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IN SILICO AND *IN VITRO* XANTHINE OXIDASE INHIBITORY ACTIVITY OF *EMBILICA OFFICINALIS* (AMLA)

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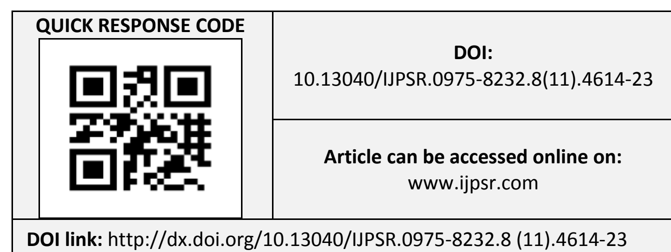
ABSTRACT: The present study on *Embilica officinalis*, a common household remedy and the present study describes the inhibition of enzyme xanthine oxidase (XO) with *in vitro* analyses and assenting *in silico* study to produce an effective phytoconstituents. The results explained the ethanolic extract of *Embilica officinalis* (EOEt) exhibited antioxidant and defensive oxidative stress and allied with its total phenol (51.33 ± 0.793 mg/g) and flavonoid content (33.51 ± 0.616 mg/g). Additionally, the DPPH scavenging activity shows significant correlation between antioxidant and XO inhibitory activities result in IC_{50} value of 40.40 ± 0.1475µg/ml. The EOEt extract probably inhibited the nitric oxide formation with increase in concentration with IC_{50} of 28.36 ± 0.1522µg/ml. The EOEt extract shows increase in dose dependent manner in percentage of XO inhibitory activity and exhibits potential IC_{50} value (352.0 ± 0.2069 µg/ml) compared to standard allopurinol (723.9 ± 0.2081µg/ml). The *in silico* docking studies of the major phytoconstituents (Phyllembilic acid B, Ethyl gallate, Gallic acid, 5-Hydroxymethylfurfural, Ascorbic acid, 1, 2, 3-benzenetriol, Ethyl alpha-d−glucopyranoside and β−cyclocitral) obtained from GC-MS analysis of EOEt extract. The inhibitory property of these active phytoconstituents may be due to the presences of synergistic effect. The result provides compelling basis for the future use of EOEt extract and its phytoconstituents in *in vivo* system for the treatment and management of gout as well as in related to all oxidative stress conditions.

INTRODUCTION: Gout is a disease which majorly affects the joints flexibility [1]. Characterization of Gout explains abnormal increased levels of uric acid in body, results in the formation of urate and deposit as tophus crystals (monosodium urate monohydrate crystals) in joints, tendons and tissues surrounding the joints. Thus it forms hyperuricemic condition and persistent to renal failure [2].



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These crystals can cause acute inflammation and induces enduring tissue damage identified by the formation of ulcer in the cartilage of joints, osteophytosis, geodic and erosive lesions and longer term it leads to chronic inflammation of synovial membrane [3, 4]. A study by Ilar Copcord in Bhigwan village shows a prevalence rate of 0.1% and it is higher in urban population of India. Besides, increase in prevalence rate of metabolic syndrome in younger population, occurrence of gout is a decade earlier in urban population. An analysis done by Mathew and Danda in Vellore district showed 15.8% of prevalence rate, mostly less than 30 years. Globally, the incidence and prevalence of gout was doubled over the last two decades [5].

The enzyme Xanthine oxidase (XO) oxidizes form xanthine to hypoxanthine and subsequently converts into uric acid by purine nucleotides catabolism [6]. During this reaction, superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) are produced [7]. The free O_2^- is transformed into H_2O_2 and O_2 either instinctively or by the enzyme superoxide dismutase. As a result, the activity of XO leads to uric acid deposition in the targeted tissues. Therefore, the released reactive oxygen species triggers the inflammatory pathways. Hence, gouty arthritis and other inflammatory diseases are related with increased hyperuricaemia by the oxidative stress [8].

Management of gout includes Nonsteroidal anti-inflammatory drugs (NSAIDs) (aspirin, ibuprofen, indomethacin); cox-2 selective inhibitors (etoricoxib); corticosteroids (prednisone); allopurinol, probencid and colchicines. Even though they are effective, forms superoxide radicals and leads to develop the adverse effects as skin allergy, rashes, diarrhea and fever. It progressively develops leukocytosis, vasculitis, hepatic and renal dysfunction, meningitis, nephritis [9]. Allopurinol is the XO inhibitor and clinically most preferable drug for treatment of gout. Nevertheless, it produces adverse effects such as hypersensitive reactions, nephrotoxicity, hepato-toxicity and Stevens - Johnson syndrome [10]. Accordingly, there is an entail of herbals possess antioxidant property to nullify the oxidative injury and inflammatory responses. It is well known that the XO inhibitors obtained in natural sources be capable of alternative to allopurinol to reduce the possible adverse effects [11].

