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EXPLORING THE ROLE OF *TERMINALIA CATAPPA* FRUIT SOURCE IN COMBATING PROTEIN IN MALNUTRITION AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

The present study investigates the anti-inflammatory activity of methanolic extracts of *Terminalia catappa* L a well-recognized medicinal plant in Ayurveda. Phytochemical tests conducted in methanolic extract contains phenols, alkaloids, tannins, flavonoid, steroids and terpenoids. The protein estimation of the extracts was reported by Lowry's method and Bradford method. The characterization size of protein is determined by SDS-PAGE. Methanolic extracts derived from fruits has been assessed for its anti- inflammatory activity by in-vitro method. In-vitro anti-inflammatory capability was evaluated using protein denaturation assay. The blind molecular docking studies using nineteen phytochemicals from the study against the protein of COX-2. The 3D protein structures were analysed, and potential drug-binding site were identified, the drug-likeness properties of ligand were assessed as well. Docking results determines the highest binding energy and inhibition constants, an active compound visualization of amino acid residues around the active compound was identified with ligplot which help in combating malnutrition and cure inflammation.

KEYWORDS: *Terminalia catappa* L, Phytochemical, Anti-inflammatory activity, Molecular docking, COX-2, Ligplot.

1. INTRODUCTION

Terminalia catappa L is a member of the Combretaceae family comprises approximately 600 species wide applicability in the traditional African, Asian, and Indian medicine.[1,2,3] *Terminalia catappa* is a shade and salt tolerant street tree, which was primarily used as an ornamental plant.[4] Tree is a tall deciduous and erect tree reaching 15-25m, trunk 1-1.5 m in diameter, often buttressed at the base. Most found on tropical and subtropical beaches.[5] In India, it is known as Malabar almond, Indian almond, and tropical almond found in Australia, Srilanka, Malaysia, Pakistan, South Asian countries.[6] vital role in coastal communities.[7] The bark leaves and fruit of the plant were used in different countries like India, Malaysia, and Philippines to cure dermatitis and for haemostatic and antipyretic purposes and in Taiwan by shredding and drying.[8] It is very rich in phytochemicals and a good source of natural antioxidants.[9] Parts of the tree, such as the leaves and fruit, are astringent, treat leprosy, The kernel of the fruit mixed with beeswax stops putrid exudation and bloody faeces, mild laxative and a galactagogue for women, diarrhoea. The young leaves are used to cure headaches and colic.[10] *T.catappa* is one of the most common plants used in Ayurveda; hence, it is considered as "King of Medicines".[11]

Fig 1: Tree and Fruit of *Terminalia catappa.*

Fruits vary greatly in shape, size, and colour, cream-coloured seed which encloses the kernel (Nut). The bark is grey to dark grey brown and shallowly fissured.[11] From the ancient period, many medicinal plants were used for inflammatory diseases. Tree has bioactive compound that is ursolic acid, oic acid and show strong anti-inflammatory activity.[12]

2. MATERIALS AND METHODS

Collection of plant sample: *Terminalia catappa* (fruits, seeds) were collected in May-June 2022 from Freedom Park, Bengaluru, India.

Processing of plant sample: The leaves, fruits, seeds, and bark of *Terminalia catappa* were properly washed and rinsed in distilled water, shade dried for 2-3 weeks. The powder was stored in airtight glass containers.

Solvent extraction: About 1 gm of each plant material 10ml of methanol. The solution is kept in shaker incubator for constant shaking to about 3hours at the speed of 85rpm and at 37^{o} C temperature. The filtrate was stored at 4^{o}C. Each extract was resuspended in the solvent and used for qualitative and quantitative analysis of phytochemicals.

Fig 2: Fruit of *T. catappa.*

Fig 3: Mesocarp, Exocarp and Seed.

3. Phytochemical screening

Tests for phytochemical constituents: Flavonoids, Steroids, Phenols, Terpenoids, Tannins, Saponins.

