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IN SILICO AND IN VITRO EXPLORATION OF ANTI-INFLAMMATORY ACTIVITY OF *ENTADA PURSAETHA* BY MOLECULAR DOCKING STUDIES

Narasimharao Bhogireddy[1], Pardhasaradhi Mathi[1], Nagavamsikrishna Ambatipudi[2], Venkata Raman Bokka[3*]

[1]Department of Biotechnology, K L University, Green fields, Vaddeswaram, Guntur Dist-522 502, AndhraPradesh, India.

[2]Department of Biochemistry, Acharya Nagarjuna University, Guntur Dist-522 510, AndhraPradesh, India.

[3]Department of Basic Sciences-Chemistry, Madanapalle institute of technology and science, Madanapalle, Chittoor Dist-517 325, AndhraPradesh, India.

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***Correspondence for Author**
Dr. Venkata Raman Bokka
Department of Basic Sciences-Chemistry, Madanapalle institute of technology and science, Madanapalle, Chittoor Dist-517 325, AndhraPradesh, India.

ABSTRACT

Back ground: Cyclooxygenase (COX), the key enzyme inside the conversion process involving arachidonic acid to prostaglandins which was involved in the pain prone process in living vertebrates. Cyclooxygenase isoforms are already identified and therefore are called COX-1 in addition to COX-2. Beneath a lot of situation, the actual COX-1 enzyme is usually developed constitutively (i.e., gastric mucosa) in contrast to COX-2 is usually inducible (i.e., sites of inflammation). Drug treatments, similar to aspirin, which hinder cyclooxygenase task, are already obtainable in much more towards the general public for approximately 100 years. A single key aim with this is usually to lay out a good anti-inflammatory molecule having a larger restorative window. This article explores the medicinal values of the *Entada pursaetha* which is having strong anti-inflammatory and analgesic properties. **Materials and methods:** Compounds were identified by GC-MS analysis through conventional extraction procedures. Identified lead compound structures were sketched by using chemsketch and marvinsketch software's. An *In silico* study has been carried out by using the software *Schrödinger maestro*. **Results and Discussion:** The compound oleoyl chloride was found to have great anti-inflammatory action [~ 80 % than

standard: Diclofenac]. The interactions of the isolated compounds has been proposed based on the docking studies.

KEY WORDS: Cyclooxygenase, GC-MS, Schrödinger, anti-inflammation.

INTRODUCTION

Damage to the human body can inspire an arrangement of chemical changes in the harmed zone. In the first stage, provocative exudates develop because of upgraded vascular porousness and lead to edema. It is followed by diapedesis or extravasation causes the migration of leukocytes and phagocytes from blood vessels to vascular tissues. In the third phase, tissue degradation is followed by fibrosis [1]. Inflammation brings about the liberation of endogenous substances like histamine, serotonin, bradykinin, prostaglandins, and so forth. Prostaglandins are universal substances that show and regulate cell and tissue reactions included in inflammation. These substances even in little amounts can evoke torment reaction. The majority of the anti-inflammatory drugs now accessible are potential inhibitors of the cyclooxygenase (COX) pathways of arachidonic acid metabolism, which generates prostaglandins and leukotrienes. Prostaglandins are hyperanalgesic, pro-pain causing agents, intense vasodialators and help erythema, edema and agony. Subsequently for treating inflammatory pain relieving and anti-inflammatory drugs are in need. These pro-inflammatory agents contributing different types of rheumatic disorders such as rheumatic fever, rheumatoid arthritis, cancer and other diseases COX-1 & 2 play major role in proinflammatory activity is inhibited by both steroidal and non-steroidal anti-inflammatory drugs (NSAID's) [2]. Two COX isoforms are known: constitutive COX-1, which is considered to be involved in intercellular signaling and homeostasis maintenance, and COX-2, mostly induced during inflammation [3, 4].

Non steroidal antiinflammatory medications can directly penetrate and initiate concerned genes through binding specifically or indirectly to atomic receptors. Because of the absence of cascading mechanism through action guided operation to limit direct activity of these drugs lead to the development of many undesirable reactions and unrelated responses. In this way it is of incredible enthusiasm to screen disconnect and mimic its system.

When steroids are taken on multiple occasions, more serious side effects may occur. It is for these reasons that the dose and duration of systemic steroids should be minimized whenever possible. Some side effects can be decreased by taking systemic steroids every other day

instead of daily, even if the total dose is the same. Many of the side effects are reversible if the steroids are stopped, while other side effects may be permanent.

People taking long-term systemic steroids should be closely checked for the symptoms, and should take preventives to forestall osteoporosis. These medications may incorporate supplemental calcium and vitamin D, along with medicines to prevent bone loss called bisphosphonates.

