Investigation of Citrullus colocynthis (stem) anti-inflammatory, anti-analgesic and anti-pyretic activities through In-vivo and in-silico approaches

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Abstract

Citrullus colocynthis is a traditional plant used as folk medicine in different diseases. The basic aim is to evaluate the pharmacological activities of bioactive compounds found in petroleum ether extract of *C. colocynthis* stem. Thirty-six rats of weight between 150-170g were used to investigate anti-inflammatory activity, anti-pyretic activity and analgesic activity. The doses were injected through the intraperitoneal cavity and muscles. The maximum anti-inflammatory activity (60%), antipyretic (100%) and analgesic (72%) has been shown by the extract at concentration of 400 mg/kg. The activities on stem part showed the level of significance ($p<0.005$) by using ANOVA. Molecular docking simulations results showed the binding interactions of catechin and palmitic acid (*C. colocynthis*) against PTGS2, TLR2 and TRPV4, respectively. Docking simulations showed that catechin and palmitic acid possessed good binding energy values inside the active regions of target proteins. Taken together, catechin and palmitic acid of *C. colocynthis* may possessed therapeutic behaviors against inflammatory, antipyretic and analgesic by targeting PTGS2, TLR2 and TRPV4.

Keywords: Inflammation, Pyrexia, Algesia, Molecular docking (MD)

Introduction

Plants are natural source of numerous bioactive compounds with a variety of diagnostic characteristics and possessed bioactive activities including anti-inflammatory, antiviral, anti-tumor, antimalarial, and analgesic [1, 2]. *Citrullus colocynthis* (*C. colocynthis*) is commonly known as bitter apple and distributed in different geographical regions such as Pakistan, India, Iran, China, Kenya and Egypt, respectively [3, 4]. The phytochemical data reported that possess different phyto-constituents in different parts such as in stem catechin, palmitic acid, 8-C-p-hydroxybenzoylisovitexin, quercetin, 5-@-stigmastin-3-beta-ol, p-cymene, cucurbitacin, succinic acid, β-sitosterol, colocynthin, and glycerol are present [5]. Whereas, leaves contain 25-*p*-coumaroyl-3′-acetyl-2-*O*-β-D-glucocucurbitacin I and 6′-acetyl-2-*O*-β-D-gluco-cucurbitacin, respectively [6]. The recent studies also showed that these bioactive compounds have different medicinal effects such as anti-inflammatory, anti-diabetic, antipyretic, anti-cancer, anti-ulcerogenic, anti-oxidant, hypo and hyperlipidemic conditions [7, 8]

Inflammation is a complex biological response of body tissues to harmful stimuli pathogens, damaged cells, or irritants, respectively. Prostaglandin-endoperoxide synthase 2 (PTGS2) is commonly used anti-inflammatory target [9]. PTGS2 is involved in arachidonic acid (AA) pathway. AA metabolism plays a key role in inflammatory response. PTGS2 converts AA into prostaglandin (PG). PG secretes white blood cell and aggravates inflammation [10]. *C. colocynthis* is having protective function causing suppression of pro-inflammatory release and inhibition of PTGS2 [9].

The Toll-like receptors (TLRs) has been identified as essential molecules to trigger innate immune system against microbes. TLR2 and TLR4 identifies and reacts against cell wall of pathogens. TLR2 is triggered in response to different ligands such as fungal components, micro bacterial and viral proteins [11]. TLRs are primarily found in immune cells including macrophages, neutrophils and lymphocytes, which perform crucial role in immunity against foreign invading pathogens. They identify the pathogen-associated-molecular-patterns (PAMPs) from bacteria and viruses. After PAMPs recognition, TLRs triggers MPAK and NF-kB signaling pathways to initiate the immune response. TLR2 induces hyperthermia (fever) as defense mechanism in response to pathogens invading the body [12].

Pain is a protective response against malfunction in any organ or imbalance of its function to harmful stimuli [13]. Chemo sensitive nociceptors are activated during pain [14] . Transient receptor potential cation channel subfamily V member 4 (TRPV4) is multimodally activated, Ca^{2+}-permeable nonselective cation channel and it initiates the pain by expressing the dorsal root ganglia (DRG) and trigeminal ganglia (TG) [15]. (TRPV4) is primary mediator of analgesic activity in albino rats [16]

The current study was designed to evaluate the therapeutic effects of phytochemicals from *C. colocynthis* (stem) as inflammatory, antipyretic and analgesic behavior though *in-vivo* and *in-silico* approaches. In vivo experiment was employed on rat model at different dose concentrations to check the inflammatory, antipyretic and analgesic activities. Furthermore, molecular docking was used to investigate the binding interactions pattern between potential inhibitors and PTGS2, TLR2 and TRPV4, respectively.

Materials and methodology

Sample collection

The *C. colocynthis* stem was collected from field area Bahawalpur, Punjab, Pakistan. The selected samples of *C. colocynthis* were further taxonomically verified from Plant Taxonomy Lab, University of Punjab, Punjab, Pakistan. Initially, *C. colocynthis* stem was dried at room temperature and retrieved in powdered form for further experiments. Thirty-six albino rats (either male or female) having average weight between 145-170 g were selected and housed in propylene cages at standard husbandry conditions (temp/food) in animal house laboratory, The University of Lahore, Punjab Pakistan.

Preparation of extracts

C. colocynthis stem was soaked in petroleum ether (non-polar solvent) in a vessel. The mixture was mildly shaken for 3-4 minutes and stored at room temperature (37 °C) for 15 days. Moreover, the mixture was filtered using Watt-man filter paper on the 16th day.

Anti-inflammatory activity model

Thirty-six selected rats were divided into three groups with equal numbers. Carrageenan is used to produce inflammation in albino rats [17]. Group 1 was a control group treated with normal saline (50, 100, 200, 400 mg/Kg). Standard group (group 2) was treated with diclofenac drug at 50, 100, 200 and 400 mg/Kg concentrations. Experimental group was treated with petroleum ether extract of *C. colocynthis* stem (50, 100, 200 and 400 mg/Kg). Initially 0.1 ml of 1% carrageenan was injected into the sub plantar region of the right hind paw to the rats of all groups.

Antipyretic activity model

Fever was induced using the brewer's yeast [17]. To study antipyretic potential rats were divided into three groups according to the anti-inflammatory model. Subcutaneously all the groups were initially inoculated with a solution of brewer's yeast and normal saline. After the interval of 20 hours, pyrexia was developed across all groups. The maximum pyrexia developed was 102.6 °F. Standard group was treated with paracetamol 50,100, 200 and 400 mg/Kg. Experimental group was treated with petroleum ether extract of *C. colocynthis* stem (50 ,100, 200 and 400 mg/Kg).

Analgesic activity model

Abdominal constriction reaction caused by acetic acid is a sensitive technique for evaluation of peripheral analgesics. Initially pain was induced in albino rats and then it was treated with petroleum ether extract of *C. colocynthis* stem. Normal saline to control group, diclofenac (50, 100, 200,400 mg/Kg) to standard group, petroleum ether extract of *C. colocynthis* stem (50, 100, 200,400 mg/Kg) to experimental design group were administered through intraperitoneal injection. After the time monetization of 1 hour the acetic acid was injected to all three groups of rats through intraperitoneal cavity. Rats were placed in separate boxes after the injection of acetic acid. Writhes were calculated using stop watch for 20 minutes.

Computational methodology

Preparation of target proteins

The three-dimensional (3D) structures of PTGS2, TRPV4 and TLR2 fromalbino rats (Rattus norvegicus) are not present in Protein Data Bank (PDB) (www.rcsb.org). Therefore, protein structures were downloaded through SWISS-PROT database in PDB format and minimized and visualized using UCSF Chimera 1.6 (Pettersen *et al*., 2004). The overall Ramachandran and stereo-chemical properties of PTGS2, TRPV4 and TLR2 were assessed by MolProbity server (Chen *et al*., 2010).

Selection and preparation of phytocompounds

A comprehensive literature mining was performed to find out different bioactive compounds present in stem region of *C. colocynthis* [18, 19] and online database (https://phytochem.nal.usda.gov/phytochem/search). Palmitic acid and catechin, the active phyto-compounds along with standard drugs (diclofenac and paracetamol) were retrieved with accession No in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The selected compounds were designed in ChemSketch (ACD/ChemSketch, version 2020.2.0, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2021) and retrieved in PDB format for further computational analysis.

Prediction of active binding sites of target proteins

The active sites of PTGS2, TRPV4 and TLR2 provide significant information regarding the functionality of mediated signaling pathways. The active binding sites of PTGS2, TRPV4 and TLR2 were predicted by using Depth Residue (http://cospi.iiserpune.ac.in/depth/), an online source which explores the probability of amino acids involved in the formation of active binding sites. Furthermore, these residues were also confirmed through literature data which justify the prediction of active binding sites [20].

