

RESEARCH ARTICLE

Identification of Proapoptotic, Anti-Inflammatory, Anti-Proliferative, Anti-Invasive and Anti-Angiogenic Targets of Essential Oils in Cardamom by Dual Reverse Virtual Screening and Binding Pose Analysis

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Abstract

Background: Cardamom (*Elettaria cardamom*), also known as “Queen of Spices”, has been traditionally used as a culinary ingredient due to its pleasant aroma and taste. In addition to this role, studies on cardamom have demonstrated cancer chemopreventive potential in *in vitro* and *in vivo* systems. Nevertheless, the precise poly-pharmacological nature of naturally occurring chemo-preventive compounds in cardamom has still not been fully demystified. **Methods:** In this study, an effort has been made to identify the proapoptotic, anti-inflammatory, anti-proliferative, anti-invasive and anti-angiogenic targets of Cardamom’s bioactive principles (eucalyptol, alpha-pinene, beta-pinene, d-limonene and geraniol) by employing a dual reverse virtual screening protocol. Experimentally proven target information of the bioactive principles was annotated from bioassay databases and compared with the virtually screened set of targets to evaluate the reliability of the computational identification. To study the molecular interaction pattern of the anti-tumor action, molecular docking simulation was performed with Auto Dock Pyrx. Interaction studies of binding pose of eucalyptol with Caspase 3 were conducted to obtain an insight into the interacting amino acids and their inter-molecular bondings. **Results:** A prioritized list of target proteins associated with multiple forms of cancer and ranked by their Fit Score (Pharm Mapper) and descending 3D score (Reverse Screen 3D) were obtained from the two independent inverse screening platforms. Molecular docking studies exploring the bioactive principle targeted action revealed that H- bonds and electrostatic interactions forms the chief contributing factor in inter-molecular interactions associated with anti-tumor activity. Eucalyptol binds to the Caspase 3 with a specific framework that is well-suited for nucleophilic attacks by polar residues inside the Caspase 3 catalytic site. **Conclusion:** This study revealed vital information about the poly-pharmacological anti-tumor mode-of-action of essential oils in cardamom. In addition, a probabilistic set of anti-tumor targets for cardamom was generated, which can be further confirmed by *in vivo* and *in vitro* experiments.

Keywords: Cardamom - eugenol - reverse pharmacophore mapping - 2D fingerprint-based Reverse virtual screening

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Introduction

Spices and their essential oils have recently been acknowledged to possess therapeutic properties and have demonstrated multiple-beneficial effects on human health. They have demonstrated a wide spectrum of therapeutic activities ranging from antioxidant, anti-inflammatory, antimicrobial, hypo-lipidemic, anti-mutagenic to anti-carcinogenic roles (Misharina et al., 2011). *Elettaria cardamomum* (Family Zingiberaceae), Cardamom, also referred as the Queen of all spices has records as aged as human history and it possess an extensive array of bioactive compounds (Aneja et al., 2009; Padmakumari et al., 2010). Cardamom has principally been used worldwide for domestic culinary functionality and in medicine. The

volatile oil present in cardamom seeds is the principal contributing factor for its characteristic aroma and therapeutic principle (Hamzaa et al., 2012).

Several research studies have documented evidence of chemo preventive actions of cardamom in *in vitro* and *in vivo* platforms. In a research study Essential oils from common dietary Indian spices (including Cardamom) were found to reduce Aflatoxin B1 (AFB1)-DNA Adduct formation in rodents, reflecting its possible anti-mutagenic behavior (Hashim et al., 1994). In another study, carrageenan-induced male albino rats feed with essential oil of *Elettaria cardamomum* seeds showed reduced inflammation (Al-Zuhair et al., 1996). Pro-apoptotic behavior of cardamom was observed in human leukemia Molt 4B and HL-60 cells in one more research

