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Phytochemical and Biomedical Analysis of β-cyclocitral Extracted from *Gallium mite* (Medicinal Herb), and its Effects on Breast Cancer Targeted Proteins

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ABSTRACT

Molecular docking PyRx software coupled with gas chromatography/mass spectrometry (GC-MS) was used to identify predicted proteins which were targeted by β-cyclocitral derived from *Gallium mite var*. reseum. Twenty-four phytochemical compounds were detected using the GC-MS technique. Physiochemical qualities of β-cyclocitral were done using Pubchem database. Pharmaceutical and pharmacokinetic properties of the extracted β-cyclocitral were evaluated using SwissADME and GeneCards databases. One hundred entries were determined in this query. Molecular docking properties were implemented using PyRx software. Results indicated that twenty-two of these entries were predicted to be candidate as target proteins in breast cancer treatment. Catepsin k, Andregone receptor, Alcohol dehydrogenase alpha chain, Cytochrome P450 17A1 and Prostaglandin E synthase with binding affinity of 6.5, 6.2, 6, 5.7, and 5.7kcal/mol, exhibited the highest binding affinity with target proteins respectively. The project results revealed that β-cyclocitral could be used as a ligand in targeting proteins evolved in breast cancer.

Keywords: Breast cancer, Gallium mite, B-cyclocitral, Molecular docking

INTRODUCTION

Breast cancer is the most malignant disorder led to death among women worldwide. The prevalence of breast cancer is proliferating in developing countries, and it has become the most common cancer among women aged 40-44 years [1]. Tomoxifen, doxorubicin, vincristine, vinblastine as anticancer pharmacologic medications were used to hinder tumor cells growth but can be led to side effects such as chemotherapy resistance in breast cancer treatment [2]. Investigations reveal that phytochemical antioxidant compounds may reduce breast cancer incidence and mortality in women [3]. Nowadays, remedies are often not very effective and are associated with undesirable side effects. Therefore, considering the lack of favorable response to treatment and the rapid development of the disease, it is necessary to find more effective drugs with less toxicity. With the help of technological advances in bioinformatics and molecular techniques, information has been obtained that will help in the early diagnosis of cancer. In addition, timely screening for some cancers helps in early detection [4]. Today, many efforts have been made to find suitable methods to treat this disease, including the discovery of new anticancer drugs (more than half of which are extracted from plants). In recent years, medicinal herbs have gained global magnitude as one of the main provenance of biologically active substances for the preparation of natural medicines for cancer treatment [5]. The anticancer effects of plants are caused by inhibiting cancer-inducing enzymes, helping to repair DNA, stimulating the production of antitumor enzymes in cells, increasing the body's immunity, and inducing antioxidant effects [4]. The genus *Galium* belongs to the family Rubiaceae. Some species of the genus possess antispasmodic, diuretic, and vulnerary effects. Plants of this genus are used for fermenting milk to cheese, and because of this utility, the plant has been named Shir-panir in Iranian culture. The aerial parts of *Galium* species are also added to different types of food as herbs or spices to improve flavor. *G.mite* var. *roseum ghahramaninejad* is distributed in Turkey, Iraq, Transcaucasus, and Iran. *G. mite* Boiss &Hohen and *G.*

subvelutinum ssp. mite (Boiss &Hohen) Ehrend are the other names for this plant. The chemical constituents of the essential oil of *G. hercynicum*, *G. humifusum*, *G. salicifolium*, and *G. serums* members of the *Galium* species have been previously investigated [6]. Monoterpenes (C10) represent one of the most studied classes of terpenoids. It accumulates at the time of root and shoots growth/development in numerous plant species and has been shown to play a defensive role against various herbivores and pathogens [7]. Monoterpenes are colorless, hydrophobic, gaseous, and volatile compounds that exhibit a strong aroma and are the main constituents of volatile compounds known as essential oils. Its presence has been reported in Euphorbiaceae, Lamiaceae, Apocynaceae, Oleaceae, Myrtaceae, Frankeniaceae, Asteraceae, and several plant families and genera. Different forms of monoterpenes are formed when DMAPP and IPP are combined to form a C10 compound. Characteristics of these by-products are the acyclic monoterpenes Linalool derivatives, monocyclic (Carvacrol and its analogs bicyclic monoterpenes (α and β pinene and 1, 8-cineole), and tricyclic monoterpenes (tricycleneane teresantanol [8]. In addition to the major classes and skeletons of monoterpenoids available, essential oil components easily undergo intra/inter-conversion enzymatically and chemically via isomerization, cyclization, oxidation, and dehydrogenation, giving rise to a new set of moieties with new pharmacological potential [9]. A well-studied example of such transformation is observed in the biotransformation of limonene in Cymbopogon species. Monoterpenes exhibit a variety of medicinal properties that have been investigated. The monoterpenes, Perillyl alcohol (POH), which is in most cherries and mints, exhibits a very high anticancer potential [10]. Carotenoids are a group of isoprenoid compounds comprising various structures. They comprise eight isoprene units, with most carotenoids derived from the linear tetraterpene phytoene. Two main classes of carotenoids can be distinguished: unoxygenated carotenoids, called carotenes (e.g. b–carotene and lycopene), and their oxygen-containing derivatives, called xanthophylls (e.g. lutein and zeaxanthin) [11].

