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Anti-inflammatory Activity of Cinnamomum camphora Explored Using Molecular Docking Studies

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Abstract: Cinnamomum is a genus of family Lauraceae, which has been recognized worldwide as an important genus due to its beneficial uses. Camphor from Cinnamomum camphora has been used for centuries, throughout the world as a remedy for treating variety of symptoms such as inflammation, infection, congestion, pain, irritation, cancer etc but many of the applications are not scientifically validated. Computational studies have shown that some of the components of Cinnamum camphora like terpenoids have suppressive effect on COX proteins. Terpenoids have been demonstrated to play an important role in immunomodulatory activity too. This work aims to study the phytochemical constituents and elucidate the molecular mechanism of anti-inflammatory activity of camphor using computational studies.

Keywords: Cinnamomum camphora, anti-inflammaty effect, molecular docking, binding energy

1. INTRODUCTION

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells or irritants and is a protective response involving immune cells, blood vessels and molecular mediators. The function of eliminate the function of inflammation is to eliminate the initial cause of cell, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process and to initiate tissue repair. The classical signs of inflammation are heat, pain, redness, swelling and loss of function. Inflammation is a generic response and therefore it is a generic response and therefore it is considered as a mechanism of innate immunity as compared to adaptive immunity which is specific for each pathogen. Too little inflammation could lead to progressive tissue destruction by the harmful stimulus and compromise the survival of the organism.

Plant extracts and essential oils, which are rich sources of antioxidant and anti-inflammatory agents, can suppress the release of inflammatory mediators and free radicals and increase antioxidant defenses. They are widely used in food, cosmetics, and medicine for their potential biological activities [1, 2].

Cinnmomum camphora is a large evergreen tree, growing up to 20 m tall. Camphor is a natural product derived from the wood of the camphor laurel (Cinnamomum camphora) tree through steam distillation and purification by sublimation, the trees used should be at least 50 years old. Camphor has a counter-irritant, rubefacient and mild analgesic action and is a major component of liniments for relief of fibrocyst, neuralgia and similar conditions[3]. It can be used as a mild expectorant; when ingested camphor has irritant and carminative properties. Camphorated-oil, a solution in oil given through intramuscular or subcutaneous way, can be used as a circulatory and respiratory stimulant, but this use is considered hazardous. . Camphor exhibits several biological properties such as antimicrobial, antibacterial, antiviral and antitussive effects, anti-mutagenic, anticancer activity, and insecticidal activity [4,5, 6]. It is also used in perfume and cosmetic industries. In addition to this, camphor is also used as an insect repellent, and pain reliever for the topical applications sine camphor is known for its antimicrobial properties. The aim of this study is to validate the anti-inflammatory properties of camphor using in-silico studies.

Molecular docking studies are used to determine the interaction of two molecules and to find the best orientation of the ligands which would form a complex with overall minimum energy. The ultimate goal of docking is the prediction of three-dimensional structure of the macromolecular complex of interest has it would occur in the living organisms.

2. MATERIALS AND METHODS:

2.1 Collection of plant material:

The plant material (Cinnamomum camphora) was collected during March 24, 2021 at 3:06 pm from Lal Bagh Botanical Garden, Bangalore. The plant was authenticated by Department of Botany, Maharani's Science College, and Bengaluru 560001.

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2.2. Extraction by distillation method

The leaves stem and bark was separated, washed well using clean water and dried at low temperature (55°C) in hot air oven. The dried leaves, stem and bark were powdered separately in a blender.10g of each powder was transferred into round-bottom flask (500ml) containing 200ml of distilled water with a distillation apparatus attached and a stir bar. Then it is placed directly on a hot plate turned to high heat. A beaker (200ml) is used as a receiving vessel to collect approximately 100ml of distillate. The bio-active molecule is extracted from the distillate with an appropriate organic solvent (ethyl acetate) of 30ml.The organic phase is dried with Magnesium sulphate and the organic solvent is removed on a hot plate at medium heat. The residue is analyzed using TLC and IR spectroscopy and compared to commercially available synthetic standards.

