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AN *IN SILICO* STUDY FOR TWO ANTI-INFLAMMATORY FLAVONOIDS OF *NERIUM OLEANDER* ON PROINFLAMMATORY RECEPTORS

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ABSTRACT: The objective was to predict the binding affinity and energy of established phytochemicals as flavonoids (Kaempferol and Chlorogenic acid) of *Nerium oleander* compared to synthetic drug (Indomethacin) against two proinflammatory cytokine receptors especially interleukins (IL-1β and IL-6) through molecular docking and interaction along with druggability assessment of these small molecules. The software, PyRx (Version 0.8) for the structure-based virtual screening to know receptor-ligand binding affinity and energy. These interleukins as receptors were obtained (PDB IDs: 2NVH and 1P9M) from the European Protein Data Bank (PDBe) and the information on selected flavonoids (phytochemicals) and one synthetic ligand (Indomethacin) were obtained from PubChem database. The prediction of pharmacokinetics, bioavailability and druglikeness for these small molecules was done by using SwissADME online tool. Present computational prediction (molecular docking) indicates that favourable binding energy (Kcal/mol) was observed in Chlorogenic acid (-7.0) followed by Kaempferol (-6.8) while Kaempferol (-7.7) followed by Chlorogenic acid (-7.1) of *N. oleander* when compared to Indomethacin (-6.7) on IL-1β and IL-6 receptors. The pharmacokinetics, bioavailability and druglikeness predictions showed Kaempferol can be suitable drug candidate. Present *in silico* study by using software, the phytoligands Chlorogenic acid and Kaempferol of *N. oleander* may be considered as lead molecules to inhibit the activity of these interleukins while drug candidate may only be considered as Kaempferol, which may prevent inflammation and pain. In future, further functional (*in vivo* and *in vitro*) assay is suggested to validate the present predictive results.

KEYWORDS: *In silico*, Proinflammatory interleukins receptors, virtual screening, Phytoligands, Medicinal plant.

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1. INTRODUCTION

The medicinal tree *Nerium oleander* Linn. is important for its phytochemicals in Chinese folk medicine [1]. This tree shows prevention of different diseases such as antinociceptive, anti-inflammatory, CNS depressant activity, etc. [1, 2, 3]. Among several phytocompounds in crude extracts of this medicinal plant are well-known for experimental study. In experimental study, the flavonoids such as kaempferol, kaempferol 3-O-β-glucopyranoside and chlorogenic acid from *Nerium oleander* are suitable phytochemicals for the prevention of pain and inflammation [4]. There are several inflammatory mediators to make reaction during inflammation. Among these, tumour necrosis factor (TNF-α) and interleukins (IL-1β and IL-6) are pro-inflammatory cytokine, inducible nitric oxide synthase (iNOS) and cyclooxygenases (COX-1 and COX-2), which increase during inflammation and cause several diseases [5, 6, 7, 8, 9]. In this context, several anti-inflammatory phytomedicines are used for pain relief and targeting specific immune and inflammatory pathways by inhibition of TNF-α, IL-1β, IL-6, iNOS, COX-1 and COX-2 [9]. The researchers observed that synthetic drugs have potent side effects when used for the inhibition of above-mentioned target receptors for pain and inflammation [9, 10, 11, 12]. In present research scenario, researchers are showing interest for medicines from plant origin or phytomedicines to target inflammatory mediators without any adverse effects [9]. According to Dragos et al. [9], there are several plant species used to relief pain and prevent inflammation, oxidative stress, etc. during joint disorders. Generally, *in silico* screening, protein or receptor is the main target to detect allosteric or inhibitory activity for drug action. Several compounds or ligands are derived from synthetic compounds or phytocompounds, which show favourable binding affinity and energy for the target. This may help in new and efficient drug development as a lead molecule(s). The virtual screening helps to detect large numbers of drug-like compounds, which are commercially available, computationally screened against targets to recognize the structure and function that are predicted to bind properly in an experiment [13, 14, 15]. Therefore, in recent trend, development of phytomedicines, *in silico* predictions play a vital role in the drug design and discovery process for pharmaceutical research. Moreover, the prediction of pharmacokinetics, bioavailability and druglikeness for small molecules has already been established by using SwissADME online tool [16, 17, 18, 19, 20]. The objective of the present study is to know the binding affinity and energy of established phytochemicals as flavonoids of *Nerium oleander* compared to synthetic medicine (Indomethacin) against two pro-inflammatory cytokine receptors (IL-1β and IL-6) through molecular docking and interaction along

with pharmacokinetics, bioavailability and druglikeness of these small molecules.

