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Hypothesis

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Insights from the docking analysis of biologically active compounds from plant *Litsea* Genus as potential COX-2 inhibitors

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Abstract:
Litsea spp of Laural family are traditionally used as herbal medicine for treating inflammation including gastroenterologia, oedema and rheumatic arthritis. Therefore, it is of interest to investigate and understand the molecular principles for such actions. Here, we have illustrated the binding of thirteen *Litsea* derived biologically active compounds against the inflammation associated target COX (cyclo-oxygenase) -2 enzymes. We compared the binding information of these compounds with a selected number of already known COX-2 inhibitors. The comparison reflected that some of these compounds such as linderol, catechin, 6'-hydroxy-2',3',4' -trimethoxy-chalcone and litseaone have better or equivalent binding features compared to already known inhibitory compounds namely celecoxib, acetylsalicylic acid, rofecoxib. Therefore, all these small compounds reported from plant *Litsea* spp were found to possess potential medicinal values with anti-inflammatory properties.

Keywords: COX-2 inhibitor, *Litsea* plant, Inflammation, Docking

Background:
The genus *Litsea* includes 200 to 400 species across the world. *Litsea glutinosa* (L.), *Litsea cubeba* (L.) and *Litsea verticillata* (L.) are some of the important species of the *Litsea* popularly used as a traditional herbal remedy for various aliments including anti-inflammatory agent **[1-2].** The species *L. cubeba* has been reported as an anti-inflammatory plant material **[1].** The species *L. rubescene* and *L. peduncalt* have long been used in traditional Chinese medicine for the treatment of gastroenterologia, oedema and rheumatic arthritis **[3].** Most *Litsea* plants contain alkaloids, flavonoids **[4],** terpenes **[5],** lactone **[6]** and volatile oils constitution **[7].** Several biologically active compounds were isolated and their structures have been elucidated by many researchers **[3, 8]**. In this current work, we illustrated the importance of these compounds as anti-inflammatory molecules by studying their binding potential with the COX (cyclo-oxygenase) enzymes. The COX enzyme exits in two isoforms (COX-1 and COX-2). The COX-1 enzyme protects the stomach lining from corrosive acids and digestive chemicals **[8].** The COX-2 enzyme binds to arachidonic acid which causes pain and inflammation **[9].** The known inhibitors such as celecoxib, robecoxib, valdecoxib of COX-2 are of the category of non-steroidal anti-inflammatory drugs (NSAID) with observed side effects ranging from pain, nausea, indigestion and lack of anti-

thrombotic activity **[1] [8]**. Thus, the need to indentify new yet better compounds to inhibit COX-2 is critical. Therefore, it is of interest to investigate the potential chemical phenomenon responsible for binding of *Litsea* derived molecules to COX-2. Here, in this communication we have presented the interaction analysis of these biologically active molecules with the COX-2 enzyme using molecular docking.

Methodology:

Litsea derived compounds and structures

Compounds selected for this experiments are; (a) 6'hydroxy- 2', 3', 4'-trimethoxy-chalcone **[10],** (b) alpinetin **[3],** (c) catechin **[8],** (d) kaempferol **[8],** (e) linderol A **[8],** (f) liteseaone A **[8],** (g) liteseaone B **[8]**, (h) flavokawin B **[3],** (i) litseaverticillol A [11], (j) pinocembrin **[3]** and (k) quercetin **[3].** These structures were drawn, and optimized using RMS gradient of 0.1 by geometry optimization. The MM2 module of ChemBio3D suite was used for optimization. The physiochemical properties of these compounds were predicted in Molsoft Browser version 3.6. **Table 1 (see supplementary material).**

Known COX-2 inhibitors

The structures of known COX-2 inhibitors such as rofecoxib, valdecoxib, celecoxib, lumiracoxib, acetylsalicylic acid (asprin), ibuprofen, nabumetone and naproxen were downloaded from DrugBank **[11].** The physiochemical properties of these inhibitors were shown in **Table 2 (see supplementary material).**

COX-2 enzyme protein structure

The 3-dimensional 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 with an inhibitor SC-558 **[12].**

Docking tool and algorithm

Molecular docking was completed using Molegro Virtual Docker (MVD) 4.02 (Lic. to CSIR-NEIST, No.3a5d-34b3). MVD uses a differential evolution algorithm. The solution to the function is the sum of intermolecular interaction energy between protein and ligand with the intra-molecular interaction energy of the ligand. The docking energy scoring function is based on a modified Piecewise linear potential (PLP) with new hydrogen bonding and electrostatics terms included.

