Vol 5 | Issue 1 | 2014 | 22-29.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

PHYTOCOMPOUNDS FROM *PHYLLANTHUS AMARUS* AS POTENTIAL COX-2 INHIBITORS

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ABSTRACT

To explore the phytocompounds isolated from *Phyllanthus amarus Schum. & Thonn.* for their selective COX-2 inhibitory activity using docking analysis. The docking of the target protein (6COX) with the phytochemical ligands was performed using the VLife MDS software. We have carried out flexible docking for sixty two structurally diverse phytocompounds isolated from *Phyllanthus amarus* for their selective COX-2 inhibitory activity. Thirty two molecular structures of phytocompounds present in the *Phyllanthus amarus* have affinity to the COX tube which were optimized for the docking study. The molecular docking scores identify the ligands that bind with similar orientation as observed with SC-558 (reference ligand) for COX. These compounds can be considered as good COX-2 inhibitors. Twelve phyto-compounds showed potent COX-2 inhibitory activity. These findings could be exploited in future for designing ligands in order to obtain novel molecules as selective COX-2 inhibitor.

Keywords: *Phyllanthus amarus Schum. & Thonn.*, Phytocompounds, COX-2 and docking.

INTRODUCTION

Phyllanthus amarus Schum. & Thonn. belongs to the family Euphorbiaceae is a small herb well known for its medicinal properties and widely used worldwide[1]. *P. amarus* is an important plant of Indian Ayurvedic system of medicine which have beneficial therapeutic potential in the management of painful disorders, hepatitis and in various diseases[1]. Phytochemical studies have shown the presence of many valuable compounds such as lignans, flavonoids, hydrolysable tannins (ellagitannins), polyphenols, triterpenes, sterols and alkaloids [2-3]. The extracts and the compounds isolated from *P. amarus* show a wide spectrum of pharmacological activities [1-3].

Discovery of the COX-2 isoenzyme led to the theory that COX-2 selective inhibition provides potent anti-inflammatory and analgesic effects of traditional NSAIDs without influencing COX-1[4]. Since most of the NSAIDs are associated with undesirable side effects such as gastrointestinal disturbances [5], new anti-inflammatory drugs are needed and complementary and alternative medicines are being sought [4]. Also COX-2 inhibitors are attractive molecular target for the development of cancer chemotherapy and neurological diseases such as Parkinson and Alzheimer's diseases [5].

Phyllanthus amarus possess potent analgesic, antinociceptic anti-inflammatory, anti-allodynic and anti-oedematogenic activity activities [1]. Recently we have reported diverse pharmacological activities of *P. amarus* standardized extracts and significant pain modulating potential. [6-11]

In support of biological and phytochemical studies of *Phyllanthus amarus* employing bioassays relevant to the analgesic and anti-inflammatory activity, as well as to assist in determining potential mechanisms of action of the various *P. amarus* extracts and their isolated phytochemical compounds we have carried out flexible docking analysis for sixty two structurally diverse phytocompounds of various class isolated from *Phyllanthus amarus* for their selective COX-2 inhibitory activity.

MATERIALS AND METHODS

Docking tool and algorithm

Molecular docking was completed using VLifeMDS version 4.1 (software licensed. to our institute). The docking algorithm BioPredicta is based on a genetic algorithm which offers a successful strategy for globally searching the docked conformer's space. Genetic algorithms allow a population of solutions to exist and in each 'generation' these can evolve by processes such 'breeding' and 'mutation'. Poor solutions are killed off, while good ones leave their offspring in future generations. Such algorithms may typically reach an excellent solution is a few tens of generations.

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Ligands Preparation [*Phyllanthus amarus* derived compounds and structures]

Sixty two compounds selected for this experiment are listed in Table 1. The various structures of phytoconstituents of phyllanthus were drawn in 2D (two dimensional) and were finally optimized for docking using VLifeMDS software in 3D (three dimensional) format.

Preparation of COX-2 enzyme protein structure [12]

The 3D structure of the COX-2 enzyme protein was downloaded from Protein Data Bank (PDB) with ID 6COX. The 6COX is a complex of COX-2 protein with SC-558 an COX2 inhibitor, which was selected as the target protein in this present screening study. Hydrogen's were added and enzyme structure was subjected to a refinement protocol in which the constraints on the enzyme were gradually removed and converted into SYBYL mol2 format. The other ligands and ions present in the protein structure were deleted. The protein moiety was checked for criss cross residues, local geometry and ramachandran plot using Biopredicta tools. For checking the local geometry following settings were set bond length 20, bond angle 20 and bond length 10 %.

