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ORIGINAL ARTICLE **OPEN ACCESS**

Anti-Inflammatory, Antioxidant and In Silico Studies of *Terminalia bellerica* Fruit Constituents

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ABSTRACT
Inflammation is the response of any tissue to injury or trauma, mediated by various signaling molecules and enzymatic pathways COX, LOX that catalyses the formation of prostaglandins and thromboxane's from arachidonic acid (AA). Terminalia bellerica fruit is used in folk medicine to treat asthma, cancer, colic, diarrhea, dysuria, headache, hypertension, inflammations and pain. Terminalia bellerica methanolic extract were screened for in vivo anti-inflammatory activity by carrageenan induced paw edema, in-vitro anti-inflammatory activity was performed by protein denaturation method and in vitro antioxidant method was performed by DPPH and radical scavenging assay. The preliminary phytochemical investigation of methanolic extract of Terminalia bellerica fruits showed the presence of phenolic compounds, alkaloids, flavonoids, steroids, terpenoids, glycosides, carbohydrates. The compounds from methanolic fruit extract have shown significant ($p<0.05$) anti-inflammatory activity. The inhibition rate of egg albumin denaturation for methanolic extract increased gradually with concentration. The isolated compounds of Terminalia bellerica fruit were subjected in silico molecular docking by mcule with protein 5UOI and 6COX, were visualized in discovery studio and screened on the basis of molecular dynamic stimulation studies which were evaluated by Ramachandran plot and Z score by prosa web. Quercetin and Kaempferol showed highest binding score and amino acids were in most favourable regions for protein 5UOI and 6cox and Z-score was below mean average that is reliable. From the above results, inhibition of COX and LOX pathway elucidated anti-inflammatory and antioxidant activities justifying its folk medicinal use in inflammation.
Keywords: *Terminalia bellerica, anti-inflammatory activity, protein denaturation, molecular docking, MCULE.*

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INTRODUCTION

Inflammation is the main body retaliation to injury caused by internal and external factors[1]. Inflammation is intricate biotic reaction of vascular tissues against hostile agents like pathogens, foreign particles, or damaged cells resulting in either acute (mild) or chronic (major). Acute (mild) inflammation is the primary response and is known by the major motion of plasma along with innate immune system, like neutrophils and phagocyte scavenging cell, blood to the damaged tissues. Chronic inflammation concerns a progressive change in the cells at inflammatory site and is noted by simultaneous demolishing and betterment of the damaged tissue[2].The complex or difficult events and agents implicated in the inflammatory reaction can persuade or aggregate reactions[3].Inflammation is a safety measure taken through the organism to cats off the injurious stimuli; but the utilization of anti-inflammatory substances may be an efficient tool in the therapeutic remedy of the diseases.

Carrageenan is used in inducing inflammation and indomethacin is now known as standard drug in our study. Acute (mild) inflammation is a step that involved the overproduction of oxidative radicals, trigger of a complex enzymes, and release of numerous inflammatory and pro-inflammatory mediators. The carrageenan bought up paw edema is a famous acute model of inflammation that is extensively used for screening new anti-inflammatory compounds. Carrageenan injection into the sub plantar surface of rat paw persuade a biphasic edema. The release of mediator's histamine along with serotonin, bradykinin and some PG's that are released by COX are for about 1 hour noticed as early phase whereas the delayed phase (after 1 h) is assigned to neutrophil infiltration, and the continuing of the prostaglandin generation

(Gilligan et *al*., 1994). Release of the neutrophil-derived free radicals, nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), and interleukin-1 β (IL-1 β) also involved in the delayed phase of carrageenan-induced acute inflammation [4].

In conventional system of drugs such as Ayurveda, Siddha, and Unani, use of herbal remedies for the treatment of ailment is well documented. One such genus is *Terminalia* belonging to *Combretaceae* family, which comprises of 200 species [5]. *Terminalia bellerica methanolic extract* is commonly rich in phenolics, flavonoids, alkaloids, triterpenoids, tannins and other compounds that elicit multiple biological, pharmacological and therapeutic activities is been reported [6].The plant *Terminalia bellerica* fruit exhibits a number of pharmacological action such as treating diabetes, cardioprotective, antitumour, hepatoprotective, protecting gastric mucosal damage, anticolitic, inhibition of free radical generation and immunostimulatory activities [7]. Furthermore, in neoteric years, different computational techniques have achieved fame to get insight into the mode of Therefore, molecular modelling (docking analysis), which is a structure-related drug design technique study was performed[8].The intent of our study is to perform anti-inflammatory activity with anti-oxidant activity, *In silico* Docking, homology modelling with Ramchandran plot and Z-score.

