International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 1161-1166
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

IN-SILICO STUDY AND DRUG TARGET FOR ANTI-INFLAMMATORY STUDY WITH COX-2 RECEPTOR (1PXX) FROM ELUCIDATED COMPOUNDS OF *MUNTINGIA CALABURA*

Pavan Kumar Chadalawada*, Pooja.Boyapati and Madhuri Latha Thadanki
Southern Institute of Medical Sciences, Department of pharmacy, Mangaldas nagar, Guntur-522001.

***Corresponding Author Email: palispavan@gmail.com**

ABSTRACT

In the present study, we have done molecular docking using Molegro Virtual Docker (MVD) on isolated moieties of ethanolic extract of Muntingia calabura and Diclofenac to compare between in silico and in vitro studies. Based on the studies, the main compounds of the ethanolic extract of Muntingia calabura are 8-hydroxy-6-methoxyflavone, 3,7-dimethoxy-5-hydroxy flavone, 2',4'-dihydroxychalcone, galangin, 3-hydroxy-1-(3,5-dimethoxy-4-hydroxy phenyl) propan-1-one and p-nitro phenol showed that Moldock Score was found to be -106.874, -102.866, -100.724, -99.7945, -97.6133, -61.9784 respectively. The Re-rank score of above said moieties was found to be -88.1573, -83.1704, -71.7152, -74.79 -86.7314, -79.8167, -52.5166 respectively. The hydrogen bonds of 8-hydroxy-6-methoxyflavone, 3, 7-dimethoxy-5-hydroxyflavone and diclofenac with amino acid of CoX-2 were Ser 3530, Tyr 3385. All the extracted compounds of Muntingia calabura was further proceeded for in-vitro anti-inflammatory activity by the human red blood cell (HRBC) membrane stabilization method. The percentage of HRBC membrane stabilization was calculated. The isolated compounds showed mild to moderate in-vitro anti-inflammatory activity. 8-hydroxy-6-methoxyflavone derived from ethanolic fraction was found to be most active among the series of isolates in comparison with diclofenac.

KEY WORDS

Muntingia calabura, anti-inflammatory activity, human red blood cell (HRBC), Molegro Virtual Docker (MVD).

INTRODUCTION

Inflammation is a critical response to dangerous potential signals, which result in damage to organs in our body. The immune system turns against the bodies' organs in diseases such as rheumatoid arthritis, lupus, ulcerative colitis, Crohn's disease and others. Both societal and economic burdens due to these painful and, in some cases, progressively debilitating conditions can take a toll on people's quality of life and create. The evidence suggests that the number of people suffering from diseases of chronic conditions such as cardiovascular diseases, diabetes, respiratory diseases, autoimmune diseases, and cancers has increased dramatically over the last three decades. Excessive and inappropriate inflammatory activity causes the increasing rates of these illnesses suggest that chronic inflammation, which in turn leads to chronic inflammatory activation in the body, can be a contributing factor in the pathology[1,2,3,4] of these diseases. There is further evidence suggesting that successful treatment of chronic inflammation (i.e., reduction of inflammation) might reduce the risk of cardiovascular disease.

Muntingia calabura, the sole species in the genus *Muntingia*, is a flowering plant native to Philippines, Brazil commonly known as Jamaica cherry, Panama berry, strawberry tree. Whole plant of *Muntingia calabura* was shown in Figure-1.

Figure No: 1 Whole plant of *Muntingia calabura*

Plants with medicinal properties are of great importance to the health of individuals and communities in general. Due to some chemical substances showing medicinal values in plants that produce a definite physiological action on the human body. The plant parts like leaves are rich in flavanoidal compounds like flavones, flavanones, flavans, and biflavans as the major constituents, possessing antidiabetic and cytotoxic activities [5, 6, 7,8,9,10,11] The leaves are used to treat headache, cold, gastric ulcer, or to attenuate the prostate gland swelling [12] [13] [14]. Other parts like roots, flowers possess medicinal values, used as emmenagogue, abortifacient, antidyspeptic, antispasmodic, diaphoretic, and to treat headaches, dyspepsia, and spasm. The whole plant is believed to possess medicinal value, various pharmacological activities such as cytotoxic, antinociceptive, antiulcer, anti-tumor, anti-inflammatory, antidiabetic etc. have been reported [15] Therefore, the main objective of this study is to find the bioactive moiety of plant *Muntingia calabura* in some solvents which could serve as an active isolate for the development of new anti-inflammatory agents.