Receptor cavity identification

The pockets or cavities (5 in number) in the COX-2 structure were identified using MVD. The minimum cavity volume is 10 $Å^3$ with a probe size of 1.20 and a grid resolution of 0.80. The maximum number of ray checks is set at 16. The side chains flexibility was relaxed by adjusting the strength and tolerance of the receptor in their nearest residues of respective cavities. The physical descriptions of the predicted cavities are given in **Table 3 (see supplementary material)**.

Molecular Docking of compounds with COX-2

The molecular docking was performed for all the small molecules (11 *Litsea* derived and 8 known COX-2 drug inhibitors) with the four (4) best predicted cavities of the receptor COX-2 model. The MolDock Score (GRID) function was used with a grid resolution (Å) of 0.30 and a binding site radius of 12 Å with respect to the origin of the respective cavities. The "MolDock SE" searching algorithm 10 runs using a maximum of 1500 iterations with a total population size of 50 was used. The energy threshold used for the minimized final orientation is 100. The Simplex evaluation with 300 maximum steps of neighbour distance factor 1 was completed.

Analysis of docked protein-ligand complex structures

The best orientation for the ligand-protein complexes were analysed, H Bonds were identified and labelled. The ligand energy was inspected and analysed using Rerank scoring (linear combination of E-inter (steric, vander Waals, H bonding and electrostatics) and E-intra (torsion, sp2-sp2, hydrogen bonding, van der Waals and electrostatics).

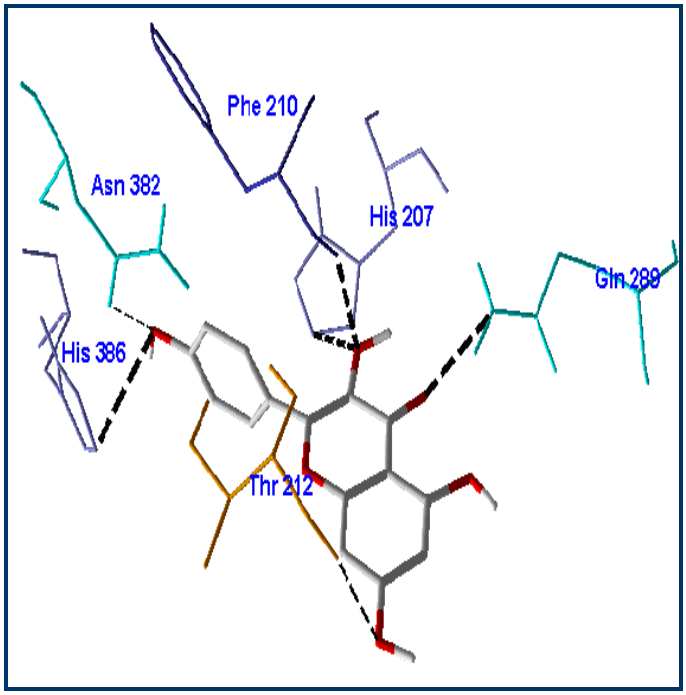

Figure 1: Kaempferol interaction with receptor, showing H Bond Interaction in black dotted line.

Discussion:

Cyclooxygenase (COX), also known as Prostaglandin (PG) H Synthase, catalyzes the conversion of arachidonic acid to the PG endoperoxidases. Two isoforms of COX are designated COX-1 and COX-2. These two isoforms have similar primary protein structure homology of 60%. COX-2 is an inducible isoform that is found and expressed mainly in inflammatory and immume cells. At the site of inflammation, COX-2 is responsible for the generation of the hyperalgesic and proinflammatory Prostaglandins. The drugs used for the inhibition of inflammations are called as COX-2 inhibitor. COX-2 inhibiting drugs were developed to provide the anti-inflammatory effects of NSAIDs with a reduced risk of gastrointestinal bleeding. Concern have found that COX-2 inhibitors could have cardiovascular and renal adverse effects similar to those of nonselective NSAIDs, such as raised systemic vascular resistance and decreased renal perfusion. The anti-inflammatory drugs alone cause over 16,500 deaths and over 103,000 hospilization per year in the United State of America. Recent research reveals that present COX-2 inhibitors have shown Hematological, Cardiovascular, Central Nervous side effects. Gastrointestinal Effects associated with NSAID therapy are common. Approximately 30-60% of NSAID users experience