2. MATERIALS AND METHODS

The present *in silico* approach is based on molecular docking and interaction to detect the efficacy of selected flavonoids in comparison with synthetic drug (Indomethacin) along with pharmacokinetics, bioavailability and druglikeness of these small molecules.

Selection of receptors

The crystal structure of receptors as interleukins viz. IL-1β (PDB ID: 2NVH) and IL-6 (PDB ID: 1P9M) reposited by Quillin et al. [21] and Boulanger et al. [22] and these were downloaded from the European protein data bank (http://www.ebi.ac.uk/pdbe/). The size of the former receptor is 1.53Å and other is 3.65Å resolutions respectively. These receptors were prepared in AutoDoc tool (version 1.5.6), developed by The Scripps Research Institute [23] and saved as .pdb file for each protein. The three-dimensional (3D) structure of each protein is depicted in Figure 1 A and B.

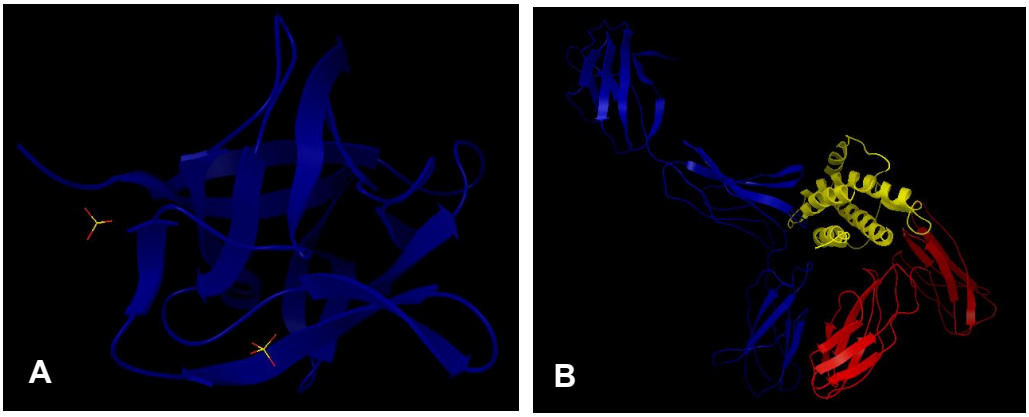


Figure 1: 3-D ribbon structure A = PDB ID: 2NVH chain A blue colour attached with two sulphate molecules; B = PDB ID: 1P9M chain A blue colour, B yellow colour and C red colour

Selection of ligands

The flavonoids viz. kaempferol and chlorogenic acid from *Nerium oleander* and Indomethacin as synthetic ligand were selected as per literature [4]. For all the selected compounds, canonical SMILES (simplified molecular-input line-entry system) string were retrieved from the PubChem database (www.ncbi.nlm.nih.gov/pubchem) and .pdb file and of each ligand was obtained from CORINA online server (http://www.mol-net.de) after inserting SMILES.

Molecular docking and interaction for receptor-ligand binding

The docking was carried out by a virtual screening method through PyRx software (Virtual Screening Tool, Version 0.8) developed by Trott and Olson [24]. All the ligands and receptors files were converted to .pdbqt file format by made macromolecule and ligand in PyRx tool. The docking site on this target protein was expressed by forming a grid box with the size values and centered position values with a grid spacing of 0.375 Å is tabulated in Table 1. The present tool predicts docking result by obtaining energy value for each ligand. Finally, all the 3 ligands were analyzed to detect binding position and energy value. The resultant structural complexes of the individual

ligand/receptor binding were finally observed in AutoDoc tool [23], to determine some specific contacts between the atoms of the test compounds and amino acids of the IL- β and IL-6.

Table 1: Grid size for studied receptor (in Å)

Receptors	Size			Position from center		
	X	Y	Z	X	Y	Z
IL-β	45.2571	39.3277	46.0013	38.4765	13.1963	68.8565
IL-6	-57.0431	175.2921	45.1406	123.0644	103.5786	55.2382

