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ANTI-INFLAMMATORY, *IN-SILICO* DOCKING AND ADME ANALYSIS OF SOME ISOLATED COMPOUNDS OF *TAGETES ERECTA* FLOWER HEADS

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Anti-inflammatory, Molecular docking, *Tagetes erecta*, ADME/T analysis

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ABSTRACT: Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents. Medicinal plants and their secondary metabolites are progressively used in the treatment of diseases as complementary medicine. In the present study methanolic extract of flower heads of *Tagetes erecta* was screened for its phytochemical constituents and then subjected for its anti-inflammatory activity by using *in-vitro* and *in-vivo* methods. The preliminary phytochemical investigation of methanolic extract of flower heads of *Tagetes erecta* showed the presence of flavonoids like quercetin, kaempferol, gallic acid, syringic acid, terpenoids like β-amyrin, erythrodiol, steroids like β-sitosterol, β-stigmasterol, phenols, and alkaloids. The methanolic extract of *Tagetes erecta* (METE) significantly ($p<0.05$) inhibited the paw oedema volume. The extract significantly ($p<0.05$) protected protein membranes from denaturation. To understand the ligand-binding affinity of the above constituents with COX-2 the constituents were subjected to molecular docking studies using ligand fit of maestro 9.1 (Schrodinger Software Inc.). Amongst the constituents identified kaempferol, quercetin, gallic acid were found to possess strong binding affinity towards COX-2, β-sitosterol, β-stigmasterol, β-amyrin, erythrodiol were found to bind to TNF-α. An *in-silico* study of these selected phytochemical constituents was also subjected to Swiss ADME, a web tool to evaluate their pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules to support drug discovery. The results revealed that the methanolic extract of flower heads of *Tagetes erecta* possesses significant anti-inflammatory activity.

INTRODUCTION: Inflammation is the tissue reaction against infectious agents like microbes, irritants or any other foreign substances. It is a part of the host defense mechanisms that are known to be involved in the inflammatory reactions associated with the release of histamine, bradykinin and prostaglandins.

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Clinically inflammation, reported by Cornelius Celsus of Rome 2000 years ago, is rubor (redness) or calor (heat) and / or dolar (pain) at the affected region because of a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells [1, 2].

Cyclooxygenases (COX) or prostaglandin-endoperoxide synthases (PGHS) are the key enzymes in the synthesis of prostaglandins, the main mediators of inflammation, pain and increased body temperature (hyperpyrexia). In human two main isoforms of COX proteins, namely Cyclooxygenases-1 (COX-1) and Cyclo-oxygenases-2 (COX-2) are found [3]. Many NSAIDs

inhibit both COX-1 and COX-2 enzymes and reduces inflammation, damaging the lining of the stomach leading to gastric problems like stomach upset, ulceration and blood bleeding from the stomach and intestine. Antagonizing specific COX-2 enzyme is an attractive therapeutic target to develop potent anti-inflammatory drugs [4]. Tumor necrosis factor α (TNF-α, also known as cachectin) is a strong pro-inflammatory cytokine that plays an important role in the immune system during inflammation, cell proliferation, differentiation and apoptosis [5]. It was first described by Carwell *et al.*, in 1975 as a cytokine which showed significant cytotoxic activity after stimulation of the immune system and thus, caused tumor necrosis. When the gene for TNF-α was cloned in 1984, a structure homology with lymphotoxin (LT)-α was found and TNF-α was included in the group of cytokines known as the TNF ligand superfamily. Its members are type II transmembrane proteins which can be expressed in both membrane-bound and secreted forms.

The search for compounds with novel properties to deal with inflammatory conditions is still in progress. Experimental induction of inflammation in animal models is essential for the advancement of our knowledge and understanding of various aspects of its pathogenesis and ultimately finding new therapies and cure. Traditionally healthful plants have served to be efficient anti-inflammatory agents for ages because of their wealthy diversity of phytochemicals.

Tagetes is a genus of annual or perennial, mostly herbaceous plants in the sunflower family (Asteraceae). Flowers of *Tagetes erecta* Linn. used traditionally from ancient times and are used in folk medicine to cure various types of diseases. The predominant phytochemical constituents identified in the flowers of *Tagetes erecta* are flavonoids like quercetin, kaempferol, terpenoids like beta- amyrin, erythrodiol, phenolics like syringic acid, gallic acid, steroids like β-sitosterol and β-stigmasterol. Being a rich source of many bioactive components and wide availability, *T. erecta* is now one of the prime targets of researchers working on the chemistry of natural products [6].