Molecular Docking of *P. amarus* compounds with COX-2

The molecular docking was performed for all the phytocompounds (ligands) from *P. amarus* with the five best predicted poses of the interaction with the COX-2 protein. VLifeMDS provides a facility to dock different ligands in protein binding sites chosen by the user. It also provides both rigid (no torsional flexibility for protein as well as ligand) and flexible (torsional flexibility to ligand with rigid protein) docking of the molecules.

Analysis of docked protein-ligand complex structures

Thirty nine optimized molecules were utilized to analyze and visualize best molecular docked poses. Before screening the ligands, the docking protocol was validated by redocking SC-558 ligand into its binding pocket within the COX-2 crystal to obtain the docked pose. The best orientations for the ligand-protein complexes were analyzed. Distinction of good or bad docked conformation is based on scoring. MDS uses fitness functions on only electrostatic and both steric and electrostatic interactions between receptor-ligand as well as Dock Score scoring function. The Dock score or X-C score as it is called compute binding affinity of a given protein ligand complex with known 3-D structure. Dock/X-C score scoring function include terms for Vander Walls interaction, hydrogen bonding, deformation penalty, hydrophobic effects. The virtual screening technique employed in this study was identifying the ligands that bind in comparable manner similar to SC-558 (reference ligand) binding for COX-2.

RESULTS

The structures of sixty two phytocompounds (ligands) were drawn in 2D and converted into 3D using VLifeMDS software. The ligands were first optimized for the docking analysis. Thirty two molecular structures of phytocompounds reported in the *Phyllanthus amarus* have affinity to the COX tube which was optimized for the final docking analysis. The molecular docking scores identify the ligands that bind with similar orientation as observed with SC-558 (reference ligand) for COX. The 2-Dimensional presentation of interaction of the reference ligand SC-558 with COX is depicted in figure 1. Twelve of the phytocompounds (ligands) make good docking poses in comparison to the reference ligand.

The protein-ligand interaction scores (total score values) obtained during docking, the docked poses obtained from visualization and the log values of the ligands are summarized in table 2.

The obtained scores are in between -73.022244 to -22.042076. As a comparison, the V Life MDS score obtained for SC-558 was -112.575282. All the ligands docked deeply within the binding pocket region suggest their shape complementarily with COX-2. The details of molecular structures and properties of the twelve phyllanthus compounds which showed good COX-2 selective inhibition are summarized in table 3.

The molecular weights of the molecules are in between 168.279 to 401.523, with molecular volume ranging from 193.245 to 369.624 of the twelve phyllanthus compounds. The XlogP values were in between 0.000 to 9.346, whereas the SlogP values were in the range of 1.278 to 6.142 of the twelve phyllanthus compounds. The 3-Dimensional presentation of the docking studies of ligand molecules with 6 COX are represented in figure 1.

Phyltetralin, Isonirtetralin and Isolintetalin showed good docking scores, maximum number of docking poses and their XlogP values were 0.000. The results suggest these compounds are potent selective COX-2 inhibitors. This study will be useful for the designing of novel COX-2 inhibitors based on the docking analysis.

Table 1. List of various classes of Phytoconstituents from *P. amarus* included in the study.

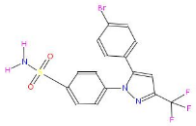
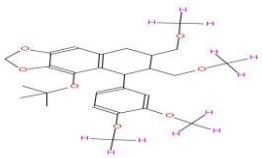
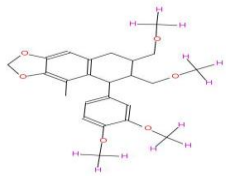
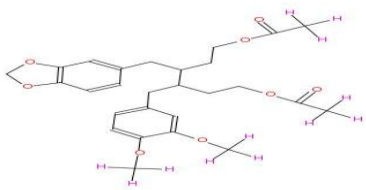
S No.	Class / Secondary metabolites	Phytoconstituents	Total number of compounds from each class
1	Lignans	Phyllanthin, Hypophyllanthin, Niranthin, Phyltetralin, Nirtetralin, Isonirtetralin, Hinokinin,Lintetralin, Isolintetralin,5- Demethylenedioxy-Niranthin,4,5-Demethoxy-Niranthin.	11
2	Flavanoids	Rutin, Astragalin, Kaempferol, Quercetin-3-O-Glucoside, Quercetin, Quercitrin.	06
3	Tannin precursors	Gallic Acid, Ellagic Acid, Gallocatechin.	03

