Molecular Docking and Drug Likeliness Study of Anticancer Potential of Camphor from Cinnamon Camphorabark

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Abstract : Camphor (Cinnamomumcamphora) is a natural product obtained from the wood of camphor tree. It has been used for centuries, throughout the world as a remedy for treating variety of symptoms such as inflammation, infection, congestion, pain, irritation, etc. The recent studies have shown that some of the components of Cinnamomumcamphora have suppressive and antimutagenic effect in number of human cancer cells without harming the healthy cells. In this paper our focus is on the use of camphor as a remedy for prevention and treatment of serious life-threatening diseases like cancer using ADMET and molecular docking studies. Camphor is a terpenoid and cyclic ketone. Many cancer strategies rely on the promotion of apoptosis in cancer cells as a means to shrink tumours, notably caspase-3 that proteolyze and induce cell death. Overexpression of procasase-3 (PC-3) has been reported in variety of cancers. The high docking score of camphor - 9.6Kcal/molwith PC-3 indicate that camphor has potential to inhibit PC-3 over expression.

Key words: Cinnamumcamphora, Caspase-3, anticancer, molecular docking,

Introduction

Natural resources including herbal plants contain a large variety of phytochemicals,as promising traditional medicine to treat chronic and infectious diseases. Amongst the surfeit of medicinal plants identified, Camphor from (C. camphora) has been widely used.Camphor is a white, crystalline substance with a strong odour and pungent taste, derived from the wood of camphor (Cinnamomumcamphora) and other related trees of laurel family. Camphor tree is native to China, India, Mongolia, Japan and Taiwan[1,2] Camphor from cinnamumcamphora have been considered as safe and effective alternatives with fewer side effects compared to synthetic agents [1]. Camphor

is obtained through steam distillation, purification and sublimation of wood, twigs and bark of the tree[3]. There are many pharmaceutical applications of camphor such as topical analgesic, antiseptic, antispasmodic, antipruritc, anti-inflammatory, anti-infective, rubefacient, contraceptive, mild expectorant, nasal decongestant, cough suppressant, etc[3-6]. Camphor is easily absorbed through the skin and can also be administrated by injection, inhalation and ingestion[3.6]. Camphor has potential cytotoxic activity. Present research has brought attention to the potential of camphor from cinnamumcamphora as anticancerous drug candidate.This finding also suggest the strong role of camphor in chemoprevention. However, further investigation is required to understand the molecular mechanism for its anti-cancerous property.

In this study, we have tried to carry out a docking study of camphor in the active of 3CPC following by the evaluation of their Lipinski's rule violation and ADME proprieties predictions.

Materials and methods

Collection of plant material

The plant material (Cinnamomumcamphora) was collected during March 24, 2023 at 3:06 pm from LalBagh Botanical Garden, Bangalore. The plant was authenticated by Department of Botany, Maharani Cluster University,Bengaluru 560001.

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 analysed using TLC and compared to commercially available synthetic standards.

Characterization by thin layer chromatography

Distillate sample of bark (C.camphora) 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 anisaldehyde (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 with the retention factor (R*f* value).

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 Whatman 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 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 and yield of extract obtained was (5.34%).

Qualitative analysis of bark extract

The different extracts of bark from C.camphora were tested for various components as follows [9].

Test for alkaloids

Small portion of solvent free extract was stirred with few drops of dilHCl 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 indicate the presence of alkaloids.

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.

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 colour was observed.

Detection for carbohydrates and glycosides

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 concentrated sulphuric acid was slowly added through the sides of the test tube, purple or violet colour appeared at the junction.

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.

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.

Test for sterols and tri terpenoids

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.

Test for proteins and amino acids

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.

Biuret test

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

Test for saponins

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.

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 colour which indicates the presence of terpenoids in the selected plant sample.

Computational Studies

Tools and Materials

In this study we retrieved the data from the biological data base like Protein Data Bank (PDB).

Pharmacokinetic profile

Swiss ADME (http://www.swissadme.ch/index.php) was used to determine the pharmacokinetic profile of the tested compounds by entering the simplified molecular input line entry system (SMILES) formula for each active substance. SMILES data were retrieved from the PubChem database. Lipinski's Rule of Five analysis was conducted to determine the compounds pharmacokinetic properties. [8-10]

Preparation of Ligands

Vandetanib drug was retrieved from website "NCBI PubChem" in SDF format and prepared for docking, geometry using Lead IT software the structure and analogues were sketched draw and generated their MOL file followed subsequent generation of their 3-D structures.Optimization of the ligands were carried out in Argus Lab 4.0 (www.arguslab.com)

Docking studies

Argus Lab is a molecular modelling, graphics and drug design program. 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 [10-11]pyMOL is a molecular modelling and structure analysis tool.

Result and Discussion

Phytonutrients analysis revealed the presence of all bioactives like alkaloids, glycosides, steroids, flavonoids, tannins, resins, phenols and terpenoids. The adsorption,metabolism, distribution, excreationand toxicity (ADMET) studies of isolated camphor from Cinnamumcamphora was predicted using Swiss ADMET. T Lipinski's rule including molecular weight, number of rotatable bonds, number of hydrogen bonds acceptor, number hydrogen bonds donor and logP were shown in (Table 2).