To understand the ligand binding properties, the above said phytochemical constituents, were subjected to molecular docking studies. These studies act as a computational tool to predict the plausible interactions between the phytochemical constituents and protein in a non-covalent fashion. An *in-silico* study of selected phytochemical constituents was performed by Swiss ADME, a web tool to evaluate their pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules to support drug discovery [7].

The present study is an attempt to predict the potential compounds of *Tagetes erecta* using a three-dimensional (3D) structure of COX-2 and TNF-α from protein data bank (PDB). Ligands known to bind with COX-2 and TNF-α were submitted to docking protocol GLIDE 5.6 (Schrodinger) to establish relationships between their biological activity and the predicted binding affinities. ADME property analysis is used to measure the safety of the compounds as drugs to treat the inflammatory disorder.

MATERIALS AND METHODS:

Preparation of Extract: The plant was identified and authenticated by a botanist of Government Degree College with voucher specimen no (GRCP/009). The powdered crude material (200 gm) was extracted with methanol by Soxhlation at the end of the extraction process the extract obtained was filtered and evaporated to a solid mass. The extract was preserved in desiccators to remove remaining moisture if present and finally stored in airtight containers for further use.

Identification of Phytochemical Constituents: Phytochemical screening of *Tagetes erecta* extract was carried out by using standard tests.

Acute Toxicity Testing: Acute toxicity study of methanolic extract of *Tagetes erecta* flower heads were carried out as per the OECD 425 guidelines.

Experimental Animals: Adult Wistar albino rats (180 - 220 g) were used for the present study. They were kept in polypropylene cages at 25 ± 2 °C, with relative humidity 45 - 55% under 12 h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed and water *ad libitum*. All the pharmacological experimental protocols were approved by the Institutional

Animal Ethics Committee (IAEC) with (Reg. No. 1175/PO/ERe/S/08/CPCSEA).

Anti-Inflammatory Activity:

***In-vitro* Studies:**

Protein Denaturation Method: Antiinflammatory activity was evaluated by the protein denaturation method. Indomethacin was used as a standard. The reaction mixture consists of 1 ml of different concentrations of extracts and indomethacin ranging from 50-1000 µg/ml and 3 ml of phosphate-buffered saline (pH 6.4) was mixed with 1 ml of egg albumin solution (1%), the reaction mixture without plant extracts was taken as control and incubated at 37 ℃ for 20 min. Denaturation was induced by keeping the reaction mixture at 90 ℃ in a water bath for two minutes. After cooling the turbidity was measured using a spectro-photometer at 660 nm. Percentage inhibition of denaturation was calculated by using the following formula [8].

$$\% \text{ Inhibition} = (At - Ac) / Ac \times 100$$

Where, Ac = Absorbance of control, At = Absorbance of a test sample.

***In-vivo* studies:**

Carrageenan Induced Rat Hind Paw Oedema Method: In the present study male Wistar rats (180 - 200 g) were divided into 6 animals in each group. Inflammation is induced by subcutaneous injection of 0.1 ml of 1% freshly prepared carrageenan in saline in the left hind paw of rats of control, test and standard groups. The paw volume of rats was measured in all groups of animals with the help of the plethysmometer during the interval of 0h, 1h, 2h, 3h, 4h and 5h after Carrageenan administration [8].

TABLE 1: STUDY DESIGN FOR CARRAGEENAN INDUCED PAW OEDEMA MODEL

Group I	Normal saline
Group II	1% Carrageenan (50 µL)
Group III	METE (200 mg/kg, bd. wt, *p.o*) + 1 % Carrageenan (50 µL)
Group IV	METE (400 mg/kg, bd. wt, *p.o*) + 1 % Carrageenan (50 µL)
Group V	Indomethacin (5 mg/kg, bd. wt, *p.o*) + 1 % Carrageenan (50 µL)

Docking Studies: The crystallographic structure of the enzymatic target COX-2 and TNF-α was obtained from the Protein Data Bank database [PDB: 5HDU, 3EDZ]. The molecular docking study was performed using Schrodinger software 5.6. The docking analysis of the compounds with COX-2 and TNF-α was carried out by ligand fit of maestro 9.1 (Schrodinger Software Inc.). The software allows us to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. The collection of enzyme-substrate complexes were identified via docking and their relative stabilities were evaluated using their binding affinities. Ligand fit was used for accurately docking ligands into protein active sites employing a cavity detection algorithm. A high-throughput screening study applied to the COX receptor is also presented in which ligand fit when combined with lig score, an internally developed scoring function, yields very good hit rates for a ligand pool seeded with known actives [9].