MATERIAL AND METHODS

Plant collection

Methodology design involves a series number of steps including selection and collection of fruits of *Terminalia bellerica* from a local market during the month of December 2016. This material was identified, authenticated by Botanist, cleaned from dirt, under shade it is dried for about fifteen days and cut into small pieces in laboratory.

Preparation of *Terminalia bellerica* fruit extraction

The powdered material of fruits of *Terminalia bellerica* was extracted by soxhlation technique with methanol.Soxhlet extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor for several times. This process involves extraction followed by evaporation of the solvent. The vapors of the solvent are taken to a condenser and the condensed liquid is returned to the drug for continuous extraction.

Preliminary phytochemical analysis of the extract

The extract was used to identify various phytoconstituents available in the fruits by preliminary phytochemical investigations.

Acute toxicity testing

Toxicity studies were performed so that one can identify the toxic effects of the *Terminalia bellerica* extract. Acute toxicity studies were carried out as per the OECD 425 guidelines. The Initial test i.e., sequential test is a Limit test were usage of maximum of 5 animals. A test dose of 2000, or exceptionally 5000 mg/kg, may be used.

Experimental protocol

Wistar albino mice (Approx. 20 to 25 gm) & albino rats (Approx 200-250 gm) were procured from Albino research, Hyderabad. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India. (Reg. No. 1175/PO/ERe/S/08/CPCSEA). The animals were housed in poly acrylic cages with not more than six animals per cage, with 12 h-light/12 h-dark cycle. Rats have free access to standard diet and drinking water ad libitum. The albino mice were permitted to habitat the preclinical laboratory environment for eight days before the start of the experiment. The care and maintenance of the albino mice were carried out as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Anti-inflammatory activity

In vitro model

1. Protein denaturation method

Protein denaturation method is *in vivo* method for the anti-inflammation evaluation. Indomethacin wasused as standard. 1 ml of various concentrations of extracts ranging from 20-100 µg/ml were present in the reaction mixture, standard Indomethacin along with 3 ml of phosphate buffered saline (pH 6.4) were added and mixed with 1 ml of egg albumin solution (1%). Control reaction mixture that is without *Terminalia bellirica* fruit extract is allowed to incubate at 37°C for twenty minutes. Then reaction mixture placed in laboratory water bath for two minutes at 90°C, this process is called denaturation. Mixture was cooled and analysed in spectrophotometer at the 660 nm for turbidity. Denaturation percentage inhibition was mathematically calculated by the formula provided below [9].

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

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

***In vivo* model**

1. Carrageenan induced rat hind paw oedema method

Albino mice were kept on fast for about 16 h with free access to water. Different concentrations of extract were administered orally to two groups of rats using an oral cannula. Animals in the standard (reference) group served with indomethacin (5 mg/kg *p.o.*), while control group of animals received normal saline. Sixty minutes later, oedema was caused into the right hind paw of albino mice by injecting of 50 microliter carrageenan solution with 1%

Study design of Carrageenan induced rat hind paw oedema method is Group –I serves as Control (Normal saline) were as Group –II receives Carrageenan (50 µl of 1% carrageenan solution), Group –III and IV receives methanolic extract of *Terminalia bellirica*(200mg/kg and 400 mg/kg, *p.o*) + 1% Carrageenan (50 µl) and Group –V receives Indomethacin (5 mg/kg, *p.o*) + 1% Carrageenan (50 µl). The thickness of injected paws was measured before and 1st, 2nd, 3rd, 4th, and 5thhr after induction of inflammation, using a Plethysmograph. Percentage protection (or inhibition) was calculated by using the formula [10].

$$\% \text{ Protection} = 1 - Vt/Vc$$

Where, Vt = Increase the paw volume in the test animal. Vc = Increase the paw volume in the control group.