MATERIALS AND TOOLS USED:

Chemicals: Ethanol, Phosphate buffer, *Alsever solution* (2% dextrose, 0.8% sodium citrate, 0.5% Citric acid and 0.42% NaCl), Hypo saline (0.42% NaCl), Human red blood cell (HRBC), Diclofenac used as reference standard for *in-vitro* antiinflammatory activity study.

In our present study we collected the authenticated plant from Guntur district. Plant material:The leaves of *Muntingia calabura* were collected from the pedakakani region in guntur district Andara Pradesh and taxonomically identified and authenticated from Dr. S. Sandhya Rani, Sims college of Life Sciences, Mangaldas Nagar, Guntur, A.P

Preparation of ethanolic extract of *Muntingia calabura* leaves: Fresh plant material was collected in bulk, washed under running tap water to remove adhering material, dried under shade and pulverized in a mechanical grinder. The coarse powder was sent through sieve no. 60#. Care was taken to select healthy plant parts.

Our present study used biological data bases like drug bank, PDB (protein data bank), Molegro Virtual Docker (MVD) and Chem3D Ultra 8.0.

EXPERIMENTAL METHODS

Docking Protocol:

Preparation of Ligand

To investigate the detailed intermolecular interactions between the analogues we carried out docking of 6 molecules using Molegro Virtual Docker (MVD), Ligand structures were drawn and optimized using MM2 force field by using Chem3D Ultra 8.0 and saved in mol format.

The ligands are imported to the workspace and preparation of them is done. The docking scores of the active constituents are compared against the standard drugs (DICLOFENAC [16]) obtained from the drug bank in. mol format.

Preparation of Enzyme

The target for docking studies is selected as α - Amylase. Docking analysis is done by initially selecting the target for the anti-inflammatory activity and followed by obtaining the 3D structure of CoX-2 receptor (1PXX) from protein data bank in a format of pdb (.pdb). Poor or missing assignments of explicit hydrogens found in PDB files and the PDB file format cannot accommodate bond order information. Therefore, proper bonds, bond orders, hybridization and charges were assigned using the MVD. The potential binding sites of both the targets were calculated using the built-in cavity detection algorithm implemented in MVD. The subset region of 25.0 Angstroms around the active side cleft was studied by search space of the simulation exploited in the docking studies. Water molecules are also taken in to consideration and the replaceable water molecules were given a score of 0.50.

Molegro Virtual Docker's docking search algorithms and scoring functions

Ligand docking studies were performed by which has recently been introduced and gained attention among medicinal chemists. MVD is a fast and flexible docking

program that gives the most likely conformation of ligand binding to a macromolecule. Mol-Dock software is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm. It has an interactive optimization technique inspired by Darwinian Evolution Theory (Evolutionary Algorithms - EA), in which a population of individuals is exposed to competitive selection that weeds out poor solutions. Recombination and mutation are used to generate new solutions. The scoring function of Mol-Dock is based on the Piecewise Linear Potential (PLP), which is a simplified potential whose parameters are fit to protein-ligand structures and a binding data scoring function that is further extended in GEMDOCK (Generic Evolutionary Method for molecular DOCK) with new hydrogen bonding term and charge schemes.

Mol-Dock Optimizer

In MVD, selected parameters were used for the guided differential evolution algorithm: number of runs =5 by checking constrain poses to cavity option), population size=50, maximum interactions =2000, cross over rate=0.9, and scaling factor=0.5. A^{o} variance-based termination scheme was selected rather than root mean square deviation (RMSD). To ensure the most suitable binding mode in the binding cavity, Pose clustering was employed, which lead to multiple binding modes.

Parameters for scoring functions

Mol-Dock score

They ignore atoms far away from the binding site by using ignore-distant-atoms option. Additionally, hydrogen bond directionality was said to check whether hydrogen bonding between potential donors and acceptors can occur. The binding site on the protein was defined as extending in X, Y & Z directions around the selected cavity with a radius of 25 Angstroms.