Fruit *Emblica officinalis* Gaertn (syn. *Phyllanthus emblica* Linn.), family Euphorbiaceae, usually known as Indian gooseberry or Amla. It is a common household remedy. The Indian system of medicine finds its use next to several ailments [12].

The preclinical studies revealed that the fruit of amla possesses greater antioxidant, anti bacterial, anti ulcer, anti tumor, anti aging and hepatoprotective properties [13 - 18]. It is also used for healing the cough, bronchitis, tuberculosis and scurvy [19 - 21]. It possesses cardiotonic, antidiabetic, antiviral, antipyretic and antiemetic activities [22 - 24]. It is also preferred in the treatment of atherosclerosis and leucorrhea [25]. The reports recommended that it contains alkaloids, phenols and tannins. The higher concentration of fruit *Embilica officinalis* contains minerals, proteins and amino acids like aspartic acid, alanine, cystine, glutamic acid, lysine, and proline. Amla is an important dietary sources of Vitamin C and highly nutritious. Additionally it also contains phyllemblin, phyllembelic acid, curcuminoides, rutin, and emblicol [26].

The current study was conceded with the aim of determining the *in vitro* xanthine oxidase inhibitory activity and antioxidant activity. To enhance the phytoconstituents analysis from ethanolic extracts of *Embilica officinalis* (EOEt). Also to perform the *in silico* docking studies using Molegro Virtual Docker (MVD) and find out the molecule with higher docking scores.

MATERIALS AND METHODS:

Plant Authentication: Fresh fruits of *Emblica officinalis* were collected from Tirupati, Chittoor district, Andhra Pradesh in the month of January 2015 and pharmacognostically identified and authenticated by Dr. Madava chetty, Assistant Botanist, Sri Venkateswara University, Tirupati.

Drugs and Chemicals: Bovine's milk, allopurinol, xanthine oxidase enzyme, ethanol, dragendorff's reagent, Hager reagent, Wagner's reagent, Mayer's reagent, lead acetate, folin ciocalteu reagent (0.5N), quercetin, catechin, aluminum chloride (1.2%), potassium acetate, ascorbic acid, Griess reagent, 1 1-diphenyl- 2- picrylhydrazyl (DPPH) reagent, saturated Na_2CO_3, sodium nitroprusside, phosphate buffer, dimethyl sulfoxide (DMSO).

Preparation of Extract: The fresh fruits of *Embilica officinalis* were washed under running water and shade dried. Finely powdered by mechanical grinder and extracted with 90% ethanol by using soxhlet apparatus maintaining temperature at 60°C. Evaporated the solvent by rotary evaporator and brownish gummy exudates were obtained [27]. The crude EOEt extract was used for evaluate antioxidant and xanthine oxidase (XO) inhibitory properties. The fraction yield of EOEt extract was calculated by using the formula.

$$\% \text{ yield} = \frac{\text{weight of crude extract}}{\text{weight of raw material}} \times 100$$

The fraction yield of ethanolic extract of *Embilica officinalis* (EOEt) was found to be 5.65% w/w.

Phytochemical Analysis: The *Embilica officinalis* ethanolic (EOEt) extract was analyzed by preliminary phytochemical screening methods [28].

Total Phenol Content: The estimation of the total phenol content was done by using folin ciocalteu reagent method. 2.5ml of saturated Na_2CO_3 was added to the pre incubated 0.5ml of EOEt extract with 0.1ml of folin ciocalteu reagent (0.5N) for 15min in optimum temperature. Measure the absorbance at 760nm using quercetin as standard [29]. The total phenol content was uttered as standard equivalent (mg/g)

Total Flavonoid Content: The total flavonoid content was estimated by using Aluminum chloride method [30]. The mixture (3.0ml) contains 1.0ml of EOEt extract, 0.5ml of aluminum chloride (1.2%) and 0.5 ml of 120 mM potassium acetate were pre-incubated in room temperature for 30 min and at 415 nm the absorbance was measured using catechin as standard. The total flavonoid content was uttered in terms of standard equivalent (mg/g).

GC-MS Analysis: The EOEt extract was analyzed by GC-MS using a Perkin-Elmer GC Clarus 500 system contains auto-sampler (AOC-20i) and gas chromatograph interfaced to a mass spectrometer. GC-MS chromatogram of EOEt extract indicated the presence of about 30 phytoconstituents respectively. The spectrum was compared with NIST library and the phytoconstituents were identified.