Antioxidant assays

1. Reducing power method (RP)

In this method, antioxidant compound forms a colour complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples. 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) were added to 1.0 mL of sample dissolved in distilled water. The resulting mixture is incubated at 50 ºC for 20 min, followed by the addition of 2.5 mL of Trichloro acetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). The absorbance is then measured at 700 nm against blank sample [11].

2. Lipid peroxidation (LPO) assay

Male swiss albino mice (weighing 20-25 g) were sacrificed by cervical dislocation. The brain was removed and then homogenized in phosphate buffer saline (pH 7.0). 1 ml of brain homogenate (10%, w/v) was added to the test extract of different concentrations. The lipid peroxidation was initiated by adding 100 µl of 15 mM $FeSO_4$ solution. After 30 min of incubation at room temperature, 0.1 ml of reaction mixture (brain homogenate + test drug) was taken in a tube containing 0.1 ml of SDS (8.1%w/v), 0.75 ml of 20% Trichloro acetic acid and 0.75 ml of 0.8% TBA solution. The volume in each tube was made up to 2 ml with distilled water and then heated on water bath at 95ºC for 60 minutes. After 60 minutes, the volume in each tube was made up to 2.5 ml and then 2.5 ml of N butanol: pyridine (5:1) was added in each tube. The reaction mixture was vortexed and centrifuged at 4000 rpm for 10 minutes. The organic layer was removed and absorbance was read at 532 nm in a UV spectrophotometer. The experiment was performed in triplicate. The percentage inhibition was calculated using the formula [12].

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

***In silico* analysis**

Molecular docking

The mechanism of binding of drug with the target protein is called docking. Docking can be used to find inhibitors for specific target proteins and thus to design new stable drugs from docking results. Docking can be calculated by binding energy (energy release during protein and ligand interaction). In this project, online mcule software was used for docking and visualized in discovery studio.

Ramachandran plot and Z score

Ramachandran plot has been generated from PROCHECK validation server which was used to access the quality of the model by looking into the allowed and disallowed regions of the plot. A Z-score value was generated from ProSA web server that can determine the overall quality of the model and its identity nearest to native nuclear magnetic resonance/X-ray crystal structures [13].

Statistical analysis

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, diabetic control and standard by using Dunnett's test. Significant values are expressed as control group (**=$p<0.01$, *=$p<0.05$), negative control (A= $p<0.01$, B = $p<0.05$) and standard (a = $p< 0.01$, b = $p< 0.05$), ns- non significant.

RESULTS

Methanolic extract of *Terminalia bellerica* fruit was explored for its *in vitro* and *in vivo* anti-inflammatory activity using suitable animal models and it's *in vitro* antioxidant activity. All the results obtained in the studied were included below

Percentage yield of METB obtained by soxhlation

The methanolic extract of *Terminalia bellerica* fruits was prepared by Soxhlation technique. The percentage yield of the extract was calculated by using the following formula. *i.e.,*

$$\% \text{ Yield of extract} = \frac{amount\ of extract\ obtained}{amount\ of\ powder\ used} * 100$$

$$= 18\ \%\ w/w$$

Preliminary Phytochemical analysis

The preliminary phytochemical investigation of methanolic extract of *Terminalia bellerica* fruits showed the presence of phenolic compounds, alkaloids, flavonoids, steroids, terpenoids, glycosides, carbohydrates etc.

Acute toxicity studies

Methanolic extract of *Terminalia bellerica* fruits was tested on albino swiss mice up to a dose of 2000mg/kg bd. wt. The animal did not exhibit any signs of toxicity or mortality up to 2000mg/kg bd. wt. Hence the extract was found to be safe up to 2000mg/kg bd. wt.

Anti-inflammatory activity

***In vitro* anti-inflammatory activity**

The *in vitro* anti-inflammatory activity was performed using protein denaturation method. The results were expressed in table 1.