***In-vitro* Anti-Inflammatory Activity:**

Human Red Blood Cell (HRBC) Membrane Stabilization Method [16]

The main content of the ethanolic extracts of *Muntingia calabura* were studied for the in-vitro anti-inflammatory activity by HRBC Membrane Stabilization Method. The active constituents of ethanolic extract of *Muntingia calabura* of 50 and 100µg concentration solutions were prepared. The drug was initially dissolved in dimethyl sulfoxide (DMSO)

Experimental Procedure

The human red blood cell (HRBC) membrane stabilization method

A healthy human volunteer blood was collected from who had not taken any NSAIDS for 2 weeks prior to the experiment and it is mixed with an equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. Isosaline was used for washing packed cells and a 10% suspension was prepared. Various concentrations of extracts were prepared (200 and 400 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hypo saline, 0.5 ml of HRBC suspension were added and incubated at 37^{0}C for 30 min and centrifuged at 3,000 rpm for 20 min. The supernatant solution content of haemoglobin was spectrophotometrically at 560 nm. Diclofenac (50 and 100 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. The HRBC membrane stabilization percentage or protection was calculated by using the following Formula,

Formula = 100 - (Abs of test solution - Abs of product control / Abs of test control) *100

RESULTS AND DISCUSSION:

Anti-inflammatory Docking studies of main content of the ethanol extract of *Muntingia calabura*

Ligand	Mol-Dock Score	Re-rank Score	H-Bond
8-hydroxy-6-methoxyflavone	-106.874	-88.1573	-2.02954
3,7-dimethoxy-5-hydroxyflavone	-102.866	-83.1704	-4.09505
DICLOFENAC	-100.792	-71.7152	-2.49836
2',4'-dihydroxychalcone	-100.724	-74.7938	-6.87869
Galangin	-99.7945	-86.7314	-5.07578
3-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl) propan-1-one	-97.6133	-79.8167	-5
p-nitrophenol	-61.9784	-52.5166	0

Binding interactions of ligands along with standard

H-Bonding interactions	Ligand
Ser 3530, Tyr 3385	8-hydroxy-6-methoxyflavone
Ser 3530, Tyr3385	3,7-dimethoxy-5-hydroxyflavone
Ser 3530, Tyr3385	DICLOFENAC

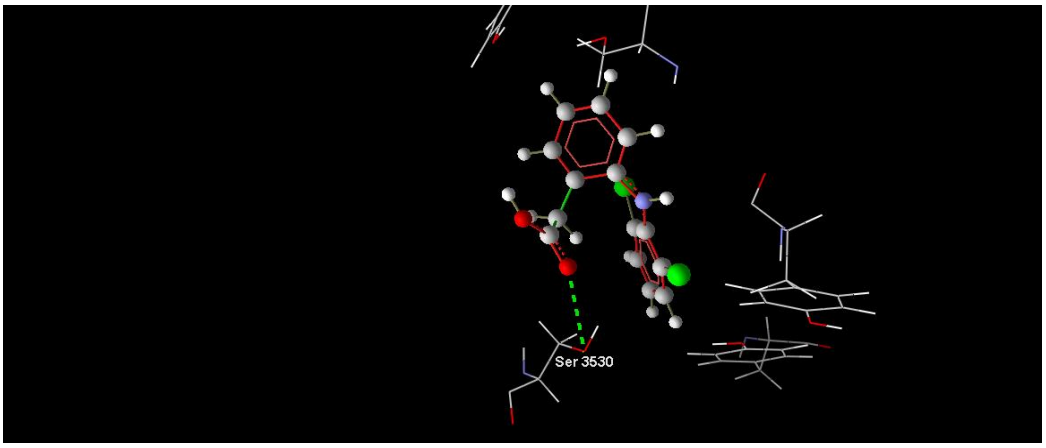

Fig No.2 MVD generated conformations for localization of diclofenac co-crystallized upon docking

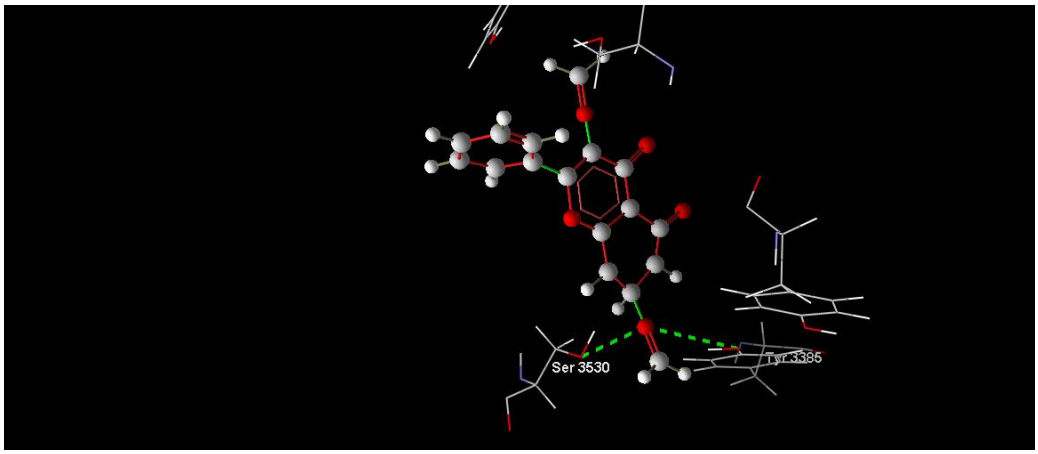

Fig No.3 1PXX shows the bonding of the molecules with the 3,7-dimethoxy-5-hydroxyflavone