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study (Moteki et al., 2002). Anti-oxidant profiling study on Cardamom demonstrated presence of medium levels (50-100 mg) of antioxidant phenolics and flavonoids. (Nair et al., 1998). Regardless of all these facts, a detailed mechanism of cancer chemoprevention of the bioactive principles of Cardamom is still to be elucidated. Essential oils being a complex mixture of up to 45 different constituents, the identification process for the most active cancer chemo preventive compounds of essential oils is laborious procedure (Delaquis et al., 2002; Djenane et al., 2011; Espina et al., 2011). Even though, several studies pertaining to mode- of-action of several essential oils have been conducted, thorough investigation into mode- of- action of individual bioactive ingredient can still bring to light technicalities of its onco-preventive mechanisms, masked by influence of other ingredients. For the majority of the bioactive principles in essential oils, the investigation strategy of characterizing their precise mode-of-action and their corresponding molecular targets is a quite demanding job. The investigation approach engaging proteomics profiling and Pharmacokinetic studies is a laborious method and is going to be a prolonged affair (Lin and Lu, 1997; Persidis, 1998; Huang et al., 2004; Hajduk et al., 2005).

In this perspective, a Reverse pharmacology protocol integrating 2D fingerprint-based Reverse virtual screening and Reverse Pharmacophore mapping has found recognition as a quick optional technique to fish-out molecular targets. The principle of 2D Fingerprint-based Reverse virtual screening is based on a search algorithm that spots probable target proteins which are expected to bind to a given compound. This search strategy explores the databases of biologically-relevant subset of ligands extracted from the RCSB Protein Data Bank (Kinnings and Jackson, 2011). The principle of Reverse Pharmacophore mapping involves placement of a given small molecule into a 'flexible conformation' into every 'Pharmacophore model' of proteins in the target list (Liu et al., 2010). Of late, a number of Insilico Reverse Screening tools have been developed by scientific community, some of them are: ReverseScreen3D (Kinnings and Jackson, 2011), INVDOCK (Chen and Ung, 2001), Tarfisdock (Li et al., 2006), PharmMapper (Liu et al., 2010), idTarget (Wang et al., 2012), Inverse Screening@tome server (Pons and Labesse, 2009). By employing these Insilico platforms, molecular targets of tea polyphenols (Zheng et al., 2011) and saffron bioactive agents (Bhattacharjee et al., 2012) have been identified successfully.

In the present study, we employ a comparative analysis of two different reverse screening approaches, i.e. 2D fingerprint-based Reverse virtual screening based on ReverseScreen3D and the Reverse Pharmacophore mapping strategy based on a PharmMapper system, to identify potential molecular targets for bioactive principles of Cardamom and its essential oil. Ranking of the putative targets identified by dual Inverse screening strategies is done based on their Fit Score (PharmMapper) and descending 3D score (ReverseScreen3D). Annotation of these putative targets is done to derive their association to the proposed anti-tumor mechanisms principles. Further exploratory studies on the binding postures of bioactive

principles of Cardamom with its respective targets is carried out to validate the outcomes of the docking simulations.

Materials and Methods

Literature survey and annotation to list the cardamom bioactive ingredients

Cardamom has a wide range of bioactive agents that gives it's overall anti-tumor activity. So literature review was done to pin point the most significant of these bioactive agents based on the percentage composition in a cardamom sample and its essential oil fraction. The figure depicts small molecular structures of Cardamom and its essential oil fraction (Figure 1).

The small molecular structures of potent bioactive principles of cardamom i.e., 1, 8-cineol (eucalyptol), d-limonene, alpha-pinene, beta-pinene, myrcene, linalool, farnesol, and geraniol were retrieved in sdf format from PubChem database. The sdf file format is converted into a mol2 format in Marvin Sketch.