Xanthine Oxidase Enzyme Preparation: The enzyme xanthine oxidase was prepared and isolated from Bovine's milk as proposed by Jun Ichi Toyama *et al* [31].

***In vitro* Xanthine Oxidase Inhibitory Activity:** The EOEt extract was used for the analysis of XO inhibition. The XO inhibitory effect was evaluated at 295 nm using UV spectrophotometer with some slight modifications [32]. Dilution of XO enzyme was made to a final concentration of 2 u/ml. 1 mM xanthine (substrate) solutions was prepared by addition 5 drops of 1.0 M NaOH to solubilize the xanthine. Dissolve EOEt extract in 1% dimethyl sulfoxide (DMSO) solution and prepared in various concentration (50 - 800 µg/ml). Allopurinol was used as a standard.

The mixture (3.2 ml) contains 1 ml test sample at various concentrations, 1ml of 0.51M phosphate buffer (pH 7.5), 100 µl XO enzyme solution. Preincubate the EOEt extract for 15 min at 37 °C. The reaction was initiated by adding 100 µl of xanthine solution. Again incubate the mixture at 37 °C for 30 min. Add 1 ml 1N HCl to stops the reaction. Measure the absorbance at 295 nm. The percentage of XO inhibitory activity was evaluated by estimate the uric acid absorbance from the mixture of control compared with the absorbance of a samples. The XO inhibition percentage can be calculate by using the formula.

$$\% \text{ XO Inhibition} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

Further, using non-linear regression analysis IC_{50} value was obtained by plotting series of various concentrations of sample against percentage of XO inhibition.

DPPH Radical Scavenging Activity: The EOEt extract scavenging activity on DPPH radical was evaluated using spectrophotometer. To prepare DPPH solution, add 12.5 mg DPPH in 50 ml of ethanol. Absorbance was measured at 517 nm. Add 100 µl of EOEt extract (20-100 µg/ml) to 1 ml of DPPH solution was added. Incubate the mixture at 37 °C for 30 min. Measure the absorbance at 517 nm. Ascorbic acid was used as standard [33]. Calculate the percentage of DPPH scavenging activity using the formula.

$$\% \text{ DPPH scavenging activity} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

Further, using non linear regression analysis the IC_{50} values were acquired and plot a series of various sample concentrations against percentage of scavenging activity.

Nitric Oxide Scavenging Activity: The EOEt extract scavenging activity on nitric oxide (NO) was examined by using Griess reagent. 100 µl of EOEt extract in various concentrations (20-100 µg/ml) was mixed with 100 µl of 10 mM sodium nitroprusside. Incubate the mixture at 25 °C for 3

hours. Add 1 ml of Griess reagent and at 546 nm absorbance was measured using standard ascorbic acid [34]. Calculate the percentile scavenging activity of NO using the formula.

$$\% \text{ NO scavenging activity} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

Using non linear regression analysis, IC_{50} values were acquired and plot a series of various sample concentrations against percentage of NO scavenging activity.

Molecular Docking Study:

Preparation of Ligand: The major active phytoconstituents of EOEt namely, Phyllembilic acid B, Ethyl gallate, Gallic acid, 5-Hydroxymethylfurfural, Ascorbic acid, 1, 2,3-benzenetriol, Ethyl alpha -d−glucopyranoside and β−cyclocitral [35-39] were selected. The 3D structures of those active constituents are retrieved from PubChem chemical databases and saved in sdf format. The ligands are imported to the workspace and preparation is done for docking studies in mol format. The docking scores of the active constituents are compared against allopurinol as a standard drug obtained from the Pubchem in sdf format [32].

Preparation of Enzyme: The enzyme Xanthine oxidase (1FIQ) was obtained from RCSB protein databank [40]. The PDB file format cannot accommodate bond order information due to deprived or misplaced assignments of unequivocal hydrogen. Thus MVD analyzer was used for assigning appropriate bond orders, bonds, charges and its hybridization. The possible binding site of the target was calculated by using algorithm (cavity detection).

The simulations search space was exploited around the active side cleft 15.0 Angstroms. Docking analysis was done by opt the Molegro Virtual Docker (MVD) software. It gives the confirmation on ligand dock to target and widely preferred by medicinal chemists [41]. The Mol-Dock score was worked on Piecewise Linear Potential (PLP), where the structure of target-ligand and its docking score function parameters are fit [42]. In an advance GEMDOCK were extended with furthermore new H-bond and its charges [43].