MATERIAL AND METHODS

Plant material procurement was performed in the field at Dena Mountain located in Kohgelooyeh and Boyer Ahmad Province, 3200 m above sea level and on the northern slope in Iran. The collected plant samples were immediately dried in the shade and divided into two parts. The first part was prepared from the plant herbarium, and the second part was transferred to the laboratory for purification and extraction. The aerial parts were air-dried at ambient temperature in the shade. In this project, we used hexane as the solvent for G.*mite* in a liquid–liquid separation technique with minor modifications. A sample of 5 mL of *G. mite* and 100 mL of solvents were mixed in a 500 mL separating funnel and shaken steadily for 15 min. After 5 min, the solvent phase was detached. The solvent phase was then harvested, and these steps were repeated twice using the same sample. Sodium thiosulfate (anhydrous) was added to the mixture extracts to eliminate moisture levels, condensed with a rotary concentrator, and purged with nitrogen gas until 1 ml was obtained. The essential oil was obtained from the aerial parts of *G.mite* var. *roseum* as a yellow liquid in 0.1 %(w /w) yields. The essential oil was analyzed by GC-MS (60–325ºC at 3ºC/min rate) in an Agilent Technology 7890A GC coupled to a 5977A-MSD instrument using an HP-5MSUI capillary column (Hexane, 30m× 0.25 mm; 0.25µmfilm thickness). The injector temperature was 240ºC; injection was performed in the split mode (1:50).

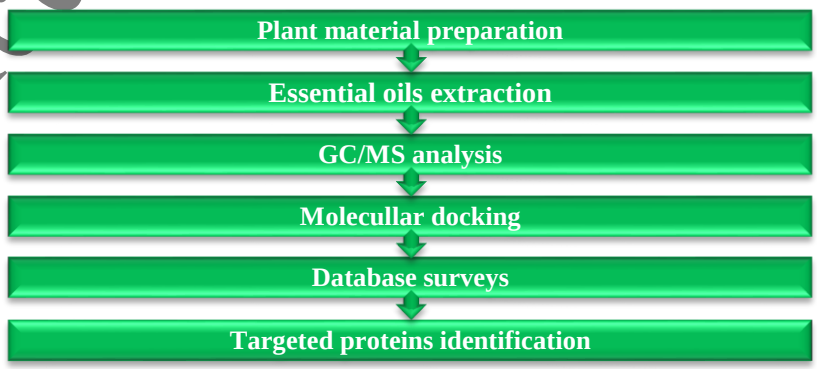


Fig. 1 Phases of *G.mite* essential oils extraction and breast cancer targeted proteins identification

Helium was used as the carrier gas at a flow rate of 1 mL/min. MS spectra were obtained using electron impact at 70 eV with a scan interval of 0.5 s and a mass range of 35–550 m/z. Figure 1 depicts the phases for essential oils and breast cancer targeted proteins identification. Figure 1 represents the steps for essential oil extraction and targeted proteins identification. Pubchem databases were used to detect physiochemical characteristic of β-cyclocitral extracted from *G.mite* solvent. In this project we used SwissADME and GeneCards databases in order to clarify proteins which targeted by β-cyclocitral extracted from *G.mite* and the evaluation of their role in breast cancer respectively. Docking analysis for the targeted proteins was conducted using PyRx software.