Putative therapeutic target identification by dual inverse screening

a. By Reverse Pharmacophore mapping: PharmMapper server employs principles of Pharmacophore mapping to spot potential molecular target candidates for a known small bioactive molecule. On submission of a small molecule as an input query, PharmMapper performs an automatic search operation to acquire the 'best mapping poses' of the query molecule against the entire Pharmacophore models in the in-house PharmTargetDB (derived from annotation of all the target information in TargetBank, BindingDB, and DrugBank). An N best-fitted hits library of aligned poses for individual targets with their respective target annotations is the outcome of these search operation (Liu et al., 2010). The mol2 files of potent bioactive principles of cardamom i.e., 1,8-cineol(eucalyptol), d-limonene, alpha-pinene, beta-pinene, myrcene, linalool, farnesol, geraniol were submitted in PharmMapper and a list of possible binding receptors were received. The list was further annotated to screen out the putative target list pertaining to anti-tumor activity. b. By 2D fingerprint-based Reverse virtual screening: ReverseScreen3D is a reverse virtual screening

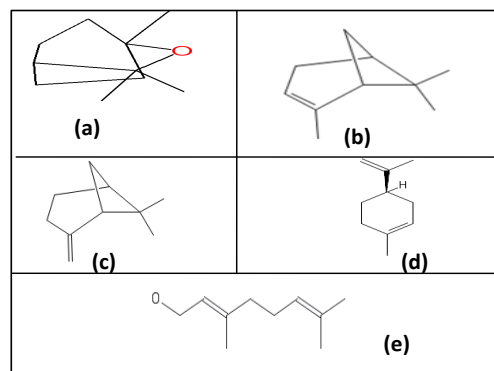


Figure 1. Structures of Cardamom's Bioactive Principles. (a) Eucalyptol, (b) Alpha-Pinene, (c) Beta-Pinene, (d) D-limonene and (e) Geraniol

tool that screens a list of putative target proteins which are probable to bind to a ligand by employing a search strategy against a database of biologically relevant ligands mined from the RCSB PDB. On submission of a small molecule as an input query in ReverseScreen3D, a set of 25 conformers of this query compound are generated. A 2D similarity search is carried out between the query compound and all ligands in the database. A single ligand with the highest 2D similarity to the query compound is selected from each unique target protein binding site in the database. A 3D structure-based ligand matching method, based on that employed by LigMatch (Kinnings and Jackson, 2009), is carried out between the query compound and each of the previously selected database ligands. In this way, each unique target protein binding site in the database can be prioritized based on either the 2D or 3D similarity score between its bound ligand and the query compound. The sdf files of potent bioactive principles of cardamom i.e., 1,8-cineol(eucalyptol),d-limonene ,alpha-pinene, beta-pinene, myrcene, linalool, farnesol, geraniol were submitted to ReverseScreen3D for 2D and 3D similarity search. The list of potential targets of Human targetome received from PharmMapper and ReverseScreen3D were further annotated to screen out a subset target list associated to anticancer mechanisms.

Therapeutic target identification from bioassay databases

The High-throughput experimental findings of the bioactive compounds (Table 1) were manually curated from three different Bioassay Databases: PubChem Bioassay (Wang et al., 2012), NPACT (Mangal et al., 2013) and Herbal Ingredients' Targets Database (Ye et al., 2011). PubChem BioAssay database contains information produced in the course of high-throughput screening experiments, biological and medicinal chemistry research, plus those mined from the literature. The data generated from human tumor cell line screening of Developmental Therapeutic Program (DTP) (Driscoll et al., 1984) at the US National Cancer Institute (NCI) was more relevant to ours study. NPACT is a curated database of Plant derived

natural compounds exhibiting anti-cancerous activity and it holds information of 1574 entries pertaining to the structure, physicochemical properties, type of cancer, cell lines used in the study and its respective inhibitory values and molecular targets. Herbal Ingredient Target Db is a curated database of protein targets of 586 active compounds from 1,300 reputable Chinese herbs having detailed information about the target action.