3.1 Protein Estimation

Bradford protein assay: The Bradford assay is a quick and sensitive method for measuring the concentrations of proteins in absorbance maximum of Coomassie Brilliant Blue G-250 dye. Prepare a 0.1 mg/ml stock solution of the standard BSA. Any 100µl and add 1.0ml of dye reagent to sample. incubate for 30 minutes, absorbance at 595 nm.

Lowry protein assay: The protein detection range is 5–100µg.[13] Prepare 100mg/ml stock solution of the standard BSA. Prepare the working solution from standard BSA of 200µg/ml of different concentrations and made final volume 1.0ml with distilled water. Add copper reagent of 5.0ml to standards and samples. After adding copper reagent incubate for 10 min at room temperature. After incubation, add 0.6ml of FC reagent to standards and samples absorbance at 600 nm.

3.4 Standard and test solutions: 10 mg of rutin and gallic acid was accurately weighed and transferred into 10 ml of volumetric flask. Add about 5 ml of diluent methanol and sonicate in ultrasonic water bath for 30 min. Cool the solution and make up the volume with methanol to obtain stock solution of 1000 ppm. 50 mg of *Terminalia catappa* extract was weighed and transferred into a 20 ml of volumetric flask. Add about 15 ml of diluent methanol and then sonicate in ultrasonic water bath for 30 min the volume with diluent methanol. Then filter through 0.45 µ syringe filter. 250 mg of Baheda SH Capsule powder was weighed and transferred into a 20 ml volumetric flask. To this 15 ml of diluent methanol was added sonicated in ultrasonic water bath for 30 min. Then filter through 0.45 µ syringe filter.

3.5 Size of Protein

Centrifugation: Centrifugation is a process used to separate the particles or the concentrated materials, such as the cell, sub-cellular organelle, viruses, and large molecules to obtain pure samples of the entire particle or material.

3.6 SDS–PAGE (Polyacrylamide Gel Electrophoresis of Proteins)

SDS-Polyacrylamide complexes from and migrate through the gels according to the size of the polypeptide. by using markers of known molecular weight of the polypeptide chain(s) can be estimated. Leave sufficient space for the stacking gel, acrylamide solution carefully with 0.1% of SDS or iso butanol. The gel can be fixed, stained with coomassie brilliant blue, and kept for overnight later destain the gel and view under fluorography.[14] (Rf) of the molecular weight markers and the unknown sample protein.

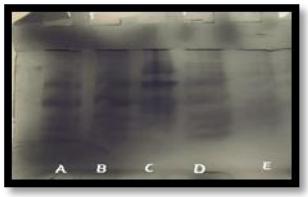

Fig 4: A and B-Mesocarp, C-Seed, D and E-Leaves.

$$R_f = \frac{\text{Migration of band (cm)}}{\text{Migration of dye front (cm)}}$$

3.7 Biological activity (in-vitro anti-inflammatory activity)

The present study has the anti-inflammatory activity of methanolic extract of *Terminalia catappa* is a valuable medicinal plant which has been valued for centuries in ayurvedic medicines. Phytochemical analysis of plant extracts revealed the presence of various biochemical compounds such as phenols, alkaloids, tannins, anthraquinone glycosides, saponins, flavonoids and terpenoids. Terpenoids and flavonoids have remarkable evaluating the in-vitro anti- inflammatory cause of inflammation. Denaturation of protein is well documented cause of inflammation.

Anti-denaturation activity: A solution of 0.2% w/v of BSA was prepared in a tris buffer saline and pH was Percentage of inhibition of denaturation =

$$\frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} * 100$$

4. Computational studies

4.1 Swiss ADMET

Drug likeliness properties of the ligand based on Swiss ADME analysis include their chemical properties like, its molecular weight being < 500 Daltons, with < 5 hydrogen bond donors, < 10 hydrogen bond acceptors and QPlogPo/w < 5. The *n*-octanol/water partition coefficient (log *P*o/w) is a key physicochemical parameter for drug discovery depicts lipophilicity indices of the ligand as within the range. The parameters measured for the ligand's solubility in water identifies the ligand to be an ideal drug.