Pharmacokinetics, bioavailability and druglikeness prediction of ligands

The predictive study of pharmacokinetics especially ADME, bioavailability and druglikeness of ligands were done through SwissADME online tool developed by Daina et al. [16]. The tool predicts bioavailability radar as per six physicochemical properties such as lipophilicity, size, polarity, insolubility, flexibility and insaturation to detect druglikeness. The ADME properties viz. passive human gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeation as well as substrate or non-substrate of the permeability glycoprotein (P-gp) as detected positive or negative in the BOILED-Egg model within the tool developed by Daina, and Zoete, [17] and Daina et al. [16]. The lipophilicity estimation (Log p/w) parameters such as iLOGP on free energies of solvation in n-octanol and water calculated by the generalized-born and solvent accessible surface area (GB/SA) model developed by Daina et al. [18], XLOGP3 is an atomistic method including corrective factors and knowledge-based library developed by Cheng et al. [25], WLOGP is an implementation of a purely atomistic method based on the fragmental system of Wildman and Crippen [26], MLOGP is an archetype of topological method relying on a linear relationship with 13 molecular descriptors implemented as per researchers [27, 28] and SILICOS-IT is an hybrid method relying on 27 fragments and 7 topological descriptors as per earlier study (http://silicos-it.be.s3-website-eu-west-1.amazonaws.com/software/filter-it/1.0.2/filter-it.html, accessed June 2016). The Lipinski (Pfizer) filter is the pioneer rule-of-five was implemented in the tool from Lipinski et al. [29] and incorporated in this tool for the prediction of druglikeness [16]. The bioavailability radar for oral bioavailability prediction as per different physico-chemical parameters was developed by SwissADME tool [16]. The ranges of each parameter was mentioned as LIPO = lipophilicity as -0.7 < XLOGP3 < +5.0; SIZE = size as molecular weight 150gm/mol < MV < 500gm/mol; POLAR = polarity as 20Å² < TPSA (topological polar surface area) < 130Å²; INSOLU = insoluble in water by log S scale 0 < Logs (ESOL) < 6; INSATU = insaturation or saturation as per fraction of carbons in the sp3 hybridization 0.3 < Fraction Csp3 < 1 and FLEX = flexibility as per rotatable bonds 0 < No. rotable bonds < 9 [16].

3. RESULTS AND DISCUSSION

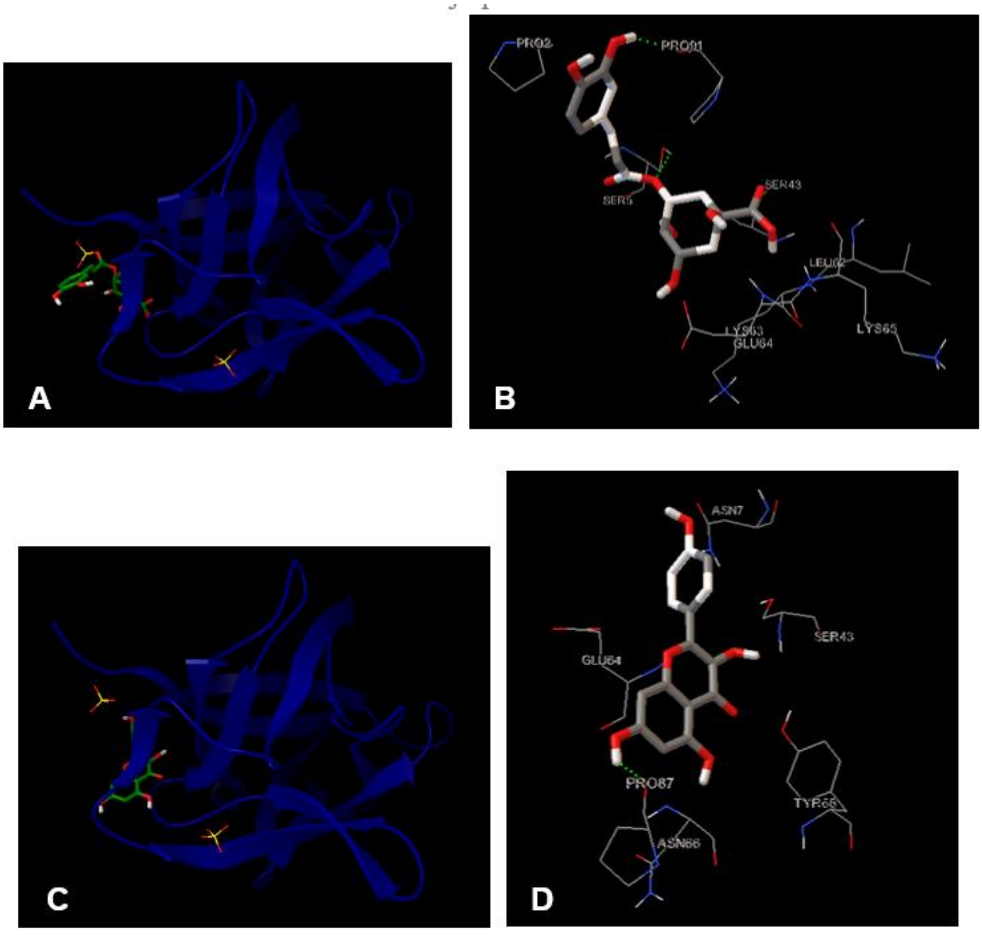
The present study was done with two proinflammatory cytokines as interleukins (IL-1β and IL-6) receptors are well-established inflammatory markers. The docking was done to detect active binding site for these targets. Present computational prediction (molecular docking) indicates that favourable binding energy (Kcal/mol) was observed in chlorogenic acid (-7.0) followed by Kaempferol (-6.8) of *N. oleander* when compared to Indomethacin (-6.7) on IL-1β receptor (Table 2). On the other hand, favourable binding energy (Kcal/mol) was observed in Kaempferol (-7.7) followed by chlorogenic acid (-7.1) of *N. oleander* when compared to Indomethacin (-6.7) on IL-6 receptor (Table 3). The interaction study of flavonoids such as Chlorogenic acid with IL-1β receptor it was observed that contact residues were PRO2, LYS63, SER43, LEU52, LYS65 and GLU34 along with two hydrogen bond contacts and residues were PRO91 and SER5 while Kaempferol with IL-1β receptor showed that contact residues were SER43, TYR68, ASN66, ASN7 and GLU64 along with one hydrogen bond contact and residue was PRO87 (Figure 2 A-D). In case of Indomethacin interaction, no hydrogen bonding was found, and contact residues were PRO91, TYR68, LEU62, LYS65 and GLU64 observed (Figure 2 E-F). The interaction study of flavonoids such as Kaempferol with IL-6 receptor showed that contact residues were ARG154, LYS153, PHE136, THR130 and GLU129 along with one hydrogen bond contact and residue was ALA152 while Chlorogenic acid with IL-6 receptor, it was observed that contact residues were ARG154, THR130, PHE136, ARG128 and GLY127 along with one hydrogen bond contact and residue was THR134 (Figure 3 A-D). In case of Indomethacin interaction, no hydrogen bonding was found, and contact residues viz. LYS252, LYS228, SER229, LEU254, ARG30, GLU278 and ARG233 were observed (Figure 3 E-F).