MolDock Optimizer: In MVD, the differential evolution algorithm was guided by the elected parameters includes number of runs is 5, population size is 50, maximum interactions are 2000, crossover rate is 0.9 and scaling factor is 0.5. Pose clustering was preferred to make certain appropriate binding mode in the selected cavity.

MolDock Score Parameters: Select the ignore-distant-atoms to disregard the atoms which are far from docking site. Furthermore, check the H-bond direction between the impending donors and acceptors. Option the cavity with a radius of 25 Å in the binding site of the target made in X, Y and Z directions.

Rerank Score: Rerank scoring functions are used to generate and predict the models for evaluating the chemical properties (*e.g.* QSAR). The rerank scoring function was computationally expensive compare to the scoring function in docking simulation. In general it was most preferable in finding the best pose in among the poses originates from the same ligand. Although the rerank score in MVD gives an approximate to the strength of the interaction, the chemical units are not calibrated and do not consider the complex contributions (entropy) in the account. The rerank score accurately rank the dissimilar poses of individual ligands. The measuring of binding affinity was used subsequently to get a rough estimate of the highest ranked poses.

DruLiTo Software: DruLiTo is open source software to can calculate different molecular properties and screen the molecules based on drug likeness rules using 'The Lipinski rule of five' (Lipinski 2004) [44].

Statistical Analysis: The *in vitro* analyses were done in triplicate. Using Graph Pad Prism software (Ver. 5.02), the results are uttered in terms of mean ± SEM with 95% confidence level.

RESULTS AND DISCUSSION: Currently using synthetic drugs are well recognized to protect from gout and oxidative stress although have their adverse effects. Therefore, the consumption of natural antioxidants through plants, food or dietary supplements in day to day life could be acts as a protective measure for such diseases. The enzyme

XO catalyzes and reduces conversion of O_2, leads to production of H_2O_2 and superoxide anion radicals, as well as OH radicals [7]. The mechanism of oxidative damage leads to develop gout. In the current study, we performed the xanthine oxidase inhibition by choosing the ethanolic extract of *Embilica officinalis* (EOEt).

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF EOET EXTRACT

Phytochemical constituents	Extract
Alkaloids	+
Phenol	+++
Tannins	++
Carbohydrate	-
Glycosides	++
Saponins	+++
Steroids	+++
Flavonoids	++
Terpenoids	-
Resins	-
Proteins	+

Key = +++ abundantly present, ++ moderately present, +fairly present, - absent.

The EOEt extract confirms the presence of phytoconstituents such as phenols, saponins and steroids in major where as flavonoids and tannins present in moderately and trace amount of alkaloids and proteins in preliminary phytochemical analysis (**Table 1**).

The presence of phenols carries redox properties, which acts as antioxidants. The presence of hydroxyl group in phenolic compounds facilitates the rapid scavenging activity of free radicals [45]. The total phenol content of EOEt extract was found to be 51.33 ± 0.793 (50.53 - 51.33 mg/g).

Flavonoids include flavones, flavanols and condensed tannins are the secondary metabolites. These flavonoids contain free OH- groups, enhances the antioxidant activity [46]. The total flavonoid content of EOEt extracts was found to be 33.51 ± 0.616 (32.894 - 34.126 mg/g).

Preliminary GC-MS analysis was made to categorize the active phytoconstituents in EOEt extract majorly liable for the antigout activity (**Table 2**). Various molecules of *Embilica officinalis* was reported with antioxidant activity [35-39].

TABLE 2: MAJOR CONSTITUENTS OF EOEt EXTRACT USING GC-MS ANALYSIS

S. No	Compound	Molecular formula	Molecular weight	R.T	Area%
1	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.111	7.19	5.24
2	Ethyl .alpha.-d-glucopyranoside	$C_8H_{16}O_6$	208.21	12.353	5.07
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	15.889	4.15
4	Pthalic acid,2-cyclohexyethyl iso butyl ester	$C_{20}H_{28}O_4$	332.44	16.209	4.87
5	β-cyclocitral	$C_{10}H_{16}O$	152.237	7.257	4.73
6	Ethyl gallate	$C_9H_{10}O_5$	198.174	20.54	3.7
7	Gallic acid	$C_7H_6O_5$	170.02	22.137	21.65
8	1,2,3-benzenetriol	$C_6H_6O_3$	126.11	21.2	4.47
9	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	$C_{16}H_{22}O_2S$	278.13	24.7	6.4
10	Beta-Sitosterol	$C_{29}H_{50}O$	414.39	26.074	7.33

As known that the molecules capable of free radical scavenging result in inhibition or prevention of oxidation and reduce the oxidative stress [8]. The EOEt extract exhibited maximum antioxidant activity by IC_{50} 40.40 ± 0.1475µg/ml as compared to ascorbic acid of 69.31 ± 0.1422µg/ml. From the results, the free radical scavenging activity of EOEt extract was augmented in concentration dependent manner (**Fig. 1**). The EOEt extract potentially inhibited the nitric oxide formation with increase in concentration with IC_{50} of 28.36 ± 0.1522 µg/ml compared with ascorbic acid of 42.87 ± 0.1457µg/ml (**Fig. 2**).