4.2 Molecular docking

Molecular docking is a method which predicts the preferred relative orientation of one molecule (key) when bound in an active site of another molecule (lock) to form a stable complex such that free energy of the overall system is minimized.[16] The first step of docking is the generation of composition of all possible conformations and orientations of the protein paired with the ligand. The second step is that the scoring function takes input and returns a number indicating favourable interaction.[17] Pharmacological management for inflammation includes nonsteroidal anti-inflammatory drugs (NSAIDS) and selective COX-2 inhibitors that inhibit PGs formation without affecting LOX activity.[18] Mankind, through trial and error, has found medicinal properties in seeds, barks, roots and leaves of certain plants and traditional knowledge has given clues to the discovery of these valuable drugs[19][20] Seeds, as a new source of anti-inflammatory and analgesic bioactive.

Study reported in literature stated the importance of dataset size such as 10,000 compounds using Flex and X[21] 44,000 compounds using Surflex.[22] highly effective in reducing the dataset to be docked.[18] Flex and X tool of the Lead IT software and Swiss ADME analysis were applied. A set of bioactives identified in seeds was screened and were finalized for docking. The 3D-coordinates for these compounds in the PDB format were obtained through drawing window of chemsketch and further explored for biological activity, which is comprised of Lipinski rules of 5, drug likeness and drug score.

In silico docking experiments were performed using Flex and X tool of the Lead IT software. The ligands were downloaded from Pubchem and H atoms were added to them as required. The molecules were then model built and minimized by running a 1000 cycles of energy minimization by steepest descent approximation and were converged to a gradient of 0.02 using the tool, Chimera UCSF 1.6.2., and the AMBERff99SB Force field for this procedure. These were then uploaded into the Flex and X docking tool of the Lead IT software.

adjusted to 6.8 using glacial acetic acid stock solutions of 10,000µg/ml of all the extracts were prepared by using ethanol as a solvent. 4 different concentrations of 1, 100, 200, and 500µg/ml were prepared by using ethanol as a solvent. 50µl (0.5ml) of each extract was transferred using 1ml micropipette. 5ml of 0.2% w/v BSA was added. The control consists of 5ml of 0.2% w/v BSA solution with 50µl ethanol. The standard consists of 100µg/ml of ibuprofen in ethanol with 5ml 0.2% w/v BSA solution. The test tubes were heated at 72°C for 5 min and cooled for 10 minutes. The absorbance of these solutions was determined by using a UV-VIS double beam spectrophotometer (ELICO SL 244) at a wavelength of 660 nm. The percentage of inhibition of precipitation was determined on a percentage basis relative to control using the formula.[15]

5. RESULTS

5.1 Phytochemical analysis: The seed, fruit, bark and leaves of *Terminalia catappa* were subjected to the phytochemical analysis to identify the presence and absence of phenols, alkaloids, tannins, anthraquinone, glycosides, Saponins, steroids, terpenoids and flavonoids.

5.2 Bradford protein assay

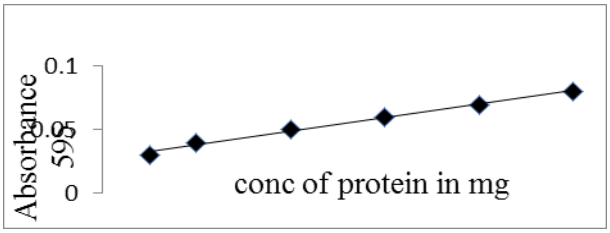

Fig 5: Standard graph of Bradford protein assay.

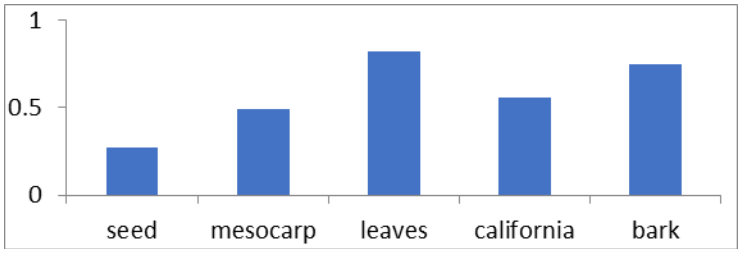

Fig 6: Absorbance of protein samples (seed, mesocarp, leaves, Califoria, bark).