Table 2: Interaction profiles of selected ligands as flavonoids from *N. oleander* along with synthetic drug after docking against IL-1β receptor

Sl. No.	Ligands	Binding energy value (Kcal/mol)	Hydrogen bond nos. and contacts	Contact residues
Phytochemicals				
1.	Chlorogenic acid	-7.0	2 nos. and PRO91 & SER5	PRO2, LYS63, SER43, LEU52, LYS65 & GLU34
2.	Kaempferol	-6.8	1 no and PRO87	SER43, TYR68, ASN66, ASN7 & GLU64
Synthetic chemical				
3.	Indomethacin	-6.7	None	PRO91, TYR68, LEU62, LYS65 & GLU64

Table 3: Interaction profiles of selected ligands as flavonoids from *N. oleander* along with synthetic drug after docking against IL-6 receptor

Sl. No.	Ligands	Binding energy value (Kcal/mol)	Hydrogen bond contacts	Contact residues
Phytochemicals				
1.	Kaempferol	-7.7	1 no. and ALA152	ARG154, LYS153, PHE136, THR130 & GLU129
2.	Chlorogenic acid	-7.1	1 no. and THR134	ARG154, THR130, PHE136, ARG128 & GLY127
Synthetic chemical				
3.	Indomethacin	-7.6	None	LYS252, LYS228, SER229, LEU254, ARG30, GLU278 & ARG233



