

Cannabinoid as Potential Aromatase Inhibitor Through Molecular Modeling and Screening for Anti-Cancer Activity

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ABSTRACT: Inhibition of aromatase (CYP450), a key enzyme in the estrogen biosynthesis, could result in regression of estrogen-dependent tumors and even prevent the promotion of breast cancer. The present research has been designed for searching a potent chemical moiety from natural sources to inhibit aromatase enzyme, the over-functionality of which causes the breast cancer. *Cannabis sativa* contains a very much promising group of cannabinoids with more than 66 compounds with reported anticancer property and for the search of a target specific potent aromatase inhibitor, 61 cannabinoids from *C. sativa* were selected. The Structures Data File (SDF) of these ligand molecules were subjected to docking studies at the binding site of aromatase X-ray crystallographic structure based on lower resolution of the protein crystal structure and higher docking accuracy, predicted by calculating the correlation between experimental activities and Glide dock scores and compared with the standard aromatase ligand androstenedione and aromatase inhibitor fadrozole with existing drug for breast cancer treatment. The best docked pose of each ligand was selected on the basis of the highest dock score related to the binding free energies of the internal dataset compounds as compared to their observed activities. Apart from the hydrogen bond formation with the oxygen present on the aromatic ring system, the other parts of the molecules are stabilized by hydrophobic interactions with non-polar amino acid residues (Ile133, Phe134, Phe221, Trp224, Ile305, Ala306, Ala307, Val369, Val370, Leu372, Val373, Met374 and Leu477). From the screening results of the cannabinoid analogs, 21 out of 61 were found to have an acceptable docking score in comparison to the standards, androstenedione and fadrozole. The pharmacokinetic filters like absorption, distribution, metabolism and excretion and toxicity (ADMET) property determination were applied to select drug-like compounds. Among them three compounds were found to reveal the most promising drug like activity, which were cannabidiol (CN 17, CBD-C1), cannabitol (CN 43, CBT) and cannabiripsol (CN 55, CBR). The anti-cancer activity of the target compounds was performed against brine shrimp lethality bioassay, where cannabidiol exhibited significant LC_{50} value of $0.348 \pm 0.002 \mu\text{g/ml}$ ($R^2 = 0.9853$) which is almost similar to vincristine sulfate ($LC_{50} = 0.316 \pm 0.003 \mu\text{g/ml}$, $R^2 = 0.9882$). Compound cannabitol also showed promising cytotoxicity $0.650 \pm 0.004 \mu\text{g/ml}$ ($R^2 = 0.9882$) in comparison to the reference standard. But cannabiripsol demonstrated relatively weaker activity $12.95 \pm 1.234 \mu\text{g/ml}$ ($R^2 = 0.9897$). It can be concluded that the lead compounds may be developed as potent aromatase inhibitor performing their further biological evaluation.

Key words: Molecular docking, chemometric analysis, cannabinoids, breast cancer, aromatase inhibitor, *Cannabis sativa*, cytotoxicity, brine shrimp lethality.

INTRODUCTION

Now a days, the most common type of cancer is breast cancer resulting with more than 234,000 new cases reported in the United States in 2015.¹ It is categorized as hormone-dependent and independent

simulation state of various hormone receptor subtypes.² In hormone-dependent cases of breast cancer, estrogen has been reported to play a crucial role through various mechanisms which can induce and proliferate the breast cancer.² This helps to provoke the intracellular signaling cascades and resulting in the development of tumors.^{3,4} Estrogens are the causes of cancer, which can synergically

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accelerate the cell division, at the time of DNA repairing phase by generating quinines via their metabolic pathway.⁵ Estrogen-dependent carcinomas were reported in near 60% of premenopausal and 75% of post-menopausal breast cancer patients.⁶ In this background, the inhibition of aromatase, the rate limiting enzyme in the estrogen biosynthesis pathway can help to control the growth and development of estrogen-dependent tumors. Basically, aromatase is a Cytochrome P450 enzyme which converts cholesterol to estradiol E2, the most biologically active estrogen.⁷ Moreover, such inhibition may reduce the estradiol level in breast tissue, which can prevent the tumor promotion process.⁵

Aminoglutethimide and testololactone are the first-generation aromatase inhibitors, related with unspecific action that leads to induction of various kind of side effects.⁸ Recent FDA- approved drugs, such as exemestane⁹ as a steroid inhibitor, letrozole¹⁰ and anastrozole¹¹ as non-steroid specific aromatase inhibitors (AIs) can efficiently decrease the recurrence rate of hormone-dependent breast cancer. Exemestane is a mechanism-based inhibitor and inhibits aromatase in an irreversible manner¹², but other AIs like letrozole and anastrozole act as competitive and reversible inhibitors. But their mechanism of action still remain unclear.^{13,14} Until today, non-steroidal aromatase inhibitors (NSAIs) are the most progressive kinds¹⁵ but these are related with few side-effects. This motivates to take a new challenge for development of a new generation of AIs based on natural compounds and their derivatives such as coumarin, lignin and flavonoids.¹⁶ It is approved that with the structural modification of isoflavanone through various approaches, a new derivative has been developed having greater ability to inhibit the aromatase activity.¹⁷