4	Tannins	1,6-Digalloylglucopyranose, 4-O-Galloylquinic acid, Geraniin, Amariin, Furosin, Geraniinic acid B,Amariinic Acid, Amarulone, Repandusinic Acid A,Corilagin, Isocorilagin, Elaeocarpusin,Repandusinic acid A (Potassium salt), Phyllanthusiin A, B, C and D, Melatonin.	18
5	Alkaloids	Phyllanthine, Isobubbialine, Nor-Securinine, Securinine, Epibubbialine, 4-Methoxy-Nor-Securinine, Dihydrosecurinine, Tetrahydrosecurinine, Securinol, Allo-Securine, 4-Methoxy Dihydrosecurinine, Phenazine.	12
6	Triterpenes	Farnesylfarnesol, Lupeol, Phyllanthenol, Phyllanthenone, Phyllantheol, Oleanolic Acid, Ursolic Acid, P-Cymene	08
7	Sterols	Amarosterol A, Amarosterol B	02
8	Volatile oil	Linalool, Phytol	02
Total number of phyto-compounds used for the docking studies			62

Table 2. Summary of results of docking analysis of SC-558 and Phyllanthus compounds.

Ligand	Maximum Score of docked pose.	Number of docked poses	XlogP	SlogP
Reference Ligand [SC-558 inhibitor]	-112.575282	-	0.000	0.564
Phyltetralin	-73.022244	05	0.000	4.168
Isonirtetralin	-67.887102	05	0.000	3.547
Isolintetalin	-48.740446	05	0.000	3.294
Linalool	-42.590642	05	2.690	3.324
Securinine	-32.941904	03	1.779	2.369
Phyllanthine	-31.578374	03	1.259	1.995
4methoxy-nor-securinine	-31.055762	03	0.964	1.278
Gallic acid	-29.839113	05	1.760	1.499
Limonene	-29.129161	02	9.346	6.142
Nor securinine	-28.405444	05	2.503	2.570
Niranthin	-25.960728	01	3.847	3.646
p-cymene	-24.795065	03	8.559	5.524

Table 3. Structures and Properties of SC-558 and phyllanthus compounds.

Ligand	Structure	Mol. Wt.	Volume
Reference Ligand [SC-558 inhibitor]		438.184	285.876
Phyltetralin		401.523	252.784
Isonirtetralin		343.443	217.160
Isolintetalin		387.453	234.646

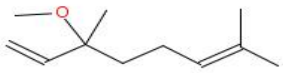
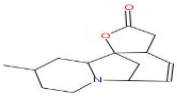
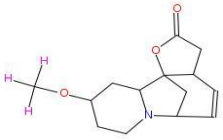
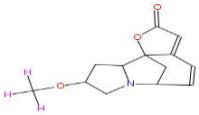
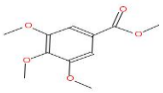
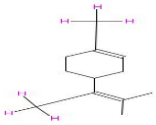
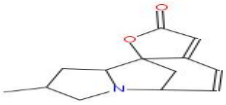
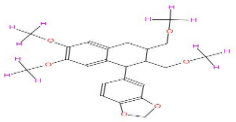
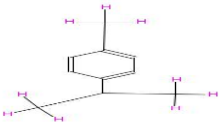
Linalool		168.279	193.245
Securinine		249.353	248.176
Phyllanthine		279.379	273.915
4methoxy-nor-securinine		249.310	235.136
Gallic acid		226.229	203.404
Limonene		248.452	289.736
Nor securinine		249.353	251.595
Niranthin		400.472	369.624
p-cymene		232.409	267.821

Figure 1. 2-Dimensional [2-D] presentation of reference ligand SC-558 with COX.