Non-steroidal anti-inflammatory drugs (NSAIDs) are constantly utilized with increasing frequency, because of their potent pain relieving impacts without having symptoms on the central neural system and furthermore because of the large number and varieties of these agents. The analgesic, anti-inflammatory properties and efficacy of NSAIDs in a wide variety of diseases have been already established. NSAIDs can restrain the inflammatory methodology and the structuring of fibrosis, due to their ability to suppress prostaglandin synthesis. It was exhibited in a rat model that breaking strength and collagen fixation at the injury site were less after use of NSAIDs than in the untreated wounds. Regardless of this, NSAIDs are generally utilized as perioperative analgesics.

In spite of the wide utilization of NSAIDS throughout the most recent century, their mechanism of activity was not completely acknowledged until 1971, when Vane published his seminal observations proposing that the capability of NSAIDS to suppress inflammation rests principally on their capacity to inhibit the cyclooxygenase (COX) enzyme. This would confine the generation of proinflammatory prostaglandins (PGs) at a site of injury. Given this, NSAIDS have been utilized by researchers throughout the previous 25 years to dissect the critical part that both the COX enzyme and the eicosanoids inferred from this pathway have in normal and abnormal physiologic states [5].

Entada pursaetha is an immense woody liana among legumes, which produces 90-150 cm long woody giant pods with 5-30 seeds. All parts of this species hold saponins and are accordingly utilized within the soap industry. This species is reported as tribal pulse [6]. Its semi ripe seeds are likewise utilized as a substitute for coffee. The plant material is used by the tribals as a wide range compound. This species might be utilized as an narcotic or as a tonic, etc, or used in curing liver troubles, allaying body pains, in warding off cold, curing eye diseases, arthritis, and paralysis [7]. This species is reported as endangered [8, 9, 10]. In recent times, there has been a deeply felt concern for the preservation and conservation of *E. pursaetha* germplasm [11] owing

to an increasing realization of its significance and usefulness. The present piece of work was carried out to investigate the tribal's knowledge and traditional uses of this species.

In silico research in medicine is thought to have the potential to speed the rate of discovery while reducing the requirement for costly lab work and clinical trials. One approach to accomplish this is by creating and screening drug candidates more effectively. This methodology differs from use of extensive high-throughput virtual screening (HTVS) labs to physically test many differing compounds a day frequently with an expected hit rate on the order of 1% or less with still less expected to be true leads after further testing. Recently docking ligands to receptors utilizing rational drug design is on the increase owing to few problems in the conventional methods of drug designing. Numerous pharmaceutical organizations use this rational drug designing in the advancement of new drugs as this computational method is less time consuming.

Molecular docking has helped important proceedings to drug discovery for long time. One main inspiration in drug discovery is the distinguishing of innovative small molecular scaffolds exhibiting high binding affinity and selectivity for the target together with a reasonable ADME (absorption, distribution, metabolism, and excretion) profile, lead and/or drug likeness. Such chemical entities are likely to have capacity to enter higher phases in the further drug development process. Molecular docking, compared to the fast and successful method of three-dimensional pharmacophore modeling is a rather complex and computer-intensive approach to find new compounds by virtual screening. Docking techniques intend to recognize correct poses of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. At the end, docking describes a procedure by which two molecules fit together in three-dimensional space. The crystalline structure of ligands bound to their target receptor is one of the most important sources to gain information about the fundamental mechanism of interaction between the parts constituting the three-dimensional complex structure.

MATERIALS AND METHODS

Extraction of *Entada pursaetha* phytochemical compounds

Entada pursaetha seeds were collected from the reserve forest of Srisailam which is located at Western Ghats of Andhra Pradesh, India. The seeds of *E. pursaetha* were collected, dried and powdered in a blender to get fine powder. The extract was prepared by using ethanol in 1:3 ratios by using soxhlet apparatus and the filtrate was concentrated under reduced pressure on

rotavapor under vacuum (BÜCHI, R-3000, Switzerland) at 40^{o}C temperature. The filtrate was used to measure the presence and absence of phytochemical compounds, anti-oxidant activity and for GC-MS studies.

Preliminary phytochemical studies

Preliminary phytochemical screening was performed to identify phytochemicals present in the ethanol. Several sophisticated techniques like thin layer chromatography, ultra violet spectroscopy, infrared spectroscopy, nuclear magnetic resonance and HPLC have been used for identification of various groups of phytochemical compounds in plant extracts. In the present investigation, the phytochemical compounds were detected by simple color tests. These extracts were subjected to preliminary phytochemical tests as described by *Mathi PS et al* [12], with slight modifications.