Upon extensive literature review of *C. sativa* as medicine, it is seen that endo-cannabinoids can act as selective inhibitors of human breast cancer cell proliferation through a growth factor-dependent mechanism.^{18,19} It has been reported that cannabinoids can act through different cellular mechanisms by inducing apoptosis, cell-cycle arrest,

or cell growth inhibition, targeting angiogenesis and cell migration.²⁰⁻²² *In-silico* screening techniques from the compound databases is presently the most popular and useful cheminformatics application in the field of drug discovery. In the current approach, the cannabinoids from the plant, *C. sativa* as a source of a lead molecule, was used for development of an aromatase inhibitor via computer based analytical techniques using ligand-based molecular docking, drug-like property analysis and ADMET prediction studies and we, here in, report the results of our *in silico* and laboratory-based studies.

MATERIALS AND METHODS

Data retrieval of molecular modeling studies.

The crystal structure of human placental aromatase cytochrome P450 in complex with androstenedione (PDB:3EQM)²³ has been retrieved from the RCSB protein data bank (<http://www.pdb.org>). The enzyme is co-crystallized with androstenedione, protoporphyrin IX containing Fe⁺³ and phosphate ion. Structure-based modeling study was performed with this crystallographic receptor (Figure 1) as clues for catalytic site grid generation in molecular docking.

Molecular docking of cannabinoids against aromatase. The molecular docking has great promise in virtual lead discovery as the scoring algorithms become more refined along with the continuous improvement in computer processing power and capabilities.²⁴ In the present study, dataset containing 61 cannabinoid compounds²⁵⁻²⁷ designated as CN (Table 1) were docked at the binding site of aromatase protein 3EQM. The protein structure was prepared by *Protein Preparation Wizard*, in tool of Schrödinger, Inc.^{28,29} All ligands in dataset were prepared in Ligprep tool (Ligprep 2013), Version 2.8, Schrodinger, LLC, New York).³⁰ Epik based ionization was used to generate all possible ionization states of the ligands in pH range of 7.0 ± 0.5 (Figure 2). These prepared molecules were subsequently docked at the active site of (PDB:3EQM) by standard precision (SP) mode in Glide (Figure 3). The best docked pose of each ligand was selected on the basis of the highest dock score.

Table 1. Docking score of the compound in the dataset.²⁵⁻²⁷

Sl. No.	Compound ID	Compound name	Docking score	Bonding interaction	Hydrophobic interaction
1	CN1	Cannabigerolic acid (CBGA)	-5.62	HEM600	Val370, Phe221, Val369, Val313, Ile305, Ala306, Leu372, Val373, Met374, Phe134, Ile133, Trp224, Leu477
2	CN2	Cannabigerolic acid monomethylether (CBGAM)	-4.41	ARG115, MET374	Val373, Phe134, Ile133, Ile305, Ala306, Phe221, Trp224, Val370, Leu372, Leu477
3	CN3	Cannabigerol (CBG)	-6.62	HEM600	Val373, Leu372, Met374, Leu477, Trp224, Phe134, Val369, Ile133, Phe221, Ile305, Ala306, Met127, Val370, Val373, Leu372
4	CN4	Cannabigerol monomethylether (CBGM)	-	-	-
5	CN5	Cannabigerovararin	-8.34	ARG115	Ile133, Ala303, Ile305, Trp224, Phe221, Val369, Leu479, Val370, Leu447, Leu372
6	CN6	Cannabigerovararin (CBGV)	-7.8	HEM600	Val370, Leu372, Met374, Phe134, Ile133, Ala306, Ile305, Phe221, Val373, Trp224, Leu477
7	CN7	Cannabichromenic acid (CBCA)	-7.73	HEM600	Ile70, Met374, Leu372, Leu477, Val373, Ile133, Ile305, Val370, Ala306, Val369, Phe221, Val313, Trp224, Phe134
8	CN8	Cannabichromene (CBC)	-	-	-
9	CN9	Cannabichromevarinic acid (CBCVA)	-7.45	HEM600	Leu372, Met374, Phe134, Val373, Ile133, Ile305, Ala306, Trp224, Phe221, Val369, Val307, Leu477
10	CN10	Cannabichromevarin (CBCV)	-8.29	HEM600	Val370, Leu372, Leu477, Phe134, Val373, Met127, Ile305, Ala306, Ile133, Phe221, Trp224
11	CN11	Cannabidiolic acid (CBDA)	-	-	-
12	CN12	Cannabidiol (CBD)	-5.98	HEM600	Met374, Leu372, Trp224, Phe134, Ile133, Ile305, Ala306, Phe221, Val370, Val373, Leu477
13	CN13	Cannabidiol monomethylether (CBDM)	-	-	-
14	CN14	Cannabidiol-C4 (CBD-C4)	-6.84	HEM600, ARG115	Val373, Met374, Phe134, Trp224, Ile133, Ala306, Ile305, Phe221, Val369, Val370, Ile479, Leu372, Ile477
15	CN15	Cannabidivarinic acid (CBDVA)	-	-	-
16	CN16	Cannabidivarin (CBDV)	-8.34	HEM600	Val373, Met374, Trp224, Phe134, Ile133, Ile305, Phe221, Val369, Val370, Leu372, Leu477
17	CN17	Cannabidiocol (CBD-C1)	-9.03	HEM600	Val373, Met374, Trp224, Phe134, Ile133, Ile305, Ala306, Phe221, Val369, Val370, Leu372, Leu477
18	CN18	Delta-9-tetrahydrocannabinolic acid A (THCA-A)	3.27	H-Bond with MET374, II-II interaction with PHE134	Val373, Met374, Trp224, Ile133, Ile305, Ala306, Phe221, Val369, Val370, Leu372, Leu477
19	CN19	Delta-9-tetrahydrocannabinolic acid B (THCA-B)	-	-	-
20	CN20	Delta-9-tetrahydrocannabinol (THC)	-	-	-
21	CN21	Delta-9-tetrahydrocannabinol-C4 (THC-C4)	-	-	-
22	CN22	Delta-9-tetrahydrocannabivarinic acid (THCVA)	-4.16	MET374, PHE134 II-II Interaction	Ile70, Val373, Met374, Trp224, Ile133, Ile305, Ala306, Phe221, Val369, Val370, Leu372, Leu477