Receptor Grid Generation and Molecular docking

The ligands have a specific binding site within proteins; therefore, grid generation is important step before going to perform docking experiment. A cubic gird box with x-axis, y-axis and z-axis values were fixed by entering particular size and numbers at active site in all three selected proteins separately. In PTGS2, grid box was adjusted as X = 26.5 Å, Y = 42 Å, and Z = 12.9 Å, whereas for TLR2 grid size was fixed at X=-1 Å, Y=29.8Å, Z=-14.8Å, respectively. Likewise, TRPV4 binding pocket dimensions were determined keeping the grid size of X=137.9 Å, Y=120 Å, Z=131 Å for palmitic acid, catechin and diclofenac. Moreover, the exhaustiveness value was fixed for all docking complexes to obtain the finest binding conformational pose of selected compounds. After ligands and proteins preparation, docking was done by using PyRx a virtual screening tool (Dallakyan & Olson, 2015). The different docking complexes were analyzed based on binding energy values (Kcal/mol) and interactive behavior such as hydrogen and hydrophobic interactions. The graphical representation of docking complexes was generated using Discovery Studio and Chimera tool, respectively.

Results

The in-*vivo* results showed the significant of *C. colocynthis* extract in albino rat models and possessed good anti-inflammatory, antipyretic and analgesic activities. The actual binding depiction of selected compounds along with standard also represented good conformational behavior within the active region of target proteins such as PTGS2, TRPV4 and TLR2, respectively.

Anti-inflammatory activity

Carrageenan induced paw edema

The petroleum ether stem extract of *C. colocynthis* showed significant results against anti-inflammatory activity. The maximum inhibition activity of *C. colocynthis* shown by concentration 400mg/Kg that was 60%. The plant showed 56, 30 and 41% at respective concentrations of 50, 100 and 200 mg/Kg. The standard group showed the inhibition activity of 37.5, 28.5, 40 and 100% at respective concentrations of 50, 100, 200 and 400 mg/kg. All the results were significant ($p<0.005$) (Figure 1).

Antipyretic activity

The petroleum ether stem extract of *C. colocynthis* showed excellent results for antipyretic activity having $p<0.005$ at concentrations of 50, 100, 200 and 400 mg/kg as compared to standard drug i.e., paracetamol. The different inhibition percentages shown by the experimental group which were treated by petroleum ether extract of stem of *C. colocynthis* were 71, 89, 22 and 100% at dose concentration of 50, 100, 200 and 400mg/kg. In the standard group, percentages inhibitions were 85, 37, 75 and 76% for dose concentration of 50, 100, 200 and 400 mg/kg of paracetamol drug. While 0% inhibition is shown by the control group after induced pyrexia (Figure 2).

Analgesic activity

The petroleum ether extract of the stem of *C. colocynthis* showed maximum analgesic activity at different concentrations. In writhing response, the inhibition activity was 72.6, 26, 53 and 62% at concentrations of 400, 50, 100 and 200 mg/Kg of stem extract of *C. colocynthis*. The standard group showed 45.5, 45, 85 and 72% at the 50, 100, 400 and 200mg/kg of diclofenac. Control group was unable to show analgesic activity after inoculation of acetic acid. All the results were significant i.e., $P<0.005$ (Figure 3). The percentage inhibition of anti-inflammatory, antipyretic and analgesic activity has been mentioned in Table 1.

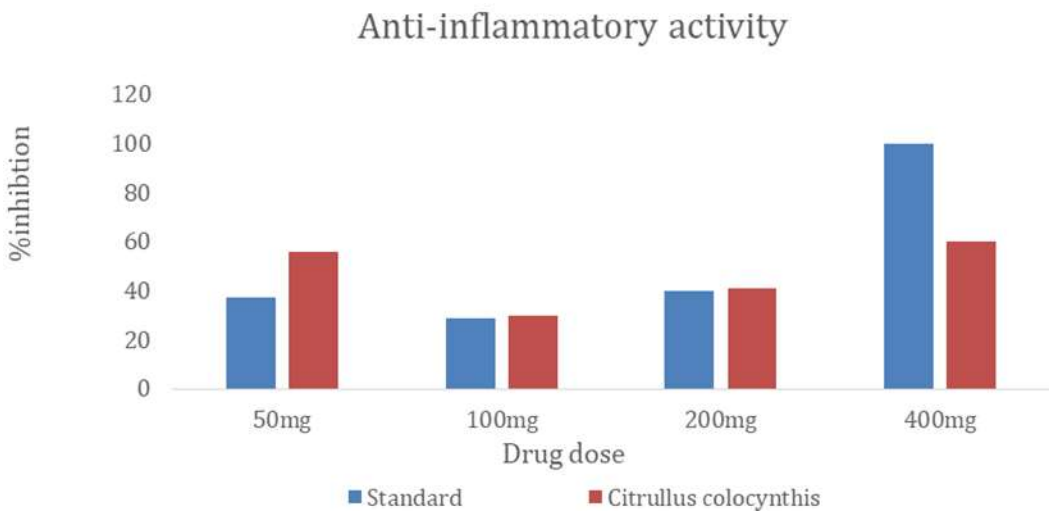

Figure 1: The percentage inhibition of petroleum ether extract of *C. colocynthis* stem againt inflammation

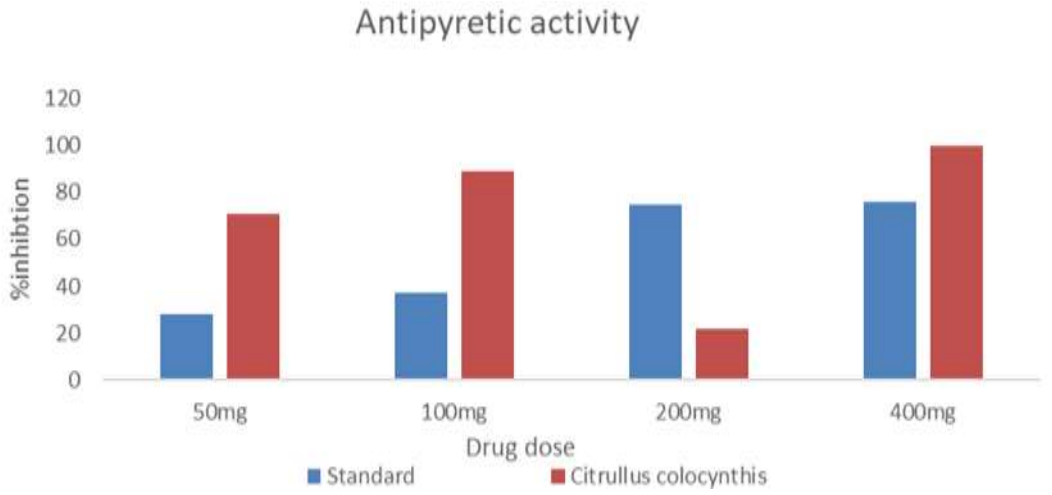

Figure 2: Percentage inhibition of petroleum ether extract of *C. colocynthis* stem against pyrexia

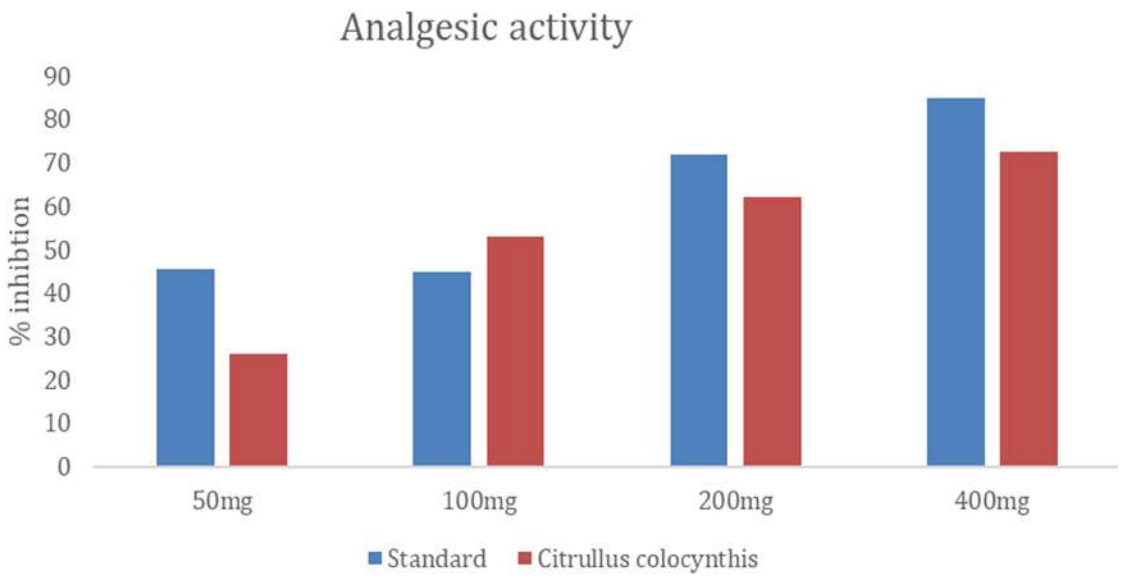

Figure 3. Percentage inhibition of petroleum ether extract of *C. colocynthis* stem against algesia

Table 1 Percentage inhibition of anti-inflammatory, antipyretic and analgesic activity by *C. colocynthis*

Group	Dose (mg/kg)	%Inhibition of anti-inflammatory activity	%Inhibition of antipyretic activity	%Inhibition of analgesic activity
Control	50	0	0	0
	100	0	0	0
	200	0	0	0
	400	0	0	0
Standard	50	37.5	28	45.5
	100	28.5	37	45
	200	40	75	72
	400	100	76	85
Stem	50	56	71	26
	100	30	89	53
	200	41	22	62
	400	60	100	72.6

Statistical analysis

All data are presented as mean ± S.E.M. Data were subjected to two-way repeated measures analysis of variance (ANOVA) and $p < 0.05$ was considered statistically significant in all analyses.