Gas Chromatography and Mass Spectroscopy (GC-MS) separation conditions

The phytochemicals were analyzed by GC-MS Agilent 5975-C Series instrument employing the electron impact (EI) mode (ionizing potential -70eV) and a capillary column (DB-5ms Agilent) (length 30 m × Diameter 0.25 mm, film thickness 0.25µm) packed with 5% phenyl dimethyl silicone) and the ion source temperature was monitored at 200°C. Further, the GC-MS settings were indicated as the initial column temperature was set at 70°C and kept hold for 2 min; the temperature was increased to 300°C at a rate of 10°C/min for 9 min, and placed in isothermal condition for 2 min. The column oven temperature was maintained at 70°C. Helium was used as carrier gas with 99.9995 % purity. Samples were injected at a temperature of about 250°C with a split ratio of 10: 1 with a flow rate of helium 1.51 ml/min. Mass scan (m/z): 45-1000, Total MS running time: 36 min. The constituents were identified after comparison with those available in the computer library (NIST ver. year 2005) attached to the instrument and reported.

Structure elucidation of Compounds in plant

Structural elucidation of principal compounds was done by GC-MS analysis. Identified lead compounds structures were characterized by using chemdraw and chemsketch software's. The three-dimensional models of the plant *E. pursaetha* molecules under investigation were built by assembling fragments from the software package chemsketch and marvin sketch.

***In-vitro* anti-inflammatory activity using bovine serum albumin (BSA) denaturation assay**

To evaluate the anti-inflammatory activity of phytochemical compounds present *in E. pursaetha*, we used an anti-denaturation of BSA assay [13]. In brief the reaction mixture consists of 0.2 ml (10mg/ml) of bovine serum albumin (BSA), 2.8ml of phosphate buffered saline (PBS, pH -6.4), and 2 ml of varying concentrations of ethanol extracts of *E. pursaetha* 50, 100, 200,400, 800, 1200, 1600, 2000, and 5000 µg ml^{-1} to a final volume of 5 ml. PBS lacking BSA served as control. The samples were then incubated at 37 ± 2^0C in an incubator for 15 min and then transferred to 70^0C water bath for 5 min. After cooling the sample, the turbidity was measured at 620 nm using spectrophotometer. The anti-inflammatory activity of phytochemical compounds was determined by plotting the percentage of inhibition with respect to control against treatment condition. In our studies we used Diclofenac as a positive anti-inflammatory drug. The percentage inhibition of protein denaturation was calculated by using the following formula. Percentage (%) of inhibition= $100 \times (v_t/v_c -1)$, Where Vt = Absorbance of test Sample, V_c = Absorbance of Control.

DPPH Free radical scavenging assay

In order to evaluate the free radical scavenging activity of the test samples, the change in optical density by DPPH radical was assessed [14]. The sample extracts were diluted with methanol to give different concentrations of the seed extracts (100, 200, 300, 400, 500, and 600 µg/mL). Then 0.2 mL of DPPH was added to 2.8 mL of the extracts at various concentrations and incubated at 37ºC for 30 min. Absorbance was measured at 517 nm. Ascorbic acid was used as reference standard. Percentage inhibition was calculated as: DPPH Scavenged (%) = [(Abs control– Abs test) / Abs control] × 100 Where Abs control is the absorbance of the control reaction and Abs test is the absorbance in the presence of the sample.

PROTEINS

Various X-ray crystal structures were available in the Protein Data Bank (http://www.rcsb.org/pdb), the COX 2 was identified with different ligands are downloaded from the database with PDB ID: 1DDX (Fig: 5A).

Docking Studies

Protien Preperation

Docking studies were conducted on the three dimensional (3D) structures of the molecular targets (1DDX) which was obtained from protein data bank. Before performing docking, hydrogen atoms and charges were added to these crystal structures and then the complex was submitted to a series of restrained, partial minimizations using the optimized potential for liquid simulations-all atom (OPLS-2005) force field. The 3D structure was then processed by use of the 'Protein Preparation module' with the 'preparation and refinement' option before docking. The missing loop in the structure was then filled in the respective protein molecule with the help of Prime. Hydrogen atoms were added and all unwanted water molecules were removed from the structure. Partial charges were assigned according to OPLS-2005 force field. Charges and atom types were assigned.

Docking using Glide extra precision

All the ligands which were prepared using LigPrep were then subjected for docking against the molecular targets using Glide extra-precision (XP) mode. The grid-enclosing box was centered to the active sites of the corresponding 3D-structures of these molecular targets. Glide XP mode determines all reasonable conformations for each low-energy conformer in the designated binding site. In the process, torsional degrees of each ligand are relaxed, though the protein conformation is fixed. During the docking process, the glide scoring function (G-Score) was used to select the best conformation for each ligand. Final G-scores were analyzed based on the conformation at which the ligands formed hydrogen bonds to at least one of the active site amino acid residues of the corresponding 3D-structures of these molecular targets with optimal binding affinity. Hereby, the data obtained from these dockings were used to analyze the molecular interactions and also to identify the residues involved in hydrogen bond formation with PDB. The glide scores and energies including vanderwaals (VDW) and electrostatic were calculated for all the ligands against (PDB).