Table 1: Protein Denaturation of methanolic Fruit Extract of *Terminalia bellerica*

S.No	Compounds	Concentration	% Inhibition	IC_{50} values
1.	METB	50	21.23±1.06	415
		100	40.22±1.04	
		200	41.84±0.95	
		400	46.24±2.06	
2.	Indomethacin	50	4.28±1.09	405
		100	8.59±1.02	
		200	23.87±1.07	
		400	48.62±1.59	

In protein denaturation assay, the METB was tested at different concentrations of 50, 100, 200, 400 µg/mL. The lowest concentration of 50 µg/mL showed a percentage inhibition of 21.23 where as the highest concentration of 400µg/mL showed a percentage inhibition of 46.24. The IC_{50} value for the METB was found to be 415µg/mL which is compared with standard ascorbic acid having IC_{50} value of 405 µg/mL.

***In vivo* model**

Carrageenan induced rat hind paw edema method

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

Table 2: Effect of Metb On Paw Volume (Ml) In Carrageenan Induced Paw Oedema Model Using Plethysmograph

Groups	Paw volume(ml)					
	0hr	1hr	2hr	3hr	4hr	5hr
Normal control	0.21± 0.03	0.22 ± 0.02	0.19 ± 0.04	0.22 ± 0.02	0.22 ± 0.03	0.20 ± 0.03
Negative control	0.19± 0.03	0.61 ± 0.04^{**A}	0.73 ± 0.02^{**A}	0.83 ± 0.03^{**A}	0.92 ± 0.04^{**A}	0.98 ± 0.04^{**A}
METB (200 mg/kg)	0.23± 0.04	0.49± 0.03^{**Ab}	0.53± 0.03^{**Aa}	0.48± 0.03^{**Aa}	0.44± 0.03^{**Aa}	0.41 ± 0.04^{**Aa}
METB (400 mg/kg)	0.21± 0.02	0.45 ± 0.02^{**Ba}	0.49± 0.03^{**Ba}	0.43± 0.02^{**Ba}	0.39 ± 0.02^{**a}	0.37 ± 0.03^{**Ba}
Indomethacin (5mg/kg)	0.22± 0.03	0.33 ± 0.03^{*a}	0.36 ± 0.02^{**a}	0.33 ± 0.03^{*a}	0.29 ± 0.02^{a}	0.24 ± 0.02^{a}

Values were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing 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), ns=non-significant.

In control group, which received saline the average initial and final paw volume of rats was found to be similar. In carrageen control group the initial and final paw volume were found to be 0.19 ± 0.03 and 0.98 ± 0.04. There was a significant decrease in paw volume after administration of METB (200 & 400mg/kg, *p.o*) and standard when compared with negative control. In screening the anti-inflammatory activity, the percentage inhibition of paw volume was considered to be an important factor. From the above results the METB and indomethacin significantly possesses anti-inflammatory activity compared to negative control group.

***In vitro*-antioxidant assays**

The methanolic extract of *Terminalia bellerica* fruits was subjected to *in vitro* antioxidant activity. *In vitro* anti-oxidant activity was performed using reducing power assay & Lipid peroxidation assays.

Reducing power assay

The *in vitro* antioxidant activity was performed using reducing power assay. The increase in absorbance with increase in concentration indicates the reducing capacity. The results were expressed in table 3.

Table 3: Reducing Power assay of methanolic fruit Extract of *Terminalia bellerica*

S.No	Compounds	Concentration	Absorbance
1.	METB	10	0.573±0.003
		20	0.610±0.006
		30	0.770±0.006
		40	0.833±0.007
		50	0.920±0.006
2	Ascorbic acid	10	0.613±0.007
		20	0.770±0.006
		30	0.850±0.006
		40	0.883±0.009
		50	0.957±0.003

In reducing power assay, the METB & Ascorbic acid were tested at different concentrations 10, 20, 30, 40 and 50µg/ml. This clearly shows with increase in concentration the absorbance also increases and increase in reductive ability of METB. Early reports also suggest that increase in absorbance indicates increase in reducing capacity. From this the METB possesses significant anti-oxidant activity. The reducing ability of a compound generally depends on the presence of reductants which have been exhibiting anti-oxidative potential by breaking the free radical chain and donating a hydrogen atom. The reducing power activity of METB might be due to presence of phenols and flavonoids in the extract with adequate number of hydroxyl groups [11].

Lipid peroxidation assay

The *in vitro* antioxidant activity was performed using Lipid peroxidation assay. The results were expressed in table 4.