Table 1 Contd.

23	CN23	Delta-9-tetrahydrocannabivarin (THCV)	-	-	-
24	CN24	Delta-9-tetrahydrocannabinorcol (THC-C1)	-	-	-
25	CN25	Delta-7-cis-iso-tetrahydrocannabivarin	-	-	-
26	CN26	Delta-8-tetrahydrocannabinolic acid (delta8-THCA)	-	-	-
27	CN27	Delta-8-tetrahydrocannabinol (delta8-THC)	-	-	-
28	CN28	Cannabicyclic acid (CBLA)	-	-	-
29	CN29	Cannabicyclol (CBL)	-	-	-
30	CN30	Cannabicyclovarin (CBLV)	-	-	-
31	CN31	Cannabielsoic acid A (CBEA-A)	-	-	-
32	CN32	Cannabielsoic acid B (CBEA-B)	-	-	-
33	CN33	Cannabielsoin (CBE)	-	-	-
34	CN34	Cannabinolic acid (CBNA)	-	-	-
35	CN35	Cannabinol (CBN)	-	-	-
36	CN36	Cannabinol methylether (CBNM)	-	-	-
37	CN37	Cannabinol-C4 (CBN-C4)	-	-	-
38	CN38	Cannabivarin (CBV)	-	-	-
39	CN39	Cannabinol-C2 (CBN-C2)	-3.93	LEU477	Val373, Met374, Trp224, Phe134, Ile133, Ala306, Phe221, Val369, Val370, Leu372
40	CN40	Cannabiorcol (CBN-C1)	-	-	-
41	CN41	Cannabinodiol (CBND)	-6.14	HEM600	Val373, Met374, Met127, Trp224, Phe134, Ile133, Ile305, Ala306, Phe221, Val369, Val370, Leu372, Leu379, Leu477
42	CN42	Cannabinodivarin (CBVD)	-6.59	HEM600	Val373, Met374, Trp224, Phe134, Ile133, Ile305, Ala306, Ala307, Phe221, Val369, Val370, Leu372, Leu477
43	CN43	Cannabitriol (CBT)	-7.86	ALA306	Val373, Met374, Trp224, Phe134, Ile133, Ile305, Phe221, Val369, Val370, Leu372, Leu477
44	CN44	10-Ethoxy-9-hydroxy-delta-6a-tetrahydrocannabinol	-	-	-
45	CN45	8,9-Dihydroxy-delta-6a-tetrahydrocannabinol	-	-	-
46	CN46	Cannabitriolvarin (CBTV)	-	-	-
47	CN47	Ethoxycannabitriolvarin (CBTVE)	-	-	-
48	CN48	Dehydrocannabifuran (DCBF)	-	-	-
49	CN49	Cannabifuran (CBF)	-	-	-
50	CN50	Cannabichromanon (CBCN)	-	-	-
51	CN51	Cannabicitran (CBT)	-	-	-
52	CN52	10-Oxo-delta-6a-tetrahydrocannabinol (OTHC)	-	-	-

Table 1 Contd.

