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Leaf phytoligands of *Annona reticulata* Linn.: molecular docking approach against proinflammatory receptors to detect antiinflammatory small molecules

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Abstract
Annona reticulata Linn. is well-known medicinal tree and contains phytochemicals to prevent several diseases through traditional knowledge. The aim of the present *in silico* study was to predict the binding affinity and energy of common phytochemicals especially saponins of *A. reticulata* compared to synthetic drugs (Ibuprofen and Indomethacin) on three proinflammatory receptors viz. tumour necrosis factor-α (TNF-α) and interleukins (IL-1β and IL-6) through molecular docking and interaction. The structure-based virtual screening was done by using PyRx tool (Version 0.8) to detect receptor-ligand binding affinity and energy. These receptors were obtained (PDB IDs: 2AZ5, 2NVH and 1P9M) from the European Protein Data Bank (ePDB) and the information on selected phytoligands (saponins) of *A. reticulata* and two synthetic ligands were obtained from PubChem database. Present molecular docking indicated that favourable binding energy was observed in Farnesyl acetate-(E,E) (-9.7 Kcal/mol) followed by Furostan and Taraxerol (-9.6 Kcal/mol) on TNF-α receptor while Kaurenoic acid (-7.2 Kcal/mol) on IL-1β receptor and Taraxerol and Furostan (-9.4 and -8.9 Kcal/mol) on IL6 receptor when compared to Indomethacin (-7.6, -6.7 and -7.5 Kcal/mol) and Ibuprofen (-6.7, -5.7 and -6.1 Kcal/mol) for these three receptors. This is a computational prediction to identify lead compound(s) for anti-inflammatory agents, which may substitute as a cost-effective natural product to prevent pain and inflammation. Future experimental assay with these natural products is suggested to validate the present predictive results.

Keywords: *In silico* study, molecular docking and interaction, proinflammatory receptors, phytoligands

1. Introduction
An important medicinal tree *Annona reticulata* Linn., commonly called custard apple and is well-known for Indian folk medicines, which prevents several diseases by its natural products [1]. The leaf phytochemicals of this plant are potent anti-inflammatory agents due to NO inhibition in LPS-activated mouse peritoneal macrophages studied by Thang *et al*. [2]. 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 and inducible nitric oxide synthase (iNOS), nitrous oxide (NO) and cyclooxygenases (COX-1 and COX-2), are proinflammatory factors, which increase during inflammation and causes several diseases [3-9]. 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]. Recently, researchers are showing interest in plant derived natural products or phytomedicines to target inflammatory mediators without any side effects [9]. Among several medicinal plants, *A. reticulata* has potent phytoconstituents to relief pain and inflammation as well as antioxidant activity [2, 10-15].
In computational prediction, the *in silico* screening is detected allosteric or inhibitory activity on protein or receptor as main target to prevent disease through drug action. Several compounds or ligands are derived from synthetic or natural products, which show favourable binding affinity and energy for the target receptor, which supports new and efficient lead molecule(s) for drug design. In general, the study through virtual screening detects large numbers of drug-like compounds in which receptor-ligand binding can be known easily for future experiment with a narrow range of compounds [16-19].
However, the development of new phytomedicines, *in silico* study is suitable for the drug discovery process in the arena of pharmaceutical research.

The present predictive study was carried out to detect the binding affinity and energy of common leaf phytochemicals as saponins of *A. reticulata* compared to synthetic medicines (Indomethacin and Ibuprofen) against three pro-inflammatory cytokine receptors viz. TNF-α and interleukins (IL-1β and IL-6) through molecular docking and interaction.

2. Materials and Methods

The present *in silico* approach is based on molecular docking and interaction to detect the efficacy of selected phytoligands (saponins) of leaf of *A. reticulata* in comparison with synthetic drug (Indomethacin and Ibuprofen).