RESULTS AND DISCUSSIONS

Phytochemical compounds spectrum data extracted from *G. mite* were compared with the stored data in GC-MS. Twenty-four total compounds were detected (table 1and figure 2). Physicochemical characteristics of β-cyclocitral obtained using the PubChem database (Table 2; Fig.3a). Target proteins evolved in breast cancer that interacted with beta-cyclocitral were determined using the SwissADME and GeneCards databases (Fig 3b). Among extracted metabolites, β-cyclocitral with retention time (R.T) 14.784 min was studied in breast cancer proteins targeting using PubChem, SwissADME, and GeneCards databases. B-cyclocitral targets nuclear receptor, oxidoreductase, secreted protein, fatty acid binding protein family, membrane receptor, enzyme, voltage-gated ion channel, and cytochrome p450 by 40%, 13.3%, 13.3%, 6.7%, 6.7%, 6.7%, 6.7%, and 6.7%, respectively, as shown in (fig. 3b). According to the SwissADME database, the water solubility of β-cyclocitral is 20 and 9 times more than that of curcumin and iboprofen, respectively. Pharmacokinetic properties demonstrated that gastrointestinal (GI) is high in β-cyclocitral. In addition, there is a blood– brain barrier (BBB) for β-cyclocitral while there is no BBB for curcumin (Table. 3). Molecular docking studies implemented using the PyRx software. The results indicated that β-cyclocitral as ligand had the best predicted molecular docking with 22 target proteins (Table 4). Among these, cathepsin K, androgen receptor, alcohol dehydrogenase alpha chain, cytochrome P450 $17A_1$, and prostaglandin E synthase with 6.5, 6.2, 6, 5.7, and 5.7 (Kcal/mol) binding affinity were identified as the best target proteins, respectively (Figure 4) and (Table 5). Previous studies depicted that drug- loaded delivery- released system (DDRS) may be utilized to deliver drugs to tumor sites through enhanced-permeability and retention effect (EPR). Commonly, to accomplish stringent anti-cancer remedy first, drugs are loaded through π-π interactions. Second, tumor cells were targeted by the DDRS through ligand-receptor interactions. Finally, anti-cancer medications are released from DDRS in the low PH of tumor cells environment for impressive tumor extirpation [12]. Cytochrome p45019A1 encodes a member of the cytochrome P450 superfamily of enzymes. Cytochrome P450 proteins are monooxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. This protein localizes to the endoplasmic reticulum and catalyzes the last steps of estrogen biosynthesis. Mutations in this gene can result in either increased or decreased aromatase activity; the associated phenotypes suggest that estrogen functions both as a sex steroid hormone and in growth or differentiation. Alternative promoter use and alternative splicing results in multiple transcript variants with different tissue specificity. [13]. Steroid 5-alpha-reductase (EC 1.3.99.5) catalyzes the conversion of testosterone into the more potent androgen dihydrotestosterone (DHT) [14]. The progesterone receptor encodes a member of the steroid receptor superfamily. The encoded protein mediates the physiological effects of progesterone, which plays a central role in reproductive events associated with the establishment and maintenance of pregnancy. This gene uses two distinct promoters and translation start sites in the first exon to produce several transcript variants, both protein coding and non-protein coding. Two of the informs (A and B) are identical except for an additional 165 amino acids found in the N-terminus of isoform B and mediate their own response genes and physiological effects with little overlap [15]. Testis-specific androgen-binding protein encodes a steroid-binding protein that was first described as a plasma protein secreted by the liver but is now thought to participate in the regulation of steroid responses. The encoded protein transport androgens and estrogens in the blood, binding each steroid molecule as a dimer formed from identical or nearly identical monomers. Polymorphisms in this gene have been associated with polycystic. Ovary syndrome and type 2 diabetes mellitus. Alternative splicing results in multiple transcript variants [16]. The androgen receptor gene is more than 90 kb long and codes for a protein that has 3 major functional domains: the N-terminal domain, DNA-binding domain, and androgen-binding domain. The protein functions as a steroid hormone- activated

transcription factor. Upon binding to the hormone ligand, the receptor dissociates from accessory proteins, translocates into the nucleus, dimerizes, and then stimulates the transcription of androgen-responsive genes. This gene contains 2 polymorphic trinucleotide repeat segments that encode polyglutamine and polyglycine tracts in the N-terminal transactivation domain of its protein. Expansion of the polyglutamine tract from normal 9–34 repeats to pathogenic 38–62 repeats causes spinal bulbar muscular atrophy (SBMA, also known as Kennedy's disease). Mutations in this gene are also associated with complete androgen insensitivity (CAIS). Alternative splicing results in multiple transcript variants encoding different isoforms [17]. The alcohol dehydrogenase alpha chain encodes a member of the alcohol dehydrogenase family. The encoded protein is the alpha subunit of class I alcohol dehydrogenase, which consists of several homo- and heterodimers of alpha, beta, and gamma subunits. Alcohol dehydrogenases catalyze the oxidation of alcohols to aldehydes. This gene is active in the liver in early fetal life but weakly active in the adult liver. This gene is found in a cluster with six additional alcohol dehydrogenase genes, including those encoding the beta and gamma subunits, on the long arm of chromosome 4. Mutations in this gene may contribute to variations in certain personality traits and substance dependence [18].