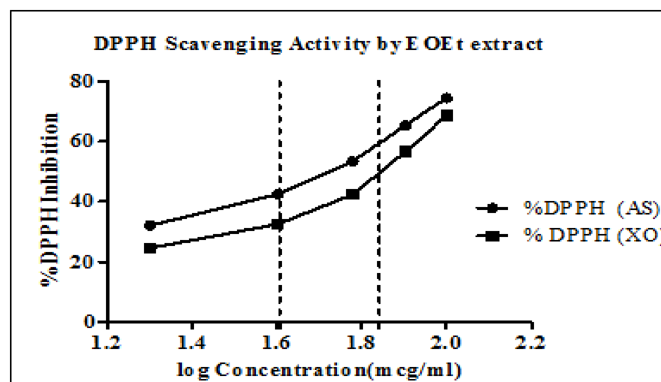

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF EOET EXTRACTS AND STANDARD ASCORBIC ACID

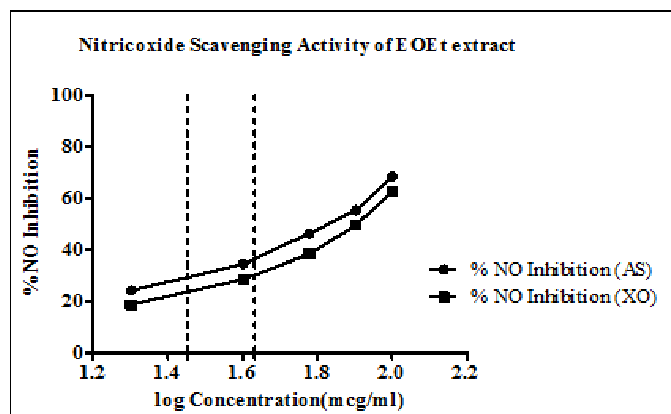

FIG. 2: NITRIC OXIDE SCAVENGING ACTIVITY OF EOET AND STANDARD ASCORBIC ACID

The results conclude that EOEt extract exhibited potential DPPH radical and Nitric oxide scavenging activities, which signifies its strong antioxidant activity when compare to standard Ascorbic acid.

***In vitro* Xanthine Oxidase Inhibitory Activity:** In general, the increase in activity of XO result in increase of uric acid levels in blood. Thus leads to result in myocardial infarction, renal stone formation and free radical mediated diseases [47].

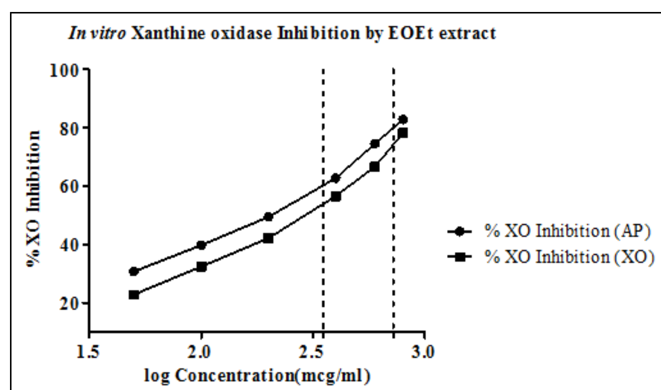

FIG. 3: *IN VITRO* XANTHINE OXIDASE INHIBITON OF EOET EXTRACT

Reports suggested that the incidence of gout was raised particularly consuming food rich in nucleic acid. XO inhibitors and uricosuric agents, commonly hypouricemia agents are used in management of gout. The EOEt extract shows the concentration dependent inhibition. XO inhibitory activity spectroscopically revealed decrease in uric acid production. The EOEt extract was found to have strong inhibitory activity (IC_{50} 352.0 ± 0.2029 µg/ml) while, considering the standard drug allopurinol (723.9 ± 0.2081µg/ml) (**Fig. 3**). In consequence, the XO inhibitory activity of EOEt extracts might be due to the presence of phenols, flavonoids, steroids and tannins.