Table.2: Physicochemical parameters of camphor

Molecular formula	$C_{10}H_{16}O$
Molecular weight (g/mol)	152.23
Number of heavy atoms	0
Number of aromatic heavy atoms	11
Number of H- bond acceptor	1
Number of H- bond donor	0
Molar refractivity	45.64
TPSA	17.A

The skin permeabilityvalue (Kp)-5.67 indicates the skin absorption of molecule. Whereas vandetanibKp value is -6.7 under the cancer clinical trial. Additionally gastrointestinal (GI) and blood brain barrier permeation indicate good absorbtion and

distribution of drug. In silico Swiss ADME prediction inhibited all cytochromes which regulate drug metabolism except CYP1A2 inhibitor. (Table3).

Table.3: Pharmacokinetic parameters of camphor

Bioavailability score	0.55
Solubility class [ESOL]	Soluble
Solubility class [Silicos-IT]	Soluble
Blood brain barrier permeation	yes
Gastrointestinal absorption	High
LogKp (Skin permeation)	-5.67
Number of PAINS	0.0
Number of Brenk structural alerts	0.0
CYP1A2 inhibitor	no
Bioavailability score	0.55
Synthetic accessibility	0.33
LogPo/w(iLOGP)	2.12
LogPo/w(XLOGP3)	2.19

Caspase-3 belonging to cysteine protease family is a key enzyme in inducing cell apoptosis. This enzyme is present as an inactive pro caspase in viable cells, which is activated during apoptosis.Molecular docking was carried out to find the types of interactions and the binding affinity of camphor with caspase-3 enzyme. The results are presented in fig.1,3a and 3b. The best energies of interaction with Caspase-3 main protease is -9.28 Kcal/mol so, these compounds could have moreinhibitory potential Caspase-3main protease than the other studied compounds. The inhibition of this protein will induce the proliferation of cancerous cells; these results show that camphor could bea interesting molecule in treatment of various types of cancers.

Fig.1: The 3D binding interaction of standard Vandetanib with Caspase-3

3CPC enzyme in binding site contains amino acids Arg 1022A, Ile 892A, Glu   917A, Asn 923A.We evaluate the designed compounds through docking techniques using

Argus lab. We docked the designed compounds on one of the crystal structures of 3CPC available through the RCSB Protein Data Bank. The scoring functions of the compound were calculated from minimized ligand protein complexes.
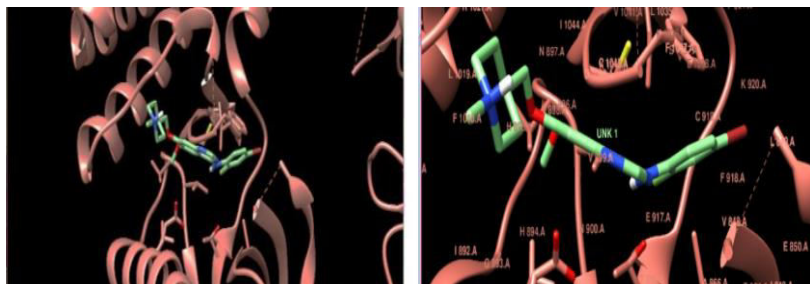

Fig.3a and 3b: The 3D binding interaction of camphor with Caspase-3

Docking studies confirm that the main interaction of standard anticancerous drug Vandetanib inhibitors with 3CPC fig.1.Chemical structure of Vandetanib shown in fig.4. The information has potential implications to understand the mechanism of 3CPC related enzymatic inhibition reactions and also applicable in the prediction of more effective inhibitors and engineering 3D structures of other enzyme as well.
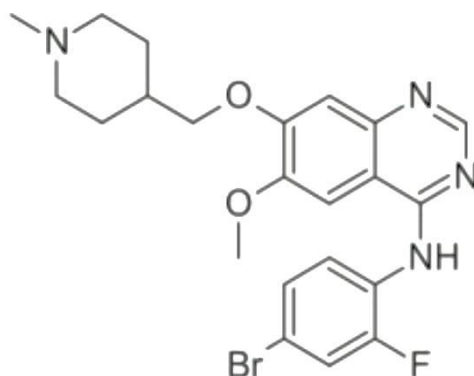

Fig.4: structure of Vandetanib

The woody bark of Cinnamomumcamphora containcamphor. Some of the studies have shown that monoterpenes have suppressive and anti-mutagenic effect in number of human cancer cells including colon cancer, gastric cancer, liver tumor, breast cancer, leukemia and others[1]. Most cancer chemotherapy treatments include highly cytotoxic drugs against proliferating cancer cells as well as healthy cells which can be harmful for the body. With a different mechanism of action, camphor with their monoterpene components can have multiple pharmacological tumor-suppressive activities, mostly without such harm.[1]

Camphor is a terpenoid and cyclic ketone. Many cancer strategies rely on the promotion of apoptosis in cancer cells as a means to shrink tumors, notably caspase-3 that proteolyze induce cell death. Overexpression of procasase-3 (PC-3) has been

reported in variety of cancers. The high docking score of camphor with PC-3 indicate that camphor has potential to inhibit PC-3 overexpression.

Among different targets to design anticancer, Caspase-3 is an important target as its cleavage and activation agentslead to apoptosis and finally cancer death[12,13]. Camphor is caspase-3 activator.Majority of anticancerous drugs target caspase-3 suggested as an important strategy for anticancer drug discovery.These findings can be the prerequisite basis for the potential development of these bioactive substances as potent anticancer drugs. However, further studies are still needed to evaluate their toxicity and safety.

Conclusion

Considering the growing number of cancer patients, Cinnamomumcamphora and its components should be investigated further as a viable option in the treatment of different types of cancer. We hope to get the attention of researchers for conducting more studies for clinical efficacy of camphor as anticancerous drug in future.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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