Comparative analysis of insilico identified targets and bioassay findings

The list of targets identified by Reverse Screening is compared with the target information obtained from different Bioassay databases so as to assess the credibility of results obtained by the dual Virtual Inverse Screening platforms.

Mapping of identified targets into respective functional sub-classes

Experimental studies on cardamom have documented evidence of the following five different mechanisms of its broad-spectrum anticancer activity: a) proapoptotic, b) anti-inflammatory, c) anti-proliferative, d) anti-invasive and e) anti-angiogenic. On the basis of these classes the identified putative therapeutic targets were mapped into their respective sub-category.

Molecular docking simulation of therapeutic targets with bioactive agents

Macromolecular protein structures of the identified target were retrieved from RCSB-Protein Data Bank and molecular docking simulation was performed using AutoDock Vina in PyRx 0.8 (Trott and Olson, 2010). This method was a prerequisite for substantiating the precise molecular mechanism and receptor targeting of cardamom bioactive molecules.

Validation of molecular docking poses

AutoDock docking simulations has demonstrated its ability to reproduce experimental binding conformations

Table 1. Potential Targets of Eucalyptol by Identified by Reverse Screening and Bioassay Databases

Sl. No	Name of Target	Implicated by PubChem Bioassay (IC50 in Micromolar units)	Implicated by Herbs Ingredient Target Database	Identified by PharmMapper (FitScore)
1	Mitogen-activated protein kinase 1	Yes (00.0316)		
2	Tumor necrosis factor		Yes	
3	Interleukin-1 beta		Yes	
4	Interleukin-4		Yes	
5	Interleukin-5		Yes	
6	Microtubule-associated protein tau	Yes (04.4668)		
7	RecQ-Like Dna Helicase 1 (RECQ1)	Yes (39.8107)		
8	Androgen Receptor	Yes (19.9526)		Yes(3.974)
9	Geminin, DNA replication inhibitor, also known as GMNN	Yes (05.8048)		
10	thyroid stimulating hormone receptor	Yes (15.8489)		
11	Collagenase 3			Yes (4.465)
12	Retinoic acid receptor gamma			Yes(4.600)
13	Cell division protein kinase 2			Yes(4.412)
14	Mitogen-activated protein kinase 14			Yes(4.328)
15	Peroxisome proliferator-activated receptor gamma			Yes(4.416)
16	Proto-oncogene tyrosine-protein kinase ABL1			Yes(4.358)
17	B-Raf proto-oncogene			Yes(4.428)

in many research studies. In agreement with this principle, the structure of Caspase 3 retrieved from RCSB-Protein Data Bank (PDB ID: 1RHR) and its inbuilt crystallized ligand is re-docked with Caspase 3. During the docking procedure the grid centre and dielectric constant has been pre-fixed at 77.1301635456(x), -20.0977700189 (y) and 99.5992935556 (z) coordinates and -0.1465 respectively. A comparative study is done on the molecular interactions pattern of the binding pose of the original Caspase 3 -Cinnamic acid complex and post-docked Caspase 3 -Cinnamic acid complex, in order to substantiate the integrity of AutoDock simulation studies. The deviation of H-bond length and RMSD (Root Mean Square Deviation) of Backbone and Heavy Atom between the 'Experimental derived Crystal Structure' and 'Theoretical docked model' was also calculated.

Results

The strong, distinctive taste and intense aromatic, resinous fragrance of Cardamom are due to the presence of oils in the seeds. The purported Onco-preventive benefits of dietary Cardamom is due to its various essential oil constituents acting together synergistically. Essential oils are basically hydrophobic liquid in nature and contain complex fusion of volatile organic compounds (VOC's). The essential oils are the end products of secondary metabolic processes and majority of their components are terpenoids in chemical nature. They are commonly monoterpenes and sesquiterpenes, plus bit of diterpenes and aromatic compounds derivatives. Having small molecular structures gives essential oils an added advantage in absorption into different parts of body. The lipophilic of essential oils permits effortless movement across the plasma membrane and by doing so they exercise their effect by interaction with intracellular proteins in intra-organelle sites. (Ka et al., 2003; Duchon, 2004; Griffiths, 2005). The therapeutic value of cardamom (stigmas of *C. sativus* L.) is established by the presence of nine key secondary metabolites: 1, 8-cineol(eucalyptol), d-limonene, alpha-pinene, beta-pinene, myrcene, linalool, farnesol, geraniol.