2.3. Characterization by thin layer chromatography

Distillate sample of bark, stem and leaf (C.camphora) and synthetic camphor is dissolved in 5% ethyl acetate. For separation process, the solution mixture to be separated is applied as a small spot about 2cm from one end of the TLC plate (stationary phase). The beaker containing n-hexane and ethyl acetate in the ratio of 7.2:2.9 is used as a solvent (mobile phase). The plate is then placed in a closed beaker containing the solvent, as the solvent in the beaker moves up, the components of the mixture moves up along the plate to different distance depending on their degree of adsorption and separation takes place. The plate is then dipped in the petri plate containing anise aldehyde (spraying reagent). Then the plate is taken out and dried in a hot air oven. Later, the TLC plate is observed under UV light. The relative adsorption of each component of the mixture is separated in the retention factor (R*f* value).

2.4. Extraction with methanol

The bark sample is washed well using clean water and dried at low temperature (55°C) in a hot air oven. The dried bark samples were powdered in a blender. 10g of powdered material was transferred into a clean conical flask containing 20ml of methanol. A flask was left for 2 days with occasional stirring. The contents of the flask were filtered through 4-fold muslin cloth followed by Whattman No.1 filter paper. The filtrate was evaporated to dryness and stored in refrigerator until use. The weight and color of bark extract was noted. The bio-active molecule is extracted from the distillate with an appropriate organic solvent. After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (R*f*) which is equal to the distance migrated over the total distance covered by the solvent. The value is found to be 0.66cm. The color of bark extract was light brownish color respectively. Yield of extract obtained was (5.34%).

2.5. Qualitative analysis of bark extract

The different extracts of bark from Cinnamomum camphora were tested for various components as follows [6,7,8].

A.Test for alkaloids:

Small portion of solvent free extract was stirred with few drops of dil HCl and filtered. (i) Mayer's test (a) 1.36 gm of mercuric chloride was dissolved in 60 ml distilled water. (b) 5 gms of potassium iodide was dissolved in20 ml of distilled water.(a) and (b) was mixed and the volume adjusted to 100ml with distilled water .Appearance of cream color precipitate with Mayer's reagents showed the presence of alkaloids.

(ii) Wagner's Test: 1.27 gm of iodine and 2 gm of potassium iodide was dissolved in 5 ml of water and make up the volume to 100ml with distilled water. Appearance of reddish brown precipitate with Wagner's reagent showed the presence of alkaloids.

(iii) Hager's test: Take 20 ml of saturated solution of picric acid and add few drops of it to 2- 3 ml of extract. A yellow color was observed.

B. Detection for carbohydrates and glycosides: (i) Molisch's test: 10 gm of alpha naphthol was dissolved in 100 ml of 95% alcohol. Extract was treated with this solution and 0.2 ml of conc. sulphuric acid was slowly added through the sides of the test tube, purple or violet color appeared at the junction. (ii) Benedicts test: The test solution was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed if reducing sugars were present. (iii) Fehling's Test: 6.932 gm of copper sulphate was dissolved in distilled water and make volume up to 100 ml (solution A). 34.6 gm of potassium sodium tartarate and 10 gm of sodium hydroxide was dissolved in distilled water and make volume up to 100 ml (solution B). Two solution was mixed in equal volume prior to use and few drop of sample was added and boiled, a brick red precipitate of cuprous oxide was formed, if reducing sugars were present.

C. Test for sterols and tri terpenoids (i) Salkowski test: Extract was treated with few drops of conc. Sulfuric acid , shake well and allowed to stand for some time, red color appear at the lower layer indicated the presence of steroids and formation of yellow colored lower layer indicated the presence of tri terpenoids.

D. Test for proteins and amino acids (i) Ninhydrin test: 1gm of ninhydrin (indane1,2,3trione hydrate) was dissolved in n-butanol and make the volume to 100ml. Extract treated with this solution gave violet colour on boiling. (ii) Biuret

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test: To 3ml test solution 4% w/v NaOH and few drops of 1% w/v copper sulphate solution were added. A blue color was observed. www

E. Test for saponins (i) Foam test: 1ml of extract was diluted with distilled water to 20ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicated the presence of saponins.

F. Test for terpenoids: 0.8g of plant sample was taken in a test tube and 10ml of methanol was poured. The mixture was shaken well and filtered to take 5ml of extract of plant sample. Then add 2ml of chloroform and mixed in a extract of selected plant sample and 3ml of sulphuric acid were added in selected sample extract.