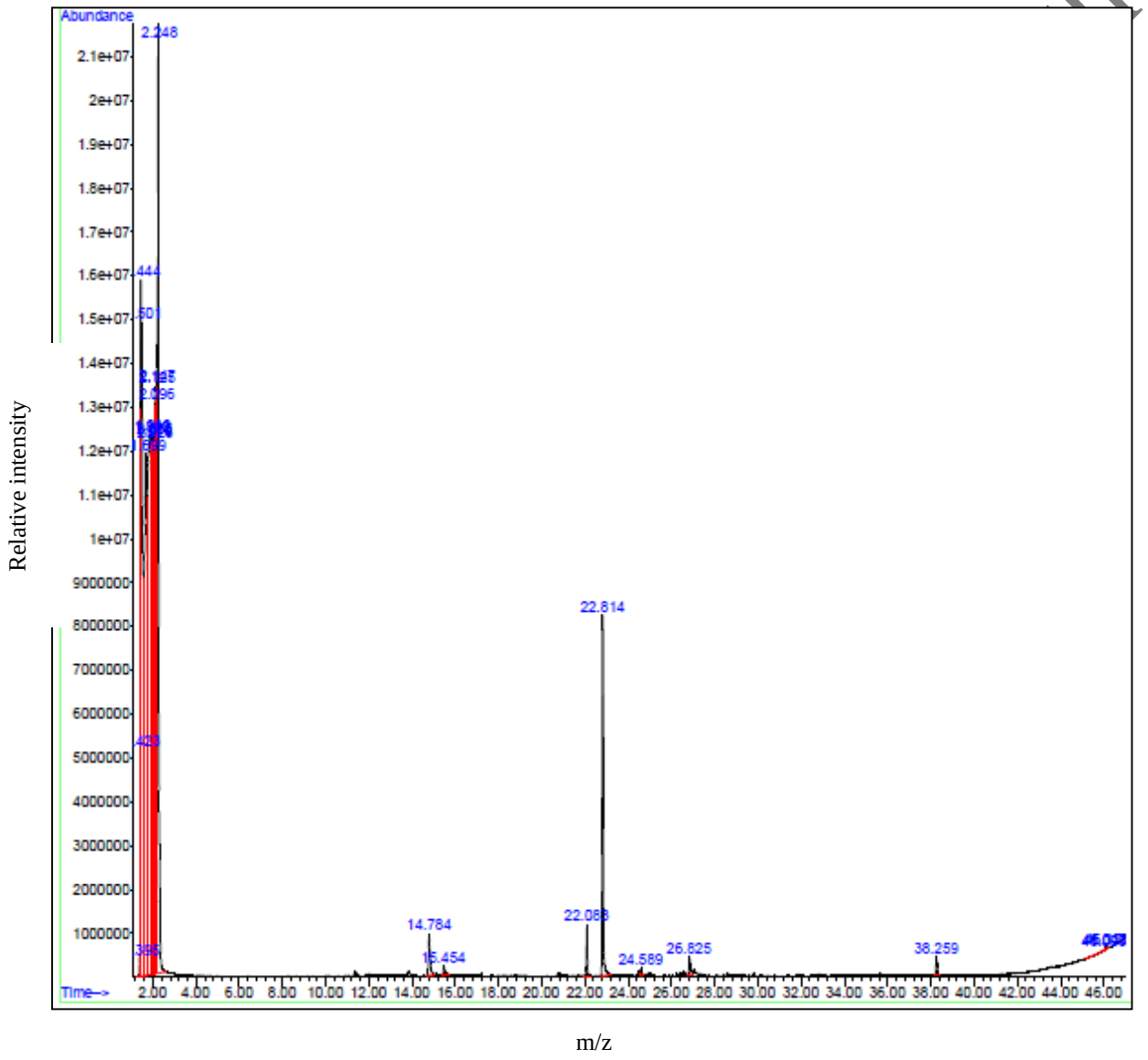


Fig. 2 GC– MS analysis of essential oils extracted from *Gallium mite*

Table 1 Total compounds extracted from *gallium mites* in GC-MS analysis

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	1.395	33	54	55	BV	404939	4535878	0.33%	0.068%
2	1.423	55	59	60	VV	5120591	44641461	3.27%	0.665%
3	1.444	60	62	64	VV	15352915	180255322	13.22%	2.686%
4	1.501	64	72	86	VV 3	14925531	885906152	64.99%	13.201%
5	1.699	86	107	109	VV	11895834	804074957	58.98%	11.982%
6	1.880	109	139	143	VV 2	12296538	1363186375	100.00%	20.313%
7	1.919	143	145	153	VV 4	12282320	435664536	31.96%	6.492%
8	1.970	153	154	157	VV 2	12207077	153894568	11.29%	2.293%
9	2.005	157	160	162	VV 2	12221508	240087100	17.61%	3.578%
10	2.026	162	164	174	VV 2	12171742	474764160	34.83%	7.074%
11	2.096	174	176	178	VV	13066108	200435030	14.70%	2.987%
12	2.125	178	181	184	VV 3	13394737	240455383	17.64%	3.583%
13	2.147	184	185	191	VV 4	13397019	315776988	23.16%	4.705%
14	2.248	191	203	271	VB 2	21638178	1016571446	74.57%	15.148%
15	14.784	2374	2394	2438	BV 2	938428	42311127	3.10%	0.630%
16	15.454	2493	2511	2554	BB 2	201729	11821375	0.87%	0.176%
17	22.083	3641	3669	3696	BB	1158708	28590680	2.10%	0.426%
18	22.814	3784	3797	3862	BB	8164319	225163793	16.52%	3.355%
19	24.589	4099	4107	4123	VV 2	154527	5913829	0.43%	0.088%
20	26.825	4481	4498	4523	PV 4	405658	19579019	1.44%	0.292%
21	38.259	6475	6496	6529	BV 3	422163	17011043	1.25%	0.253%
22	46.005	7704	7850	7852	PV 10	10865	-1446798	-0.11%	-0.022%
23	46.043	7852	7857	7861	PV 7	30199	539206	0.04%	0.008%
24	46.176	7861	7880	7885	VB 7	12392	1193012	0.09%	0.018%