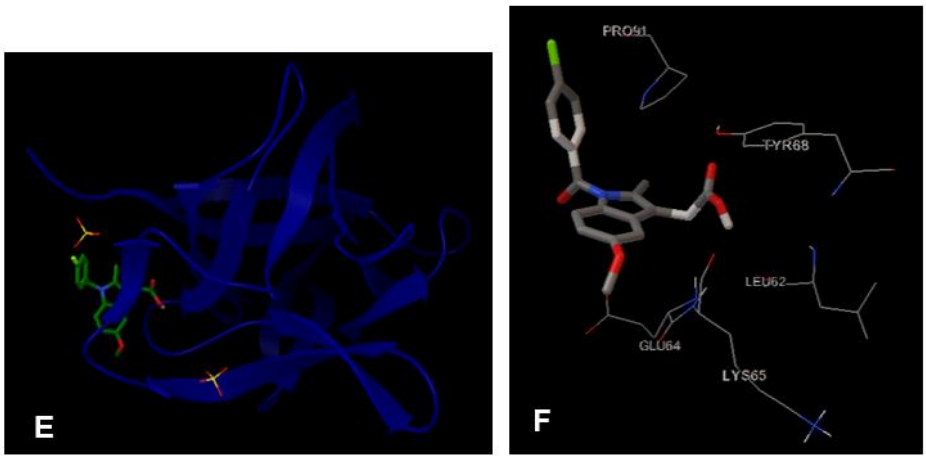
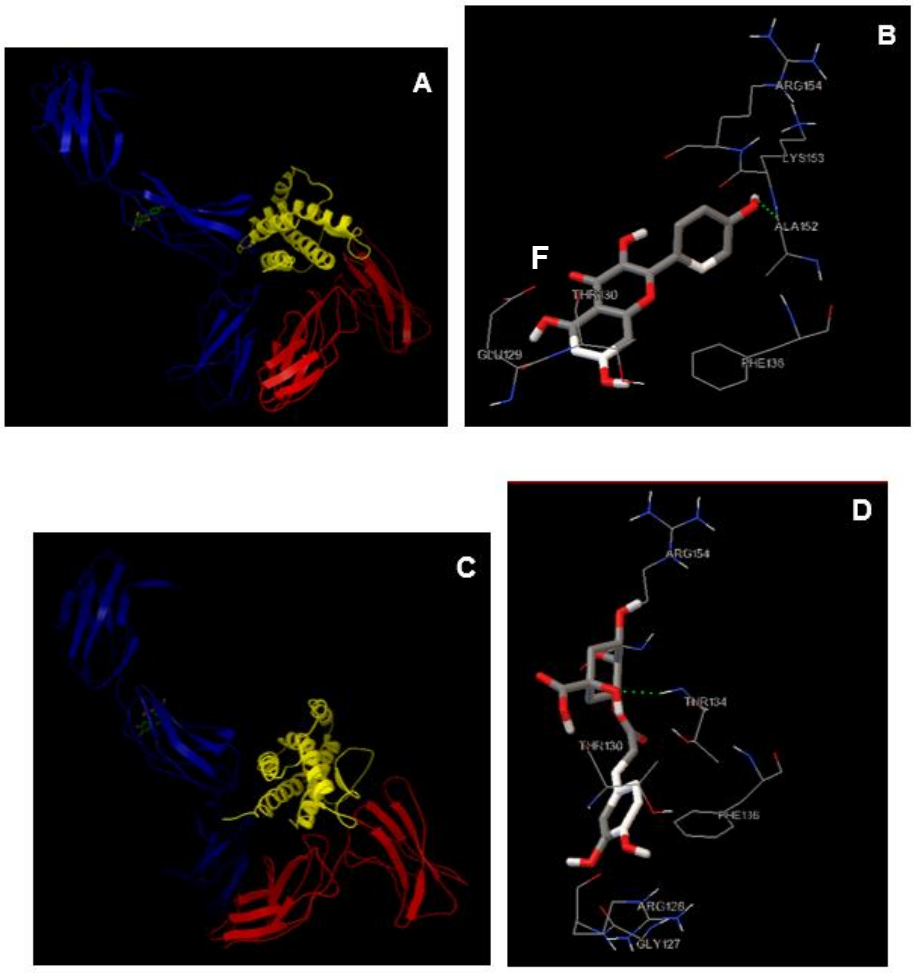


Figure 2: Chlorogenic acid docking pose (A) and interaction (B), Kaempferol docking pose (C) and interaction (D) and Indomethacin docking pose (E) and interaction (F) on IL-1β (PDB ID: 2NVH) receptor



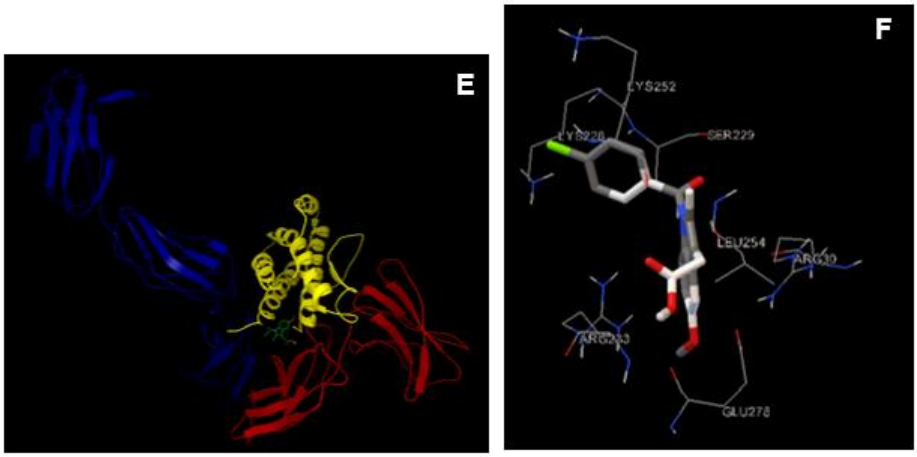


Figure 3: Kaempferol docking pose (A) and interaction (B), Chlorogenic acid docking pose (C) and interaction (D) and Indomethacin docking pose (E) and interaction (F) on IL-6 (PDB ID: 1P9M) receptor