2.1 Selection of receptors

The three-dimensional (3-D) crystal structure of receptors TNF-α (PDB ID: 2az5) and interleukins viz. IL-1β (PDB ID: 2NVH) and IL-6 (PDB ID: 1P9M) were selected for the present predictive study, which have been submitted by He *et al*. [20], Quillin *et al*. [21] and Boulanger *et al*. [22]. All these receptors were downloaded from the European protein data bank (http://www.ebi.ac.uk/pdbe/). The size of the former receptor is 2.1Å resolution and others are 1.53Å and 3.65Å resolutions respectively. These receptors were prepared in Auto Doc tool (version 1.5.6), developed by The Scripps Research Institute [23] and saved each receptor separately as. PDB file for *in silico* study. The 3-D structure of each receptor is exhibited in Figure 1A, B and C.

2.2 Selection of ligands

The leaf phytoligands especially saponins of *A. reticulata* as well as Indomethacin and Ibuprofen as synthetic ligands were selected as per different research articles [2, 12-15]. For all the selected compounds, canonical SMILES (Simplified molecular-input line-entry system) string were taken from the PubChem database (www.ncbi.nlm.nih.gov/pubchem) and PDB file of each ligand was obtained from CORINA online server (https://www.mn-am.com/online_demos/corina_demo) after incorporating SMILES in the particular area of interface.

2.3 *In silico* study for receptor-ligand binding

The *in silico* study with special reference to molecular docking was carried out by using PyRx tool (Ver 0.8) developed by Trott and Olson [24]. In the first step of screening, all the ligands and receptors files were converted to made macromolecule and ligand in this tool. Next step was formed a grid box with the size and centered position values with a grid spacing of 0.375 Å for each target receptor in the docking site (Table 1). The present tool was predicted the energy value for each ligand during docking. In last step, all the phytoligands and synthetic ligands were analysed to detect energy value and receptor-ligand binding position. As per favourable binding energy of ligand on each receptor, the receptor-ligand binding interaction was visualized and screened in Auto Doc tool [23] to know some specific contacts between the atoms of the test compounds and amino acids of the studied receptors viz. TNF-α, IL- β and IL-6.

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

Receptors	Size			Position from centre		
	X	Y	Z	X	Y	Z
TNF-α	71.6928	67.4813	71.3257	-13.6907	71.6033	26.9992
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

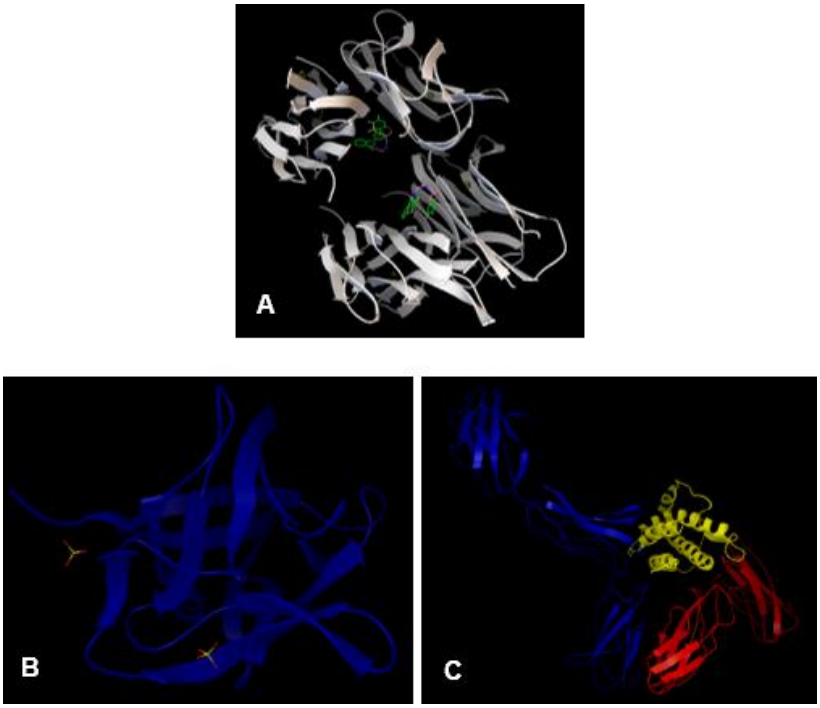


Fig 1: 3-D ribbon structures {A = 2AZ5 attached with inhibitory ligands (6,7-Dimethyl-3-[(Methyl{2-[Methyl({1-[3-(Trifluoromethyl)Phenyl]-1H-Indol-3-YL}Methyl)Amino]Ethyl}amino)Methyl]-4H-Chromen-4-One) in Chain A and Chain C (307) as line structures; B = PDB ID: 2NVH chain A blue colour attached with two sulphate molecules; C = PDB ID: 1P9M chain A blue colour, B yellow colour and C red colour}