The Glide XP (Extra Precision), a ligand docking program of the software Schrödinger version 9.1 used in the present study for predicting protein-ligand binding modes and ranking ligands via high-throughput virtual screening utilizes scoring functions XP GlideScore, to rank-order compounds. The docking process involves a conformational search for a compound which complements a target binding site, with the aim of identifying the best matching binding pose. The Glide docking algorithm performs a series of hierarchical

searches for locations of possible ligand affinity within the binding site of a receptor. The stability of the docked ligand–protein complex is due to hydrogen bonding and vanderwaal interactions. The glide score glide energy value, H-bonds and vanderwaals contacts to the receptor were visualized in the XP Visualizer using default settings to analyze the binding modes of the ligands to receptor.

RESULTS AND DISCUSSION

Identification of phytochemical constituents

Biochemical analysis showed the presence (+) and absence (−) of phytochemical excipients in ethanol extract represented in Table: 1. All the excipients present in the ethanol extract except steroids. Whereas steroids are the only phytoconstituent absent. Flavonoids and Glycosides are most abundantly present in the extract.

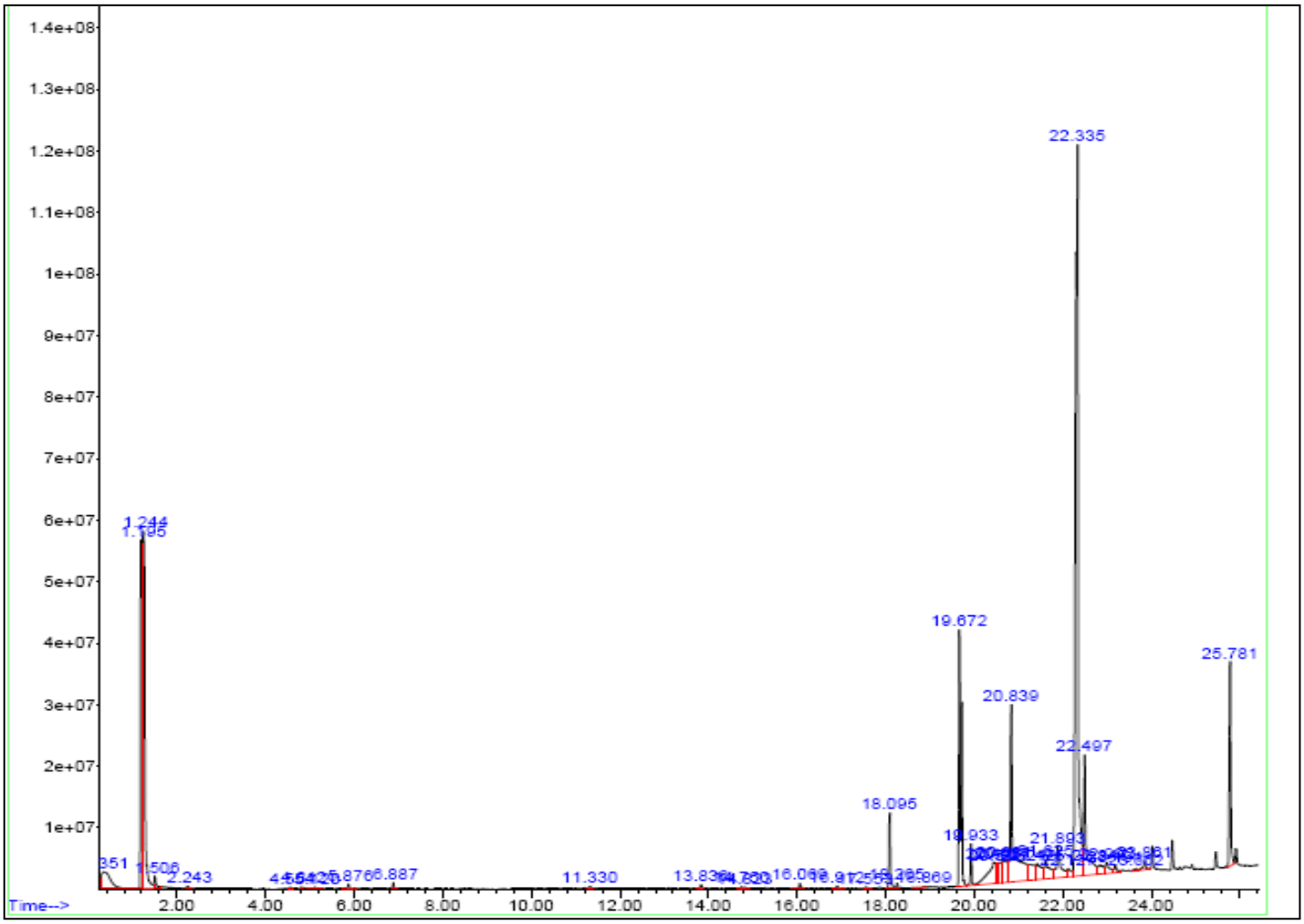
Table: 1

Biochemical tests	***E. pursaetha***
Alkaloids	+
Steroids	−
Flavonoids	+ +
Saponins	+
Terpenoids	+
Polyphenols	+
Tannins	+
Glycosides	+ +

