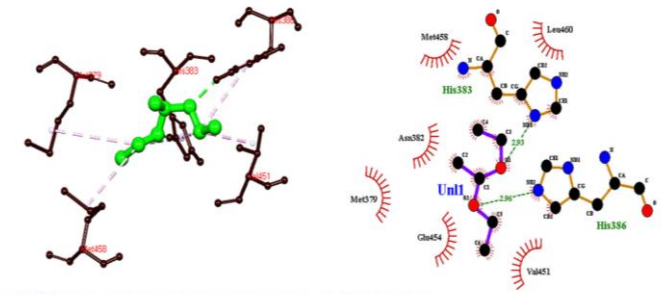


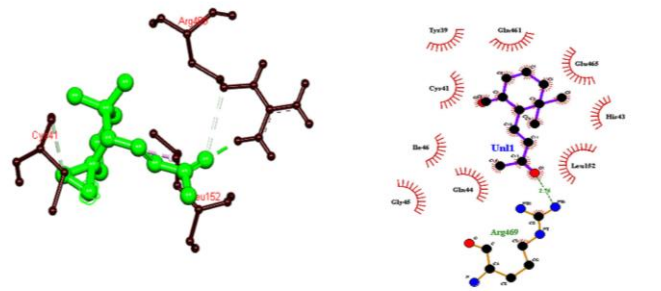
Figure 2 3D structures of bioactive compounds in *B. herbacea* plant

The anti-inflammatory effects of the identified compounds based on the ADME study were examined utilizing Autodock 4.2. molecular docking experiments against the COX-1 protein. The following six compounds were considered for consideration based on the ADME analysis: The structures of the ligands that were selected were obtained from the Pubchem database. The absolute optimum conformation on docking energy for both ligands and protein was discovered among the numerous binding poses at the active site. 1-Acetal; 3-buten-2-one, 4-(5,5 -dimethyl-1-oxaspiro[2.5]oct-4-yl); 1, 3-diformyl -2- chloro-5-isopropylbenzene; 7 - Methoxy-2, 2-dimethyl-2 H – 1 - benzothiopyran; (1Ar)-3-buten-2-one; (1Ar)-3-buten-2-one; (Acetyloxy) methyl-1a alpha, 1bbeta, 4, 4a, 5, 7a alpha, 7b, 8, 9, 9a-decahydro - 1, 1, 6, 8 alpha – tetra methyl - 1H-cyclopropa[3,4]benz[1,2-e] azulene-4abeta,5beta,7balpha,9beta,9aalpha-pentol 9,9a-diacetate and (2E,6E,10S,11S) (2E,6E,10S,11S) The interaction of -7 Ethyl-10,11-dihydroxy-3,11-dimethyl-2,6-tridecadienoic acid methyl ester with COX-1 resulted in docking energies of -3.73, -6.90, -6.45, -6.94, -5.46, and -5.24 kcal/mol (Tab 2).

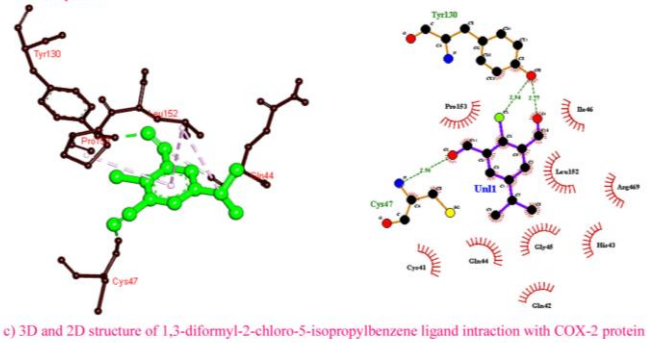
It has been demonstrated by docking 7-Methoxy-2,2-dimethyl-2H-1-benzothiopyran with COX-1 that it is the most effective inhibitor, but it does not interact with COX-1 via hydrogen bonding (Fig. 3D). Afterward, the combination of 3-buten- 2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl), and COX-1 revealed one hydrogen bond with residue ARG469 (Fig. 3B), with a bond length of 2.76Å. 1,3-diformyl-2-chloro- 5-isopropylbenzene COX-1 interacted (Fig. 3C)with residues of CYS47 and TYR130 through two hydrogen bonds with bond lengths of 2.96 and 2.77Å, respectively. (1Ar)-3-(Acetyloxy) methyl-1a alpha, 1b beta, 4, 4a, 5, 7a alpha, 7b, 8, 9a-decahydro-1,1,6,8alpha-tetramethyl-1H- cyclopropa[3-4 The combination of azulene-4abeta, 5beta, 7b alpha, 9beta, 9a alpha-pentol 9, 9a-diacetate with COX-1 also revealed four hydrogen bonds with residues of ARG49, ASP135, ASP135 and ILE 137 (Fig. 3E), with bond lengths of 2.97, 2.78, 2.97, and 3.09Å, respectively. The interaction of (2E,6E,10S,11S)-7 Ethyl-10,11-dihydroxy-3,11-dimethyl-2,6-tridecadienoic acid methyl ester with COX-1 also revealed two hydrogen bonds with residues of CYS41 and CYS41, with bond lengths of 2.76 and 2.80Å, respectively (Fig. 3F). Acetal with COX-1 also showed two hydrogen bonds with residues of HIS383 and HIS 386 (Fig. 3A) with a bond length of 2.93 and 2.96 Å, respectively.