The result of qualitative analysis of bark extract of C.camphora indicates the formation of reddish brown color which indicates the presence of terpenoids in the selected plant sample

3.1. COMPUTATIONAL STUDIES

An electronic literature search using Pub Med and Google scholar were conducted. We searched for randomized controlled trials and prospective cohort studies of camphor in population effect by inflammatory and cancer. We also searched for in-vitro and animal (in-vivo) experiments that explored camphor proposed mechanism of action but none of the studies have explained the underlying molecular mechanism of anti-inflammatory and anti-cancerous effect of camphor.

3.2. Tools and Materials:

In our study we retrieved the data from the biological data base like Protein Data Bank (PDB), Pub Chm. In-silico studies were carried out using software and online tool like pyMOL, ADME prediction, Argus Lab.

- Protein Data Bank (PDB) is a repository for the 3-D structural data of large bio- NCBI Pub Chem is a chemical compound data base that provides information on biological activities of small molecule
- pyMOL is a molecular modelling and structure analysis tool
- ADME is used in pharmacokinetics and pharmacology for absorption, distribution, metabolism and elimination. These properties describe the disposition of a drug like compound within the molecules, such as protein, DNA and RNA
- Argus Lab is a molecular modelling, graphics and drug design program.
- Using Lead IT software the structure and analogs were sketched draw and generated their MOL file followed subsequent generation of their 3-D structures by using tool.
- Web Lab viewer lite program a molecule format converter in to PDB, appropriate force field applied to them and then optimization was carried out using Argus lab.

3.3. Ligands

Ligands such as Drugs (Pharacetamol, Diclophenac) were retrieved from website "NCBI PubChem" in SDF format and prepared for docking, geometry. Optimization of the ligands were carried out in Argus Lab 4.0 (http://www.arguslab.com)

3.4. Docking studies

Argus Lab is a molecular modelling, graphics and drug design program. It was developed Dr.Mark Thompson of planaria software. Geometric optimization of the target protein was performed. The location of the respective amino acids in active site of enzymes was chosen to serve as binding site for ligands (table 1). The Argus Lab was selected has docking engine to carry out the docking analysis [9,10].

Table 1: Amino acids composition of protein

Enzyme	Amino acids present in binding site
COX-1	Leu 352, Gly 526, Val 525, Trp 387, Ser 353, Ala 527.

3.5. Toxicity studies:

The toxicity of ligands were carried out by ADME. Based on following ADME properties, drugs were selected further to perform docking studies since they had no side effects.

3.5.1. ADME Results: The following ADME properties of ligands were obtained

Table 2: Physicochemical properties of the ligands

Ligand name	M.F	M.W (g/mol)	Heavy Atoms	Rotatable bonds	H-bond Acceptor	H-bond donar	MR	TPSA (A^2)

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Paracetamol	C8H9NO2	151.16	11	2	2	2	42.78	49.33
Diclofenac	C14H11C12NO2	296.15	19	4	2	2	77.55	49.33
Camphor	C10H16O	152.23	11	0	1	0	45.64	17.07

Table 3: Lipophilicity properties of the ligands

Ligand Name	Log Po/W (Ilogp)	Log Po/W (XLOGP3)	Log Po/W (WLOGP)	Log Po/W (MLOGP)	Log Po/W (SILICOS-IT)	Consensus Log Po/W
Paracetamol	1.21	0.46	1.16	0.91	0.89	0.93
Diclofenac	2.13	4.40	4.36	3.84	3.84	3.69
Camphor	2.12	2.19	2.40	2.30	2.85	2.37

Table 4: Pharmacokinetic parameters for the ligands and their solubility in water

Ligand name	P-glycoprotein Substrate	GI-tract absorption	Log kp(skin permeation) ($cm/^2$)	Log S(ESOL) solubility in water
Paracetamol	No	High	-6.90	-2.19
Diclofenac	No	High	-4.98	-5.15
Camphor	No	High	-5.67	-2.60

Table 5: Drug likeliness of the ligand

Ligand Name	Lipinski	Ghose	Veber	egan	muegge	Lead-like	Bioavailability Score
Paracetamol	Yes	No	Yes	Yes	No	No	0.55
Diclophenac	Yes	Yes	Yes	Yes	Yes	No	0.56
Camphor	Yes	No	Yes	Yes	No	No	0.55