GC-MS analysis

The spectrum profile of the GC-MS data of the *Entada* was compared with the known compounds stored in the NIST library (Fig: 1). Results showed six major peaks along with remaining other phytochemical constituents which was reported in Table: 2.

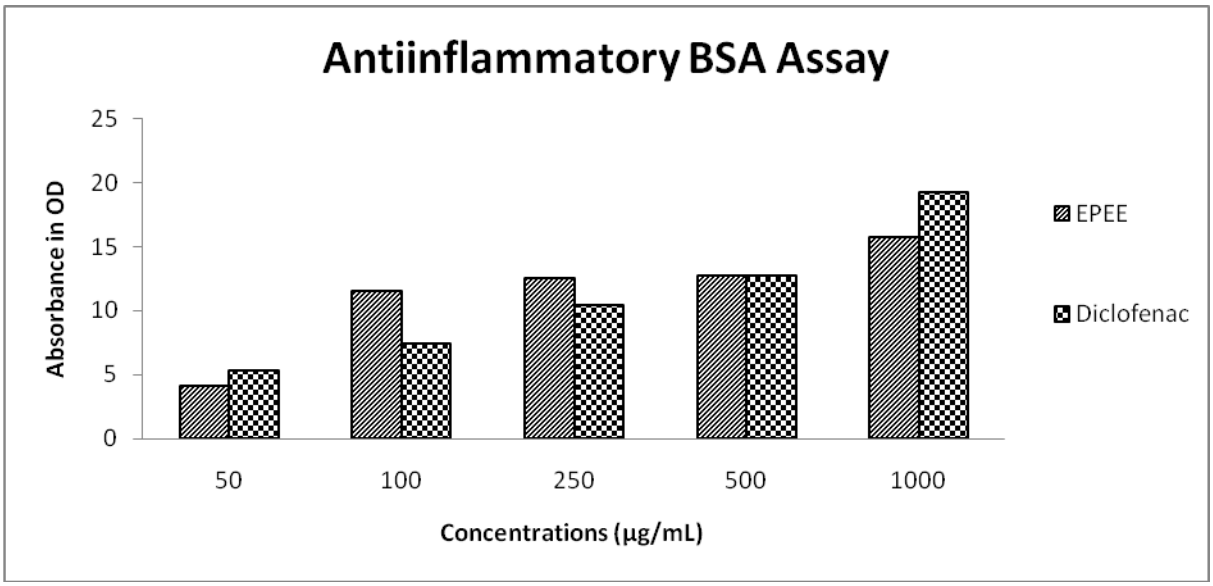
Figure: 1



Anti-inflammatory activity of EPEE

Anti-inflammatory effect of EPEE was evaluated by measuring percent inhibition of Bovine Serum Albumin denaturation (BSA). Our results confirm that EPEE inhibits the denaturation of BSA in dose dependent manner throughout the concentration range of 50-1000 µg/mL. The percent inhibition of BSA denaturation is enhanced with increase in the concentration of the plant extract. Diclofenac (50-1000 µg/mL) was used as reference drug which also demonstrate concentration dependent inhibition of protein denaturation. At the concentration of 500 µg/mL diclofenac standard as exhibited equal absorbance with EPEE. However, at higher concentration, the effect of Diclofenac was found to be less as compared with EPEE (Fig: 2).

Figure: 2.



DPPH Free radical scavenging assay

The radical scavenging activity of *E. pursaetha* and standard based on DPPH assay is depicted in Fig. 3. Ethanol seed extracts showed IC_{50} values of 350 µg/mL. IC_{50} value of ascorbic acid was found to be 8 µg/mL shown in Fig. 4. As lower IC_{50} values indicate higher scavenging activity. However, ascorbic acid displays significant (p <0.05) scavenging activity.