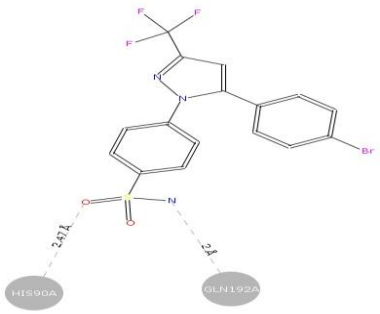
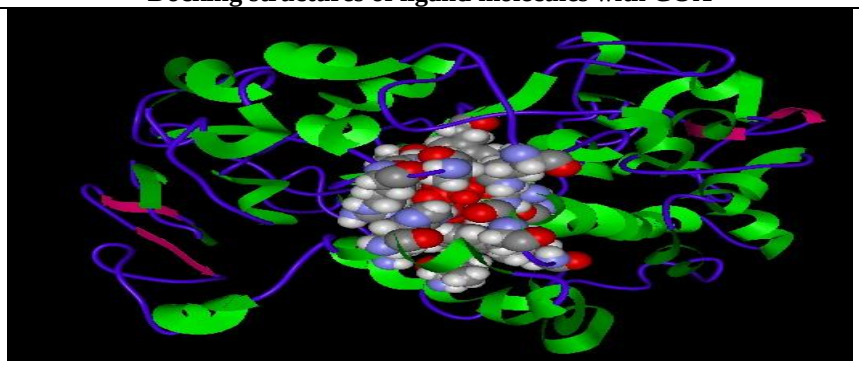
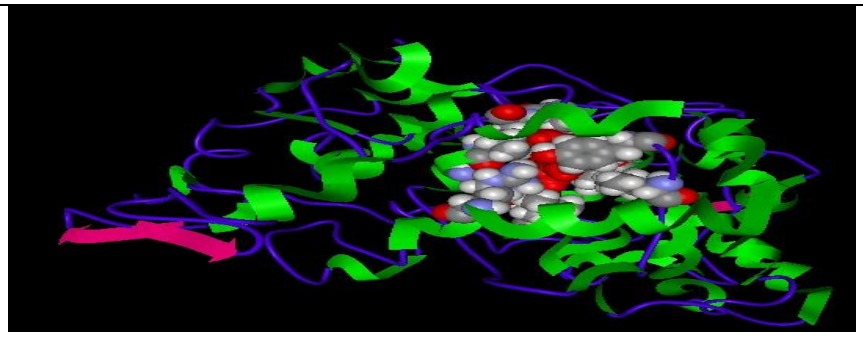
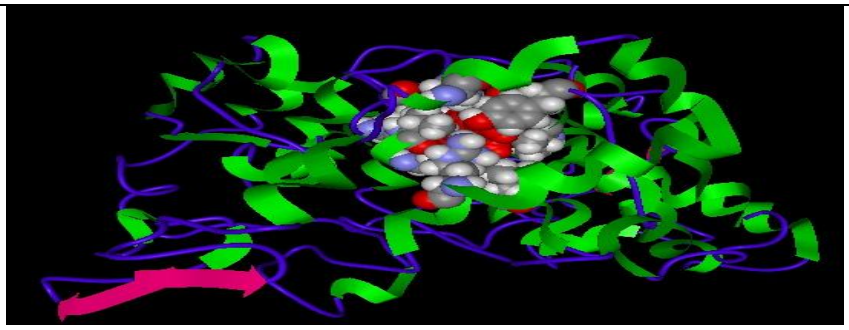
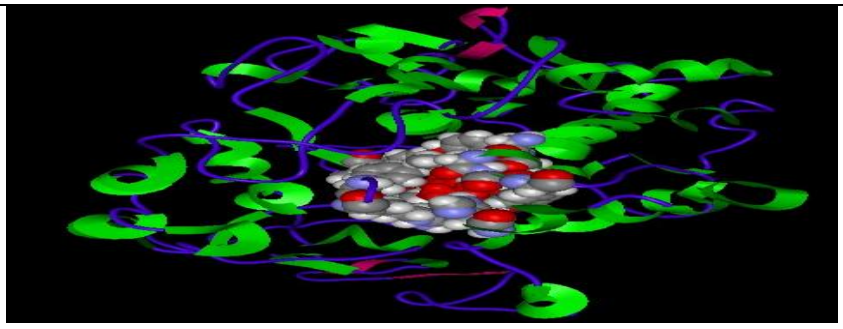