Docking Protocol:

Protein Preparation: The crystal structure of COX-2 and TNF-α (5HDU and 3EDZ) was prepared and the active site was identified. The ligands and crystallographic water molecules were removed from the protein and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the CHARMM force field.

Ligand Preparation: The three-dimensional structures of compounds were downloaded in .sdf format from Pub Chem and Chemdraw database. Hydrogen Bonds were added and the energy was minimized using the CHARMM force field. G score, Hydrogen bond, Rotatable bond penalty, a lipophilic term derived from the hydrophobic grid for the isolated compounds from *Tagetes erecta*.

Docking Studies: The active site of the protein was first identified and it is defined as the binding site. The binding sites were defined based on the ligands already present in the PDB file (*i.e.* Cyclooxygenase binding site region) which were followed by site sphere definition. The determination of the ligand-binding affinity was calculated using the lig Score and dock score to estimate the ligand-binding energies. Apart from

these other input parameters for docking were set as default options [10].

ADME Studies: An *in-silico* study of Isolated compounds from *Tagetes erecta* was done for the prediction of ADME properties, Molecular weight, Total polar surface area (TPSA), ILOG P, number of rotatable bonds, number of hydrogen donor and acceptor atoms were calculated on the basis of Lipinski's rule of five (Lipinski *et al.*, 2001).

In the present study, ADME was done by utilizing a web-based program (www.swissadme.ch). This software computes physicochemical descriptors as well as predicts pharmacokinetic properties and the drug-like nature of one or multiple small molecules (BBB, Cyp, Pgp). The compounds with positive values can cross readily in the BBB, while compounds with negative values are poorly distributed to the brain [11].

Statistical Analysis: All the values were expressed as mean ± standard error of the mean. The data were statistically analyzed by one-way analysis of variance followed by Dunnett's test and values $p < 0.05$ were considered to be significant.

RESULTS:

Preliminary Phytochemical Analysis: The preliminary phytochemical investigation of methanolic extract of flower heads of *Tagetes erecta* showed the presence of flavonoids terpenoids, steroids, phenols, and alkaloids. The results are shown in **Table 2**.

TABLE 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemical Constituents	Results
Flavonoids	++
Terpenoids	++
Steroids	++
Phenols	++
Alkaloids	++

Acute Toxicity Studies: Using the methanolic extract of *Tagetes erecta* acute toxicity studies were performed as per OECD guidelines 425.

The oral administration of methanolic flower extract of *Tagetes erecta* did not exhibit any signs of toxicity and mortality even up to 2000 mg/kg. bd. wt. All animals were safe even after 14 days of observation.

***In-vitro* Anti-Inflammatory Activity:** The *in-vitro* anti-inflammatory activity was performed using the protein denaturation method. The results were expressed in **Table 3**.

TABLE 3: IC_{50} VALUES OF *IN-VITRO* PROTEIN DENATURATION OF METE FLOWER HEADS

S. no.	Compounds	IC_{50} Value
1	METE	3.96
2	Indomethacin	4.14

In protein denaturation assay, the METE was tested at different concentrations like 50, 100, 200, 400, 600, 800 and 1000 µg/ml. The lowest conc. of 50 µg/ml showed a percentage inhibition of 20.27 whereas the highest concentration of 1000 µg/ml showed a percentage inhibition of 87.63. The IC_{50} value for the METE was found to be 3.96 µg/ml which is compared with standard indomethacin having an IC_{50} value of 4.14 µg/ml respectively.

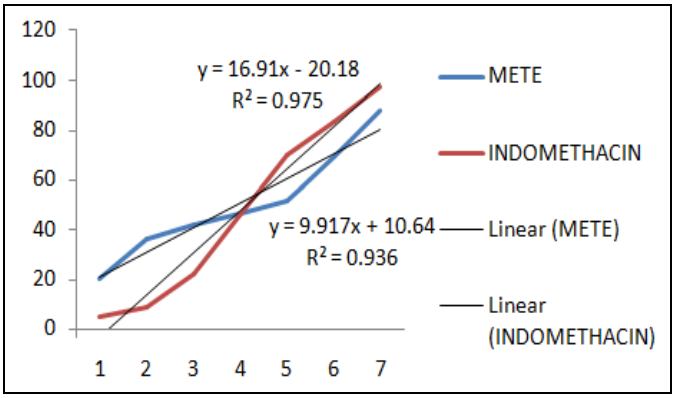

FIG. 1: IC_{50} VALUES OF *IN-VITRO* PROTEIN DENATURATION OF METE

***In-vivo* Anti-Inflammatory Activity:**

Carrageenan Induced Rat Hind Paw Oedema Model: Carrageenan induced rat hind paw oedema model is ubiquitously used model to determine anti-inflammatory activity and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation. In the control group which received saline the initial paw volume of rats was found to be 0.21 ± 0.04 and the paw volume after 5 h of drug administration was found to be 0.20 ± 0.03. In the carrageen control group, the initial and final paw volume was found to be 0.22 ± 0.03 and 0.92 ± 0.04.