Eucalyptol is cyclic ether and a monoterpene and about 90% of essential oils in some spices contain Eucalyptol (Miyazawa et al., 2001). α -Pinene is an organic compound of the terpene class and is an alkene containing a reactive four-membered ring. Myrcene, or β -myrcene, is an olefinic natural monoterpene. Limonene has been classified under cyclic terpene class and it's a colourless liquid hydrocarbon in chemical nature. There are a lot of investigative studies on the metabolism of these monoterpenes based essential oils in mammals and those studies postulate that monoterpenes displays an abundant oral bioavailability (Kodama et al. 1974; 1976). Table 1 depicts the Therapeutic targets of eucalyptol identified by Reverse Screening and Bioassay databases.

Pro-apoptotic targets of essential oils in cardamom

Regulation of apoptotic process is done by means of through two different signaling pathways. On activation of effector Caspase 3, these two pathways unite to

form a global loop structure (Watters and Lavin, 1999). Caspase-3 is directly activated by extrinsic (also called the type 1) pathway through enzymatic reactions mediated by activated forms of Caspase-8 molecules. Release of mitochondrial cytochrome c and formation of apoptosome are key factors in regulation of intrinsic (also called the type 2) pathway. The apoptotic process in the type 2 pathway is coordinated by 3 local signaling modules, which are: a) death ligand binding and Caspase-8 activation; b) Activation of Bax modulated by 'BH3 only proteins'; c) Activation of post-mitochondrial events following Ca^{2+} release (Raychaudhuri, 2010). Reverse screening protocol employed in our study has identified that essential oils in cardamom i.e.1, 8-cineol (eucalyptol); beta-pinene; geraniol provide the pro-apoptotic activity by binding to Caspase 3 and Caspase 7 and its further activation. It is also been identified that Geraniol binds to mitochondria Dihydroorotate dehydrogenase which plays a key role in positive regulation of apoptotic process. In de novo pyrimidine synthesis pathway, DHODH, a ubiquitous FMN flavoenzyme, takes part in the rate-limiting step. In addition to this role, this enzyme is only one in this pathway to possess an N-terminal localisation signal which restricts it to mitochondria's inner membrane/matrix side (Nagy et al., 1992; Rawls et al., 2000). This processes mentioned above encourages cell death via apoptosis and so gives cardamom its overall onco-preventive activity.

Anti-inflammatory targets of essential oils in cardamom

Inflammation is key contributing factor in a multiple-step carcinogenesis process, starting from tumor initiation, promotion, and its progression. Despite the fact that acute inflammation plays a significant role in defense response; chronic inflammation can still lead to cancer (Rakoff-Nahoum, 2006). In last two decades, path-breaking discoveries have been brought to light a number of pro-inflammatory gene products having role in inhibition of proliferation, angiogenesis, invasion, and metastasis (Aggarwal et al., 2006). Many of the key components of inflammatory pathways such as free radicals, cytokines, NF- κ B, signal transducer and activator of transcription-3 (STAT-3), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), prostaglandins, and vascular endothelial growth factor (VEGF), have demonstrated their contribution in the progression of multi-forms of cancer (Yadav et al., 2010). Cardamom exerts its anti-cancer effects through the modulation of multiple signaling pathways, one of which is the inflammatory signaling pathway. There is a significant contribution of TNF-in regulatory machinery of immune cells and in advancement of systemic inflammation. Impairment of a regulatory mechanism in TNF- α production has been shown to cause a variety of inflammatory diseases, as well as cancer (Bruzzo et al., 2007). Reverse screening protocol in our study identified that key components of essential oil in cardamom (i.e.1,8-cineol(eucalyptol);beta-pinene; geraniol) provides anti-inflammatory activity by binding to Tumor necrosis factor, Interleukin-1 beta; Interleukin-4; Interleukin-5. Alpha and beta pinene; d-limonene; geraniol; eucalyptol binds to VEGF; Estradio 17-beta dehydrogenase-1, Collagenase 3.