Docking Analysis: Molecular docking analysis is considered as a significant parameter to estimate the mode of ligand interaction against its targeted protein. This makes us to recognize the binding and inhibition mechanism of ligands. The virtual docking analysis was performed by using the MVD software. The capability of phytoconstituents binding with the targets is expressed in terms of Mol Dock Score. The Mol Dock Score and the rerank scoring are the parameters used for docking analysis and based on the Mol Dock score the phytoconstituents are ranked. The ligand contains the highest Mol Dock and Rerank score exhibits a strong affinity towards its target.

Docking analysis of phytoconstituents from *Embilica officinalis* targeted on Xanthine oxidase (1FIQ) was ranked based on the Mol Dock Score (**Table 3**), Rerank Score (**Table 4**) and H-Bond (**Table 5**). The binding prototype of the ligands was analyzed using the ligand energy inspector tool built-in in the MVD. It was found that the binding patterns are similar to the standard drug allopurinol and poses maximum Mol Dock Score as well as the rerank score when compared to standard allopurinol. The structure 1FIQ has in total 3 chains (A, B and C chains) and contains 219, 350 and 763 residues respectively. It was determined that the chain C plays major role in binding of phytoconstituents including the standard drug.

TABLE 3: *IN-SILICO* DOCKING ANALYSIS OF PHYTOCONSTITUENTS FROM *EMBILICA OFFICINALIS* USING XANTHINE OXIDASE (PDB ID: 1FIQ) RANKING BASED ON MOLDOCK SCORE

S. No	Name	Ligand	Mol Dock Score	Rerank Score	H Bond
1	[00]pthalic acid,2-cyclohexyethyl iso butyl ester	pthalic acid,2-cyclohexyethyl iso butyl ester	-111.034	-89.413	-0.66101
2	[00]Phyllembilic acid B	Phyllembilic acid B	-106.323	-83.9944	-8.28648
3	[00]Ethyl gallate	Ethyl gallate	-104.682	-82.4419	-14.6986
4	[01](2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	-85.0129	-36.5367	0
5	[00]Gallic acid	Gallic acid	-84.2159	-75.0968	-10.4171

6	[00]allopurinol	Allopurinol	-81.1169	-62.2797	-9.57585
7	[01]5-Hydroxymethylfurfural	5-Hydroxymethylfurfural	-78.2766	-64.2736	-5.49062
8	[00]Ascorbic acid	Ascorbic acid	-77.6209	-70.8325	-11.9986
9	[00]1,2,3-benzenetriol	1,2,3-benzenetriol	-74.5034	-63.8592	-13.4972
10	[01]Ethyl .alpha.-d-glucopyranoside	Ethyl .alpha.-d-glucopyranoside	-72.4726	-69.7967	-10.5286
11	[01]β cyclocitral	β cyclocitral	-60.0489	-52.5232	0

TABLE 4: *IN-SILICO* DOCKING ANALYSIS OF PHYTOCONSTITUENTS FROM *EMBILICA OFFICINALIS* USING XANTHINE OXIDASE (PDB ID: 1FIQ) RANKING BASED ON RENANK SCORE

S. No	Name	Ligand	Mol Dock Score	Rerank Score	HBond
1	[01]pthalic acid,2-cyclohexyethyl iso butyl ester	pthalic acid,2-cyclohexyethyl iso butyl ester	-110.223	-90.8424	0
2	[00]Phyllembilic acid B	Phyllembilic acid B	-106.323	-83.9944	-8.28648
3	[00]Ethyl gallate	Ethyl gallate	-104.682	-82.4419	-14.6986
4	[00]Gallic acid	Gallic acid	-84.2159	-75.0968	-10.4171
5	[00]Ascorbic acid	Ascorbic acid	-77.6209	-70.8325	-11.9986
6	[00]Ethyl .alpha.-d-glucopyranoside	Ethyl .alpha.-d-glucopyranoside	-70.6506	-70.7162	-5.4208
7	[02](2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	-73.665	-67.236	0
8	[01]5-Hydroxymethylfurfural	5-Hydroxymethylfurfural	-78.2766	-64.2736	-5.49062
9	[00]1,2,3-benzenetriol	1,2,3-benzenetriol	-74.5034	-63.8592	-13.4972
10	[00]allopurinol	Allopurinol	-81.1169	-62.2797	-9.57585
11	[01]β cyclocitral	β cyclocitral	-60.0489	-52.5232	0