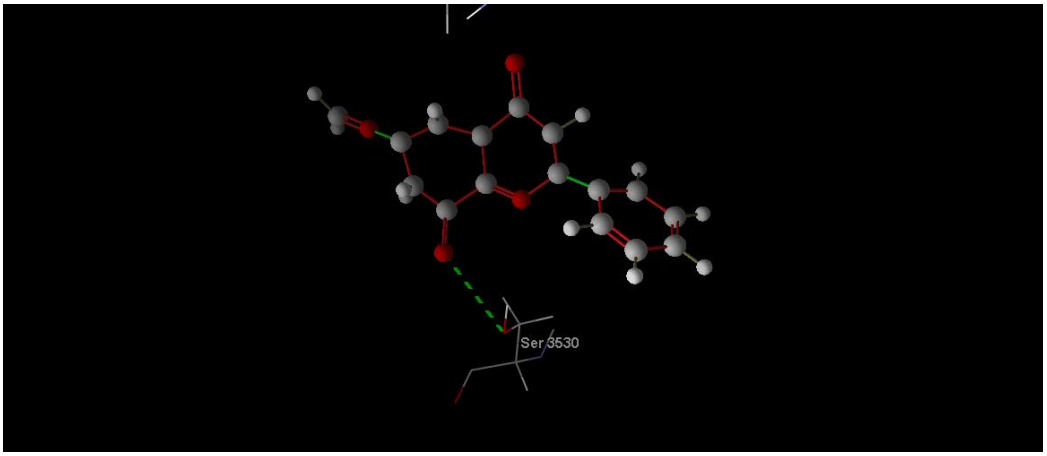

Fig no.4 1PXX shows the bonding of the molecules with the 8-hydroxy-6-methoxyflavone

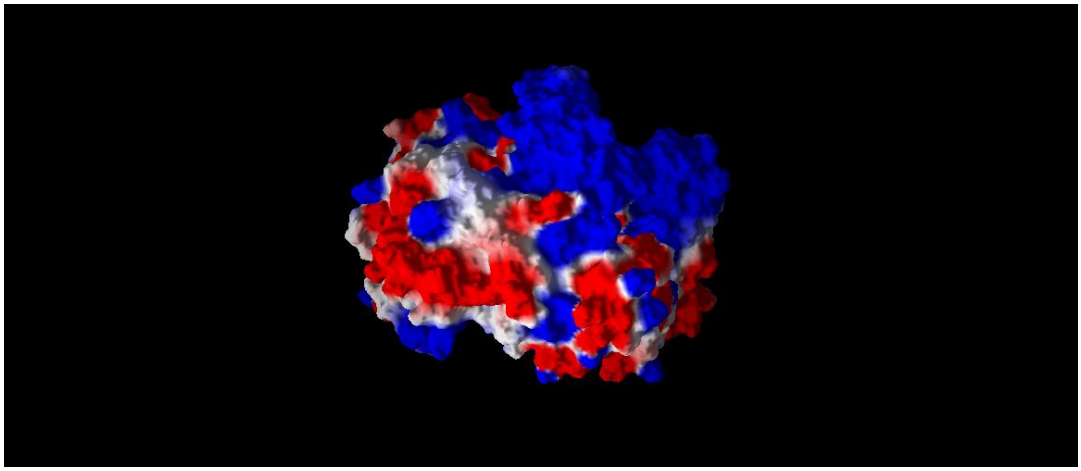

Fig no.5 Protein with free surface in 1PXX CoX-2 inhibitor

In-vitro* anti-inflammatory activity of main content of the ethanol extract of *Muntingia calabura

S. No	Name of the moiety	% of Inhibition Doses (µg/ml)	
		50	100
1	8-hydroxy-6-methoxyflavone	63.9	79.2
2	3,7-dimethoxy-5-hydroxyflavone	63.7	78.1
3	2',4'-dihydroxychalcone	62.1	69.1
4	Galangin	50.2	54.2
5	3-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl) propan-1-one	52.3	57.9
6	p-nitrophenol	41.1	42.2
7	DICLOFENAC (STANDARD)	63.7	78.9