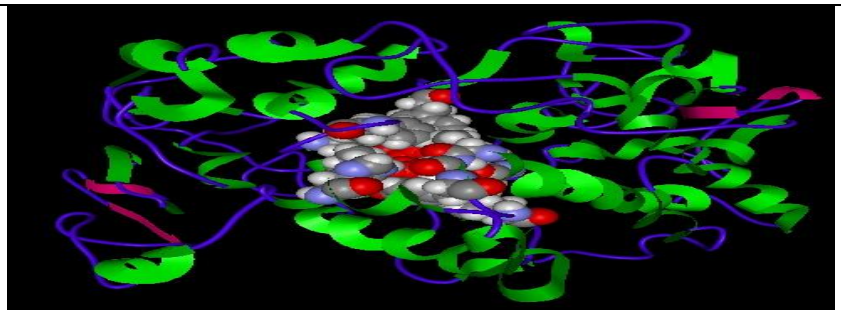
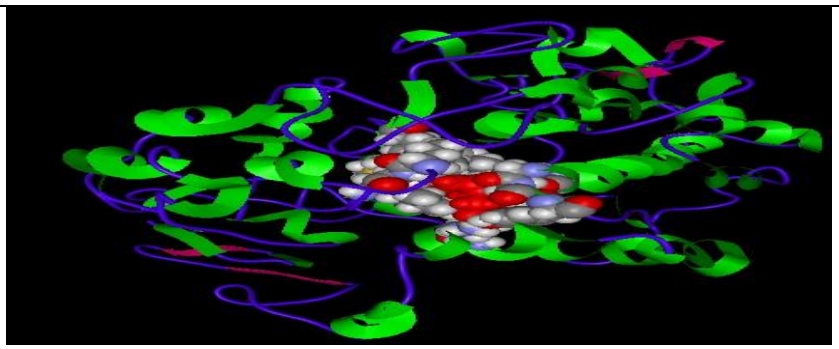
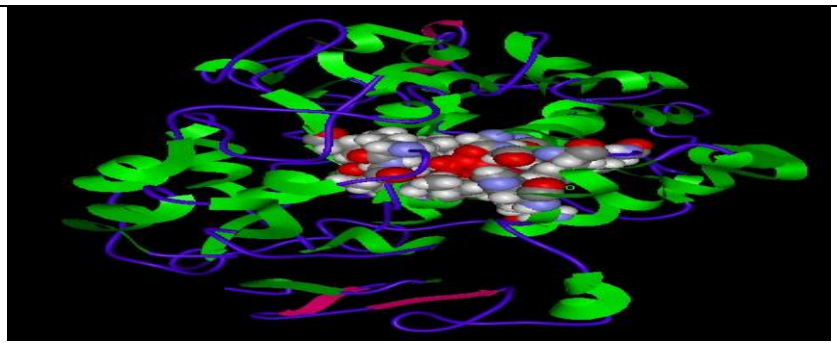
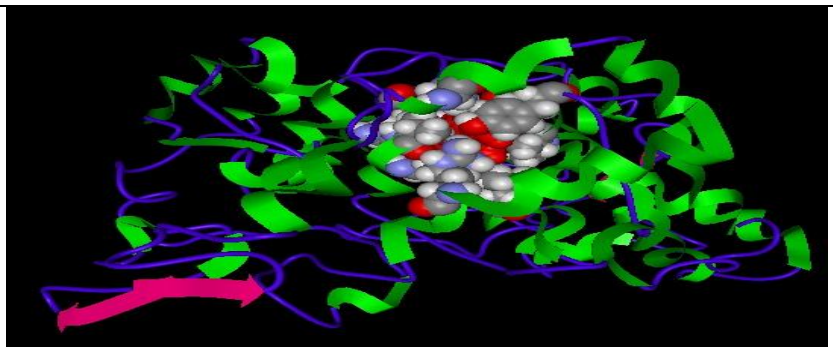