3. Results and Discussion

The present study was done with one receptor as TNF-α and 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) indicated that favourable higher binding energy was observed in Farnesyl acetate-(E,E) (-9.7 Kcal/mol) followed by Furostan and Taraxerol (-9.6 Kcal/mol) of *A. reticulata* when compared to Indomethacin (-7.6 Kcal/mol) and Ibuprofen (-6.7 Kcal/mol) against TNF-α receptor (Table 2). On the other hand, favourable higher binding energy was observed in Kaurenoic acid (-7.2 Kcal/mol) of *A. reticulata* when compared to Indomethacin (-6.7 Kcal/mol) and Ibuprofen (-5.7 Kcal/mol) against IL-1β receptor (Table 3) and favourable higher binding energy was observed in Taraxerol and Furostan (-9.4 and -8.9 Kcal/mol) of *A. reticulata* when compared to synthetic medicines viz. Indomethacin (-7.5 Kcal/mol) and Ibuprofen (-6.1 Kcal/mol) against IL-6 receptor (Table 4).

Table 2: Binding energy values of selected phytochemicals of *A. reticulata* and synthetic drug against TNF-α receptor (PDB ID: 2AZ5)

S. No.	Ligands	Binding energy (Kcal/mol)
Phytochemicals		
1.	Farnesyl acetate-(E, E)	-9.7
2.	Furostan	-9.6
3.	Taraxerol	-9.6
4.	Cycloartenol	-9.0
5.	Mitoflaxone	-8.8
6.	β-sitosterol	-8.6
7.	Kaurenoic acid	-8.2
8.	Annonaretin A	-7.8
9.	ar-Turmerone	-7.7
10.	γ-terpinene	-6.4
11.	Piracetam	-4.6
Synthetic drugs		
1.	Indomethacin	-7.6
2.	Ibuprofen	-6.7

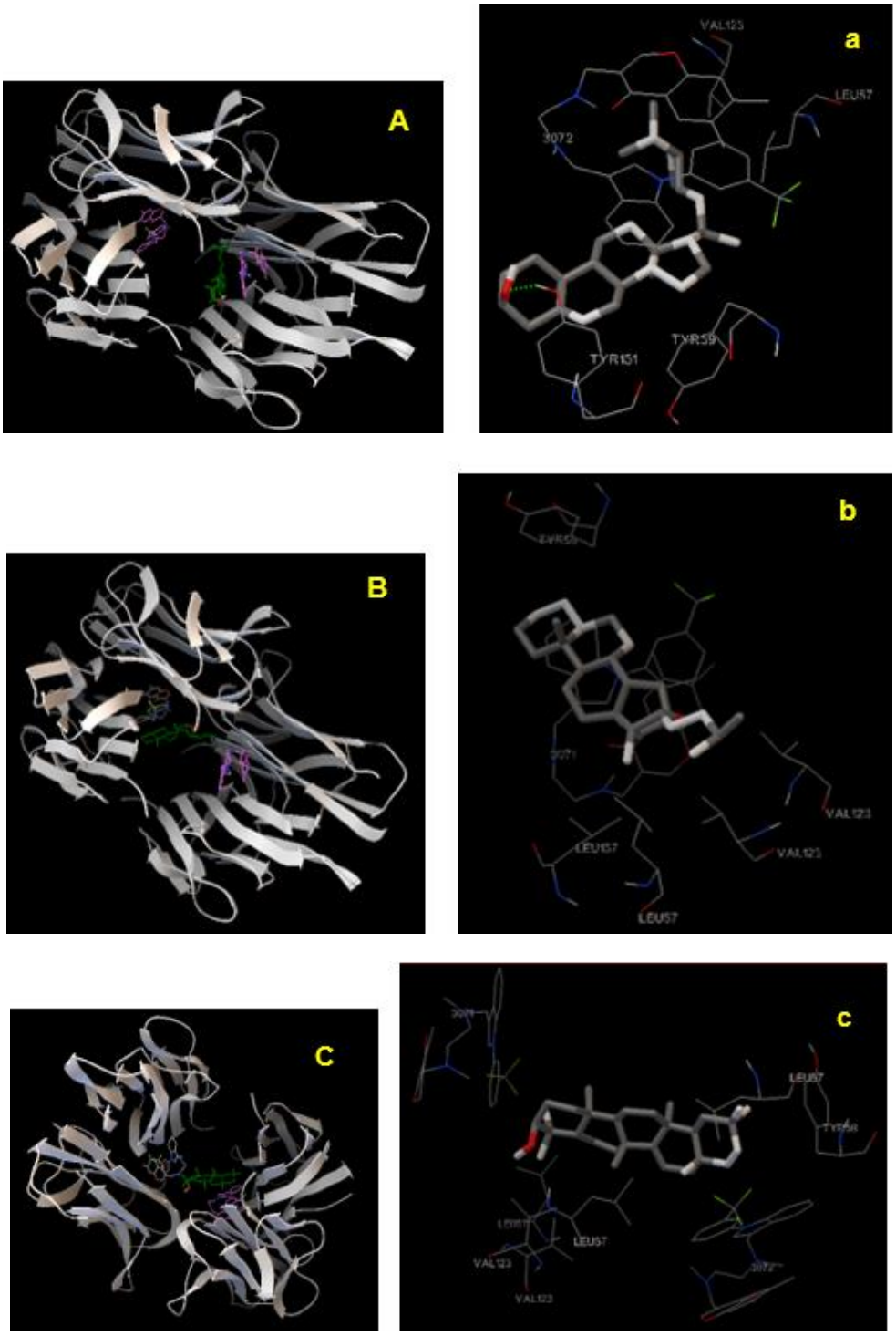
Table 3: Binding energy values of selected phytochemicals of *A. reticulata* and synthetic drug against IL-1β receptor (PDB ID: 2NVH)