Rable 4: Lipid peroxidation assay of methanolic flower extract of *Terminalia bellerica*

S.No	Compounds	Concentration	% Inhibition	IC_{50} values
1.	METB	10	16.12±0.58	39.25
		20	19.58±0.83	
		30	36.21±0.86	
		40	54.26±0.89	
		50	70.12±1.06	
2.	Ascorbic acid	10	12.68±1.15	34.22
		20	24.25±1.84	
		30	55.27±1.72	
		40	68.42±2.32	
		50	79.13±1.75	

In lipid peroxidation assay, the METB was tested at different concentrations like 50, 100, 150, 200, and 250 µg/mL. Currently, lipid peroxidation is considered as the main molecular mechanisms involved in oxidative damage to cell structures and in the toxicity process that lead to cell death [14]. Oxidative stress is understood as an imbalance situation with increased oxidants or decreased anti-oxidants. The reducing property of METB indicates they can be used as electron donors who reduce the oxidised intermediates of lipid peroxidation processes; therefore, it can be used as an anti-oxidant. The phenolic compounds identified in the METB might have supressed lipid peroxidation through different mechanisms like free radical quenching, electron transfer, radical addition or radical recombination [15]. From the above results the METB possesses anti-inflammatory, anti-oxidant activities.

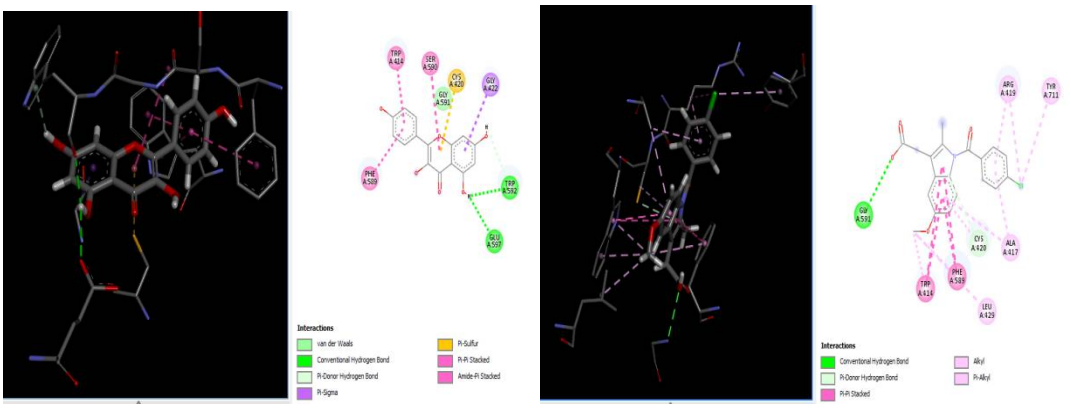
***In silico* analysis**

Molecular docking:

To perform docking, initially the protein was downloaded from PDB and molecular docking performed in mCule software and visualized in discovery studio. Some compounds have good binding ability with LOX inhibitor (PDB ID: 5UOI) and COX inhibitor (PDB ID: 6COX) were given [Figure 1&2]. Docking Results with glide score (Table5)

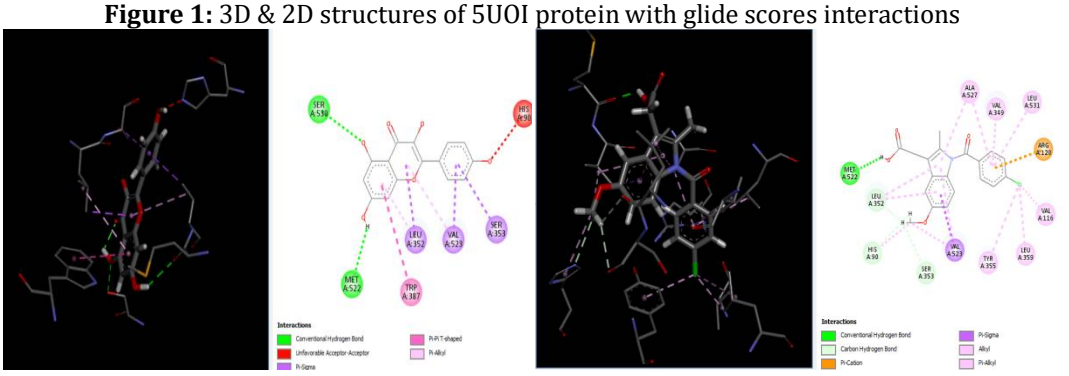
Table 5: Glide scores of *Terminalia bellerica* constituents with 5UOI and 6COX protein