Anti-proliferative, anti-invasive and anti-angiogenic targets of essential oils in cardamom

Growth factors and their receptors have a decisive role in maintenance of normal processes of growth and differentiation. A disorganized expression of these molecules can direct abnormal growth and development, whose consequence can be malignant transformation. Besides, amplification of expression level of growth factors, for e.g. Transforming growth factor- α (TGF- α), can direct the cells to non-cancerous diseases such as psoriasis. Growth factors have demonstrated profound role in anti-proliferative, anti-invasive and anti-angiogenic actions (Elder et al., 1989; 1990; De Jong et al, 2001; Zhou et al., 2011). Epidermal growth factor receptor (EGFR) over-expression in a noticeable signature in cancer cell proliferation (Bianco et al., 2002; Ciardiello et al., 2002; Janne et al., 2002; Fang-jun et al., 2008). Through Reverse Screening protocol we have been able to find many growth factor potential targets of bioactive principles of Cardamom. In this context, alpha-pinene has been identified to bind to Basic fibroblast growth factor receptor 1; gernaol has been identified to bind with Basic fibroblast growth factor, Placenta growth factor; alpha-pinene and beta-pinene has been identified to bind to Epidermal Growth Factor. Binding to these growth factors may be rationale behind the evident anti-angiogenesis mechanism of cardamom. At different phases of the cell cycle different Cyclins play its part. In the G1 phase of cell cycle, the entry is regulated by binding of three D type cyclins (cyclin D1, cyclin D2, cyclin D3)

to CDK4 and to CDK6 and CDK-cyclin D complexes. (Vermilion et al., 2003). In our study we identified that D-limonene and beta-pinene cause G1 cell cycle arrest by targeting Cyclin-A2 and cyclin dependent kinases 2 and thus inhibiting proliferation. Table 2 displays the list of potential therapeutic targets identified for all the bioactive principles of Cardamom.

In addition to the above targets, many more other targets give Cardamom its holistic cancer-preventive activity. In our study we identified glutathione-S-transferase, a Phase II detoxifying enzyme as a key target for many of the bioactive principles of cardamom such as Alpha-pinene; beta-pinene and d-limonene. In addition to the above targets, Cytochrome P450 and p53 MAP Kinase are also identified as a key for Alpha-pinene, beta-pinene, d-limonene and 1, 8-cineol (eucalyptol), alpha-pinene, d-limonene respectively.

Analysis of molecular docking with caspase 3

In Caspase family of cysteine proteases, two diverse classes of enzymes are generally implicated in apoptosis process: the initiator and executioner caspases. At the top of the signaling pathway, Caspase-2, -8, -9, and -10, are located which forms the initiator cascade system. The principle functionality of initiator Caspases is activation of the Executioner caspases i.e. caspase-3, -6, and -7. The primary responsibility of executioner Caspases is the regulation of physiological and morphological changes associated with an apoptotic process. Apoptotic processes are vital key-points in diverse set of processes in a normal cell, which includes tissue homeostasis, development of fetus and immune system maintenance activity (Denault et al., 2002). The activation of Caspase-3 is modulated by caspase-8 and caspase-9 in the upstream region of the signaling cascade. Since, Caspase 3 serves as a meeting point of diverse signaling pathways, there is a significant visibility of Caspase 3 in apoptosis assays (Reference). The primary role of Caspase 3 is catalytic proteolysis of the majority of cellular polypeptides (e.g. poly (ADP-ribose) polymerase (PARP)), directing to apoptotic phenotypic expression (Chang et al., 2000; Nicholson et al., 2004).