The METE at a dose of 200 and 400 mg/kg bd. wt, the initial and final paw volume after 5 h were 0.21 ± 0.02, 0.38 ± 0.04, 0.23 ± 0.04, 0.31 ± 0.04. The initial and final paw volume of the indomethacin

treated group was 0.20 ± 0.05 and 0.22 ± 0.03. There was a significant decrease in paw volume after administration of METE (200 & 400 mg/kg, p.o) and standard when compared with the negative control.

TABLE 4: EFFECT OF METE ON PAW VOLUME (ml) IN CARRAGEENAN INDUCED PAW OEDEMA MODEL USING PLETHYSMOGRAPH

Groups	Paw volume (ml)					
	0 h	1 h	2 h	3 h	4 h	5 h
Normal control	0.21±0.04	0.21±0.03	0.22±0.04	0.19±0.02	0.23±0.05	0.20±0.03
Negative control	0.22±0.03	0.65±0.05$^{**, A}$	0.73±0.02$^{**, A}$	0.85±0.04$^{**, A}$	0.88±0.04$^{**, A}$	0.92±0.04^{**A}
METE (200 mg/kg)	0.21±0.02	0.53±0.03^{**,Bb}	0.5±0.03$^{**,A, a}$	0.45±0.03$^{**,A, a}$	0.41±0.02$^{*B, a}$	0.38±0.04$^{**B, a}$
METE (400 mg/kg)	0.23±0.04	0.49±0.02$^{**,B, b}$	0.45±0.03$^{*,B, a}$	0.41±0.02$^{**,B, a}$	0.37±0.03^{a}	0.31±0.04^{a}
Indomethacin (5 mg/kg)	0.20±0.05	0.35±0.03^{*,a}	0.33±0.02^{*,a}	0.30±0.04^{*,a}	0.26±0.05^{a}	0.22±0.03^{a}

Values were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnet's test by comparing it with control, negative control & standard. Significant values are expressed as control group (**p<0.01, *p<0.05), negative control (a=p<0.01, b=p<0.05) & standard (A=p<0.01, B p<0.05).

TABLE 5: EFFECT OF METE ON PAW VOLUME (ml) & % INHIBITION IN CARRAGEENAN INDUCED PAW OEDEMA AT 3 H USING PLETHYSMOGRAPH

Group	Paw volume (ml)	Inhibition (%)
Negative control	0.85 ± 0.04	--
METE (200 mg/kg)	0.45 ± 0.03	61.90
METE (400 mg/kg)	0.41 ± 0.02	71.42
Indomethacin (5 mg/kg)	0.30 ± 0.04	84.12

The acute anti-inflammatory effect of METE was evaluated using the carrageenan-induced paw oedema model. The paw volume of the animals in the negative control group was found to be 0.85 ± 0.04. METE at two dose levels 200 & 400 mg/kg, bd. wt the paw volume was found to be 0.45 & 0.41 and the paw volume of the standard drug-treated animal was 0.30. But in screening the anti-inflammatory activity, the percentage inhibition of paw volume was considered to be an important factor. In the present study, the paw volume at 3 h is measured. The METE at two dose levels 200 & 400 mg/kg, bd. wt, the percentage of inhibition was found to be 61.90 & 71.42. The percentage inhibition of indomethacin treated group was found to be 84.12. From the above results, the METE significantly possesses anti-inflammatory activity.

Docking Results:

Flavonoid Docking Studies:

Quercetin (Total Score -7.98): Demonstrated hydrogen bonding interactions with Gln 73, Asp 289.