S. No.	Ligands	Binding energy (Kcal/mol)
Phytochemicals		
1.	Kaurenoic acid	-7.2
2.	Furostan	-7.1
3.	Taraxerol	-7.0
4.	Farnesyl acetate-(E, E)	-7.0
5.	Cycloartenol	-7.0
6.	β-sitosterol	-6.8
7.	Mitoflaxone	-6.8
8.	Annonaretin A	-6.2
9.	ar-Turmerone	-5.8
10.	Piracetam	-5.1
11.	γ-terpinene	-4.8
Synthetic drugs		
1.	Indomethacin	-6.7
2.	Ibuprofen	-5.7

Table 4: Binding energy values of selected phytochemicals of *A. reticulata* and synthetic drug against IL-6 receptor (PDB ID: 1P9M)

S. No.	Ligands	Binding energy (Kcal/mol)
Phytochemicals		
1.	Taraxerol	-9.4
2.	Furostan	-8.9
3.	Cycloartenol	-8.7
4.	β-sitosterol	-8.6
5.	Farnesyl acetate-(E,E)	-8.6
6.	Kaurenoic acid	-8.2
7.	Mitoflaxone	-7.4
8.	Annonaretin A	-7.3
9.	ar-Turmerone	-6.4
10.	γ-terpinene	-4.9
11.	Piracetam	-4.5
Synthetic drugs		
1.	Indomethacin	-7.5
2.	Ibuprofen	-6.1

The interaction study of saponins such as Farnesyl acetate-(E,E), contact residues were VAL123, LEU57 and TYR59 at C chain along with one hydrogen bond contact and residue was TYR151, Furostan contact residues were TYR59, VAL123, LEU57 and LEU157 at chain A and VAL123 at chain A without hydrogen bonding and Taraxerol contact residues were LEU57 and VAL123 at chain C while TYR59, LEU57 and VAL123 at chain B but synthetic drugs viz. Indomethacin the contact residues were LEU57 and VAL123 at chain C and LEU57 chain A without hydrogen bonding while Ibuprofen contact residues were TYR59 and LEU57 at chain A and LEU57 and chain C without hydrogen bonding for TNF-α receptor (Fig 2 A-E and a-e).