Caspase 3 contains 147 amino acid residues and has 21% helical (3 helices; 32 residues) and 23% beta sheet (12 strands; 34 residues) arrangement. Even though, a plentiful of success stories has demonstrated Auto Dock's competence in duplicating experimental binding structures, it still debatable whether the theory fits for specific biological systems. For examining the consistency of the docking simulation, the original ligand (Cinnamic acid methyl ester) from a ligand- Caspase 3 complex structure is detached from the PDB file and re-docked into the ligand binding site via the identical docking approach as eucalyptol. Investigation of micro-environment and inter-molecular interactions was done by superimposing the Post-docked complex structure with the RCSB-PDB crystallographic structure. On vigilant investigation of binding poses of the two conformations, it was revealed that the original ligand (Cinnamic acid methyl ester) forms 7 hydrogen bonds; 1 bond with Trp348 (Chain B), Ser339 (Chain B) and His237 (Chain A) and 2 bonds each with

Table 2. Potential Therapeutic Targets of Bioactive Agents in Cardamom

Target Name	A	B	C	D	E
cAMP-dependent protein kinase catalytic subunit alpha	✓	✓	✓	✓	
Cell division protein kinase 2	✓	✓		✓	
MAPK 10	✓		✓	✓	
Tyrosine-protein phosphatase non-receptor type 11				✓	
VEGFR2	✓		✓	✓	
VitaminD3 receptor	✓	✓	✓	✓	
Retinoic acid receptor RXR-beta	✓		✓	✓	
Retinoic acid receptor RXR-alpha	✓	✓	✓	✓	
Estrogen receptor beta	✓	✓		✓	
Nuclear receptor ROR-alpha			✓	✓	✓
Cyclin A2			✓	✓	✓
Estradiol 17-beta-dehydrogenase 1	✓			✓	
Dihydroorotate dehydrogenase, mitochondrial				✓	
Endothelial proteinC receptor				✓	
Beta-secretase 1				✓	
Serine/threonine-protein phosphatase PP1-gamma catalytic subunit				✓	
Glutathione S-transferase A1		✓	✓	✓	
Amine oxidase (flavin-containing) B				✓	
Glutathione S-transferase P				✓	
Basic fibroblast growth factor.					✓
Caspase 3.	✓		✓	✓	
Heat shock protein HSP90alpha			✓	✓	
Carbonic anhydrase 2				✓	
Dehydroorotate dehydrogenase, mitochondria				✓	
Deoxycytidine kinase				✓	
Neutrophil elastase (Leukocyte elastase)				✓	
Angiopoietin-1 receptor	✓		✓	✓	

*A: 1,8-cineol(eucalyptol), B: alpha-pinene, C: beta-pinene, D: d-limonene, E: geraniol