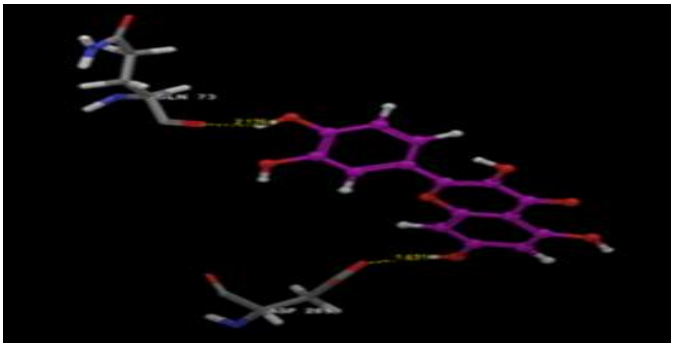

FIG. 2: HYDROGEN BINDING INTERACTIONS OF QUERCETIN WITH PDB ID: 5HDU

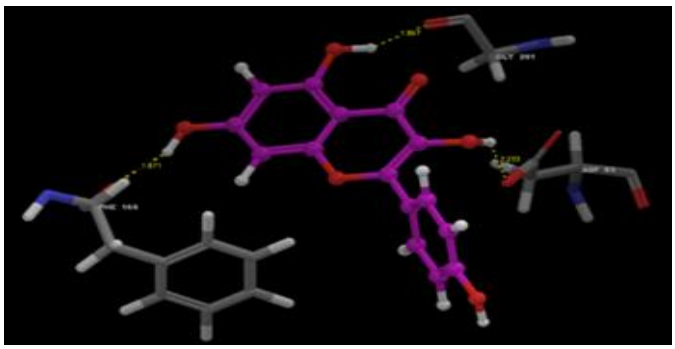

FIG. 3: HYDROGEN BINDING INTERACTIONS OF KAEMPFEROL WITH PDB ID: 5HDU

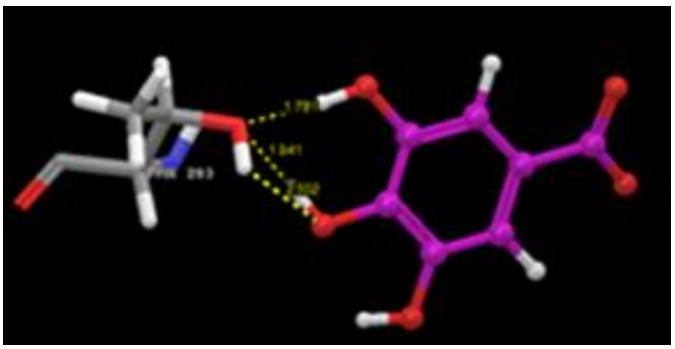

FIG. 4: HYDROGEN BINDING INTERACTIONS OF GALLIC ACID WITH PDB ID: 5HD

Kaempferol (Total Score -8.04): Demonstrated hydrogen bonding interactions with Gly 291, Asp 93, Phe 169.

Gallic Acid (Total Score -5.57): Demonstrated hydrogen bonding interactions with Thr 293.

Triterpenoids Docking Studies:

B-Stigmaterol (Total Score -4.24): Demonstrated hydrogen bonding interactions with SER 355.

Erythrodiol (Total Score -4.51): Demonstrated hydrogen bonding interactions with PRO 437.

Indomethacin (Total Score -5.89): Demonstrated hydrogen bonding interactions with HIE 415.