53	CN53	Delta-9-cis-tetrahydrocannabinol (cis-THC)	-7.43	LEU477	Val373, Met374, Trp224, Phe134, Ile133, Phe221, Val369, Val370, Leu372, Leu479.
54	CN54	3,4,5,6-Tetrahydro-7-hydroxy-alpha-alpha-2-trimethyl-9-n-propyl-2,6-methano-2H-1-benzoxocin-5-methanol (OH-iso-HHCV)	-5.02	LEU477	Val373, Met374, Trp224, Phe134, Ile133, Ile305, Ala307,Ala306, Phe221, Val369, Val370, Leu372
55	CN55	Cannabiripsol(CBR)	-6.93	HEM600	Val373, Met374, Trp224, Phe134, Ile133, Ile305, Ala306, Ala307, Phe221, Val369, Val370, Leu372, Leu477
56	CN56	Trihydroxy-delta-9-tetrahydrocannabinol (triOH-THC)	-	-	-
57	CN57	Delta-9-tetrahydrocannabinolic acid-C4-A (THCA-C4-A)	-4.18	MET374, PHE134 II-II interaction	Val373, Trp224, Phe134, Ile133, Ile305, Ala306, Phe221, Val369, Val370, Leu372, Leu477
58	CN58	Delta-9-tetrahydrocannabinolic acid-C4-B (THCA-C4-B)	-	-	-
59	CN59	Delta-9-tetrahydrocannabinolic acid-A (THCA-C1-A)	-	-	-
60	CN60	Delta-9-tetrahydrocannabinolic acid-B (THCA-C1-B)	-	-	-
61	CN61	Nabilone	-	-	-
62	AR1	Androstenedione	-6.67	MET374, II-II interaction TRP224	Val373, Trp224, Phe134, Ile133, Ile305, Ala306, Phe221, Val369, Val370, Leu372, Leu477
63	AR2	Fadrozole	-3.06	MET374, II-II interaction TRP224	Val373, Met374, Trp224, Phe134, Ile133, Ile305, Ala306, Phe221, Val369, Val370, Leu372, Leu477

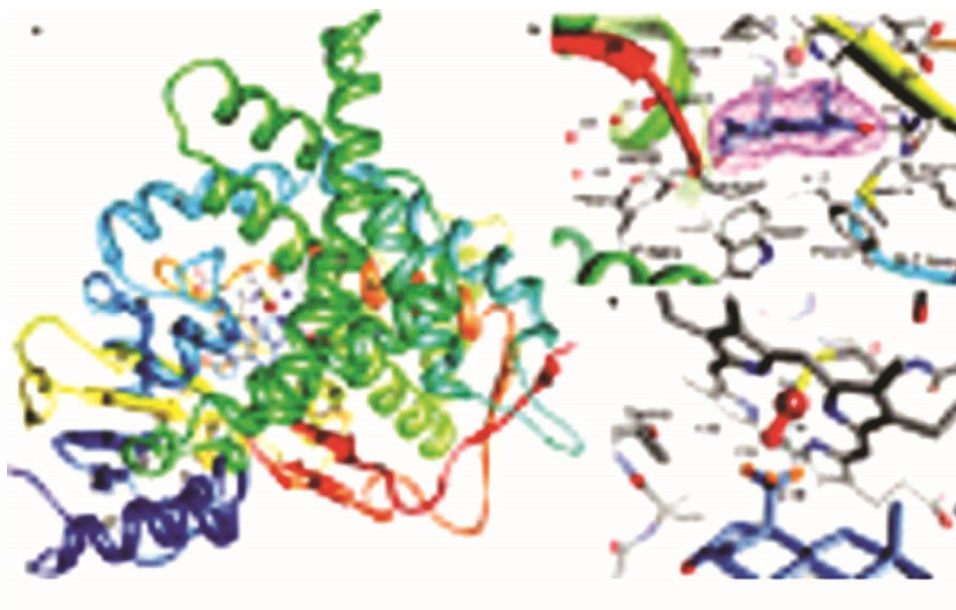


Figure 1. Structure of the PDB:3EQM protein.

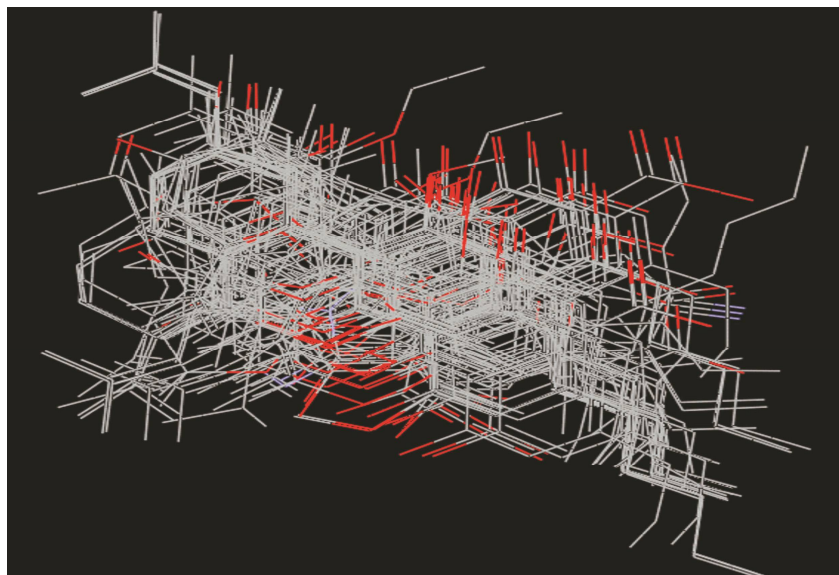


Figure 2. Ionized Ligands (Cannabinoids and standard) prepared for docking (displayed in line style) (ash-carbon, red- oxygen, blue-nitrogen; hydrogen- not shown).