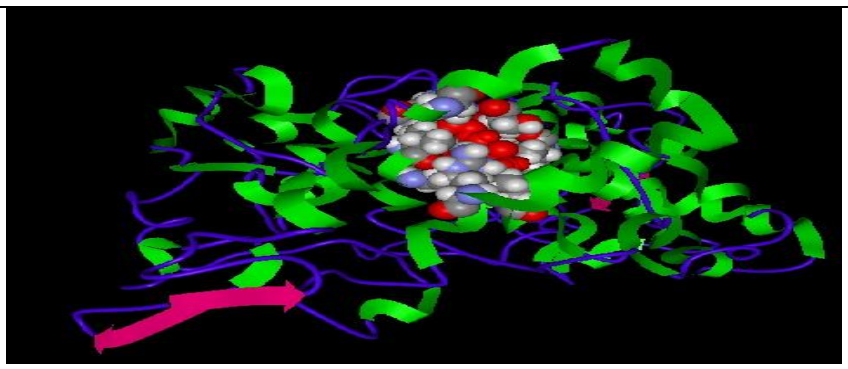
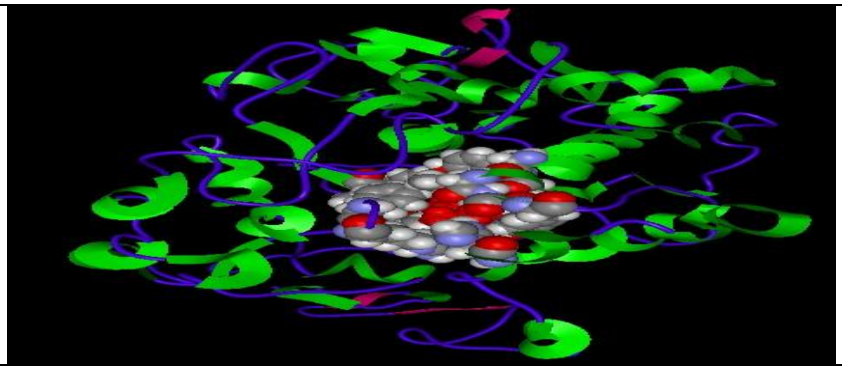
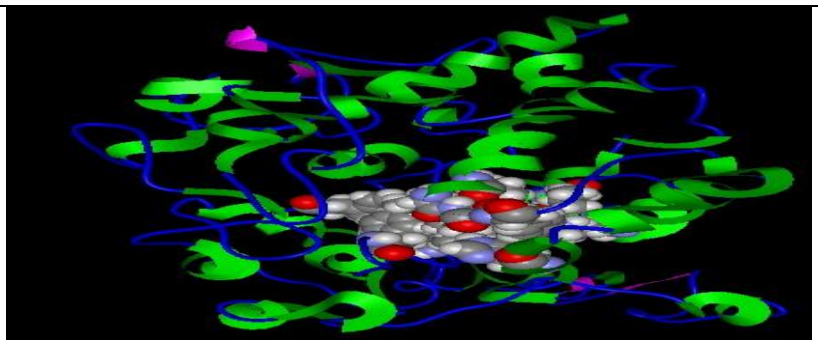
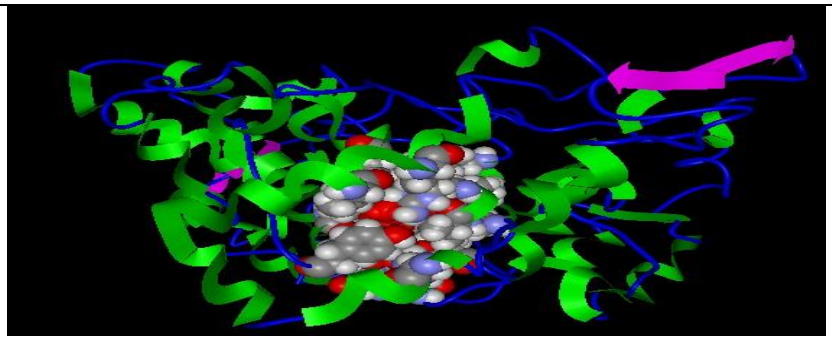
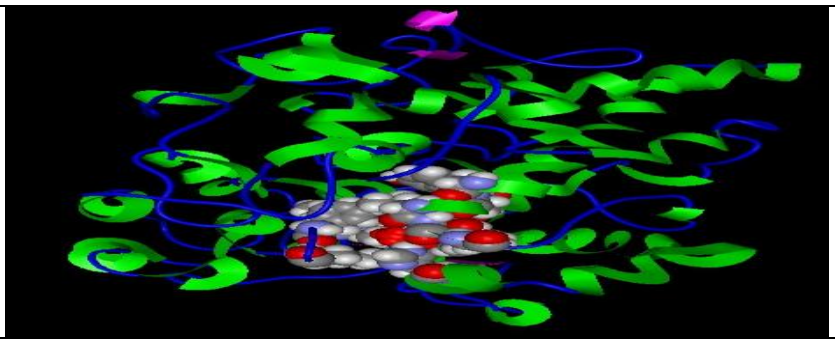