Table 6: Medicinal chemistry of ligand

Ligand Name	PAINS	Brenk	Synthetic Accessibility
Paracetamol	0	1	1.00
Diclofenac	0	0	2.23
Camphor	0	0	3.22

RESULTS:

The preliminary phytochemical screening of ethanolic extracts (96%) showed tha Cinnamomum camphora contains tannins, terpenoids , flavonoids and carbohydrates but it free from resins , anthraquinons , alkaloids and saponins. Docking was performed between ligands and selected amino acids of enzymes and their binding energy (least energy) values are indicated in the (fig 1,2 and 2) and interactions below

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Fig.1: Camphor interaction with COX-1 active site

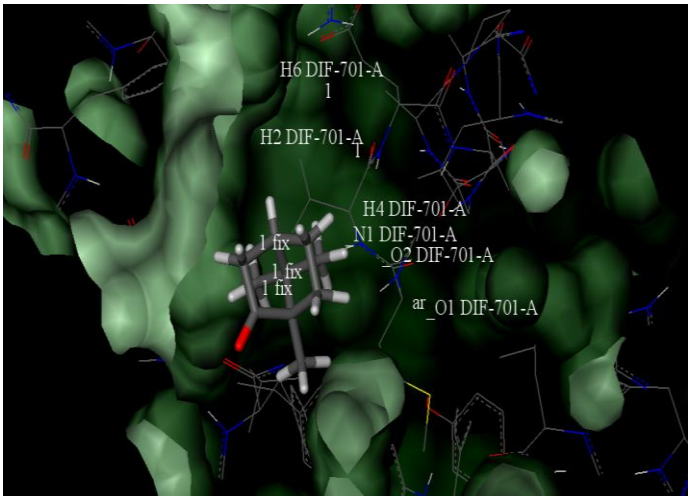

Fig 2: 3D interaction of camphor and COX-1

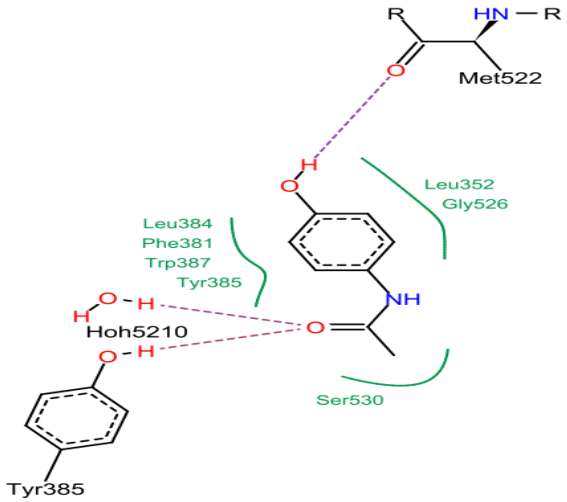

Fig. 3: Active pocket of COX-1 bound to camphor

DISSCUSION

Docking was established between camphor and COX protein using Argus lab. We have docked the camphor molecule as ligand on one of the crystal structures of cyclooxygenase-1 (PDB entry ICX2). The scoring functions of the compound were calculated from minimized ligand protein complexes. Before docking drug likeliness properties of camphor analysed which is similar to that of paracetamol and diclofenac,(table 3,4,5) thereby isolated ligand satisfies Lipinski Rule of 5 for drug likeliness with high GI absorption and lipid permeability. Docking analysis reveals that camphor binds with -9.28 kilocalories/mole which is similar to that of paracetamol and diclofenac.

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CONCLUSION

Docking analysis reveals the interaction between camphor and COX-1Ser 530 and tyrosine 385 . Camphor binds to COX protein with hydrogen bond and hydrophobic interactions with the binding pockets made by hydroxyl groups. The information has potential implications to understand the mechanism of COX-1 enzymatic inhibition.
Camphor is widely available, in-expensive and having least side-effects. So, it can be replaced with commercially available expensive drugs, by establishing its action with further clinical trials.

AUTHOR'S NOTE

The authors declare that there is no conflict of intrest regarding the publication of this article. Authors confirm that paper was free of plagiarism.

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