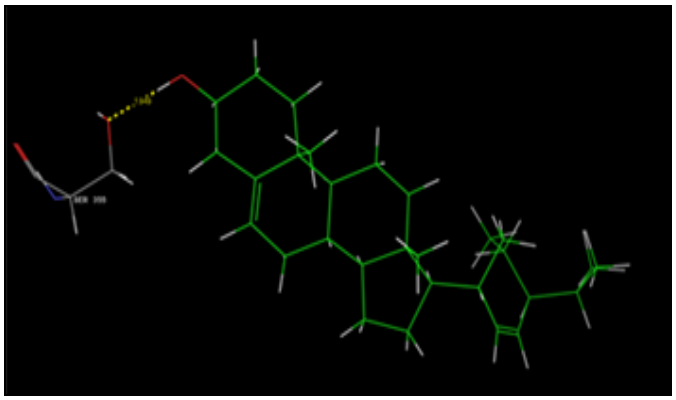

FIG. 5: HYDROGEN BINDING INTERACTIONS OF B-STIGMATEROL WITH PDB ID: 3EDZ

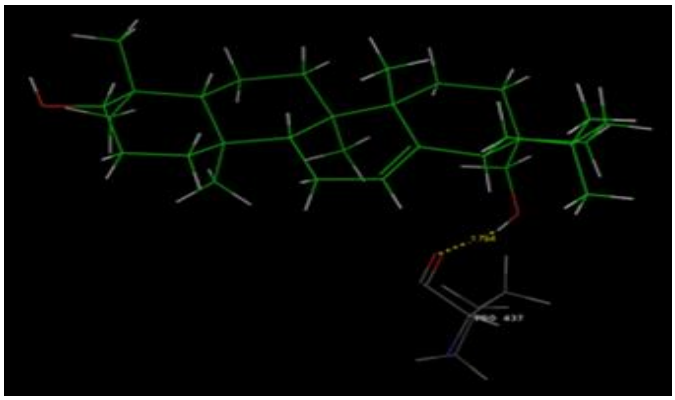

FIG. 6: HYDROGEN BINDING INTERACTIONS OF ERYTHRODIOL WITH PDB ID: 3EDZ

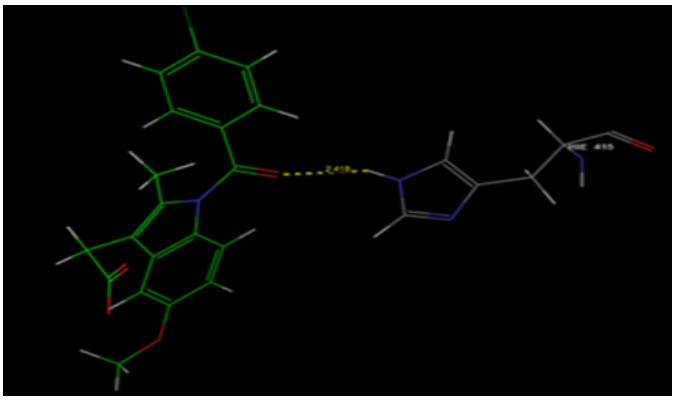

FIG. 7: HYDROGEN BINDING INTERACTIONS OF INDOMETHACIN WITH PDB ID: 3EDZ

TABLE 6: DOCKING RESULTS OF FLAVONOIDS FROM METE AGAINST COX-2 (PDB ID: 5HDU)

S. no.	Name of the compound	Glide score	Lipophilicity	H bond
1	Kaempferol	-8.04	-3.06	-1.92
2	Quercetin	-7.98	-2.58	-2.33
3	Gallic acid	-5.57	-1.99	-1.44
4	Syringic acid	-4.54	-1.54	-0.48
5	Indomethacin	-5.01	-2.27	0.00

TABLE 7: DOCKING RESULTS OF TRITERPENOIDS FROM METE AGAINST TNF-A (PDB ID: 3EDZ)

S. no.	Name of the compound	Glide score	Lipophilicity	H bond
1	β- Sitosterol	-4.90	-2.87	0
2	β- stigmasterol	-4.24	-1.21	-0.70
3	B- Amyrin	-4.42	-2.45	0
4	Erythrodiol	-4.51	-2.31	-0.70
5	Indomethacin	-5.89	-3.31	-0.06

Pharmacokinetic Parameters:

TABLE 8: ADME PROFILE OF SELECTED FLAVONOIDS AND TRITERPENOIDS OF METE

Molecule	Pubchem CID	Mol. Wt. g/mol	TPSA Å²	I LOGP	H bond acceptors	H bond donors	Lipinski violations
Quercetin	5280343	302.24	131.36	1.63	7	5	0
Kaempferol	5280863	286.24	111.13	1.7	6	4	0

Gallic acid	370	170.12	97.99	0.21	5	4	0
Syringic acid	10742	198.17	75.99	1.66	5	2	0
β-amyrin	73145	426.72	20.23	4.75	1	1	1
β-sitosterol	222284	414.71	20.23	4.79	1	1	1
β-stigmasterol	5280794	412.69	20.23	4.96	1	1	1
Erythrodiol	101761	442.72	40.46	4.5	2	2	1
Indomethacin	3715	357.79	68.53	2.76	4	1	0