TABLE 5: *IN-SILICO* DOCKING ANALYSIS OF PHYTOCONSTITUENTS FROM *EMBILICA OFFICINALIS* USING XANTHINE OXIDASE (PDB ID: 1FIQ) RANKING BASED ON HYDROGEN BOND

S. No	Name	Ligand	Mol Dock Score	Rerank Score	HBond
1	[00]Ethyl gallate	Ethyl gallate	-104.682	-82.4419	-14.6986
2	[00]1,2,3-benzenetriol	1,2,3-benzenetriol	-74.5034	-63.8592	-13.4972
3	[00]Ascorbic acid	Ascorbic acid	-77.6209	-70.8325	-11.9986
4	[01]Ethyl .alpha.-d-glucopyranoside	Ethyl .alpha.-d-glucopyranoside	-72.4726	-69.7967	-10.5286
5	[00]Gallic acid	Gallic acid	-84.2159	-75.0968	-10.4171
6	[00]allopurinol	Allopurinol	-81.1169	-62.2797	-9.57585
7	[00]Phyllembilic acid B	Phyllembilic acid B	-106.323	-83.9944	-8.28648
8	[01]5-Hydroxymethylfurfural	5-Hydroxymethylfurfural	-78.2766	-64.2736	-5.49062
9	[00]β cyclocitral	β cyclocitral	-58.0654	-44.3482	-1.01345
10	[00](2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	-84.2529	-60.1683	-0.9038
11	[00]pthalic acid,2-cyclohexyethyl iso butyl ester	pthalic acid,2-cyclohexyethyl iso butyl ester	-111.034	-89.413	-0.66101

The residues in the 1FIQ chain C which are involved in the binding to the standard drug Allopurinol and Phyllembilic acid B are Ala 258, Gly 260, Gly 350, Ile 353, Asn 261, Thr 262, Ala 356, Asn 261, Thr 262, Ala 346, Gly 260, Ser 347, Asr 351, Gly 350, Gly 349, Glu 263, Val 258, and Ile 264. The binding pattern for Allopurinol, Phyllembilic acid B, Gallic acid and (2, 6, 6-Trimethyl- cyclohex- 1- enylmethane- sulfonyl) benzene are represented in the **Fig. 4, 5, 6** and **7** respectively.

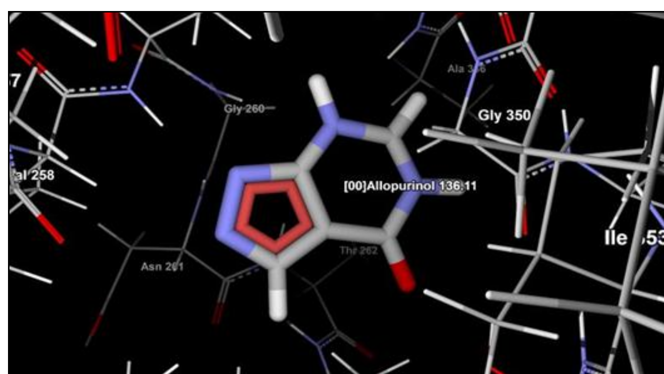

FIG. 4: DOCKED VIEW OF ALLOPURINOL WITH THE ENZYME XANTHINE OXIDASE (1FIQ)

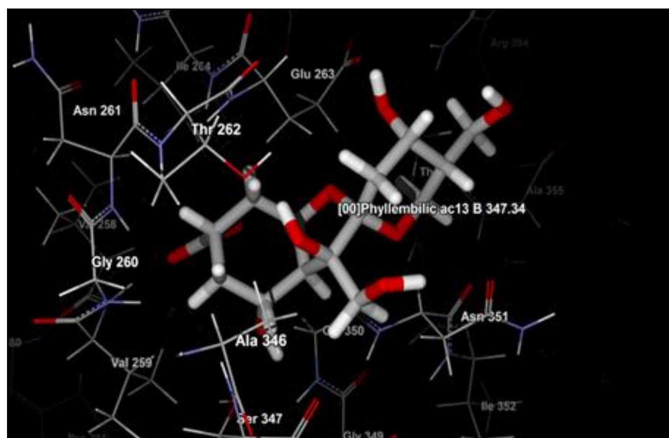

FIG. 5: DOCKED VIEW OF PHYEMBILIC ACID B WITH THE ENZYME XANTHINE OXIDASE (1FIQ)