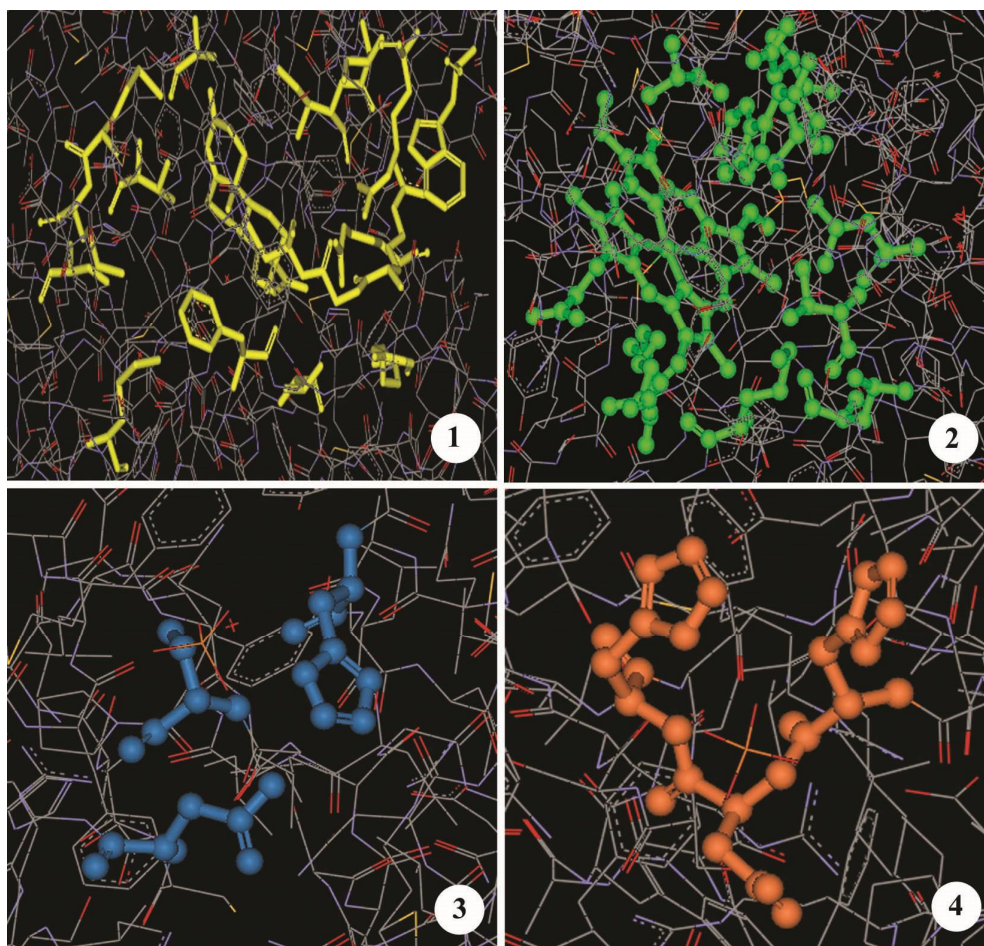


Figure 3. Zone of active sites of the aromatase enzyme docked with molecules. (Yellow-Active site 1 (HEM), Green-Active site 2 (HEM), Blue- Active site 3, two PO4, Orange- Active site 4, co-crystal ligand).

Drug-likeness and pharmacokinetic prediction (ADMET) analysis. In drug discovery technique, the concept of greater drug-likeness was used for post virtual screening of a chemical database. The *in silico* approach consists of a physicochemical filters like Lipinski's rule of five³¹ or pharmacokinetic filters like absorption, distribution, metabolism and excretion and toxicity (ADMET) property were applied to select drug-like compounds.³² Promising drug candidates often fail due to unfavorable ADMET properties. Computational QSAR models for predicting ADMET properties were used for prediction of ADMET parameters.³³ Before selecting the hit compounds, the ADMET properties of compounds were predicted using the QikProp 3.9 module³⁴ to check molecular weight, logPo/w, logHERG (potential hERG channel blockage leads to QT syndrome), and human oral absorption of the compounds.

Collection of plant sample and extraction. The plant material, *Cannabis sativa* (Family-Cannabaceae) was collected with the help of Department of Narcotics Control, Government of the Peoples's Republic of Bangladesh after getting prior permission. According to th Department of Narcotics Control, the sample was collected from Kushtia district, Bangladesh. The weight of the dried sample when received, was 550 gram. The sample was verified by the National Herbarium of Bangladesh with an accession number (DACB 38696).