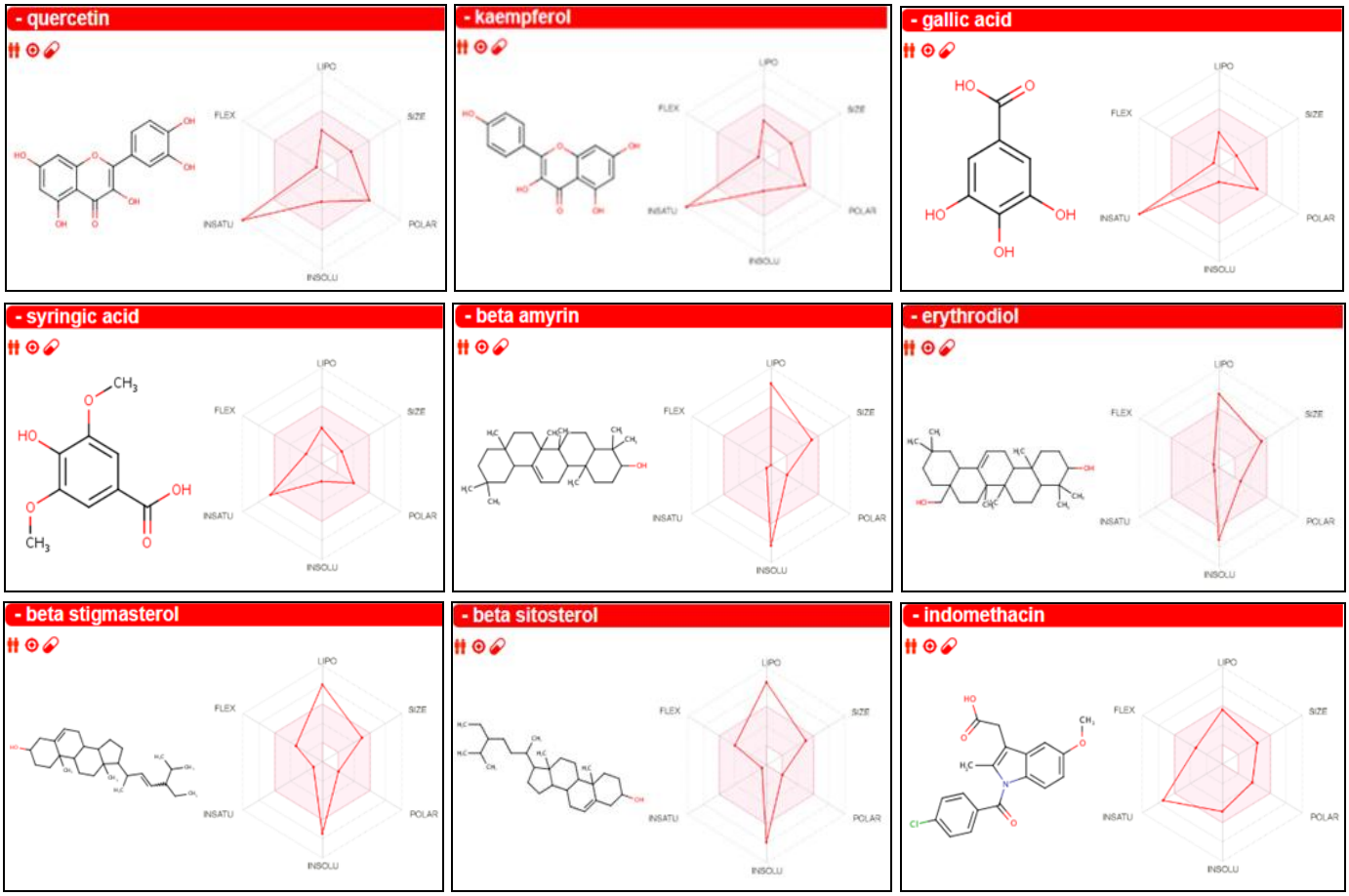

FIG. 8: LIPINSKI VIOLATIONS OF FLAVONOIDS AND TRITERPENOIDS OF METE AND INDOMETHACIN

DISCUSSION: Flavonoids belong to a group of natural compounds and occur as aglycone, glycosides, and other derivatives. The flavonoids are categorized into flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids. Flavonoids exert their anti-inflammatory activity by various mechanisms, *i.e.*, inhibition of phospholipase A2, COX and LOX.

Other mechanisms include inhibition of histamine release, phosphodiesterase, protein kinases, and activation of transcriptase. Quercetin is reported as a strong inhibitor of both COX-2 and 5-LOX [12]. Terpenoids are classified into hemi, mono, sesqui, di, sester, tri and tetraterpenoids. Large numbers of terpenoids have been tested for anti-inflammatory properties. The anti-inflammatory activity of 1, 8-Cineol, a monoterpene oxide, is correlated to inhibition of leukotriene B4, prostaglandin E2 TNF-α, interleukin, and thromboxane [13].