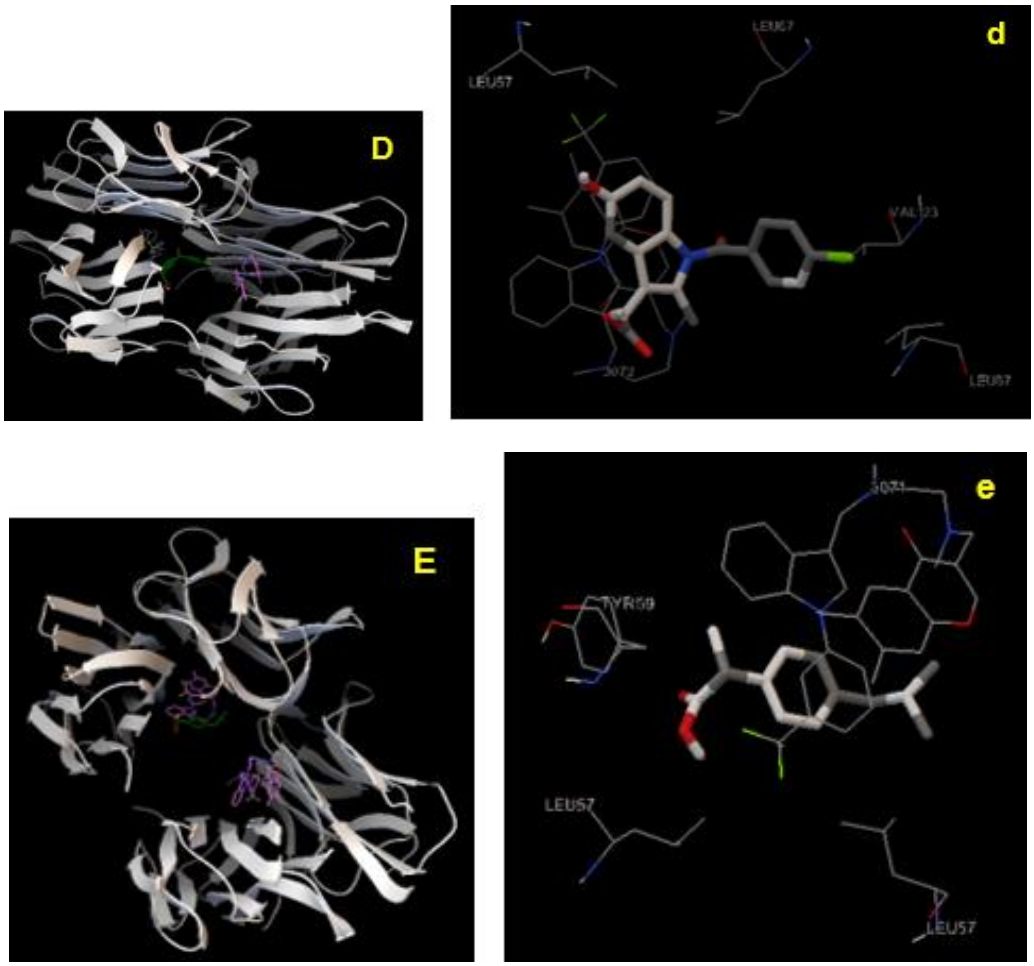
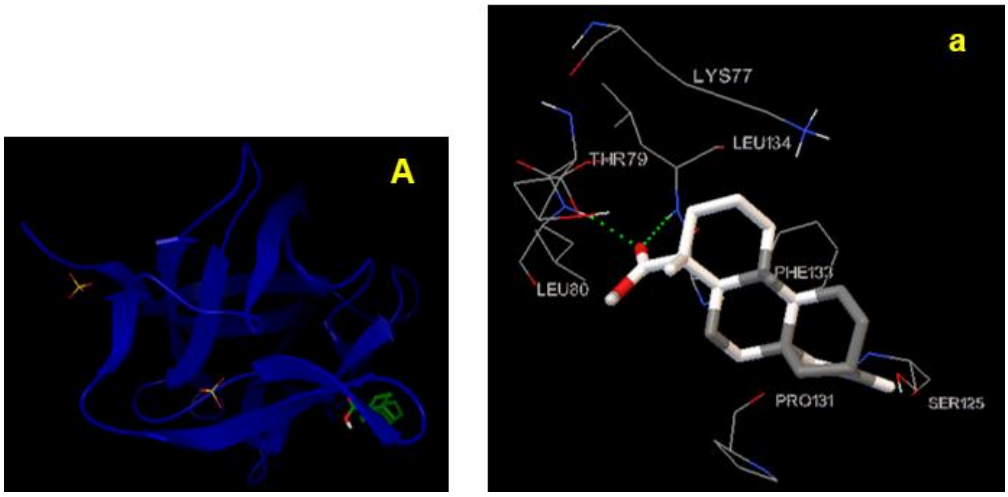