In-silico analysis

Proteins structure assessment

PTGS2 belongs to the peroxidase family, comprises two chains (A and B) and 604 amino acids having molecular mass of 69. 1kDa.The VADAR 1.8 structure analysis of PTGS2 consists of f 44% α-helices, 13% β-sheets, 42% coils and 26% turns, respectively. The Ramachandran plots and values of PTGS2 indicated that 95.99% of PTGS2 amino acids were present in preferred region and 3.47% residues were in allowed region. TLR2 is another target protein consists of a single chain (A) having 704 amino acids with molecular weight of 89.4kDa. The structural analysis revealed that TLR2 is composed of 12% α-helices, 39% β-sheets, 47% coils and 24% turns. The Ramachandran plots and values of TLR2 indicated that 90.68% of protein amino acids were present in preferred region and 8.04% residues were lie in allowed region. TRPV4 has four chains (A, B, C and D) having 871 amino acids with molecular weight of 98.01kDa. TRPV4 consists of 49% α-helices, 8% β-sheets, 41% coils and 24% turns, respectively. The Ramachandran plots and values of TRPV4 indicated that 94.99% of TRPV4residues were present in preferred region and 4.07% residues were lie in allowed region. The Ramachandran graphs of all selected proteins are mentioned in Figure 4.

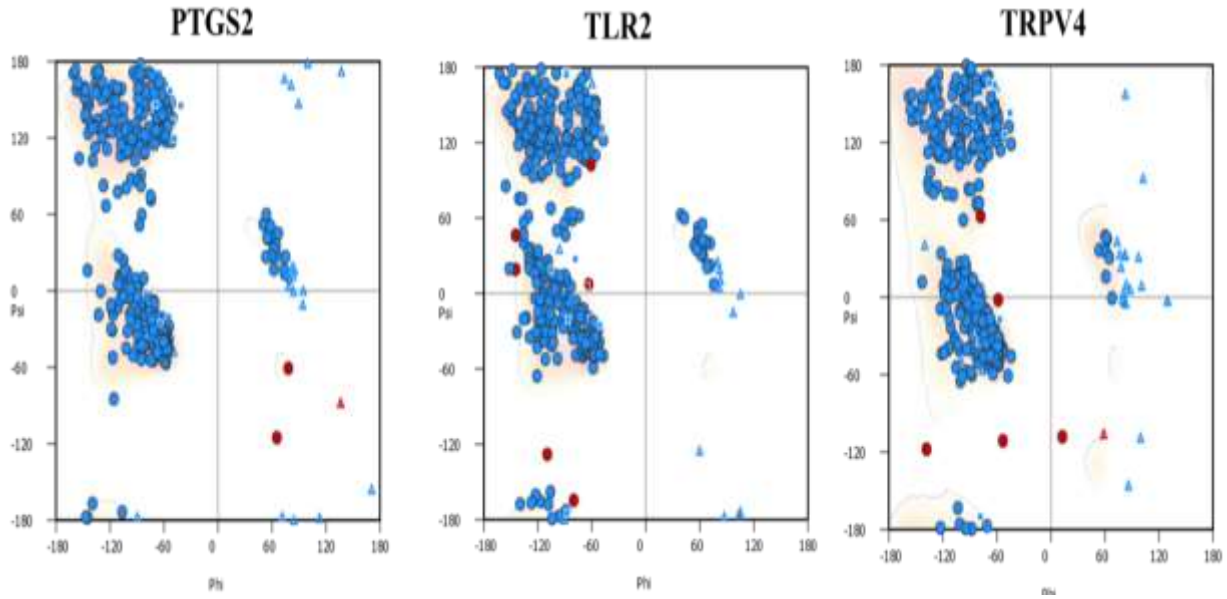

Figure 4. Ramachandran plots of target proteins

Chemistry of phytochemicals

Palmitic acid ($C_{16}H_{32}O_2$) (PubChem ID 985) is a saturated fatty acid having 16-carbon backbone atoms with molecular weight 256.42 g/mol. Palmitic acid possess anti-inflammatory, antipyretic and analgesic activities (https://phytochem.nal.usda.gov/phytochem/search). Catechin ($C_{15}H_{14}O_6$) (PubChem ID 73160), is a phenolic compound having molecular weight of 290.27 g/mol. It has been observed that catechin is effective in chemo-preventive, anti-apoptotic, anti-inflammatory, and neuro-protective properties in clinical disorders [21]. Paracetamol ($C_8H_9NO_2$) is a standard drug used in antipyretic activity having molecular weight of 155.19 g/mol [22]. Diclofenac ($C_{15}H_{14}O_6$) is used in anti-inflammatory and analgesic activities having molecular weight of 290.27 g/mol [23].The 2-D structure of selected ligands has been mentioned in Figure 5.

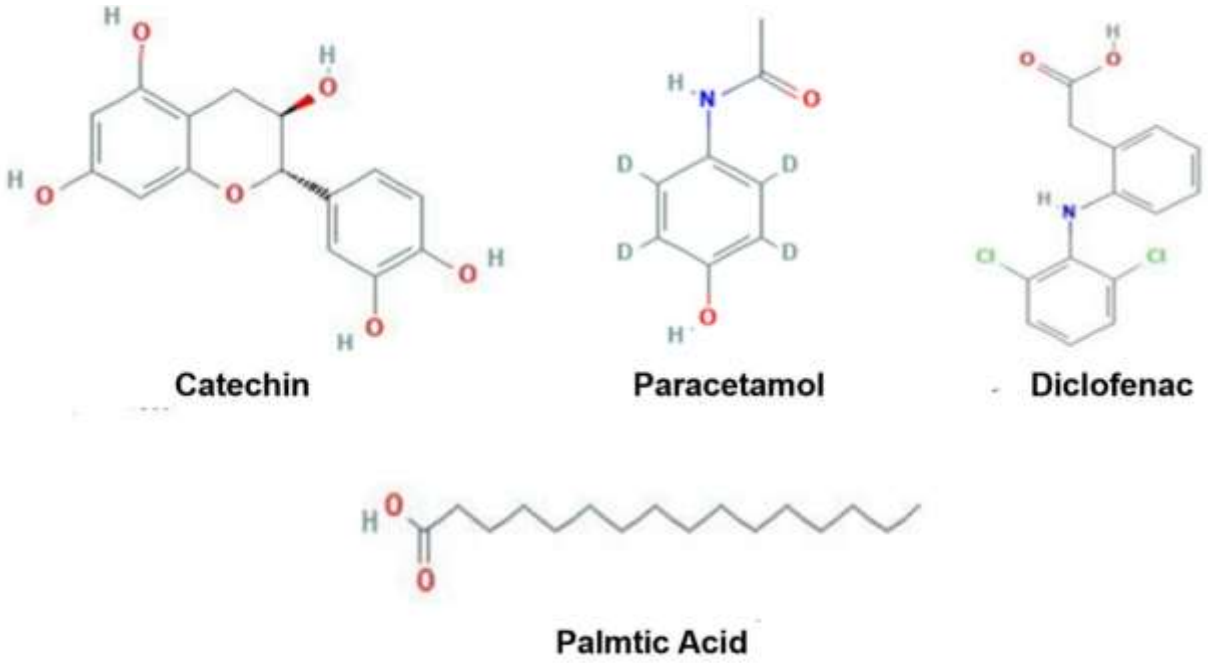

Figure 5 Two dimensional(2-D) images of selected ligands

Active site prediction of PTGS2, TLR2 and TRPV4

Active site is the functional part of proteins as it catalyzes the chemical reaction[24]. Figure 6 (A, B) showed the PTGS2 structure in surface format and probability of amino acids involved in the formation of binding pocket. The Depth Residue results showed that active site of PTGS2 contained 13 amino acids Val 335, Leu 338, Ser 339, Tyr-341, Phe-353, Glu-366, Phe-367, Asn-368, Tyr-371, His-372, Trp-373, His-374and Leu-377, respectively. The selected amino acids present in the formation of active site has probability value range from 0.4 to 1.0. Four major peaks

were observed in the PTGS2 prediction showed active binding regions. First peak appeared at Pro-139 possessed highest probability value of 0.82. Whereas in second peak three amino acids Gln-189, His-190 and His-192 showed good probability values from 0.62-1.0. In third peak consist which is consist of 10 amino acids Val-335, Leu-338, Glu-366, Phe-367, Asn-368, Tyr-371, His-372, Trp-373, His-374, Leu-377 having probability of 0.64 to 1.0 to form active site. Fourth peak consists of 6 amino acids Val-509, Ala-513, Ser-516, Lys-518, Gly-519, leu-520. Third peak was selected as active site as same amino acids were found in active site composition from literature studies [20]