Sum of corrected areas: 6710925641

Table 2 Physicochemical characteristics of β-cyclocitral

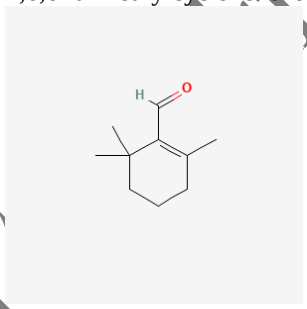
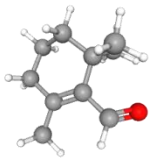
PubChem (CID)	9895
Compound name	B-cyclocitral
IUPAC name	2,6,6- trimethylcyclohexene-1- carbaldehyde
2D structure	
3D structure	
Molecular weight	152.23 g/mol
Topological polar surface area	17.1 (Å)
Canonical smiles	CC1=C(C(CCC1)(C)C)C=O

Table 3 Comparing between β-cyclocitral, Curcumin and ibuprofen according to solubility (mg/ml), gastrointestinal absorption (GI), and blood– brain barrier (BBB) indexes.

PubChem(CID)	Formula	Compound name	Canonical smiles	Solubility mg/ml	GI	BBB
9895	C10H16O	B-cyclocitral	CC1=C(C(CCC1)(C)C)C=O	0.859	high	Yes
969516	C21H20O6	Curcumin	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O	0.0422	high	No
3672	C13H18O2	Ibuprofen	CC(C)CC1=CC=C(C=C1)C(C)C(=O)O	0.0909	high	Yes

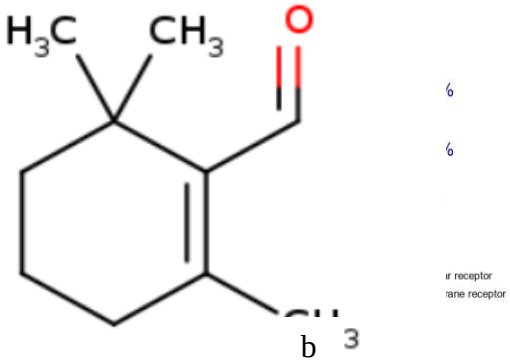


Fig. 3 Chemical structure depiction of β-cyclocitral (a). Prediction of targeted proteins by β-cyclocitral (b).

Table 4 Breast cancer targeted proteins identified by PyRx software

Row	Target	Common name	UniProt ID	ChEMBLID	Target class	Binding affinity(kcal/mol)
1	Cytochrome P450 19A1	CYP19A1	P11511	CHEMBL1978	Cytochrome p450	0
2	Steroid 5-alpha reductase 1	SRD5A1	P18405	CHEMBL1787	Oxidoreductase	-5.5
3	Progesterone receptor	PGR	P06401	CHEMBL208	Nuclear receptor	-4.7
4	Testis-specific androgen-binding protein	SHBG	P04278	CHEMBL3305	Secreted protein	-4.6
5	Androgen Receptor	AR	P10275	CHEMBL1871	Nuclear receptor	-6.2
6	Alcohol dehydrogenase alpha chain	ADH1A	P07327	CHEMBL1970	Oxidoreductase	-6
7	Prostaglandin E synthase	PTGES	O14684	CHEMBL5658	Enzyme	-5.7
8	Cytochrome P450 17A1	CYP17A1	P05093	CHEMBL3522	Cytochrome P450	-5.7
9	Protein tyrosine phosphatase 2C	PTPN11	Q06124	CHEMBL3864	Phosphatase	-4.3
10	Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase	-4.9
11	Cathepsin D	CTSD	P07339	CHEMBL2581	Protease	-5.2
12	Estrogen receptor alpha	ESR1	P03372	CHEMBL206	Nuclear receptor	-4.8
13	Estrogen receptor beta	ESR2	Q92731	CHEMBL242	Nuclear receptor	-5.6
14	Fatty acid-binding protein in adipocytes	FABP4	P15090	CHEMBL2083	The fatty acid binding protein family	-5.5
15	Fatty acid-binding protein muscle	FABP3	P05413	CHEMBL3344	The fatty acid binding protein family	-4.8
16	Poly [ADP-ribose] polymerase-1	PARP1	P09874	CHEMBL3105	Enzyme	-5.3
17	Cathepsin K	CTSK	P43235	CHEMBL268	Protease	-6.5
18	Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	-5
19	Thymidylate synthase	TYMS	P04818	CHEMBL1952	Transferase	-5.6
20	Zinc finger protein GLI2	GLI2	P10070	CHEMBL5119	Transcription factor	-4.3
21	DNA polymerase beta	POLB	P06746	CHEMBL2392	Enzyme	-5.4
22	Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase	-4.1