Table 4 describes the predictive values for pharmacokinetics, bioavailability and druglikeness data on studied phyto and synthetic ligands. The small molecules Kaempferol and Indomethacin showed high absorption rate while Chlorogenic acid obtained low absorption rate for GI absorption. No blood-brain permeability was obtained for Kaempferol and Chlorogenic acid, but Indomethacin showed penetration. Higher negative value obtained lower skin permeation (log Kp, cm/s) as Chlorogenic acid (-8.76) followed by Kaempferol (-6.70) and Indomethacin (-5.45). In case of metabolism, these three small molecules did not observe p-glycoprotein substrate while for cytochrome p450 as CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 inhibitors, Kaempferol and Indomethacin obtained as inhibitor and Chlorogenic acid as non-inhibitors for CYP1A2, Indomethacin obtained inhibitor while Kaempferol and Chlorogenic acid showed non-inhibitor for CYP2C19 and CYP2C9, and Kaempferol obtained inhibitor while Chlorogenic acid and Indomethacin showed non-inhibitor for CYP2D6 and CYP3A4. The prediction of bioavailability and druglikeness, it was observed that highest bioavailability score for Indomethacin (0.56) followed by Kaempferol (0.55) and lowest for Chlorogenic acid (0.11) was obtained. The water solubility was obtained higher in Chlorogenic acid, moderate in Indomethacin and just soluble for Kaempferol (Table 4).

Table 4: Pharmacokinetics, bioavailability and druglikeness prediction of phyto and synthetic small molecules

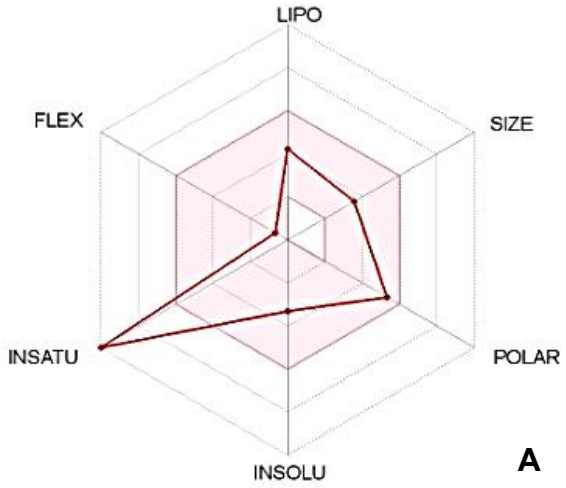
Pharmacokinetics							
Sl. No.	**Ligands**	**Gastro intestinal absorption**	**Blood-brain permeant**	**P-glycoprotein substrate**	**CYP450 1A2 inhibitor**	**CYP450 2C19 inhibitor**	**CYP450 2C9 inhibitor**
1.	Kaempferol	High	No	No	Yes	No	No
2.	Chlorogenic acid	Low	No	No	No	No	No
3.	Indomethacin	High	Yes	No	Yes	Yes	Yes

Pharmacokinetics					Bioavailability		
Sl. No.	**Ligands**	**CYP450 2D6 inhibitor**	**CYP450 3A4 inhibitor**	**Skin permeation as log Kp**	**Bioavailability score**	**Water solubility as logS**	**iLOGP**
1.	Kaempferol	Yes	Yes	-6.70	0.55	Soluble	1.70
2.	Chlorogenic acid	No	No	-8.76	0.11	Very soluble	0.96
3.	Indomethacin	No	No	-5.45	0.56	Moderately soluble	2.76