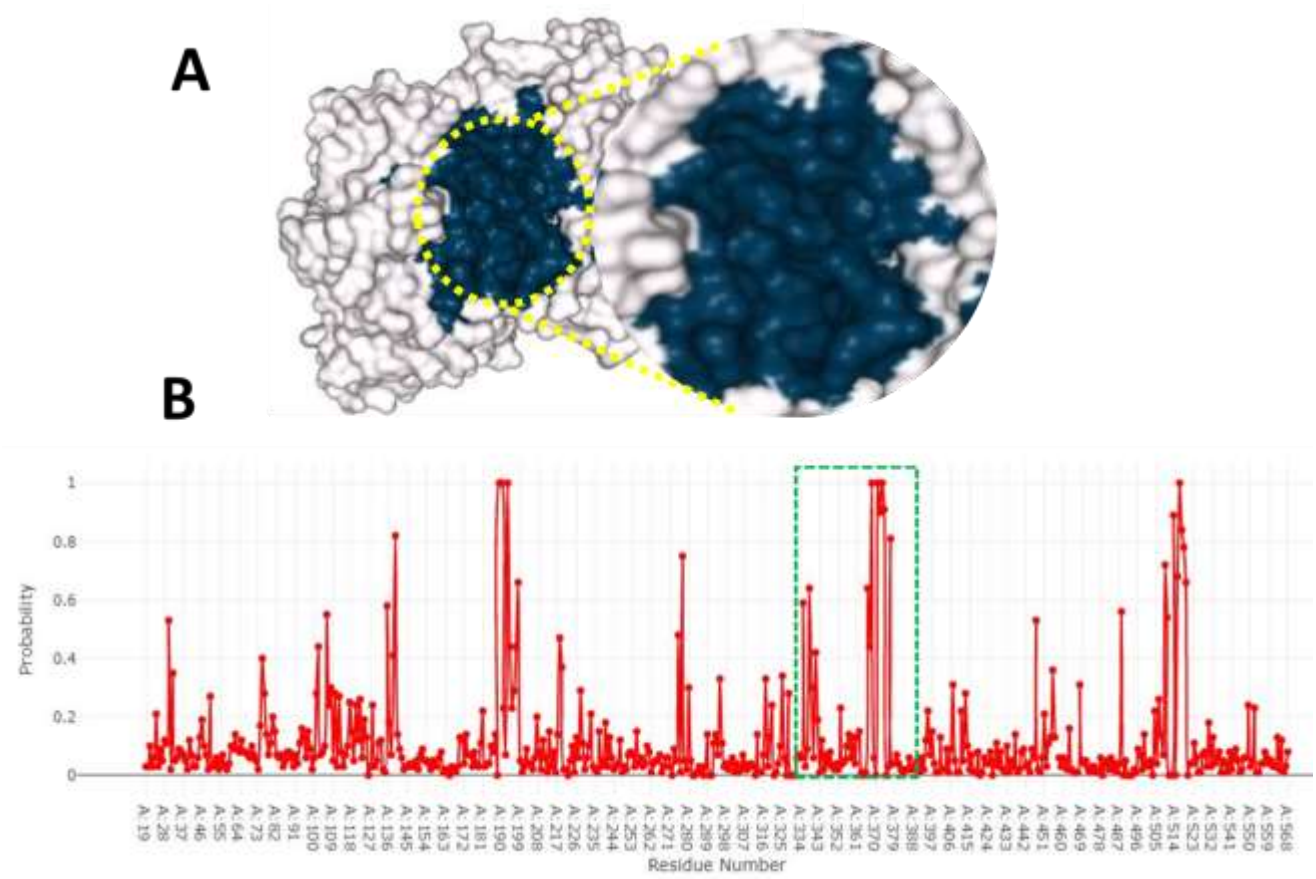

Figure 6: Binding site of PTGS2 obtained from Depth Residue tool.

The binding pocket of TLR2 comprises of 12 amino acids (Phe-266, Asn-267, Leu-273, Phe-284, Leu-289, Thr-311, Ile-314, Leu-317, Phe-325, Thr-330, Val-331and Leu-335). These binding

pocket residues have good probability values in the range from 0.4 to 1 value (Figure 7 A, B). The binding pocket consists of five peaks. First peak appeared at Phe-266, second at Leu-289, third at Ile-314, fourth at Leu-317 and fifth at Val-331. The fifth peak was selected as active site.

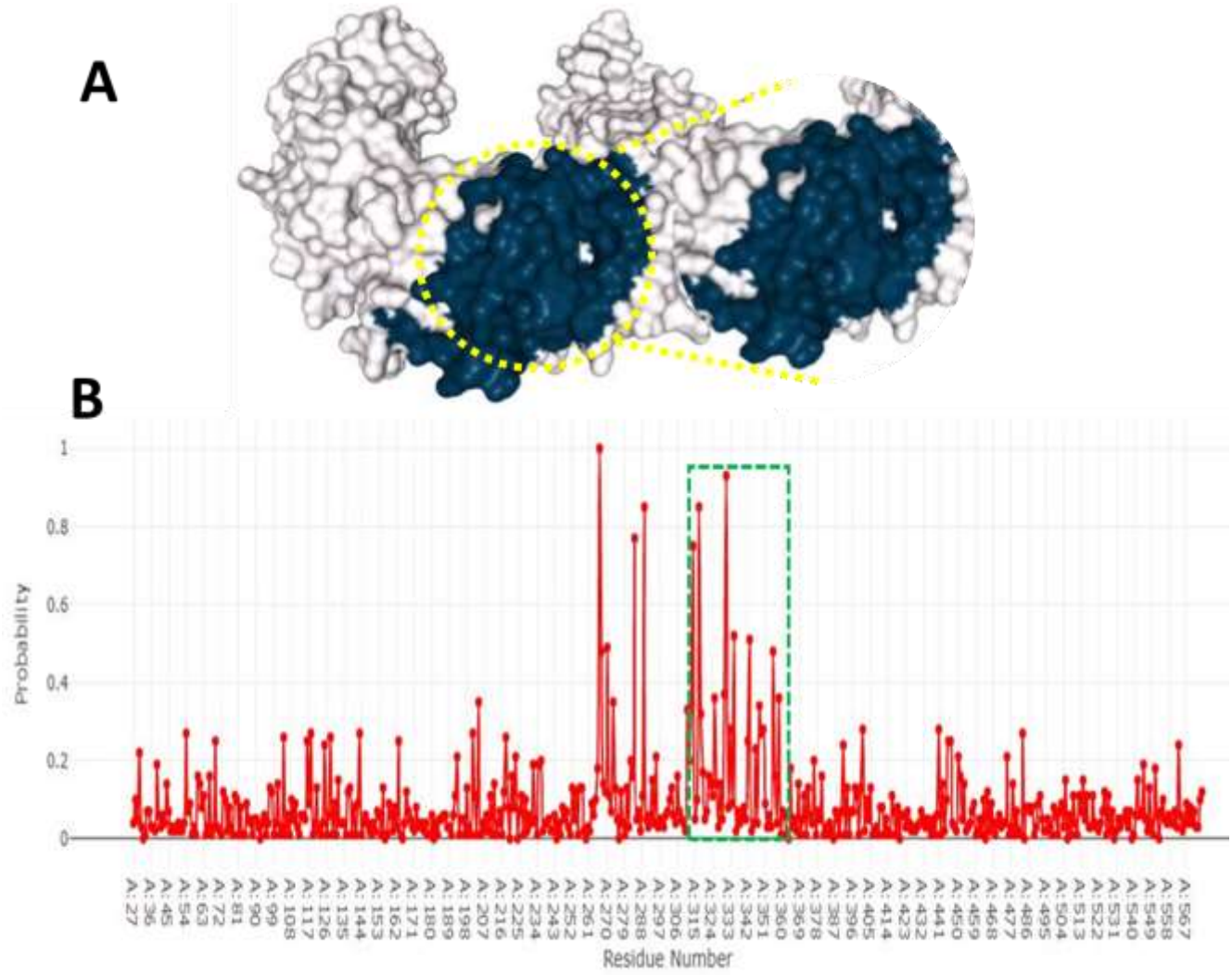

Figure 7: Binding site of TLR2 obtained from Depth Residue tool.