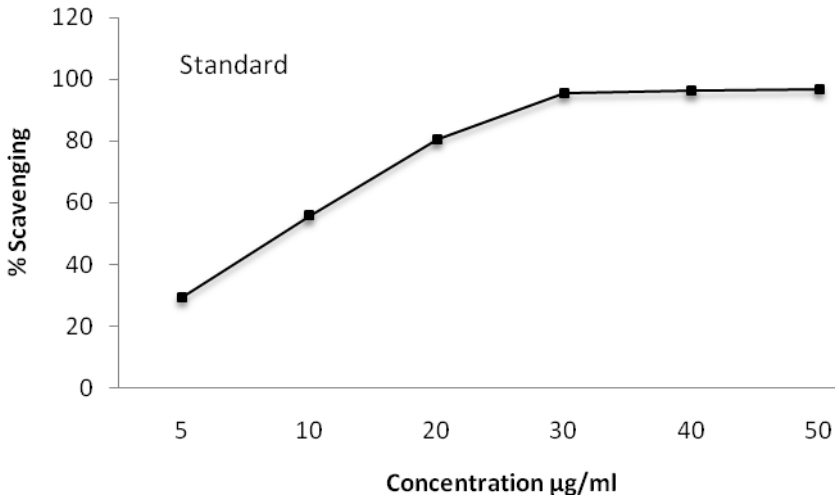


Fig.3

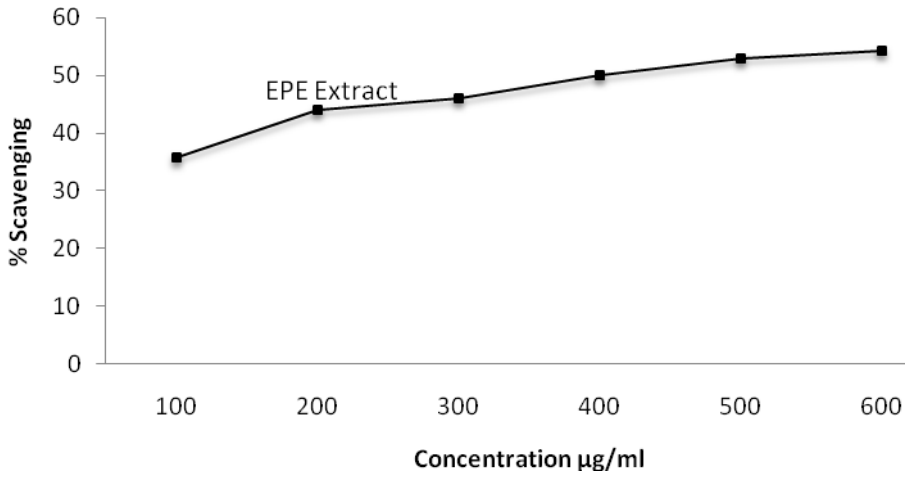


Fig.4

Table: 2 *Entada* plant compounds using the ethanol extracts

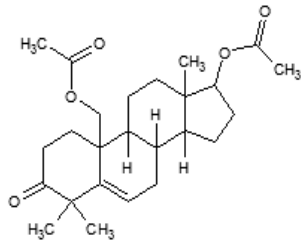
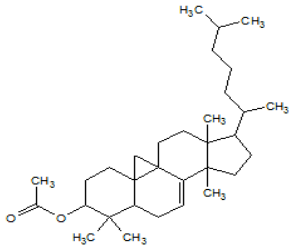
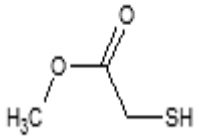
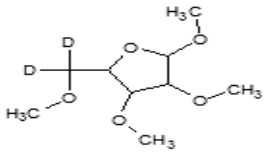
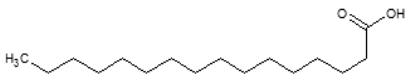
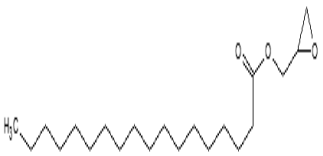
 ANDROST-5-EN-3-ONE, 17,19-BIS(ACETYLOXY)-4,4-DIMETHYL-, (17.BETA.)	 9,19-CYCLO-9.BETA.-LANOST-7-EN-3.BETA.-OL, ACETATE	 METHYL THIOGLYCOLATE
 METHYL-2,3,5-TRI-O-METHYL-.BETA.-D-ARABOFURANOSIDE-5,5-D2	 HEXADECANOIC ACID	 GLYCIDOL STEARATE