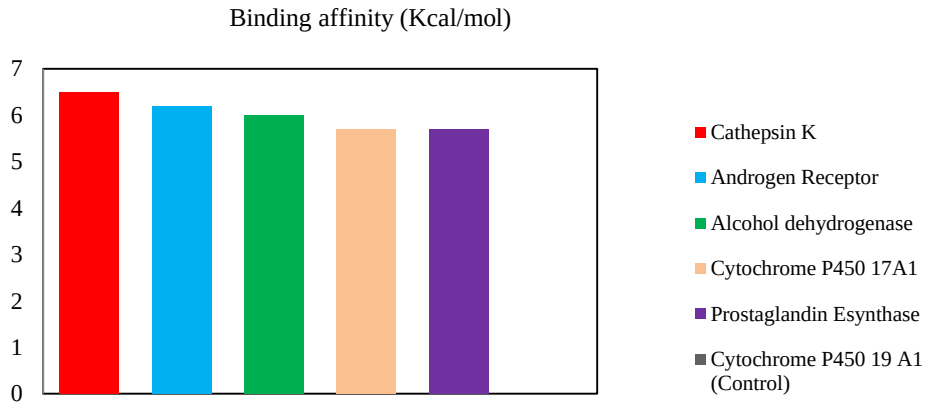
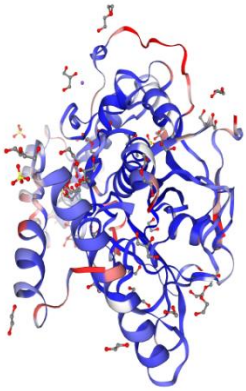
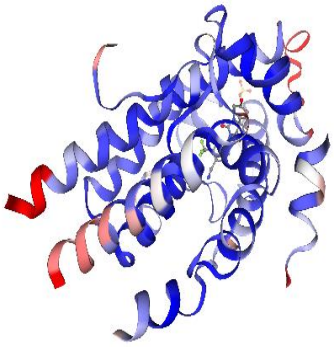
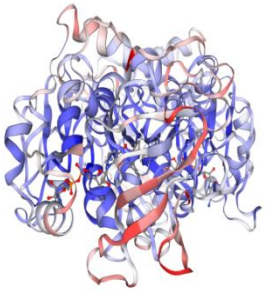
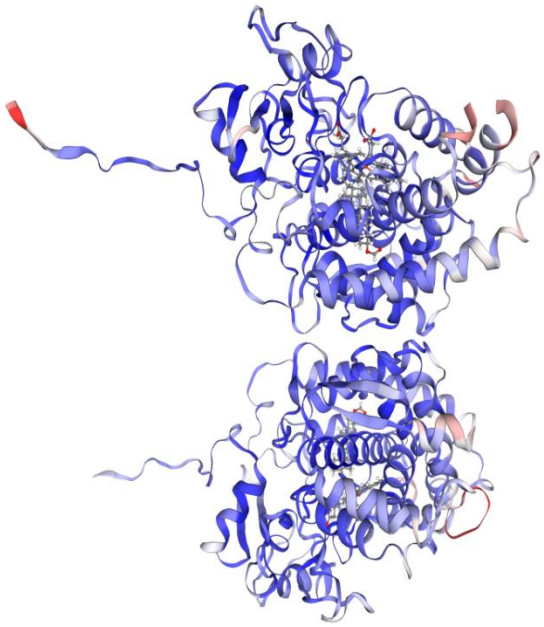
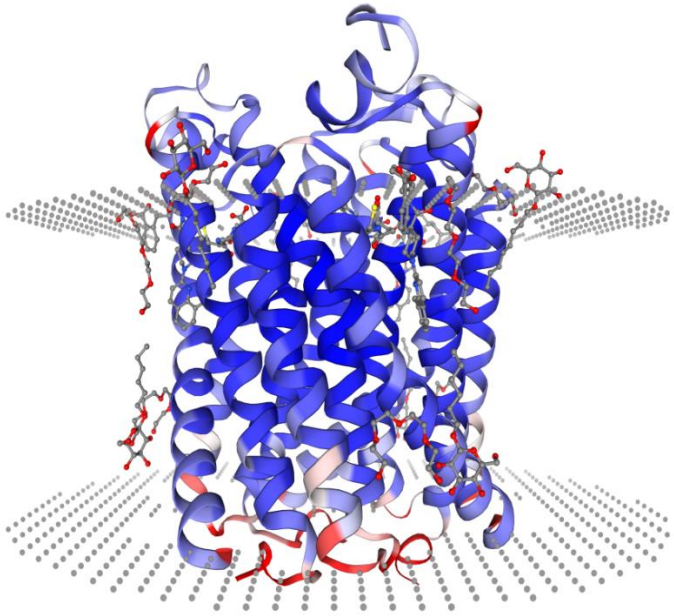