The dried sample was then grinded into powder and sieved to yield a finer grade. Then it was weighed, divided into three portions and packed for extraction. Soxhlet apparatus³⁵, a very solvent efficient way of extraction by recycling a small amount of solvent, was used for the extraction. On the basis of the solubility of the target compounds, ethanol was used and 10 cycles were run for three portions and combined. The non-soluble portion of the extracted solid remaining in the thimble was dried and returned to the Department of Narcotics Control as per the terms and condition.

The alcoholic soluble extract was dried over baked magnesium sulfate and subjected to rotary

evaporation. After solvent evaporation, a gummy mass of about 40 gram was obtained. The gummy material was transferred to Claisen distillation flask for fractional distillation. A good number of oily fractions were obtained at different temperature and subjected for mass spectroscopic studies regarding molecular identification of the target compounds.

Cytotoxicity assay of the fractionated compounds. The bioactivity of the fractionated compounds was evaluated by the brine shrimp lethality test.³⁶ Eleven washed and numbered test tubes were taken for each sample. Two mg of each fraction was weighed and dissolved in 1000 μ l dimethyl sulfoxide (DMSO). Then 800 μ l of the dissolved fractions were put inside the first test tube making 1000 μ l. From this, 500 μ l was transfer to second tube and made the solution 10mL giving a concentration of 40 μ g/ml in first tube. The transfered 500 μ l was made 1000 μ l in second test tube by adding DMSO. Then again 500 μ l from the second was taken to third test tube and giving a concentration of 20 μ g/ml in second test tube. The same process was repeated for all the test tubes and the 11th one was left blank with only DMSO in it. Then, 10 brine shrimp larvae were counted and put into each of them and the final volume was adjusted to 10 mL by adding artificial sea saltwater. Thus, the concentrations of the fractionated compounds were 40-, 20-, 10-, 5-, 2.5-, 1.25-, 0.625-, 0.3125-, 0.1563- and 0.0781 μ g/ml.

Then the test tubes were incubated at room temperature for 24 hrs. The tubes were then examined under a magnifying glass and the number of dead nauplii in each tube counted. Vincristine sulfate was used as a positive control in all experiments. The concentration of vincristine sulfate was same as that of test samples. Experiments were conducted for 3 times and the average values were used to determine the LC₅₀ value for each of the fraction.

Statistics. The experimental results of the brine shrimp lethality assay were expressed as the mean value \pm SD (n = 3). The one-way ANOVA test was performed to determine the significant mean

difference ($n = 3$) between the samples followed by Tukey's multiple comparisons test by using graph pad prism 6.0.

RESULTS AND DISCUSSION

Molecular docking study. Docking score and catalytic interactions (Ala306, Val370, Leu372, Val373, Met374, Leu377, Leu477 and Hem600) were observed and compared between ligand compounds and receptors and the standard drugs fadrozole (AR1) and androstenedione (AR2). All the compounds (high and low activity) formed at least one hydrogen bond with Met 374. But the difference in the inhibitory activity between different compounds depend on the steric clashes of the compounds in the active site with important amino-acid residues as well as most importantly with the iron atom of the heme moiety. Apart from the hydrogen bond formation with the oxygen present on the aromatic ring system, other parts of the molecules were stabilized by hydrophobic interactions with the non-polar amino acids (Ala306, Trp224, Val369, Val370, Ile133, Phe134). This is in agreement with previous observations with non-steroidal aromatase inhibitors.³⁷

Molecular docking has the potential to predict accurate binding affinities of screening hits as well as potentially reveal lead structures with novel modes of binding interaction.²³ After docking all dataset ligands along with two standard drug, fadrozole (AR1) and androstenedione (AR2), docking scores were obtained which represents their 'affinity' or 'potentiality' for binding aromatase. Out of 61 cannabinoids, 21 compounds (CN1, CN2, CN3, CN5, CN6, CN7, CN9, CN10, CN12, CN14, CN16, CN17, CN22, CN39, CN41, CN42, CN43, CN53, CN54, CN55 and CN57) showed a very close score to standard aromatase inhibitor (AR1 and AR2). The clustered column chart of their docking scores (Table 1) is shown in Figure 4.

Finally it is seen that only 14 compounds have docked score greater than 6 which are more nearer with the AR1 compound. Upon analysis of the docking results, it was seen that compounds which

bound with the heme showed a good docking score, and their hydrophobic interaction was similar to the standard compound. The only difference is that there was no interaction with the heme in case of the standard compounds (AR1 and AR2). It is reported that the hydrophobic residues and porphyrin rings of haem pack tightly, forming a cavity in shape to the bound steroid, androstenedione (AR2). But it is established that close interaction with the haem Fe^{+3} (3.42 \AA) in the active site of the enzyme is very much essential requirement for the aromatase inhibition.³⁵ Similarly, the researchers proved the importance of molecular hydrophobicity for inhibition of aromatase enzyme.³⁸ It is seemed that compounds that bind with the heme showed better anticancer property than