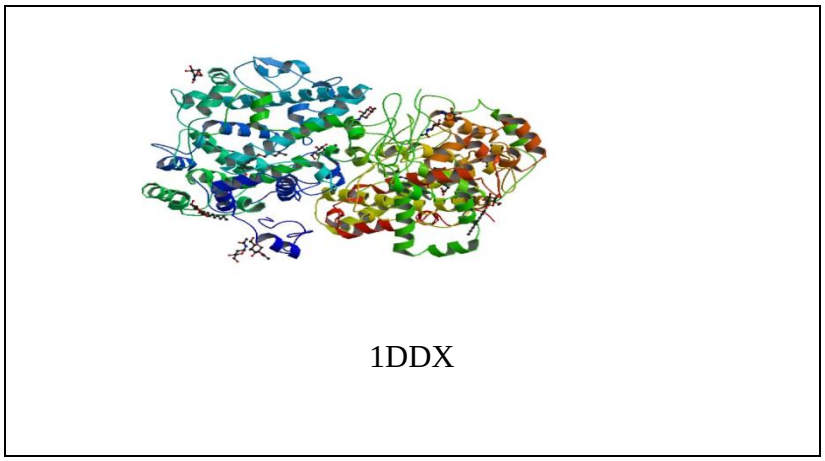
Table: 3 ADMET analysis of EPEE

MOLECULES	mol_MW	QPlog Po/w	QPlogS	QPlog HERG	QP PCaco	QPlog BB	QP PMDCK	%Human Oral Absorption	Rule Of Five
ANDROST-5-EN-3-ONE, 17,19-BIS(ACETYLOXY)-4,4-DIMETHYL-, (17.BETA.)	300.911	6.469	-7.216	-5.585	2995.096	-0.802	4069.552	100	1
9, 19-CYCLO-9.BETA. -LANOST-7-EN-3.BETA	468.762	8.485	-9.783	-4.781	3980.615	-0.229	2202.04	100	1
METHYL THIOGLYCOLATE	106.139	0.569	-0.75	-2.523	1486.405	-0.034	1913.231	87.051	0
METHYL-2,3,5-TRI-O-METHYL-.BETA.-D-AR	206.238	-0.794	1.839	-3.491	9906.038	-2.797	5899.293	93.814	0
2-HYDROXY-3-[(9E)-9-OCTADECENOYLOXY]P	620.995	12.393	-14.941	-7.697	1114.818	-3.176	556.381	100	2
GLYCIDOL STEARATE	340.545	6.304	-7.036	-5.826	2984.973	-1.2	1613.26	100	1

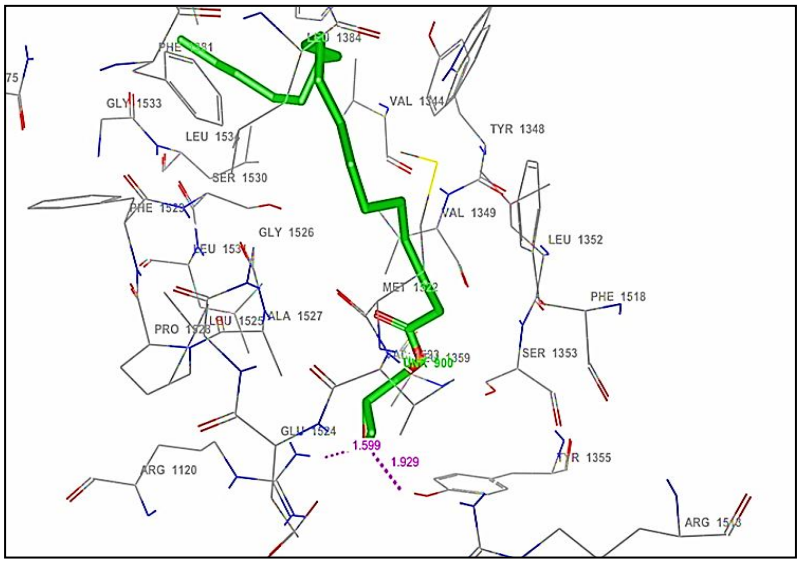
Table: 4 Docking results with 1DDX of EPEE

LIGAND	GScore	Lipophilic EvdW	PhobEn	HBond	Electro	Sitemap
DICLOFENAC	-6.76	-5.89	-2.7	-0.27	-0.27	-0.4
OCTADECANEDIOIC ACID	-9.6	-5.61	-2.27	-1.45	-0.63	-0.4
GLYCIDOL STEARATE	-11	-7.38	-2.7	-0.86	-0.5	-0.38
ANDROST-5-EN-3-ONE	-11.78	-7.22	-2.7	-0.7	-0.1	-1.1