The binding pocket of TRPV4 consists of nine amino acids (Ala-330, Tyr-346, Ser-372, Val-385, His-388, His-401, Arg-404, Trp-409, Asp-425). All the amino acid selected for active site have probability greater than 0.2 to 0. 8 (Figure 7 A, B). It consists of major six peaks. The first peak appeared at Leu-154, second at Ala-330, third at Asp-425, fourth at Lys-467, fifth at Ile 604, sixth at Thr-739. The amino acids between second and third peak were selected as active site

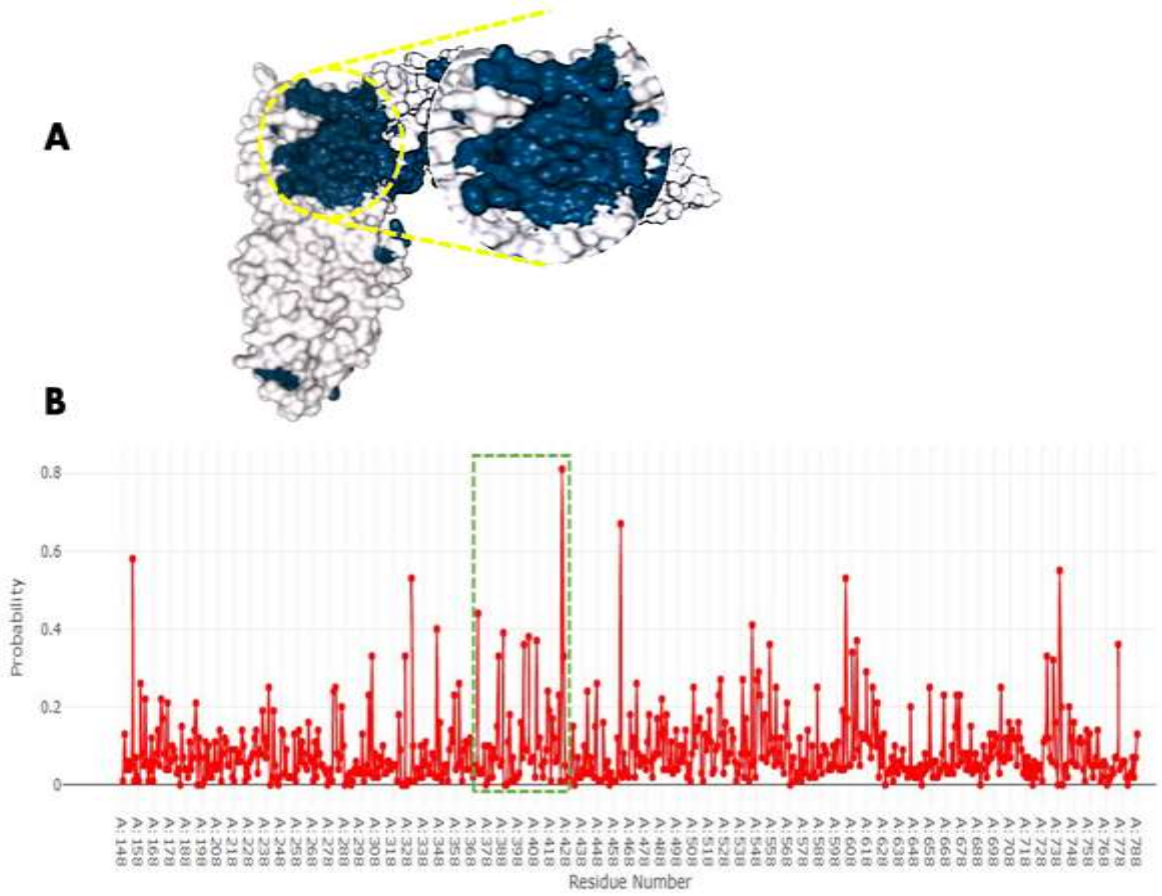

Figure 8: Binding site of TRPV4 obtained from Depth Residue tool.

Binding pockets analysis and ligands interactions.

Docking results showed that all ligands were confined in the active binding region of target proteins (PTGS2, TLR2 and TRPV4). The superimposition results of all three docking complexes showed that ligands bind in similar conformational behavior and have similar binding interactions pattern () as shown by results

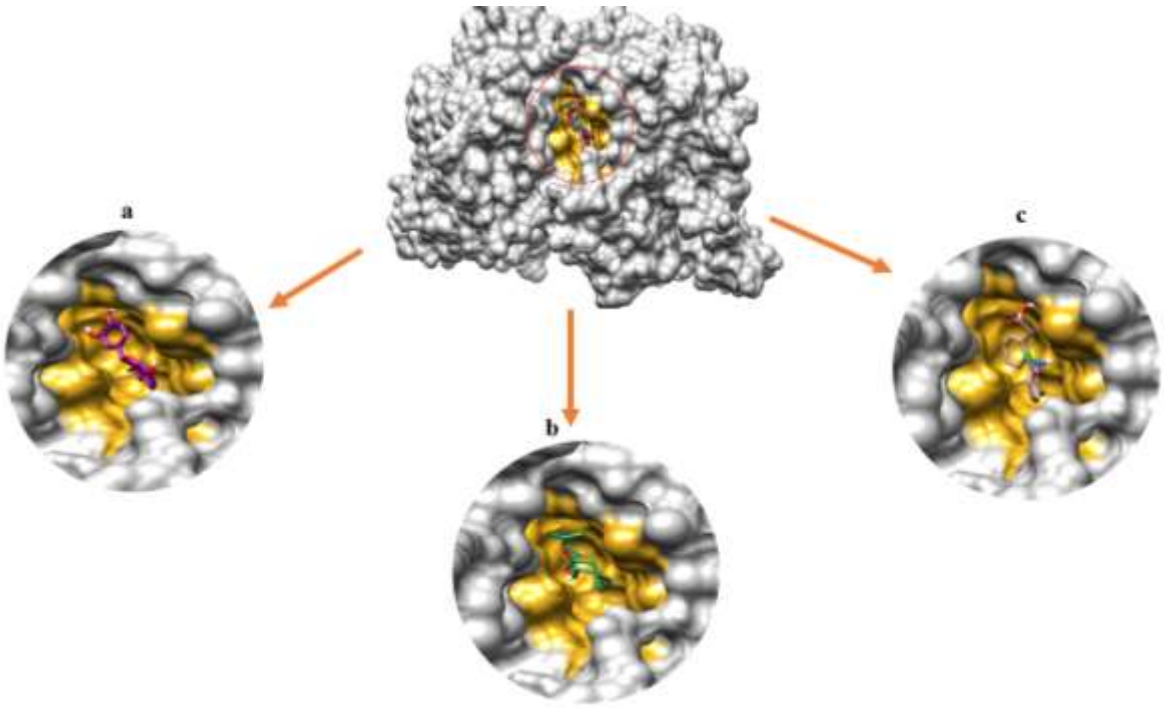

Figure 9: (a) catechin, **(b)** palmitic acid and **(c)** diclofenac binding conformation against PTGS2.