Fig 2: Binding pose and interaction of different phyto and synthetic ligands (A & a = Farnesyl acetate-(E, E); B & b = Furostan; C & c = Taraxerol; D & d = Indomethacin and E & E = Ibuprofen) on TNF-α receptor

The interaction study of saponins such as Kaurenoic acid contact residues were LYS177, THR79, PHE133, PRO131 and SER125 along with two hydrogen bond contacts and residue were LEU134 and LEU80 but synthetic drugs viz. Indomethacin the contact residues were PRO2, PRO91, TYR68 and GLU64 without hydrogen bonding while Ibuprofen contact residues were LYS77, PHE133, SER125, MET130, PRO131 and THR79 and two hydrogen bonding residues were LEU134 and LEU80 on IL-1β receptor (Fig 3 A-C and a-c).



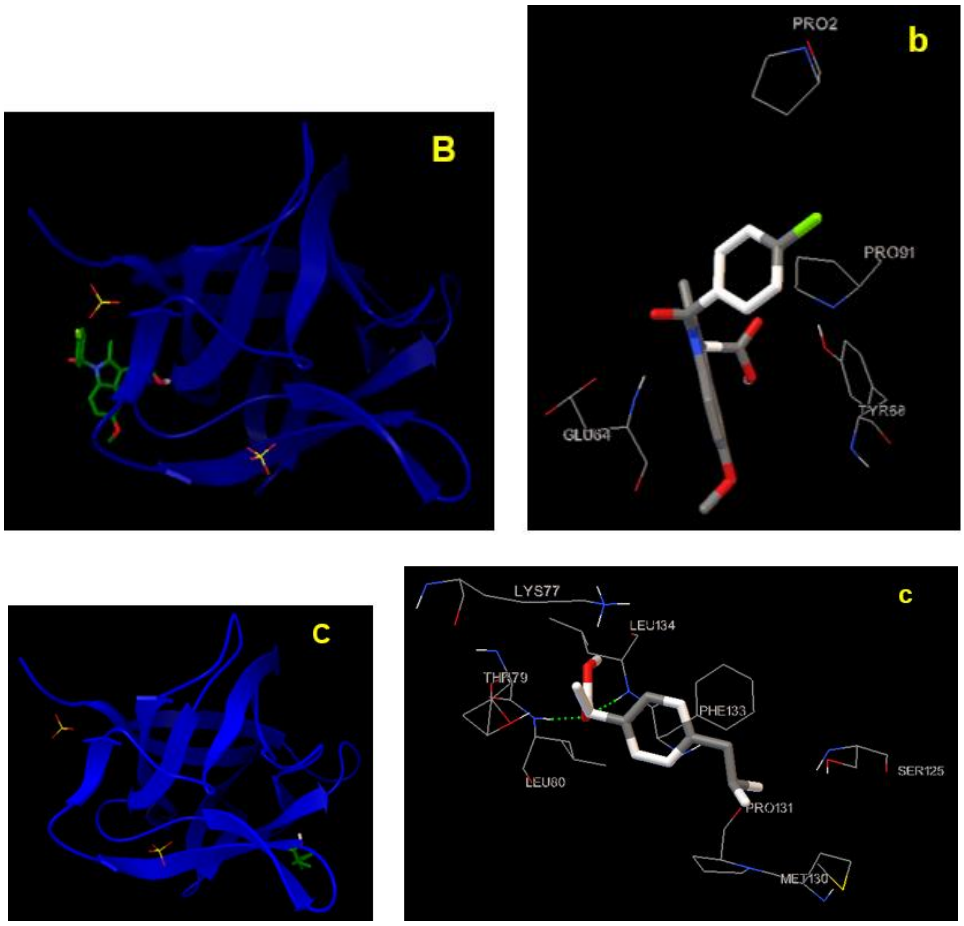