5.3 Lowry Protein Assay

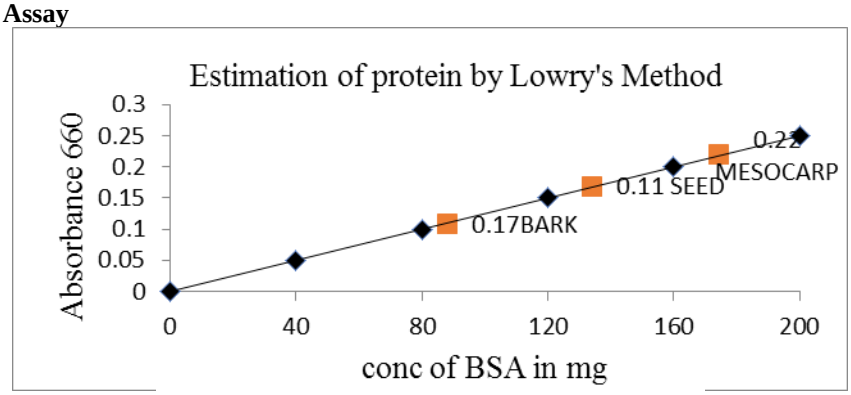

Fig 7: Standard graph of Lowry's protein assay.

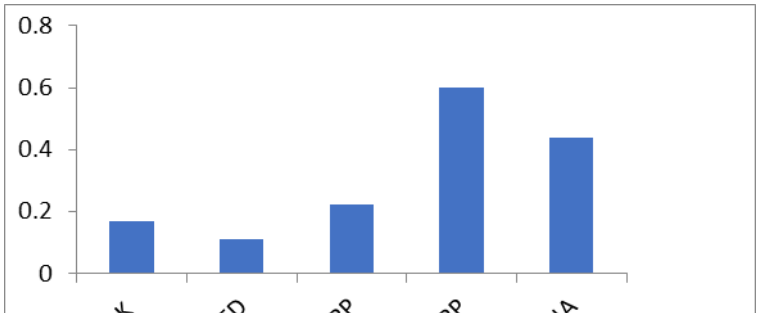

Fig 8: Absorbance of protein samples (seed, bark mesocarp, exocarp, California).

5.6 SDS-PAGE

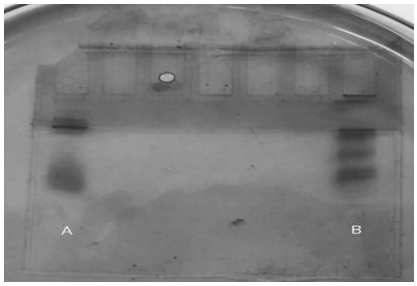

Fig 9: A= Markers (BSA and Lysozyme) B=Seed.

5.7 In-vitro Anti-inflammatory activity

Table 1: Anti-denaturation of BSA in presence of *Terminalia catappa* methanolic extracts.

Sample name	Type of sample	Concentration(µg/ml)	%Inhibition
Ibuprofen (Std)	-	100µl	22.06
California	Methanolic	100µl	23.0
Seed	Methanolic	100µl	57.5
Mesocarp	Methanolic	100µl	38.0

Terminalia catappa extract showed significant anti-inflammatory activity. Ibuprofen, a standard anti-inflammatory drug showed the inhibition 22.06% at the concentration of 100µg /ml maximum percentage of inhibition 57.5 was observed from methanol extract.

3.0 Protein Purification

Sample15g were finely homogenized by using 150ml of 0.1M Potassium phosphate buffer (pH 7).The sample suspension was centrifuged at 6000rpm for 10 mins and supernatant were collected.

Salt precipitation: Extracted sample 150ml was used for the salt precipitation. 66.3g (70%) of ammonium salt was added pinch by pinch to the sample, ice cold condition was maintained and continuous stirring at 950 rpm on magnetic stirrer. Overnight stored samples at ice cold condition were centrifuged at 10,000rpm for 10 mins. The pellets were dissolved in 10 ml of 10mM tris buffer.