Protein denaturation is a process in which proteins lose their tertiary and secondary structure by application of external stress or compounds such as strong acid or base a concentrated inorganic salt, an organic solvent or heat. Denaturation of protein is a well-documented cause of inflammation [8]. In the present study, the anti-inflammatory activity of *in-vitro* METE can be attributed to its polyphenol contents, flavonoids and saponins. These can bind cations and able to protect the protein-membrane from denaturation. Carrageenan-induced rat paw oedema is a ubiquitously used test to determine anti-inflammatory activity and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or

damage to the inflamed paw. Several studies have been reported that flavonoids inhibit pro-inflammatory enzymes, such as cyclooxygenase-2, lipoxygenase and inducible NO synthase, inhibition of NF-Kb and activating protein-1 (AP-1) and activation of MAPK, protein kinase C. METE is known to possess various flavonoids and phenols as active constituents. These phytochemical constituents in METE might be responsible for its anti-inflammatory activity [8].

In-silico molecular docking studies with COX-2 and TNF–α provides more insight into the binding modes of ligands with their active sites. Glide Score is an empirical scoring function that approximates the ligand binding free energy. It should be used to rank the poses of different ligands. As it simulates binding free energy, more negative values represent tighter binders [14]. For Glide SP, scores of -10 or lower usually represent good binding. For some targets (*e.g.,* with shallow active sites or predominantly hydrophobic interactions), scores of -8 or -9 might be very good. In the present study, nine compounds namely flavonoids like quercetin, kaempferol, gallic acid, syringic acid and triterpenoids namely β-amyrin, β-sitosterol, β-stigmasterol, Erythrodiol and standard drug indomethacin were subjected to docking studies.

The flavonoids were found to inhibit COX-2 and triterpenoids were found to inhibit TNF- α. The results were tabulated in **Table 4** and **5**. Out of all the flavonoids, the results have shown that kaempferol and quercetin were found to possess a high binding affinity with COX-2. Apart from the glide score, the protein-ligand binding energy is also analyzed, where it was observed that kaempferol interacted with the active site pocket of the COX-2 (5HDU) with a binding energy of -8.04 kcal/mol (more negative value indicates better binding affinity), which was highest when compared among other ligands. The glide scores of beta amyrin, beta-sitosterol, beta stigmasterol, erythrodiol and the standard drug indomethacin were found to be ideal. The glide score of any compound less than – 10 is considered as good binding. The protein-ligand binding energy of triterpenoids was also analyzed, where it was observed that triterpenoids interacted with the active site pocket of the TNF- α (3EDZ).

Hydrogen bonding is an exchange reaction whereby the hydrogen bond donors and acceptors of the free protein and ligand break their hydrogen bonds with water and form new ones in the protein-ligand complex [15]. The H-bond per-residue interaction term is the sum of the individual H-bond scores for H-bonds between the ligand and a given residue. The more negative the score, the stronger the H-bonding. The scores are influenced by the types of atoms involved in the H-bonds, and the geometries of the H-bonds. The hydrogen bond penalty (HBP) explains the change of hydrogen bonding energy in the binding process. The length of the bond is determined by the number of bonded electrons. The higher the bond order the stronger the pull between the two atoms and the shorter the bond length. Generally, the length of the bond between two atoms is approximately the sum of the covalent radii of the two atoms.

Lipinski's rule of five is to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it an orally active drug in humans. In the present study, all the selected flavonoids and Indomethacin has zero violations and triterpenoids were found to possess one violation out of five. Any compound with zero violation clearly indicates the probability of its higher oral bioavailability. Lipinski violations of flavonoids, triterpenoids and standard indo-methacin were depicted in **Fig. 2**.

Topological polar surface area (TPSA) allows the prediction of transport properties of drug candidates in the intestines and blood-brain barrier. The TPSA score in all the flavonoids and triterpenoids of METE was found to be less than 140 which clearly indicated better permeability into the tissues [16].

CONCLUSION: Anti-inflammatory activity of METE, high-throughput screening using Swiss ADME followed by molecular docking using Schrodinger has proved to be useful in finding some possible lead compounds. The docking score determined in this study can be correlated with biological activities. The detailed analysis of the resulted COX-2, TNF-α ligands may improve our knowledge in understanding the binding interactions in detail.

Swiss ADME web tool enables the computation of key physicochemical, pharmacokinetic, drug-like and related parameters for one or multiple molecules. Through the study conducted, it could be concluded that the aqueous solubility of the compound should be increased along with the fraction of sp^3 hybridized carbon atoms. The molecule should not be the inhibitor of metabolizing enzymes and so further modifications need to be done on the lead structure. The most potent derivatives in this study could be subjected to further pharmacological evaluations to develop highly potent anti-inflammatory drugs.

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