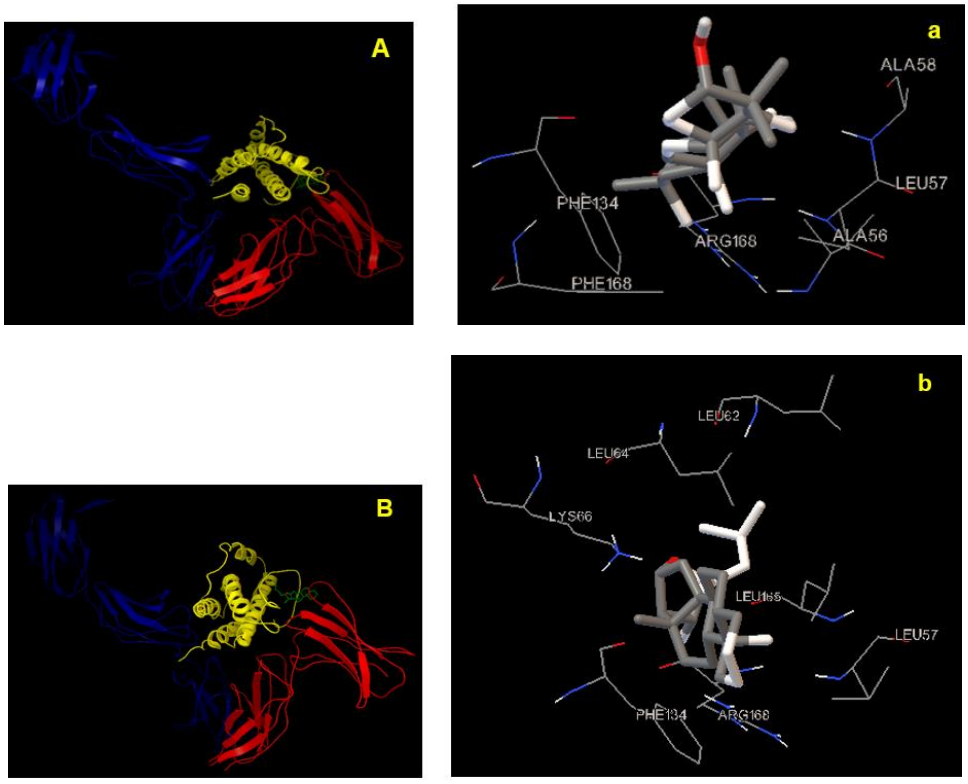


Fig 3: Binding pose and interaction of different phyto and synthetic ligands (A & a = Kaurenoic acid; B & b = Indomethacin and C & c = Ibuprofen) on IL-1β receptor

The interaction study of saponins such as Taraxerol contact residues were ALA58, LEU57, ALA56, ARG168, PHE168 and PHE134 and Furostan contact residues were LEU62, LEU64, LYS66, LEU165, LEU57, ARG168 and PHE134 without any hydrogen bond contact but synthetic drugs viz. Indomethacin the contact residues were ALA58, PHE134, PHE168 and SER166 with one hydrogen bonding residue was LEU57 while Ibuprofen contact residues were PHE134, PHE168, GLN190 and LEU57 without hydrogen bonding on IL-6 receptor (Fig 4 A-C and a-c).