Constituents	Glide scores (KcaL/moL)	
	5UOI	6COX
Kaempferol	-9.4	-8.0
Quercetin	-8.8	-8.1
Ellagic acid	-8.6	-7.4
Hexahydroxydiphenic acid	-7.7	-6.6
Syringic acid	-6.3	-5.9
Rhamnose	-6.3	-5.7
Galactose	-6.2	-5.7
Methyl ester	-6.5	-4.8
Mannitol	-5.8	-5.1
Indomethacin	-8.7	-8.5



a) Kaempferol -9.4 **b)**Indomethacin -8.7

Figure 1: 3D & 2D structures of 5UOI protein with glide scores interactions



a) Quercetin -8.1 b) Indomethacin -8.5

Figure 2: 3D & 2D structures of 6COX protein with Glide scores with interactions

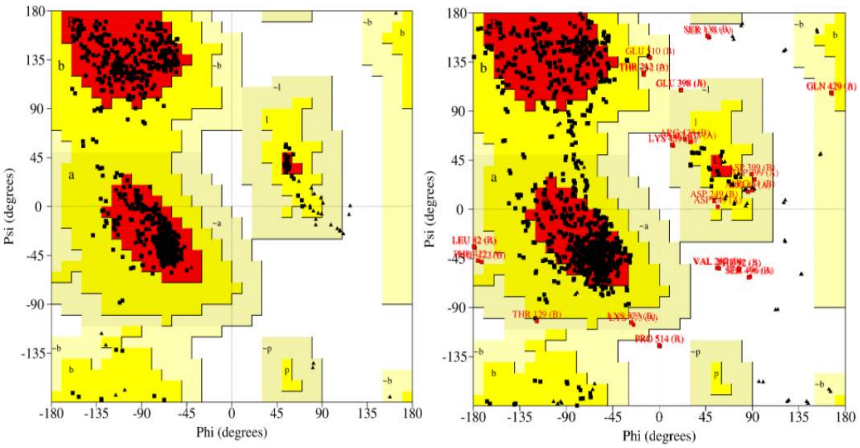
The more negative the Glidescore the more favourable is the binding. G score = glide score,

Ramachandran plot Analysis

Protein 5UOI and 6COX were analysed for Ramachandran plot to know amino acid presence in different regions of respective protein tabulated in table 6 and pictorial representation by figure 3.

Table 6: Ramachandran Plot Status with Protein 5UOI and 6COX

Residues	5UOI	6COX
Most favourable region (%)	74.1	92.4
Additional allowed regions (%)	22.5	7.6
Generously allowed regions (%)	2.5	0.00
Disallowed regions (%)	0.8	0.00

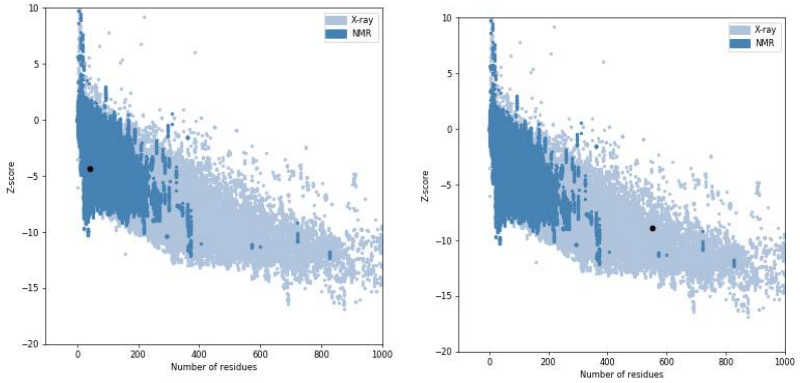


a) 5UOI b)6COX

Figure 3:Ramachandran plot of protein 5UOI and 6COX

Z score by Prosa web

The quality of the model was checked by ProSAWeb Server. Groups of structures are distinguished by different colours (NMR with dark blue and x ray with light blue). This plot can be used to check whether the zscore of the protein are within the range of scores typically found for proteins of similar size belonging to one of these groups. It can be seen in (Figure 4) that Z-scores value of the obtained model is located within the space of proteins determined By X ray. The values obtained are below average