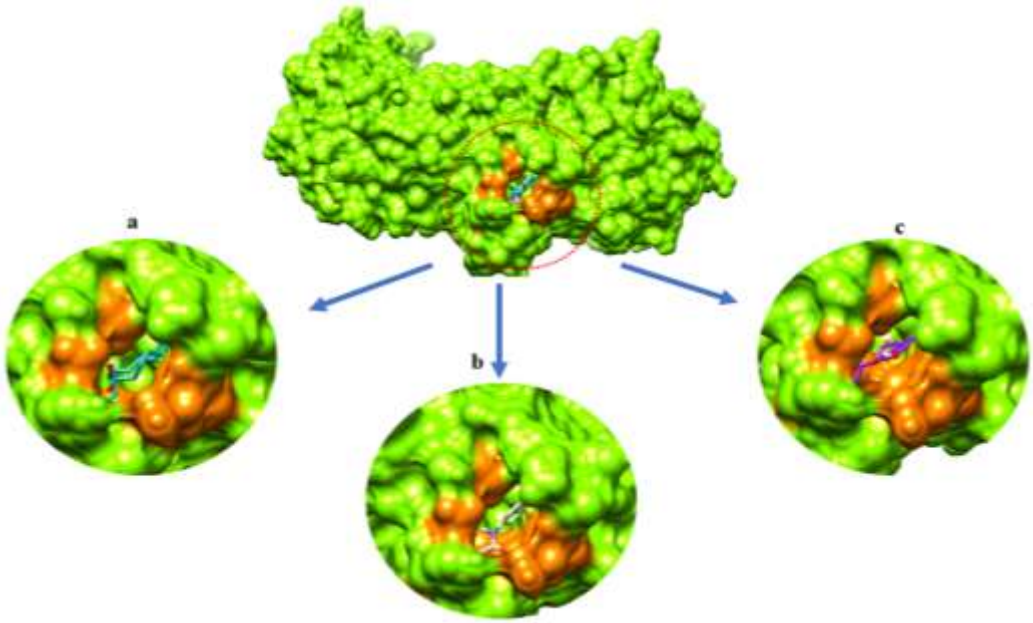

Figure 10. (a) catechin, **(b)** palmitic acid and **(c)** diclofenac binding conformation against TLR2.

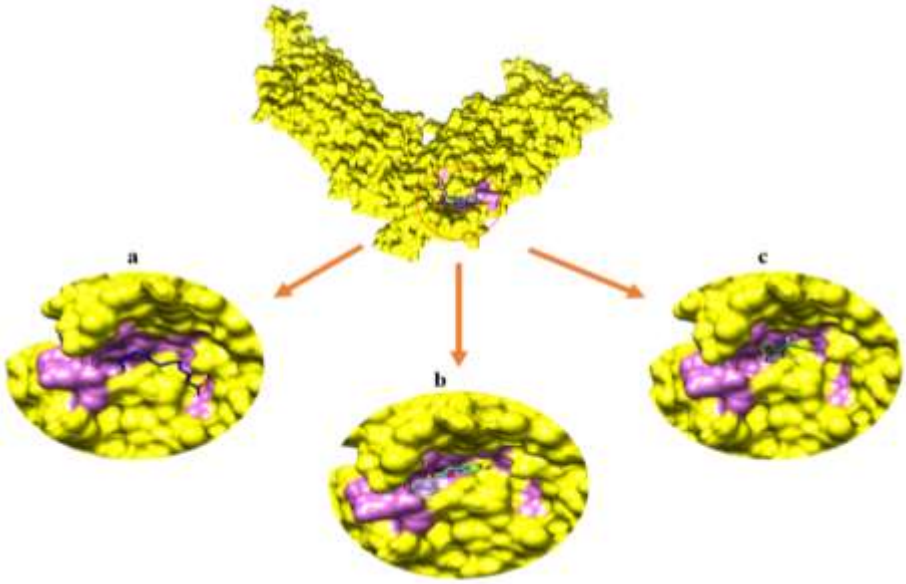

Figure 11 (a) catechin, **(b)** palmitic acid and **(c)** diclofenac binding conformation against TRPV4.

Binding Energies

The standard drug diclofenac and the plant's bioactive compounds (palmitic acid and catechin,) were docked to PTGS2 using PyRx. The top docking energy was -7.7Kcal/mol by catechin and palmitic acid showed a binding score of -5.5Kcal/mol, while standard drug diclofenac showed the binding score of -7.2 Kcal/mol

The docking results of paracetamol and the plant's bioactive compounds (palmitic acid and catechin) with TLR2 demonstrated their binding energies. The top docking energy was -8.2 Kcal/mol by catechin and palmitic acid showed binding score of -6.2Kcal/mol, while standard drug paracetamol showed the binding score of -5.9 Kcal/mol

The standard drug diclofenac and the plant's bioactive compounds (palmitic acid and catechin) were docked to TRPV4 using PyRx. The top docking energy was -8.8Kcal/mol by catechin and palmitic acid showed a binding score of -5.6 Kcal/mol, while standard drug diclofenac showed the binding score of -6.8 Kcal/mol.

Table 2: it represents the binding energies of all the ligands with target proteins.

Proteins	**Binding Energies Kcal/mol**			
	Catechin	**Palmitic acid**	**Diclofenac**	**Paracetamol**
PTGS2	-7.7	-5.5	-7.2	___
TLR2	-8.2	-6.2	___	-5.9
TRPV4	-8.8	-5.6	-6.8	___

Hydrogen bonding analysis against PTGS2

Hydrogen and hydrophobic interactions are used to evaluate the bonding interactions of docking complexes. In catechin-PTGS2 docking complex, five Hydrogen bonds has been observed at different residual positions within the active region of target protein, Ala-185, Asn-368, Tyr-371, His-372 and Trp-373. The hydroxyl group at position number 21 of catechin shares the hydrogen bond with oxygen of Ala-185, hydroxyl group at position 19 of catechin shares two hydrogen bonds with oxygen of Asn-368 and hydrogen of His-372 respectively, another hydroxyl group at position 12 of catechin makes a hydrogen bond with oxygen of Tyr-371, hydroxyl group at position 20 of catechin also makes hydrogen bond with oxygen of Trp-373. In palmitic acid-PTGS2 docking results, couple of hydrogen bonds was observed between hydroxyl group of palmitic acid and hydrogen atom of Gly-189, while standard drug Diclofenac formed 2 hydrogen bonds between NH group of diclofenac and hydrogen of Tyr-371 and hydroxyl group of catechin with NH group of Trp-373 residues respectively.

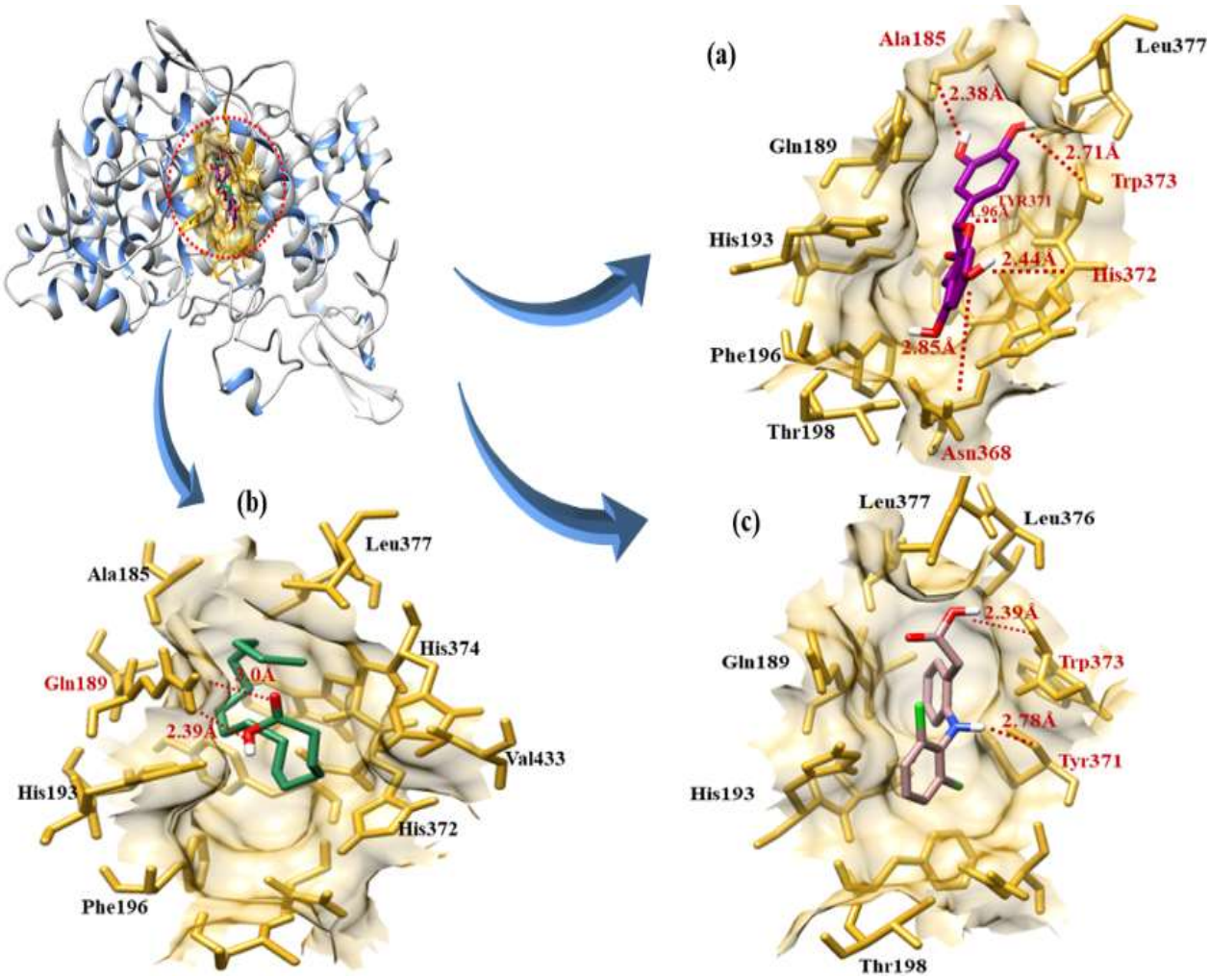

Figure 12. Docking complex

This figure shows binding interactions between target protein (PTGS2) and three ligands. The target protein is indicated with the color gray and cornflower blue in ribbon line format. The binding active amino acids all around ligands are outlined in golden color. Hydrogen bonds are also shown between TLR2 amino acids and ligands at different distances, respectively. The red dotted lines indicate the binding distance in angstrom (Å).