Activation of Dialysis membrane:100ml of distilled water was boiled and dialysis membrane Cellulose acetate membrane 6cm was placed into it and continued boiling for 10 min. Sodium bicarbonate 1g was added and boiled for 10 min. the membrane was transferred in a fresh boiling water and boiled for 10 min and cooled. The deionized water was changed frequently for every half hour. The dialysis was performed for 2 hrs.

5.8 Computational Studies

5.8.1 Swiss ADME

Serial no.	Compound Name	Molecular weight	Formula	H –bond acceptors	H – bond donars	TPSA	Lipinski drug likeliness	Synthetic accesability	Water partition co-efficient (Log *Po*/w)	Pubchem CID
1	Brevifolin-carboxylic acid	292.20 g/mol	C13H8O8	8	4	145.27 Å²	0 violation	3.54	0.04	9838995
2	Gallic acid	170.12 g/mol	C7H6O5	5	4	97.99 Å²	0 violation	1.22	-0.21	370
3	Leucocyanidin	306.27 g/mol	C15H14O7	7	6	130.61 Å²	1 violation: NH or OH>5	3.76	0.07	71629

Table 2: Swiss ADME data of the following compounds in fruit of *T. catappa*.

Serial no.	Compound name	Formula	Molecular weight	H –bond acceptors	H – bond donars	TPSA	Lipinski drug likeliness	Synthetic accesability	Water partition co-efficient (Log *Po*/w)	Pubchem CID
1	Arachidic acid	C20H40O2	312.53 g/mol	2	1	37.30 Å²	1 violation:	2.77	6.62	10467
2	Linoleic acid	C18H32O2	280.45 g/mol	2	1	37.30 Å²	0 violation	0		5280450
3	Niacin	C6H5NO2	123.11 g/mol	3	1	50.19 Å²	0 violation	1.00	0.32	938
4	Oleic acid	C18H34O2	282.46 g/mol	2	1	37.30 Å²	1 violation:	3.07	5.65	445639
5	Palmitic acid	C16H32O2	256.42 g/mol	2	1	37.30 Å²	0 violation	2.31	5.20	985
6	Stearic acid	C18H36O2	254.41 g/mol	2	1	37.30 Å²	1 violation:	2.54	5.93	5281

Table 3: Swiss ADME data of the following compounds in seed of *T. catapp T. catappa* seed.

i) Arachidic acid.

Table 4: Binding affinity of *T.catappa* seed.

Ligand	Binding affinity	Rmsd/ub	Rmsd/lb
Arachidic acid	-6.3	0	0
Linoleic acid	-5.1	0	0
Nicotinic acid	-5.7	0	0
Oleic acid	-5.4	0	0
Palmitic acid	-6	0	0
Stearic acid	-7	0	0

***Terminalia catappa* Fruit**

Table 5: Binding affinity of *T.catappa* Fruit.

Ligand	Binding affinity	Rmsd/ub	Rmsd/lb
Bervifolin carboxylic acid	-8.8	0	0
Gallic acid	-7.1	0	0
Leucocyanidin	-9	0	0

5.8.2 Molecular Docking

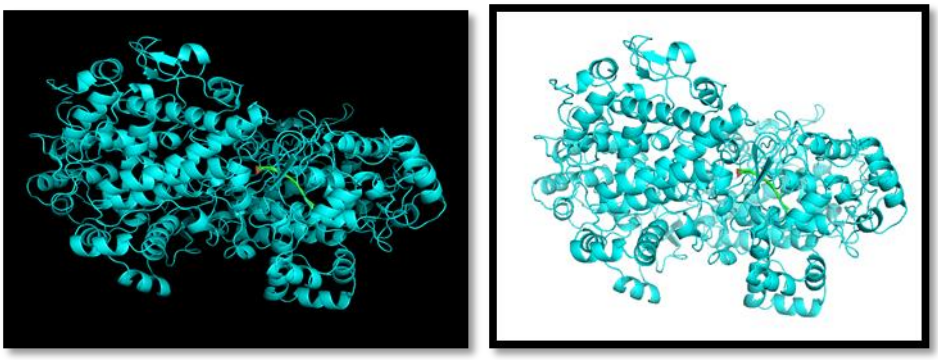

Fig 10: Protein-Ligand Interaction.