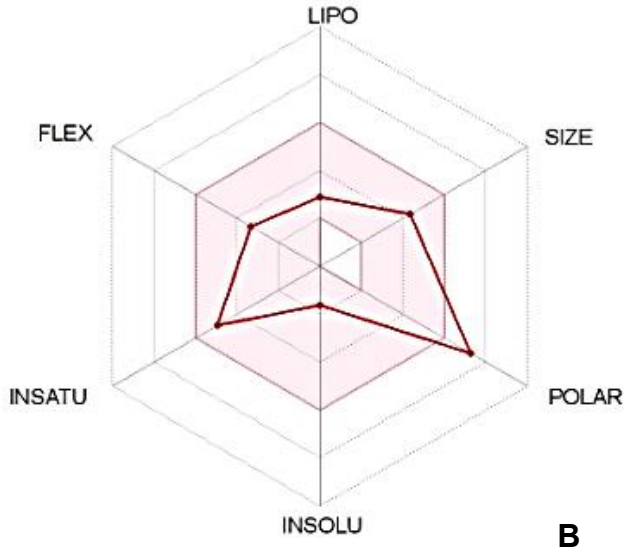
Bioavailability					Druglikeness	
Sl. No.	**Ligands**	**XLOGP3**	**WLOGP**	**MLOGP**	**Lipinski rule**	**Lead-likeness**
1.	Kaempferol	1.90	2.28	-0.03	Yes; 0 violation	Yes
2.	Chlorogenic acid	-0.42	-0.75	-1.05	Yes; 1 violation	No; 1 violation
3.	Indomethacin	4.27	3.93	3.30	Yes; 0 violation	No; 2 violations

Figure 4 A-C exhibits the bioavailability radar for oral bioavailability prediction in each small molecule. In case of LIPO as XLOGP3 value was obtained 4.27 for Indomethacin, 1.90 for Kaempferol and -0.42 for Chlorogenic acid; SIZE as molecular weight (gm/mol) was showed 357.79 for Indomethacin, 354.31 for Chlorogenic acid and 286.24 for Kaempferol; POLAR as TPSA (Å^2) 68.53 for Indomethacin, 164.75 for Chlorogenic acid and 111.13 for Kaempferol; INSOLU Logs (ESOL) -3.31 (soluble) for Kaempferol, -1.62 (very soluble) for Chlorogenic acid and -4.86 (moderately soluble) for Indomethacin; INSATU = insaturation as per Csp3 0.00 for Kaempferol,

0.38 for Chlorogenic acid and for 0.16 Indomethacin and FLEX as per no. of rotable bonds 5 nos. for Indomethacin and for Chlorogenic acid and 1 no for Kaempferol. In case of BOILED-Egg model, it was obtained that only Indomethacin has the capability of blood-brain barrier penetration among other two small molecules while Indomethacin and Kaempferol showed high penetration power of gastro-intestinal absorption but low absorption rate for Chlorogenic acid. All small molecules were found PGP negative as non-substrate in predictive model (Figure 5 A-C).



A



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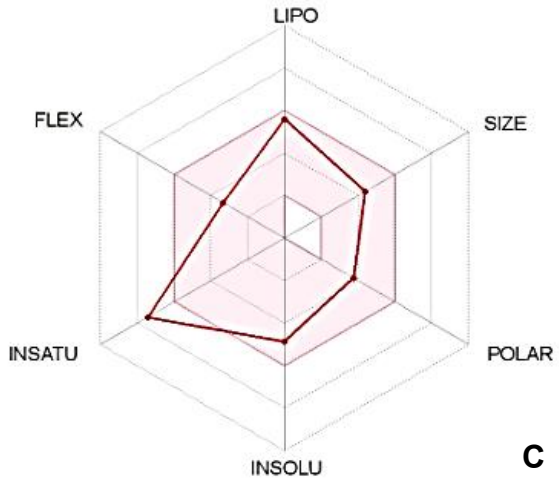

Figure 4: Bioavailability radar (pink area depicts optimal range of particular property) for studied small molecules as A = Kaempferol; B = Chlorogenic acid and C = Indomethacin (LIPO = lipophilicity as XLOGP3; SIZE = size as molecular weight; POLAR = polarity as TPSA (topological polar surface area); INSOLU = insolubility in water by log S scale; INSATU = insaturation as per fraction of carbons in the sp3 hybridization and FLEX = flexibility as per rotatable bonds