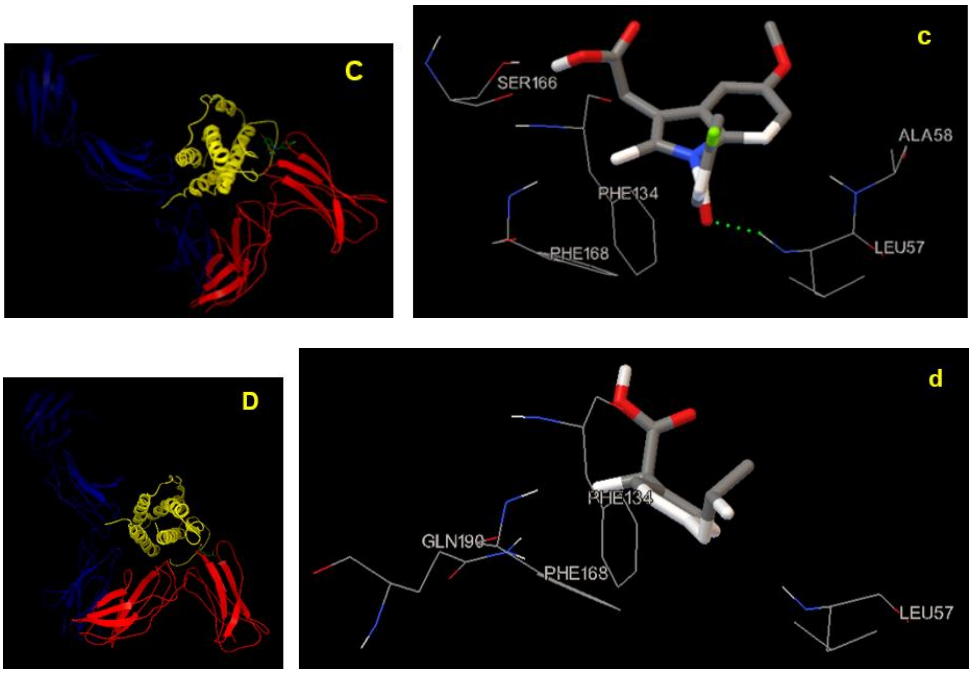


Fig 4: Binding pose and interaction of different phyto and synthetic ligands (A & a = Taraxerol; B & b = Furostan; C & c = Indomethacin and D & d = Ibuprofen) on IL-6 receptor

The computational prediction through molecular docking and interactions were identified the exact ligand (s) for known target proinflammatory receptors such as TNF-α, IL-1β and IL-6 [25]. The molecular docking is an interesting predictive study for new drug development for the efficient therapy by using natural product(s). Several researchers have emphasized on phytoligands for specific inflammatory target to obtain Gibb's free energy bindings in *in silico* screening [26]. The proinflammatory cytokines as TNF-α and 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 drugs viz. Indomethacin and Ibuprofen [19, 25, 27]. In the present prediction, the inhibition of these three receptors is primary concern to detect anti-inflammatory lead (s) for the prevention of immunomodulation. Previous studies revealed that TNF-α, IL-1β and IL-6 are released during inflammation [25, 27-30].

In the present computational prediction, saponins such as Farnesyl acetate-(E, E), Kaurenoic acid, Taraxerol, Furostan of *A. reticulata* are favourable in relation to binding energy and molecular interaction with amino acids of these target receptors when compared to Indomethacin and Ibuprofen, which is an evidence of other experimental study that saponins extracted from *Panax notoginseng* are suitable anti-inflammatory natural products [31]. In other study, kaurenoic acid inhibited NO production, prostaglandin E2 release, cyclooxygenase-2, and inducible nitric oxide synthase expression in LPS-induced RAW264.7 macrophages [32]. On the other hand, Dash *et al*. [33] mentioned in a research that leaf extract of *A. squamosa* is (Family member of Annonaceae) potential for anti-inflammatory and analgesic activity.

4. Conclusion

It is concluded that natural products saponins such as Farnesyl acetate-(E, E), Kaurenoic acid, Taraxerol and Furostan of *A. reticulata* obtained favourable binding energy values after molecular docking prediction in comparison with Indomethacin and Ibuprofen. These phytoligands showed favourable binding affinity and active site binding on TNF-α, IL-1β and IL-6, which may be competitive inhibition against studied receptors when compared to established synthetic ligands. The predictive results revealed these saponins of leaf can be suitable lead compound(s) individually or combination for new anti-inflammatory drug candidate(s). However, it is suggested further *in vitro* and *in vivo* toxicological and pharmacological assay with these receptors and extracted saponins from *A. reticulata* for anti-inflammatory and analgesic properties to validate the present predictions.

5. Acknowledgement

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Conflict of interest

Authors declare none.

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