Figure 2. 3-Dimensional [3-D] presentation of Docking studies of ligand molecules with 6COX.

Ligand	Docking structures of ligand molecules with COX
Reference Ligand [SC-558 inhibitor]	
Phyltetralin	
Isonirtetralin	
Isolintetalin	
Linalool	

Securinine	
Phyllanthine	
4methoxy-nor-securinine	
Gallic acid	
Limonene	

Nor- securinine	
Niranthin	
p-cymene	

DISCUSSION

The present study describes COX-2 screening studies of the reported phytoconstituents from *P. amarus* by applying molecular docking technique for the first time. SC-558 used as a reference ligand is a diaryl heterocyclic inhibitor with a 1,900-fold selectivity for COX-2 over COX-1. While the Thirty nine molecular structures of phytocompounds present in the phyllanthus species have affinity to the COX system that have been docked and their obtained scores identify that these ligands bind with the similar orientation as observed with SC-558 for COX-2.

Kassuya et al have investigated the anti-allodynic and anti-oedematogenic effects of the hexanic extract, lignan-rich fraction and purified lignans from Phyllanthus *amarus* in the inflammatory and neuropathic models of nociception [13]. Kiemer et al, have investigated potential anti-inflammatory properties of standardized *P. amarus* extracts concerning a potential influence of P. amarus on endotoxin induced nitric oxide synthase (iNOS), cyclooxygenase (COX-2), and cytokine production in vivo and in vitro [14].

We have postulated from our observations that the anti-inflammatory activity of Phyllanthus species could be due to its membrane stabilizing action and inhibition of protein denaturation [6-7]. We have examined some of the mechanisms underlying the analgesic effects of the extracts of *Phyllanthus amarus* & *Phyllanthus fraternus* for their central and peripheral activities [8]. In addition, we also investigated the action of both the species against capsaicin-mediated pain and formalin-induced nociception in mice [9]. The data of our study also suggest that their antinociceptive action is unrelated to central depressor action, interaction with α-adrenergic receptor or interaction with L-arginine nitric oxide pathway [9].

Molecular Docking of reported molecules from *P. amarus* like such as Phyltetralin, Isonirtetralin, Isolintetalin, Linalool, Securinine, Phyllanthine, 4methoxy-nor-securinine, Gallic acid, Limonene, Nor have securinine, Niranthin and p-cymene clearly reflected the binding of these molecules with COX-2 receptor model. These compounds showed better binding features in terms of energy scores in comparison to the reference ligand. These compounds could be considered as good COX-2 inhibitors. Thus, the significance of these plant derived medicinal compounds is highlighted by using docking analysis.

The current study dealt with the in silico investigation for alternative potent COX-2 inhibitor with minimum side effects. The simulation reflects that the molecules of *P. amarus* are being more effectively

interacting with COX-2, which is evident by the dock scores and also these are smaller in size than the existing COX-2 inhibitors. The results indicate that the above mentioned molecules are predicted to be bioactive. Together, it can be predicted that the COX inhibitors in *P. amarus* might be responsible, at least in part, for the anti-inflammatory activity of this traditional medicine.

Although a comprehensive analysis of the available data would go far beyond the scope of the present work, the actual basic idea of the work coincides with the hypotheses which can summarize the potential of various phyllanthus species, their standardized extracts and isolated compounds can act as a strategy for treatment of inflammatory hyperalgesia.

CONCLUSION

Twelve phyto-compounds showed potent COX-2 inhibitory activity. These findings could be exploited for future ligand design in order to obtain novel derivatives as selective COX-2 inhibitor.

ACKNOWLEDGEMENTS

The authors are thankful to UGC [University Grants Commission] for providing fellowship under RGNFS [Grant reference no.- F. 16-1917(SC)/2010 (SA-III)] to ARC for his doctoral studies.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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