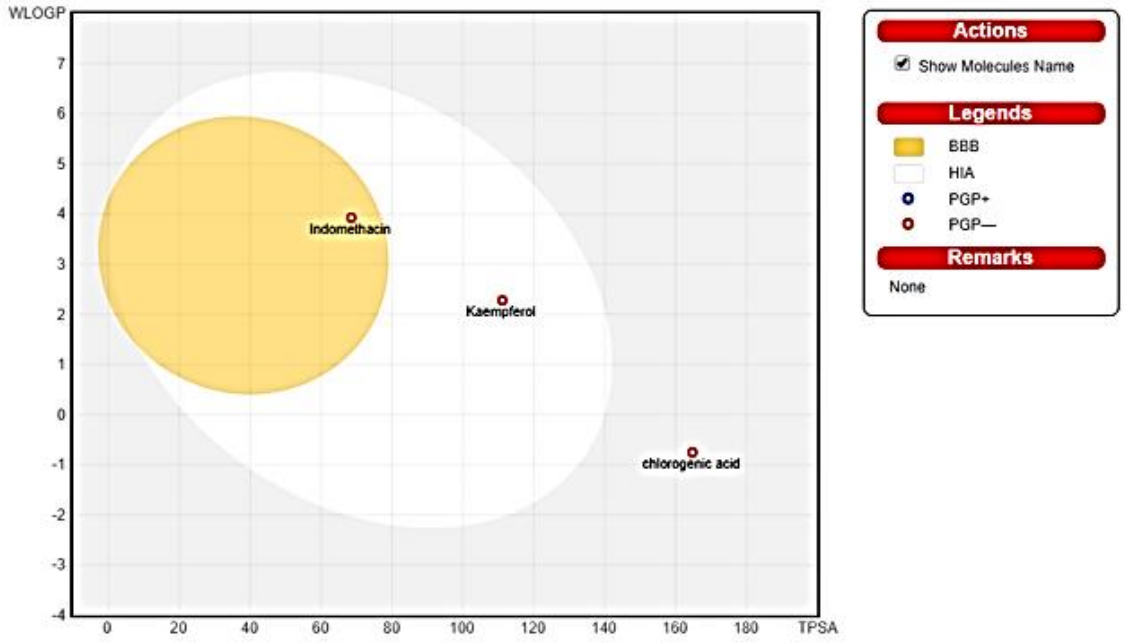

Figure 5: The BOILED-Egg represents for intuitive evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB) in function of the position of the small molecules in the WLOGP-versus-TPSA graph

The present study through molecular docking detects the exact ligand(s) for known target receptor. The molecular docking is widely used for new drug designing for therapeutic purposes. In other words, different ligands are screened to obtain Gibb's free energy bindings for affinity of drug towards the known target [30]. The proinflammatory cytokines as interleukins viz. IL-1β and IL-6

were selected as target receptor to detect inhibitory properties by lead small molecule(s) from natural products predicted as anti-inflammatory phytoligands in comparison with synthetic drug (Indomethacin). Interestingly, the inhibition of these receptors is primary concern to prevent pain and inflammation. As mentioned in earlier studies that IL-1β and IL-6 induced during chronic inflammation [30, 31, 32]. In the present *in silico* study flavonoids such as Kaempferol and Chlorogenic acid of *N. oleander* are suitable in relation to binding energy and molecular interaction with amino acids of target receptors when compared to Indomethacin, which is an evidence of experimental study that flavonoids are suitable for anti-inflammation [4]. In other study, a flavonoid Rutin is an inhibitory potential for different inflammatory mediator targets [30], and other flavonoids prevented inflammatory diseases [33]. Besides these, the prediction of pharmacokinetics with special reference to ADME, bioavailability and drug like properties of small molecules are an important research interest by using SwissADME online tool for new drug design [15, 16, 17, 18, 19]. It was well-known that the physicochemical properties such as solubility and lipophilicity prediction are also detected the small molecule whether progressing a successful drug candidate [19, 34]. The present predictions for small molecules from natural products as two flavonoids of *N. oleander*, it was observed that favourable higher binding energy was observed in chlorogenic acid (-7.0 Kcal/mol) followed by Kaempferol (-6.8 Kcal/mol) against IL-1β receptor and Kaempferol (-7.7 Kcal/mol) followed by chlorogenic acid (-7.1 Kcal/mol) against IL-6 receptor in comparison with synthetic drug Indomethacin (-6.7 Kcal/mol). But Kaempferol showed suitable predictive data on physicochemical properties, pharmacokinetics, bioavailability and druglikeness. Interestingly, the Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) has already been proposed as an accurate predictive model, which helps by computational prediction of the lipophilicity and polarity of small molecules [16]. In overall predictive results, Kaempferol can be suitable drug candidate from *N. olendar* as per bioavailability radar and BOILED-Egg representation. Furthermore, these predictive results should be validated by *in vitro* and *in vivo* functional and pharmacological assay for the prevention of pain and inflammation.

4. CONCLUSION

As per the docking binding energy values prediction, both natural phytoligands such as Kaempferol and chlorogenic acid have good binding affinity towards IL-1β and IL-6 compared to synthetic ligand (Indomethacin). These two phytoligands showed binding in the active site, which may be competitive inhibition against studied receptors when compared to established synthetic ligand. Besides docking, the prediction of pharmacokinetics with special reference to ADME, bioavailability and drug like properties of two small molecules, Kaempferol can be lead compound for new drug candidate. However, it is suggesting further *in vitro* and *in vivo* assay for anti-inflammation and analgesic to validate the present predictions.

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CONFLICT OF INTEREST

Authors declare none.

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