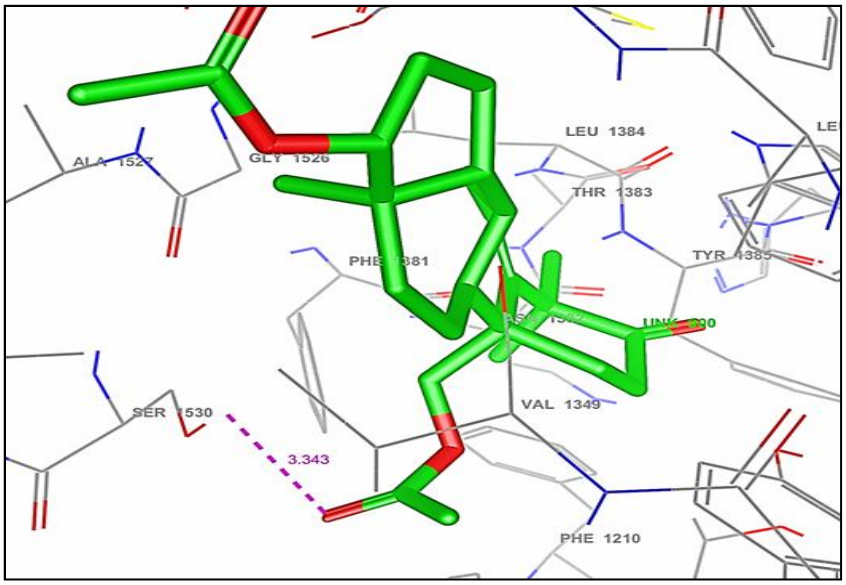
Figure-5. for all A, B, and C



A) Protein structures from pdb: 1DDX



B) 1DDX with Glycidol sterate



C) 1DDX with ANDROST-5-EN-3-ONE

***In silico* ADME analysis: (QikProp)**

Anticipate pharmacokinetic properties utilizing the Qikprop module of the Schrödinger 2009 software. Qikprop settings figure out which molecules are flagged as being dissimilar to other 95 % of the known drugs. Predicted critical ADMET properties, for example, permeability through MDCK cells (Qplogmdck), Qikprop predicted log IC_{50} esteem for blockage of K+ channels (Qplogherg), Qikprop anticipated gut-blood barrier (QPPCaco) and violations of the Lipinski's principle of five (LROF) were accounted for in Table 3. The amount of stars shows the deviations from the 95 % of the known medications. Percent of Human Oral absorption is focused around number of metabolites, number of rotatable bonds, logP, solvency and cell penetrability.

As per Lipinski's rule of five, QikProp was utilized to evaluate the drug-likeness of the lead molecules by assessing their physicochemical properties. Their molecular weights were < 500 Daltons with < 5 hydrogen bond donors, < 10 hydrogen bond acceptors and a log p of < 5. These properties are well within the adequate range of the Lipinski rule for drug-like molecules. These compounds were further assessed for their drug-like behavior through analysis of pharmacokinetic parameters needed for absorption, distribution, metabolism, excretion and toxicity (ADMET) by use of QikProp. For the two bioactive compounds, the partition coefficient (QPlogPo/w) and water solubility (QPlogS), critical for estimation of absorption and distribution of drugs inside the body, ranged between ˜ 0.7 and ˜ 2043, cell

permeability (QPP_{Caco}), a key factor governing drug metabolism and its access to biological membranes, ranged from 0.004 to 2050, while the bioavailability and toxicity were from ˜ 3.4 to ˜ 0.4. Overall, the percentage human oral absorption for the compounds ranged from ˜ 57 to ˜ 100%. All these pharmacokinetic parameters are within the acceptable range defined for human use (Table-3), thereby indicating the selected drugs-like compounds their potential as drug- like molecules could be a potential inhibitor of therapeutic targets of COX-2 and further analysis can be performed through various experimental studies. Among various commercially available drugs, ADME properties of these compounds are under satisfactory range.

Docking into 1DDX

Molecular docking has been done to understand the binding mode with the seed ingredients upon COX-2, and also to obtain facts with regard to our research. We now have employed extra precision glide docking (Glide XP) which usually docks ligands flexibly. Docking simulations of octadecanoic acid, glycedol sterate and androsterone-5- into 1DDX as well as with standard molecule diclofenac produced G-score along with other energetics from the Table-4. This docking evaluation of diclofenac as standard at COX-2 active site demonstrates interactions. This carboxylic group of diclofenac interacts having SER1530, this specific hydrogen interact at residue SER1530 is often noticed with regard to octadecanoic acidity and androstene. For glycidol sterate two hydrogen bonding interactions ended up at GLU1542 and TYR1355 (Fig: 5B & C). This information obviously suggests these seed derivatives have high affinity to productive active site of COX-2 enzyme.

CONCLUSION

The seeds of *E. pursaetha* found to have potential source for several bioactive compounds identified by GC-MS. Three compounds such as *Octadecanedioic acid, Glycidol stearate,* and *Androst-5-En-3-one*, establish promising anti-inflammatory properties in the molecular docking and *in vitro* studies experimentally confirmed positive results . It justifies the usage of this seed as a folklore medicine for preventing inflammation associated disorders such as arthritis.

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