Fig. 4 Comparison of breast cancer target proteins and β-cyclocitral as ligand according to docking affinity

Table 5 Breast cancer targeted proteins morphology and size

Row	Protein name	Morphology	Size (Å)
1	Cathepsin K		2.20
2	Androgen receptor		2.40

3	Alcohol dehydrogenase		2.50
4	Cytochrome P450 17A1		2.60

5	Prostaglandin E synthase		3.50

The protein encoded by prostaglandin E synthase is a glutathione-dependent prostaglandin E synthase. The expression of this gene is induced by the pro-inflammatory cytokine interleukin 1 beta (IL1B). Its expression can also be induced by the tumor suppressor protein TP53 and may be involved in TP53-induced apoptosis. Knockout studies in mice demonstrated that this gene contributes to the pathogenesis of collagen-induced arthritis and mediates acute pain during inflammatory responses [19]. Cytochrome P450 17A1 encodes a member of the cytochrome P450 superfamily of enzymes. Cytochrome P450 proteins are monooxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. This protein localizes to the endoplasmic reticulum. It has both 17-alpha-hydroxylase and 17, 20-lyase activities and is a key enzyme in the steroidogenic pathway that produces progestins, mineralcorticoids, glucocorticoids, androgens, and estrogens. Mutations in this gene are associated with isolated steroid-17 alpha-hydroxylase deficiency, 17-alpha-hydroxylase/17, 20-lyase deficiency, pseudohermaphroditism, and adrenal hyperplasia [20]. The protein tyrosine phosphatase 2C protein encoded by this gene is a member of the protein tyrosine phosphatase (PTP) family. PTPs are signaling molecules that regulate a variety of cellular processes, including cell growth, differentiation, mitotic cycle, and oncogenic transformation. This PTP contains two tandem Src homology-2 domains, which function as phosphotyrosine- binding domains and mediate the interaction of this PTP with its substrates. PTP is widely expressed in most tissues and plays a regulatory role in various cell signaling events that are important for a diversity of cell functions, such as mitogenic activation, metabolic control, transcription regulation, and cell migration. Mutations in this gene are a cause of syndrome and acute myeloid leukemia [21]. Cyclooxygenase-1 is one of two genes encoding similar enzymes that catalyze the conversion of arachidonate to prostaglandin. The encoded protein regulates angiogenesis in endothelial cells and is inhibited by non-steroidal anti-inflammatory drugs such as aspirin. Based on its ability to function as both a cyclooxygenase and a peroxidase, the encoded protein has been identified as a moonlighting protein. This protein may promote cell proliferation during tumor progression. Alternative splicing results in multiple transcript variants [22]. The cathepsin D gene encodes a member of the A1 family of peptidases. The encoded protein is proteolytically processed to generate multiple protein products. These products include cathepsin D light and heavy chains, which heterodimerize to form the mature enzyme. This enzyme exhibits pepsin-like activity and plays a role in protein turnover and the proteolytic activation of hormones and growth factors. Mutations in this gene play a causal role in neuronal ceroid lipofuscinosis-10 and may be involved in the pathogenesis of several other diseases, including breast cancer and possibly Alzheimer's disease [23]. Estrogen receptor alpha encodes an estrogen