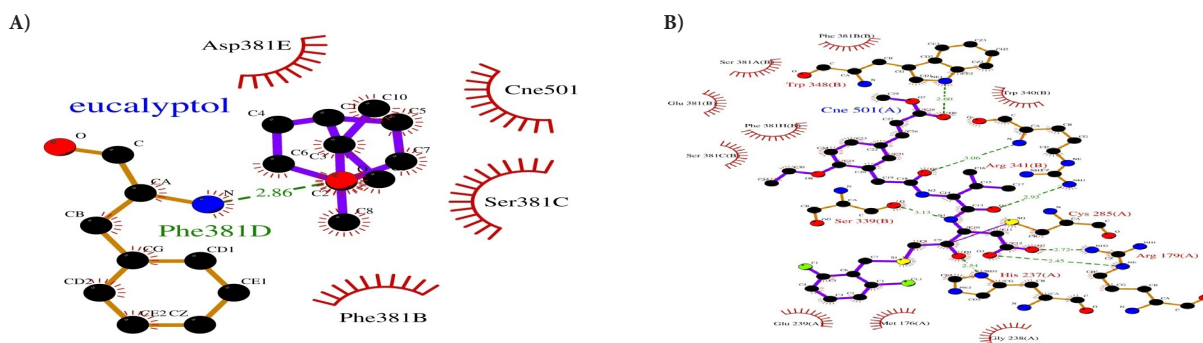


Figure 2. Micro Environment of the Binding Site of Cinnamic acid Methyl Ester/Eucalyptol and Caspase 3. a) The original ligand Cinnamic acid methyl ester forms 7 hydrogen bonds with residues Trp348 (Chain B), Ser339 (Chain B) & His237 (Chain A), Arg 341 (Chain B) and Arg 179 (Chain A) (green colour dotted lines), b) Eucalyptol forms bifurcated H-bonds with SER381 and PHE381 having in the catalytic cavity where the original ligand Cinnamic acid methyl ester is located

Arg 341 (Chain B) and Arg 179 (Chain A) in the both conformations (Figure 2(a)). Computation of deviation of H-bond lengths between docked pose and crystal structure complex gave a deviation of less than 0.14 Å. The calculated Heavy atom's RMSD (Root-Mean-Square Deviations) for Cinnamic acid methyl ester between the docked poses and the crystallographic coordinates was between 0.2-1.67Å. The above results illustrates that Auto Dock simulation are capable enough to replicate experimental binding structure of ligand-Caspase3 complex.

Binding mode between caspase 3 and eucalyptol

Figure 2(b) demonstrates the post-docked conformation of eucalyptol with Caspase 3, having binding free energy -4.21 kcal/mol. It observed that Eucalyptol binds to Caspase 3 in the same catalytic site where the original substrate Cinnamic acid methyl ester was located. In this figure we can also notice that two benzene ring planes of eucalyptol are in the active cavity composed of electrical amino acids such as Phe381D, Phe38B, Ser381C, Asp381E, etc. represented in brown colour. Eucalyptol forms bifurcated H-bonds with SER381 and PHE381 having bond length of 2.86 Å as shown in the Figure 2 (b). Investigation of the binding mode evidently exhibits an interactions pattern mediated by electrostatic and hydrogen bonds play a key role in the binding of eucalyptol and Caspase 3 and suggests that eucalyptol act as an inhibitor in the catalytic site of Caspase 3.

Discussion

A variety of dietary essential oils in Spices have been shown to be effective in the chemoprevention and chemotherapy of cancer. In recent times, a number of clinical trials are being conducted on chemotherapeutic terpenoids. In this study, a fruitful attempt has been made to characterize the proapoptotic, anti-inflammatory, anti-proliferative, anti-invasive and anti-angiogenic targets of bioactive principles in cardamom using comparative Inverse screening approach using ReverseScreen3D and PharmMapper. Our results throw light on some interesting facts about targetability of cardamom bioactive agents. First of all, a set of receptors of Cardamom bioactive constituents recognized by our method have been well

established in experiments settings as seen in various bioassay databases. Furthermore, other key receptors identified by us also fall under conventional clinical mode-of-action i.e. anti-proliferative, anti-invasive and anti-angiogenic. Validation of the target detection methodology is done by conducting comparative analysis of binding mode of identified receptor Caspase 3 with Eucalyptol and its co-crystallized ligand. Analysis of binding conformation revealed that interaction caused by electrostatic forces and intermolecular H-Bonds aids in the binding of eucalyptol to the Caspase 3 catalytic site. In vivo and in vitro bioassays in this direction will throw further light on the targetability of cardamom ingredients to the characterized set of therapeutic targets

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