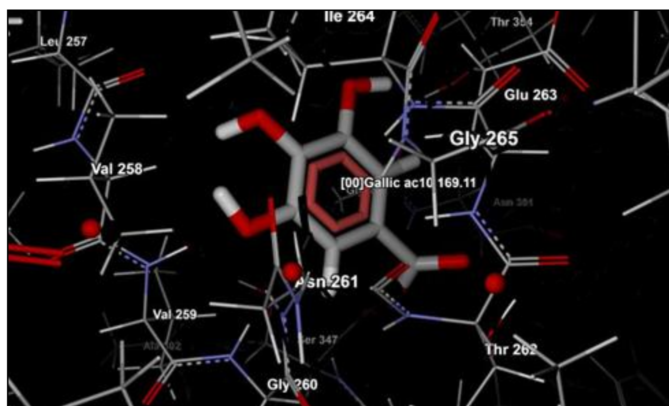

FIG. 6: DOCKED VIEW OF GALLIC ACID WITH THE ENZYME XANTHINE OXIDASE (1FIQ)

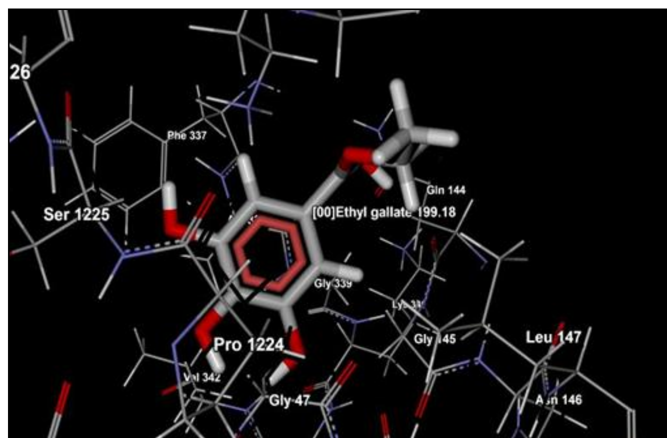

FIG. 7: DOCKED VIEW OF ETHYL GALLATE WITH THE ENZYME XANTHINE OXIDASE (1FIQ)

Our results demonstrated that the phyllembilic acid B was most potent inhibitor of Xanthine oxidase when compared to standard drug allopurinol, whereas ethyl gallate, gallic acid, 5-hydroxymethylfurfural and ascorbic acid showed good inhibitory activity. The phytoconstituents was evaluated for possessing the 'drug likeness properties' by following the 'The Lipinski rule of five' in DruLiTo software. This helps to find the potential lead molecule for antigout property. The results of phytoconstituents are represented in **Table 6**.

TABLE 6: LIPINSKI RULE OF FIVE USING DRULITO SOFTWARE

S. No	Compounds	MW	log p	HBA	HBD
1	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	278.13	4.444	2	0
2	5-Hydroxymethylfurfural	127.04	-0.252	2	2
3	Allopurinol	136.04	-0.58	4	2
4	Ascorbic acid	177.04	-0.878	6	5
5	Beta- Sitosterol	414.39	11.595	1	1
8	Ethyl .alpha.-d-glucopyranoside	208.09	-1.184	6	4
9	Ethyl gallate	199.06	0.152	2	4
10	Gallic acid	169.01	-0.58	0	3
12	Phyllembilic acid B	347.13	-2.87	7	5
15	1,2,3-benzenetriol	126.03	1.002	3	3
16	pthalic acid, 2-cyclohexyethyl iso butyl ester	332.2	4.718	4	0
17	β-cyclocitral	152.12	2.564	1	0

This rule was based on 90% values of the drug's distributions and applies only to absorption of compounds by passive diffusion. The compounds which are actively transported through cell membranes by protein transporters are exceptions to this rule. The criteria of Lipinski rules are widely used to predict not only the absorption of compounds but also overall drug-likeness. Except Beta-Sitosterol all other phytoconstituents follows Lipinski's rule. Consequently, we suggest that the synergistic effect of EOEt extract on XO inhibitory activity might be because of existence of active phenols, flavonoid, steroids and tannins according to our results of *in vitro* and *in silico* methods.

CONCLUSION: In conclusion, the results obtained from ethanolic extract *of Embilica officinalis* (EOEt) expressed as a strong antioxidant and XO inhibitory activities. The *in silico* docking studies confirmed the antioxidant property of the

active phytoconstituents obtained in GC-MS analysis. It is also suggested that the synergistic effect of phytoconstituents of this extract confirms the xanthine oxidase inhibitory property. In consequence, the EOEt extract approaches to manage the gout as a whole and shows a fine enzyme inhibitory and antioxidant activities. Further studies can be performed to evaluate the *in-vivo* antigout activity and also isolation of active phytoconstituents of the fruit responsible from the activity.

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