some abdominal discomfort or dyspepsia. The current study dealt with the *in silico* investigation for alternative potent COX-2 inhibitor also with minimum side effects. The simulation reflects that Linderol, one of the molecules of *Litsea* being more effectively interacting, which is evident by the rerank score, and is smaller than the existing COX-2 inhibitors. Beside, linderol, most other compounds of *litsea* also analysed to be more potent to become a drug molecule inhibiting COX-2 inhibitor. The interaction was analyzed by the formation of the hydrogen bond, where it reflected that linderol forms four hydrogen bonds on interaction with best cavity. These hydrogen bonds are formed with residues Tyr385, Trp387, His388 and Thr206. The docked poses were analyzed on the basis of Rerank score followed by MolDock and HBond (Hydrogen Bond Interaction) Score. In the best Cavity, most of the *litsea* molecules showed better score against the receptor then that of existing COX-2 inhibitors shown in **Table 4 (see supplementary material)**. Kaempferol, on the other hand a molecule of *Litsea,* although having a low rerank score than linderol shows better stability by formation of seven hydrogen bond in the same cavity **(Figure 1).** However, this interaction might result into permanent inhibition of the COX-2 until the protein molecule gets degraded. The other compounds of *Litsea,* Catechin, Pinacembrin and Litseaverticillol also showed a better docking simulation and interaction analysis, than the existing COX-2 inhibitor such as Celecoxib, Lumiracoxib which is evident from the rerank score and interaction analysis. The molecule 6'-hydroxy-2', 3', 4'-trimethoxy-chalcone scored moderate range of dock result, but six different interactions were noticed. In the cavity 2 of the receptor molecule most of the *litsea* compounds have shown significant docking but poor than that of the best cavity of the receptor. The active compound such as 6'-hydroxy-2',3',4'-trimethoxy-chalcone, Flavokawin, Alpinetin, Litseaone and Lumiracoxib have been predicted to be more potent through comparative analysis in this docking simulation experiment than the existing COX-2 inhibitors. Since the compounds were reported from plant source and the different parts of the *Litsea* are already been used as anti-inflammatory agent as herbal treatment. Hence, these compounds hold lots of promise to develop as an inhibitor of COX-2.

Conclusion:

Molecular Docking of *Litsea* derived selected molecules have clearly reflected the binding of these molecules such as linderol, catechin, 6'-hydroxy-2', 3', 4'- trimethoxy-chalcone and litseaone against COX-2 receptor Fmodel. These compounds showed better binding features in terms of energy scores in comparison to already known inhibitors such as e celecoxib, acetylsalicylic acid and rofecoxib. Thus, the significance of these plant derived medicinal compounds is highlighted to using docking analysis.

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Supplementary material:

Table 1: *Litsea* Compound showing good docking score

SN	Structure	Formula	Mol. Wt	HBA	HBD	RotB	ClogP
1	6'-hydroxy-2',3',4'-trimethoxy-chalcone	$C_{18}H_{18}O_5$	314.33	5	1	6	3.241
2	Catechin	$C_{15}H_{14}O_6$	290.079	6	5	1	0.533
3	Kaempferol	$C_{15}H_{10}O_6$	286.047	6	4	1	2.099
4	Linderol A	$C_{26}H_{30}O_5$	422.210	5	2	6	6.097

NB: HBA: Hydrogen Bond Acceptor, HBD: Hydrogen Bond Donor, MW: Molecular Weight, Clog*P:* Calculated log P, NRB: Number of rotatable bond**.**

Table 2: Physiochemical parameters of COX-2 inhibitor

SN	Structure	Formula	Mol. Wt	HBA	HBD	RotB	ClogP
1	Rofecoxib	$C_{17}H_{14}O_4S$	314.06	4	0	3	1.798
2	Valdecoxib	$C_{16}H_{14}N_2O_3S$	314.36	4	2	3	1.831
3	Celecoxib	$C_{17}H_{14}F_3N_3O_2S$	381.075	3	2	4	4.372

NB: HBA: Hydrogen Bond Acceptor, HBD: Hydrogen Bond Donor, MW: Molecular Weight, Clog*P:* Calculated log P, NRB: Number of rotatable bond**.**

Table 3: List of Predicted Cavities

Cavity	Position			Volume (Å^3)	Surface (Å^2)
	X	Y	Z		
Cavity 1	23.750	36.555	31.079	377.856	1120
Cavity 2	23.992	23.659	46.989	82.944	243.200
Cavity 3	40.931	14.159	45.073	72.192	281.600

Table 4: The Docking Result (Arranged on the basis of Rerank Score)

SN	**Ligand**	**MolDock Score**	**Rerank Score**	**HBond**
1	Linderol A	-134.01	-87.058	-8.359
2	flavokawin B	-112.03	-87.204	-5.006
3	6'-hydroxy-2',3',4'-trimethoxy-chalcone	-117.372	-85.210	-6.489
4	Catechin	-97.595	-82.886	-12.615
5	Pinocembrin	-86.121	-75.847	-9.413
6	Kaempferol	-83.574	-74.714	-22.350
7	Celecoxib (COX-2 inhibitor)	-99.966	-74.170	-3.524
8	Acetylsalicylic acid (asprine) (COX-2 inhibitor)	-15.995	96.144	6.3162