Main constituents of ethanolic extract of *Muntingia calabura* was employed for *in-vitro* anti-inflammatory activity. All the extracts showed moderate to superior *in-vitro* anti-inflammatory activity. Percentage (%) of Inhibition lysosomal enzymes or lysosomal membrane stabilization of 8-hydroxy-6-methoxyflavone (89.9%) is greater than diclofenac (89.1%) and found to be most active among the series of extracts., Ethanolic extract of 3,7-dimethoxy-5-hydroxy flavone (79.5%),2',4'-dihydroxychalcone(73.4%),3-hydroxy-1-(3,5dimethoxy-4-hydroxyphenyl)propan-1-one (66.2%) and p-nitrophenol (58.7) respectively.

CONCLUSION:

The main content of the ethanolic extract of *Muntingia calabura* were docked by MVD software and was co-related with *in-vitro* anti-inflammatory activity by using diclofenac as standard. 8-hydroxy-6-methoxy flavone has better binding sites, its Moldock score and Rerank scores found to be -106.874 and -88.1573 respectively. Hence, further investigations are necessary to develop new lead compound for the treatment of inflammation.

CONFLICTS OF INTEREST

Nil

REFERENCE

1) El-Gabalawy H, Guenther L C and Bernstein C N. Epidemiology of immune-mediated inflammatory diseases: incidence, prevalence, natural history, and comorbidities. *The Journal of rheum Supplement*, 2010; 85:2-10.
2) Kolb H and Mandrup-Poulsen T. The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. *Diabetologia*, 2010; 53(1):10-20.
3) Miller A H, Malteic V, Raison C.L. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry*. 2009; 65(9):732-41.
4) Mallbris L, Akre O, Granath F, Yin L, Lindelöf B, Ekbom A, et al. Increased risk for cardiovascular mortality in psoriasis inpatients but not in outpatients. *Eur J Epidemiol* 2004; 19:225–30.
5) Lim Dr T. K. "*Muntingia calabura*". Edible Medicinal and Non-Medicinal Plants. *Springer*. 2012; 486–492.

6) Smith Jr C.E. In: Flora of Panama, part VI". Annals of the Missouri Botanical Garden. Missouri Botanical Garden. *Elaeocarpaceae*. 1965; 52 (4): 494–495.
7) Nelson G. The Trees of Florida: A Reference and Field Guide. *Pineapple Press Inc*. 2010; 268–269.
8) Fosberg FR, Sachet M-H, Oliver RL. A geographical checklist of the Micronesian dicotyledonae. *Micronesica*. 1979; 15:1-295.
9) Perez GRM, Zavala SMA, Perez GC. Antidiabetic effect of compounds isolated from plants. *Phytomedicine*. 1998; 5: 55-75.
10) Nshimo CM, Pezzuto JM, Kinghorn AD, Farnsworth NR.Cytotoxic constituents of *Muntingia calabura* leaves and stems Thailand. *Int J of Pharmacognosy*. 1993; 31: 77.
11) Morton JF. "Jamaica cherry," in Fruits of Warm Climates, JF Morton, Ed., JFMorton, Miami, Fla, USA, 1987; 65–69.

Received:09.08.18, Accepted:10.09.18, Published:01.10.2018

12) Jensen M. "Trees commonly cultivated in South East Asia: an illustrated field guide," in FAO Corporate Document Repository, Craftsman Press, Bangkok, Thailand, 2nd edition, 1999.
13) Verheij EWM. Coronel RE. Plant Resources of South East Asia: Edible Fruits and Nuts, PROSEA., 1992; Bogor, Indonesia, 2nd edition.
14) Mahmood N. D, Nasir N. L. M, Rofiee M. S, Tohid S. M, Ching S. M, Teh L. K, Zakaria, Z. A. *Muntingia calabura*: a review of its traditional uses, chemical properties, and pharmacological observations. *Pharmaceutical biology*. (2014); 52(12): 1598-1623.
15) Hurley JV. Acute inflammation. Edinburgh, London: Churchill Livingstone. 1972.
16) Ramana H, Vasanthi R, Pavan Kumar CH. Synthesis, characterization and biological evaluation of novel isatin derivatives. *World J Pharm Res*, 2015; 4(09):905-915.

***Corresponding Author:**
Pavan Kumar Chadalawada*
Email: palispavan@gmail.com