a) 3D and 2D structure of Acetal ligand intraction with COX-2 protein



b) 3D and 2D structure of 3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl) ligand intraction with COX-2 protein



c) 3D and 2D structure of 1,3-diformyl-2-chloro-5-isopropylbenzene ligand intraction with COX-2 protein

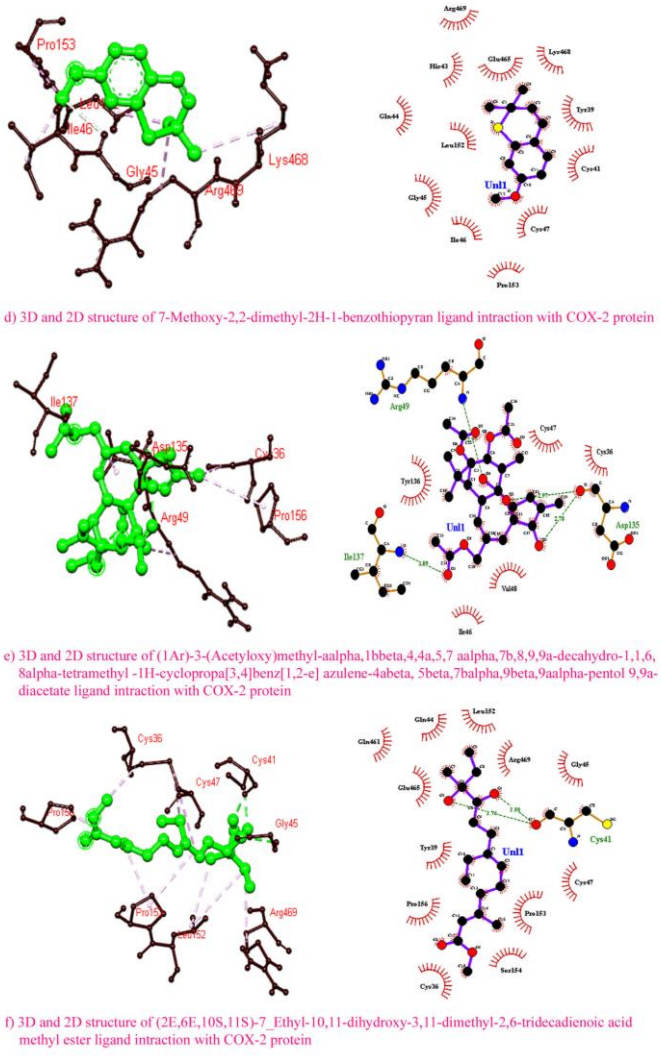


Figure 3 3D and 2D structure of target protein and selected ligands interaction

According to published data, docking of synthetic compounds indicated three basic binding patterns in general. Researchers in the current study firmly believe that COX-1 Protein is more important than previous studies. Because of the bonding in the hydrophobic pocket, COX-1 inhibitors like SC-558 should be used with caution. According to the research findings, the phenylsulphonamide filled the side pocket, bound to His90, and interacted with Arg513, another critical residue in COX-1 inhibitors (**Kurumbail *et al*., 1996**).

In another study, docking of Diclofenac revealed that its orientation renders the side pocket residues inaccessible, preventing access to the hydrophilic pocket of the COX-1 protein. It also revealed that the phenylacetic acid moiety is oriented towards Tyr385 and Ser530, resulting in H-bonding interactions with these two amino acids. Ibuprofen and naproxen were found to interact with the COX-1 enzyme when docked directly into the enzyme's active site. According to the research, the deposit 120 with which it interacts has been identified as Arg120 and Tyr355 (**Llorens *et al*., 2002**). Prodigiosin and cycloprodigiosin affect the active site conformation of COX-1 protein by combining at regions other than the existing active sites and also produce anti- inflammatory effects. Additionally, the present investigation demonstrated that the two compounds, namely (1) 7-Methoxy-2,2-dimethyl-2H-1-benzothiopyran (2) 3-buten-2- one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl), had a significant effect on the active sites of the COX-1 protein.

CONCLUSION

The GC-MS analysis of *B. herbacea* yielded 21 compounds, which were then subjected to an analysis of their drug-likeness properties. Based on the ADME analysis, six compounds were identified as superior to the other compounds in the group. The docking analysis of these six molecules was performed with Autodock 4.2. Last but not least, two compounds, 7-Methoxy-2,2-dimethyl-2H-1-benzothiopyran and 3-buten- 2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl), have a significant effect on the COX-1 enzyme. This study was indeed able to identify the phytochemical responsible for the anti-inflammatory action of *B. herbacea*, which is well-known for its anti-inflammatory properties.

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