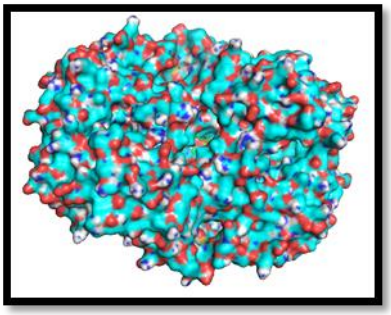

Fig 11: Interaction of Oleic acid with COX-2

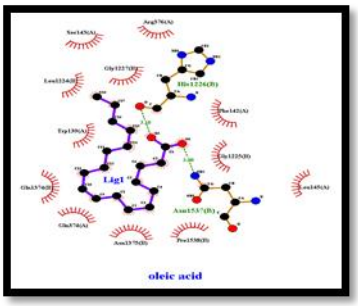

Fig 12: Ligplot interaction of Oleic acid with COX-2.

6. DISCUSSION

The seed, fruit of *Terminalia catappa* were subjected to the phytochemical analysis to identify the presence and absence of phenols, alkaloids, tannins, anthraquinone, glycosides, Saponins, steroids, terpenoids and flavonoids. The phytochemical analysis result in Fig-5-6 and revealed presence of phenols, saponins, tannins, steroids, terpenoids and flavonoids in the *Terminalia catappa* seed, fruit. In Lowry's method, fig 7,8 the concentration of protein in the methanolic extracts of mesocarp shows the highest protein concentration of 172 mg/ml, bark has 134mg/ml and seed has 88mg/ml and is compared with California almond which has 310mg/ml. The amount of phenols present in *T. catappa* fruit is 1.244 and mesocarp 4.745 mg/g. SDS-Polyacrylamide complexes form and migrate through the gels according to the size of the polypeptide in fig 9. The estimated molecular weight of protein in crude methonolic extract of *T. catappa* seed is approximately found to be 63kDa. The data of our studies suggests that the extract showed significant anti-inflammatory activity. Denaturation of protein is well documented cause of inflammation. There are several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. Ibuprofen, a standard anti-inflammatory drug showed the inhibition 22.06% at the concentration of 100µg /ml. maximum percentage of inhibition 57.5 was observed from methanol extract.

In the present study, in silico ADME properties of the isolated flavonoids, steroids, terpenoids, phenolics and tannins from *T. catappa* was performed using the Swiss ADME web tool. Table-2-5 Fig 10-12. suggests that many of the studied ligands satisfy all the Lipinski's drug parameters without any violation suggesting all these ligands could be active drugs. Molecular docking studies using phytochemicals from *Terminalia catappa* L against the protein of COX-2. The 3D protein structures were analysed, and potential drug-binding site were identified, the drug-likeness properties of ligand were assessed. Docking results determines the highest binding energy and inhibition constants an active compound visualization of amino acid residues around the active compound was identified with ligplot. The main objective of the molecular docking analysis of phytochemical compounds is to find a molecule that displays a strong binding affinity to the target protein COX-2 and to build a stable complex. The molecular docking study was performed between the target molecule COX-2 and the ligands using PyRx software. From the results obtained, the findings in Oleanolic acid indicates that Arg1061 (B), Lys1083 (B) and in quercetin

the findings indicate that Ala151(A) serve as binding residues in the COX-2 protein. It can serve as a possible drug candidate against anti-inflammatory activity relative to all other compounds.

7. CONCLUSION

Our present work aims at evaluating the in-vitro anti-inflammatory activity of *Terminalia catappa* by protein denaturation method. Denaturation of protein is a well-documented cause of inflammation. The data of our studies suggests that *Terminalia catappa* extract showed significant activity, therefore our studies support the isolation and use of active constituents of *T. catappa* in treating inflammation. Molecular docking is to give a prediction of the ligand-receptor complex structure using computational methods. This computational analysis reveals that quercetin and oleanolic acid showed better scores with good binding contacts. Therefore, we recommend that this analogue is suitable in further exploration using in-vivo studies.

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