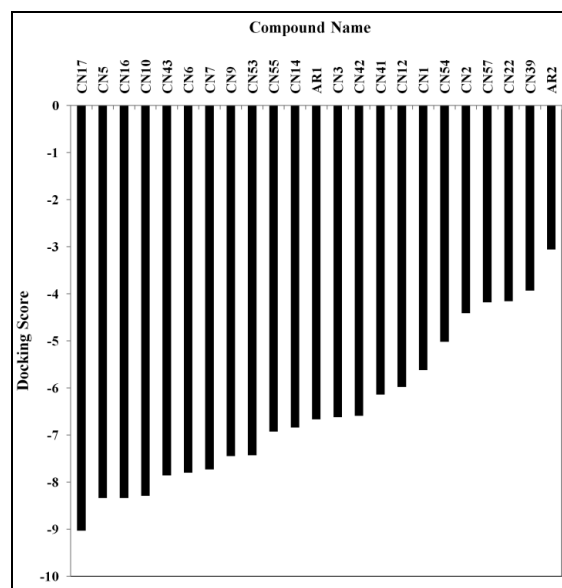


Figure 4. Clustered column chart indicating the docking scores of 21 cannabinoids (CNs) and standards (AR1 and AR2).

others. It is due to the fact that after binding to the heme, the oxygen carrying capacity of the heme will be reduced, which causes the cell death that is the only purpose of the anticancer drugs. So, this docking analysis with their interaction between receptor and ligand molecules approved the previous study.

Drug-likeness and ADMET study. Finally, all 14 compounds were checked for their drug-likeness through the Lipinski filter rule and only the 3 compounds such as cannabidiol (CN 17, CBD-

C1), cannabitrinol (CN 43, CBT) and cannabiripsol (CN 55, CBR) (Figure 5) had been passed (Table 2) and then they were used for further ADMET test by Qikprop.

ADMET analysis³⁴, which yielded 3 active hit molecules, showed the all the acceptable

pharmacokinetic parameters, such as molecular weight, logPo/w, logHERG and human oral absorption, which are essential for drug-likeness prediction, are shown in Table 3. The binding interaction of the promising molecules are represented in Figure 6.

Table 2. Analysis of drug-like property.

Drug-like	Failed compounds
CN17 Passed	CN3 Lipinski Failure: partition coefficient > 5
CN43 Passed	CN5 Lipinski Failure: partition coefficient > 5
CN55 Passed	CN6 Lipinski Failure: partition coefficient > 5
AR1 Passed	CN7 Lipinski Failure: partition coefficient > 5
AR2 Passed	CN9 Lipinski Failure: partition coefficient > 5
	CN10 Lipinski Failure: partition coefficient > 5
	CN14 Lipinski Failure: partition coefficient > 5
	CN16 Lipinski Failure: partition coefficient > 5
	CN41 Lipinski Failure: partition coefficient > 5
	CN42 Lipinski Failure: partition coefficient > 5
	CN53 Lipinski Failure: partition coefficient > 5

Table 3. The ADMET profiles of the promising compounds.

Hit molecule	Mol_MW ^a	logPo/w ^b	logHERG ^c	PCaco ^d	%HumanOralAbsorption ^e	RuleOffFive ^f
CN43	346.47	3.95	-4.74	1384.19	100	0
CN17	258.36	3.85	-4.17	3062.65	100	0
CN55	348.48	3.93	-4.71	1131.26	100	0

^aMolecular weight (acceptable range <500). ^bPredicted octanol/water partition coefficient log p (acceptable range from -2.0 to 6.5). ^cPredicted value for blockage of HERG K⁺ channels (concern below -6.5). ^dPredicted Caco-2 cell permeability in nm/s (acceptable range: <25 is poor and >500 is great). ^ePercentage of human oral absorption (<25% is poor and >80% is high). ^fRule of five (no. of violations of Lipinski's rule of five: 0 is good and 4 is bad).

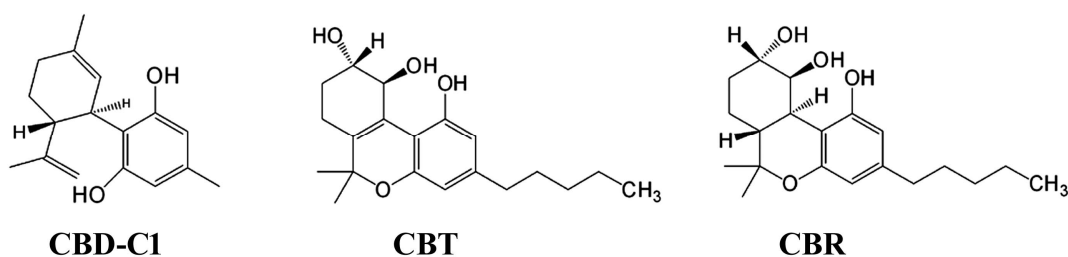


Figure 5. Structures of cannabidiol (CBD-C1), cannabitrinol (CBT) and cannabiripsol (CBR).