Table 3: It represents the bond distance between amino acids and ligands viewed in docking complex of PTGS2

Ligands	Amino acids	Bond distance (Å)	Nature of Bond
Catechin	Ala-185	2.83	Hydrogen Bond
	Asn-368	2.85	Hydrogen Bond
	Tyr-371	1.96	Hydrogen Bond
	His-372	2.44	Hydrogen Bond
	Trp-373	2.71	Hydrogen Bond
Palmitic acid	Gly-189	3.0	Hydrogen Bond
	Gly-189	2.39	Hydrogen Bond
Diclofenac	Tyr-371	2.8	Hydrogen Bond
	Trp-373	2.0	Hydrogen Bond

Phytochemical and TLR2 binding analysis

Catechin-TLR2 docking results, single Hydrogen bond has been observed with Asp-263. The bond is shared between hydroxyl group at position 17 of catechin and oxygen of Asp-263. In TLR2 docking results, another single hydrogen -bond is formed between palmitic acid and TLR2 at residual position Asp-327. This bond is shared between hydroxyl group of palmitic acid and oxygen of Asp-327. And one hydrogen bond at residue Gln-321 was imputed between paracetamol and the TLR2 binding site. Oxygen from paracetamol contributes this hydrogen bond with hydrogen of Gln-321.

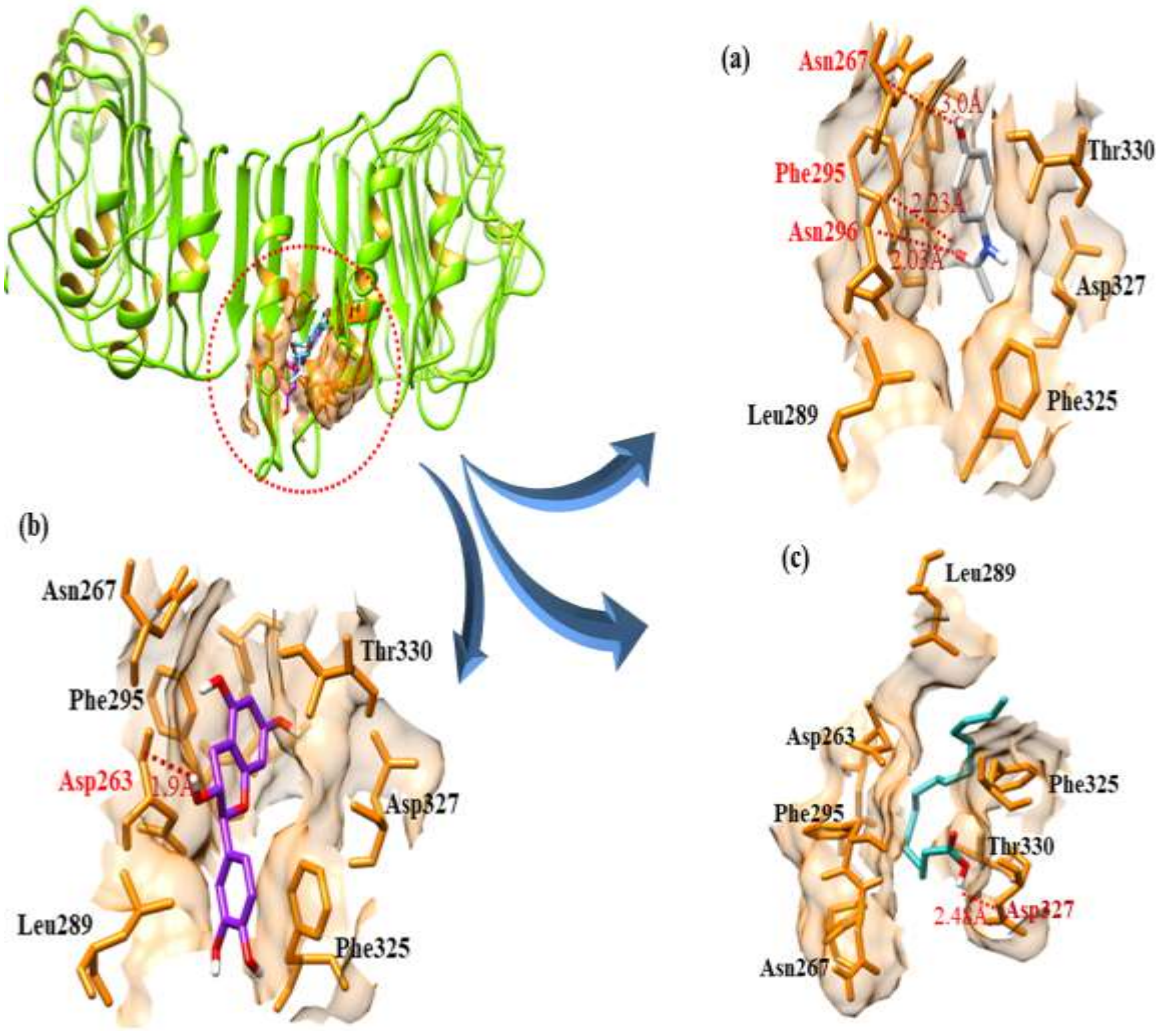

Figure 13: This figure shows binding interactions between target protein (TLR2) and three ligands. The target protein is indicated with the color chartreuse and goldenrod in ribbon line format. The binding active amino acids all around ligands are outlined in an orange color. Hydrogen bonds are also shown between TLR2 amino acids and ligands at different distances, respectively. The red dotted lines indicate the binding distance in angstrom (Å).

Table 4: It represents the bond distance between amino acids and ligands viewed in docking complex of TRPV4

Ligands	Amino acids	Bond distance (Å)	Bond nature
Catechin	Asn-263	1.9	Hydrogen Bond
Palmitic acid	Asp-327	2.48Å	Hydrogen Bond
Paracetamol	Gly-321	2.8Å	Hydrogen Bond

Docking results with TRPV4

The best pose found for Catechin in the enzymatic pocket of TRPV4 was stabilized by the formation of two hydrogen bonds (H-bonds) with Ser-360 and Ser-768. The oxygen atom at position 20 and hydroxyl group at position 21 from catechin formed the 2 hydrogen bonds with oxygen of Ser-360 and hydroxyl group of Ser-768 respectively. Similarly, oxygen of palmitic acid showed a single H-bond with hydroxyl group of serine residue at 372 position number, while standard drug diclofenac interacted with the same enzymatic pocket by establishing a single H-bonds with Ser-768 and a hydrophobic bond with Arg-775. The oxygen atom of diclofenac makes hydrogen bond with NH group of Ser-768.