receptor and ligand-activated transcription factor. The canonical protein contains an N-terminal ligand-independent transactivation domain, a central DNA binding domain, a hinge domain, and a C-terminal ligand-dependent transactivation domain. The protein localizes to the nucleus, where it may form either a homodimer or a heterodimer with estrogen receptor 2. The protein encoded by this gene regulates the transcription of many estrogen-inducible genes that play a role in growth, metabolism, sexual development, gestation, and other reproductive functions and is expressed in many non-reproductive tissues. The receptor encoded by this gene plays a key role in breast endometrial and osteoporosis. This gene is reported to have dozens of transcript variants because of the use of alternate promoters and alternative splicing; however, the full-length nature of many of these variants remains uncertain [24]. Estrogen receptor beta encodes a member of the family of estrogen receptors and the superfamily of nuclear receptor transcription factors. The gene product contains an N-terminal DNA-binding domain and a C-terminal ligand-binding domain and is localized to the nucleus, cytoplasm, and mitochondria. Upon binding to 17-estradiol or related ligands, the encoded protein forms homo- or heterodimers that interact with specific DNA sequences to activate transcription. Some isoforms dominantly inhibit the activity of other estrogen receptor family members. Several alternatively spliced transcript variants of this gene have been described, but the full-length nature of some of these variants has not been fully characterized [25]. Fatty acid-binding protein adipocyte encodes the fatty acid-binding protein found in adipocytes. Fatty acid-binding proteins are a family of small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. It is thought that FABP roles include fatty acid uptake, transport, and metabolism [26]. FABP muscle belongs to a multigene family. FABPs are divided into at least three distinct types: hepatic, intestinal, and cardia. They form 14-15 KD proteins and are thought to participate in the uptake, intracellular metabolism, and/or transport of long-chain fatty acids. They may also be responsible for modulating cell growth and proliferation. The fatty acid-binding protein 3 gene contains four exons and its function is to arrest the growth of mammary epithelial cells. This gene is a candidate tumor suppressor gene for human breast cancer. Alternative splicing results in multiple transcript variants [27]. Poly [ADP-ribose] polymerase-1 encodes a chromatin-associated enzyme, poly (ADP-ribosyl) transferase, which modifies various nuclear proteins by poly (ADP-ribosylation). The modification is dependent on DNA and is involved in the regulation of various important cellular processes, such as differentiation, proliferation, and tumor transformation, as well as in the regulation of the molecular events involved in the recovery of cells from DNA damage. In addition, this enzyme may be the site of mutation in Fanconi anemia and may participate in the pathophysiology of type I diabetes [28]. Cathepsin K, the protein encoded by this gene, is a lysosomal cysteine proteinase involved in bone remodeling and resorption. This protein, a member of the peptidase C1 protein family, is predominantly expressed in osteoclasts. However, the encoded protein is also expressed in a significant fraction of human breast cancers, where it could contribute to tumor invasiveness. Mutations in this gene cause pycnodysostosis, an autosomal recessive disease characterized by osteosclerosis and short stature [29]. Carbonic anhydrase II, the protein encoded by this gene, is one of several isozymes of carbonic anhydrase, which catalyzes the reversible hydration of carbon dioxide. Defects in this enzyme are associated with osteopetrosis and renal tubular acidosis. Two transcript variants encoding different isoforms have been identified for this gene [30]. Thymidylate synthase catalyzes the methylation of deoxyuridylate to deoxythymidylate using 10-methylenetetrahydrofolate (methylene-THF) as a cofactor. This function maintains the thymidine-5-prime monophosphate (dTMP) pool, which is critical for DNA replication and repair. This enzyme has been of interest as a target for cancer chemotherapeutic agents. It is considered as the primary site of action of 5-fluorouracil, 5-fluoro-2-prime-deoxyuridine, and some folate analogs. Expression of this gene and that of a naturally occurring antisense transcript, mitochondrial enolase superfamily member 1 (GeneID: 55556), vary inversely when cell growth progresses from the late-log to plateau phase. Polymorphisms in this gene may be associated with the etiology of neoplasia, including breast cancer, and response to chemotherapy [31). Zinc finger protein GLI2 encodes a protein that belongs to the C2H2-type zinc finger protein subclass of the Gli family. Members of this subclass are transcription factors that bind DNA through zinc finger motifs. These motifs contain conserved H– C links. Gli family zinc finger proteins are mediators of sonic hedgehog (Shh) signaling and are implicated as potent oncogenes in embryonal carcinoma cells. The protein encoded by this gene localizes in the cytoplasm and activates patched Drosophila homolog (PTCH) gene expression. It is also thought to play a role during embryogenesis. The encoded protein is associated

with several phenotypes- Greig cephalo-polysyndactyly syndrome, Pallister– Hall syndrome, preaxial polydactyly type IV, and postaxial polydactyly types A1 and B [32]. The protein encoded by this gene is a DNA polymerase involved in base excision and repair, also called gap-filling DNA synthesis. The encoded protein, acting as a monomer, is normally found in the cytoplasm, but it translocates to the nucleus upon DNA damage. Several transcript variants of this gene exist, but the full-length nature of only one has been described to date [33]. Arachidonate 5-lipoxygenase encodes a member of the lipoxygenase gene family and plays a dual role in the synthesis of leukotrienes from arachidonic acid. The encoded protein, which is expressed specifically in bone marrow-derived cells, catalyzes the conversion of arachidonic acid to 5(S)-hydroperoxy-6-Trans-8, 11, and 14-cis-eicosatetraenoic acid and further to the allylic epoxide 5(S)-Trans-7, 9-trans-11, and 14-cis-eicosatetrenoic acid (leukotriene A4). Leukotrienes are important mediators of several inflammatory and allergic conditions. Mutations in the promoter region of this gene lead to a diminished response to antileukotriene drugs used for treating asthma and may also be associated with atherosclerosis and several cancers. Alternatively, spliced transcript variants encoding different isoforms have been found for this gene [34].

CONCLUSION

In conclusion, the findings of this project revealed that β-cyclocitral extracted from *G.mite var.reseum* has the potential to target breast cancer proteins. A number of twenty-two target proteins involved in breast cancer were identified. Among which, five proteins had the highest binding affinity and consequently the probability to pair with β-cyclocitral. More studies are needed to clarify the medicinal and pharmaceutical perspectives of the photochemical compounds derived from *G.mite*.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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