a) 5UOI -4.31 b) 6COX- 8.86

Figure 4: Z-Score of protein 5UOI and 6COX

DISCUSSION

Phytochemical screening of methanolic extract of *Terminalia bellerica* fruits showed the presence of various phytoconstituents like phenolic compounds, terpenoids, flavonoids, steroids, carotenoids & fruit contain hexahydroxydiphenic acid, methyl ester, beta sitosterol, gallic acid, ellagic acid, ethyl gallate, glucose, chebulagic acid, mannitol, galactose and rhamnose. Carrageenan- induced rat hind paw oedema is ubiquitously used test to determine anti-inflammatory activity [16]. Various mediators like histamine, serotonin and bradykinin, prostaglandins (PGs), cytokines TNF-α, IL-1, and IL-6 were detectable [17]. Swelling is with the elevated production of prostaglandins, has been attributed to the induction of inducible cyclooxygenase (COX-2) in the hind paw [18]. Another important mediator in acute inflammation is nitric oxide (NO) that contributes to tissue injury and inflammation-induced oedema and hyperplasia [19]. Several studies have been reported that phenols like syringic acid and gallic acid and flavonoids such as quercetin, kaempferol inhibit proinflammatory enzymes (COX-2, lipooxygenase and inducible NO synthase, inhibition of NF-KB and AP-1 and activation of MAPK, PKC[20]. Denaturation of protein is a well-documented cause of inflammation [21]. *In vitro* anti-inflammatory activity of METB can be attributed to its terpenoids& flavonoids presence they have the ability to bind to the cations and able to protect the protein membrane from denaturation [22].

METB was explored for its antioxidant activity against reducing power assay and lipid peroxidation assay. The reducing ability of a compound generally depends on the electron donating capacity due to presence of various active constituents in METB like phenolics, triterpenoids and flavonoids [23]. Lipid peroxidation is autocatalytic complex process which involves eventual destruction of membrane lipids and cause per oxidative tissue damage in inflammation [14].

The compounds present in METB, are docked with protein LOX inhibitor (5UOI) and PG's synthase inhibitor(6COX) and Ramachandran plot is analysed. Quercerin, Kampferoland indomethacin showed good docking score when compared to other compounds. Lipoxygenase (LOX) is a kind of rate-limiting enzyme in the process of arachidonic acid metabolism into leukotriene which mediates the occurrence of inflammation. The inhibition of LOX can reduce LT, thereby producing an anti-inflammatory effect [24]. Inhibition of COX-2 has generally been considered the basis for the anti-inflammatory effects of NSAIDs. 6COX Ramachandran plot showed 92.4% of amino acids in mostly allowed regions and 74.1% with 5UOI protein. In order to facilitate interpretation of the Z-score of the specified protein, its particular value is displayed in a plot that contains the Z scores of all experimentally determined protein chains in current structure [25]. The values are -4.321 and -8.86 which suggests that the obtained model is reliable for 5UOI and more below average for protein 6COX. In the present study the superposition of kaempferol, quercetin structure and other compounds docking found with LOX inhibitor (5UOI) and PG's synthase inhibitor(6COX) protein have validated the accuracy of our docking study, Ramachandran plot and Z score that resulted anti-inflammatory and antioxidant activity.

CONCLUSION

In the present study the anti-inflammatory and anti-oxidant potential of METB was evaluated. Methanolic extract of *Terminalia bellerica* fruits has lowered the edema formation induced by carrageenan. In *in vitro* anti-inflammatory activity the presence of phenols, flavonoids, terpenoids and steroids might have protected the secondary and tertiary structure of the protein, thus preventing the denaturation of proteins which might contribute to its anti-inflammatory activity. The extract significantly scavenged the free radicals in dose dependent manner and *Insilico* analysis of compounds by protein 5UOI and 6COX elucidated anti-inflammatory activity and antioxidant activity.

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CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

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