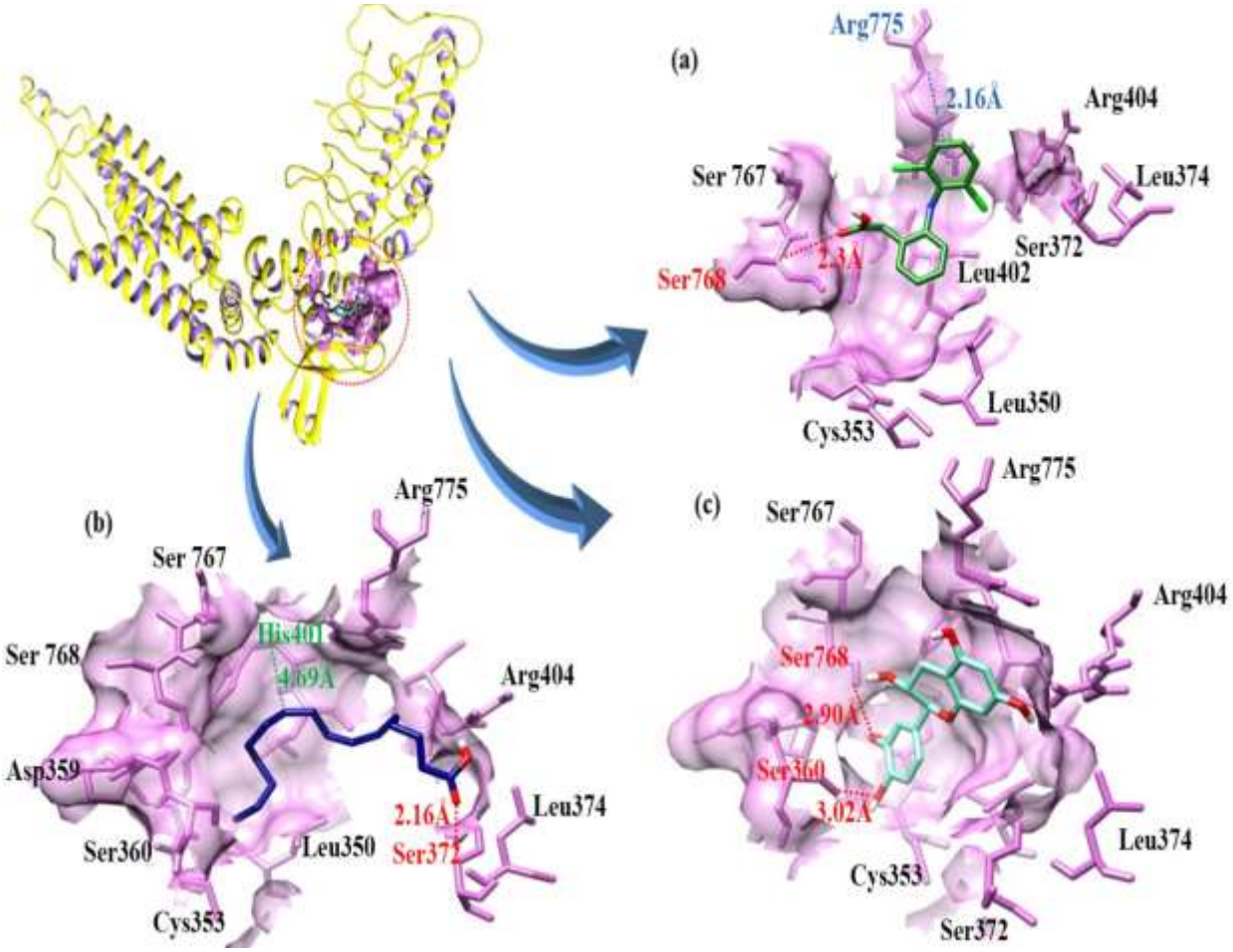

Figure 14: This figure shows binding interactions between target protein (TRPV4) and three ligands. The target protein is indicated with the color yellow and orchid in ribbon line format. The binding active amino acids all around ligands are outlined in an orchid color. Hydrogen bonds are also shown between TLR2

amino acids and ligands at different distances, respectively. The red dotted lines indicate hydrogen bond while light blue dotted lines indicate the hydrophobic interactions indicate the binding distance in angstrom (Å).

Table 5: It represents the bond distance between amino acids and ligands viewed in docking complex of TRPV4

Ligands	**Amino acids**	**Bond distance (Å)**	**Bond nature**
Catechin	Ser-360	3.02	Hydrogen Bond
	Ser-768	2.90Å	Hydrogen Bond
Palmitic acid	Ser-372	2.16Å	Hydrogen Bond
Diclofenac	Ser-768	2.3Å	Hydrogen Bond
	Arg-775	4.15Å	Hydrophobic Bond

Table 6: Common interactive residues of docking complexes.

Ligands	PGTS2	TLR2	TRPV4
Palmitic acid	Gln189, Ala185, His193, Phe196, His372, Leu377, His374, Val433,	Asp263, Asn267, Leu289, Phe295, Phe325, Asp327, Thr330	Leu350, Cys353, Asp369, Ser360, Leu374, His401, Arg404, Ser767, Ser768, Arg775
Catechin	Ala 185, Gln189, His193, Phe196, Thr198, Asn368, Tyr371 His372, Trp373, Leu377	Asp263, Asn267, Leu289, Phe295, Phe325, Asp327, Thr330	Cys353, Ser360, Ser372, Leu374, Arg404, Ser767, Ser768, Arg775
Diclofenac	Ala188, Gln189, His193, Phe196, Thr198, Lue277, Asn368, Tyr371, His372, Trp373, Leu376		Leu350, Cys353, Pro358, Ser372, Leu374, Phe375, Arg392, His401, Leu402, Arg404, Ser767, Ser768, Arg775
Paracetamol		Asn267, Leu289, Gly293, Phe295, Asn296, Gln321, Tyr323, Phe325, Asp327, Thr330,	

Discussion

Carrageenan induced rat paw edema, pyrexia induced by brewer yeast and writhing caused by acetic acid are used to study anti-inflammatory, antipyretic and analgesic activities respectively [25]. In our study, the petroleum ether extract of *Citrullus colocynthis* stem was used to evaluate the anti-inflammatory, analgesic and antipyretic activities on albino rats.

In an experimental analysis, the anti-inflammatory potential of *Citrullus colocynthis* fruit (obtained from Palestine) in different extract were examined using carrageenan induced paw edema keeping Voltarin Emulgel® as standard drug. The pharmacological analysis demonstrated that induced inflammation was reduced by 45.39% in aqueous extract, 54.11% hydrolyzed extract and acetylated extract inhibition percentage for inflammation was 64.95% in rats [26]. In presented study, it was found that petroleum ether stem extract of *Citrullus colocynthis* at dose of 400mg/kg showed significant results i.e., $p<0.005$ and percentage inhibition was 60% as compared to standard drug (diclofenac).

Carrageenan induced rat paw edema is used to study anti-inflammatory activity. Carrageenan causes inflammation in 2 phases. 1st one is the release of inflammatory agents such as serotonin, histamine and kinins. Second is the release of prostaglandins in 2 to 3 hours after carrageenan injections which are thought to be the main cause of inflammation [27]. The prostaglandins are released by PTGS2. *Citrullus colocynthis* has a variety and number of bioactive compounds having different therapeutic applications. In presented, palmitic acid and catechins found in the stem of *Citrullus colocynthis* were analyzed *in-silico* studies with PTGS2. It is observed that palmitic acid and catechin has a significant contribution in anti-inflammatory activity by inhibiting the PTGS2. The docking results showed catechin has binding affinity of -7.8Kcal/mol and palmitic acid of -5.5 Kcal/mol and that of standard drug diclofenac is -7.2 Kcal/mol.

Fever is a complex physiological response induced by an aseptic stimulus or any infection. The rise in body temperature occurs by release of cytokines that sends messages to AH/POA to induce fever. These cytokines are released by TLR2 [28]. In an experimental analysis the ethanolic extract of fruit of *Citrullus colocynthis* was found to have significant antipyretic activity. The ethanolic extract behaved like paracetamol blocking the prostaglandins and reducing the fever [29]. In presented research, petroleum ether extract of *Citrullus colocynthis* showed significant results to inhibit the pyrexia. Maximum activity was shown by the dose of 400 mg/kg which was 100%. Catechins and palmitic acid blocked the active site of TLR2 like paracetamol inhibiting the pyrexia. The binding values of catechin and palmitic acid were better than paracetamol i.e., -8.2 Kcal/mol and 6.2Kcal/mol respectively while paracetamol has binding energy of -5.5Kcal/mol.

Abdominal constriction reaction caused by acetic acid is a sensitive technique for evaluation of peripheral analgesics. Acetic acid induces discomfort by activating endogenous compounds such as serotonin, histamine, bradykinins and prostaglandins[30]. The acetic acid produces the

sensation of pain in the abdomen by activating TRPV4 [31]. In an experimental analysis the fruit and seed extract of *Citrullus colocynthis* showed the analgesic activity having significant results without any side effects [32]. In our research work it was found that petroleum ether extract of stem part of *Citrullus colocynthis* exhibits significant results ($p<0.005$) for analgesic activity. Maximum activity is shown by the dose of 400mg/kg which was 72%. Moreover, the catechin and palmitic acid were found to inhibit the active site of TRPV4 through *in-silico* analysis. Catechin have binding energy of -8.8 Kcal/mol for TRPV4 which were a lot better than standard drug diclofenac i.e., -6.2Kcal/mol. Following this palmitic acid has binding energy of -5.8 Kcal/mol for TRPV4.

Conclusion.

From our research findings, it was found that petroleum ether extract of *Citrullus colocynthis* can be used in allopathy to treat inflammation, pain and fever. The anti-inflammatory, antipyretic and analgesic activities are possessed by *Citrullus colocynthis* due to its phytoconstituent composition. The docking results and analysis of the interactions of palmitic acid and catechin with different proteins indicates their ability to bind with several targets involved in immunomodulation, suggesting clues to modern medicines.

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