The target compounds are cannabidiol (CN 17), cannabitrinol (CN 43) and cannabiripsol (CN 55). *C. sativa* was collected from Department of Narcotics Control as mentioned earlier. The dried sample was extracted with ethanol in a Soxhlet apparatus. The alcoholic portion (extract) was dried over baked

magnesium sulfate and evaporate to dryness. The gummy crude mass obtained after evaporation of solvent was transferred to Claisen distillation flask for fractional distillation. A number of oily fractions were obtained at different temperature and subjected for mass spectroscopic analysis. The spectrometry

was employed to analyze the precursor ions under investigation. The ions were identified as cannabidiol (CN 17), cannabitril (CN 43) and cannabiripsol (CN 55) with m/z values 259.352, 347.485 and 349.365, respectively for the corresponding pseudomolecular ion as $[M+H]^+$.

Cytotoxicity assay of the target compounds.

The cytotoxicity of cannabidiol (CN 17), cannabitril (CN 43) and cannabiripsol (CN 55) were evaluated through brine shrimp lethality bioassay

technique where vincristine sulfate was used as positive control. Cannabidiol exhibited LC_{50} value of $0.348 \pm 0.002 \mu\text{g/ml}$ ($R^2 = 0.985$) which is almost similar to vincristine sulfate ($LC_{50} = 0.316 \pm 0.003 \mu\text{g/ml}$, $R^2 = 0.988$), whereas cannabitril also showed promising cytotoxicity with LC_{50} of $0.650 \pm 0.004 \mu\text{g/ml}$ ($R^2 = 0.988$) in comparison to reference standard. However, cannabiripsol exhibited relatively weaker activity with LC_{50} of $12.95 \pm 1.234 \mu\text{g/ml}$ ($R^2 = 0.990$) (Table 4).

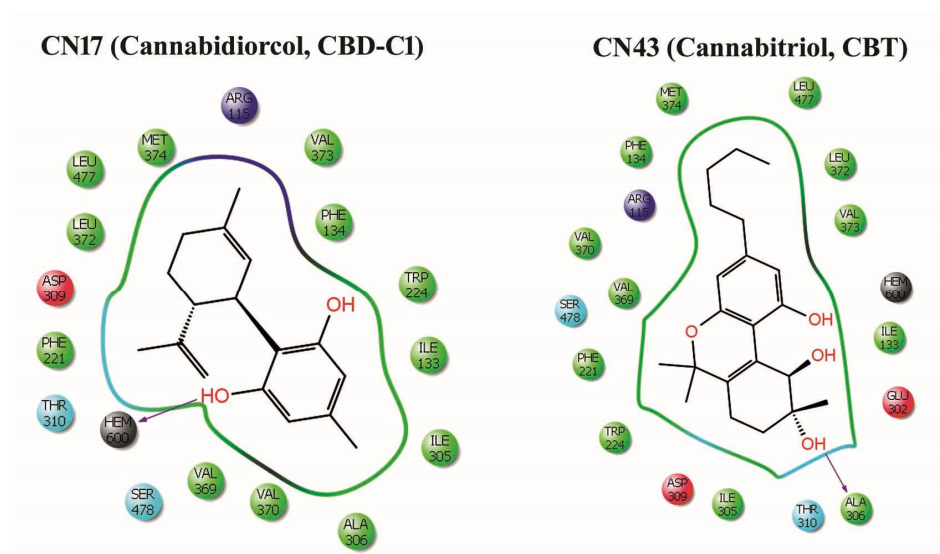


Figure 6. Binding interactions of the promising CN 17 (Cannabidiol, CBD-C1) and CN 43 (Cannabitril, CBT) molecules at the catalytic site.

Table 4. LC_{50} value of cannabidiol (CN 17), cannabitril (CN 43) and cannabiripsol (CN 55) and standard from brine shrimp lethality bioassay.

Test samples	Regression line	R^2	LC_{50} ($\mu\text{g/ml}$) (Mean \pm SD)
Cannabidiol	$y = 4.5389x + 7.164$	$R^2 = 0.985$	0.348 ± 0.002
Cannabitril	$y = 36.003x + 63.016$	$R^2 = 0.988$	0.650 ± 0.004
Cannabiripsol	$y = 40.342x - 2.112$	$R^2 = 0.990$	12.95 ± 1.234
Vincristine sulphate	$y = 37.89x + 68.695$	$R^2 = 0.988$	0.316 ± 0.003

CONCLUSION

Breast cancer has become a life threatening disease to every human being in the recent years. In the present study, the selection of new cannabinoids as an anticancer drug through virtual screening technique and docking study helped us to explore some new molecules after their ADMET checking.

Finally, three molecules, cannabidiol, cannabitril and cannabiripsol have been proposed as better candidates than the other. Subsequently, this has been evaluated in the laboratory by brine shrimp lethality assay and these three compounds have been proved to have significant cytotoxic activity.^{36,39} From this observation, it can be concluded that the lead

compounds may be developed as potent aromatase inhibitors for performing their biological evaluation.

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CONFLICT OF INTEREST

There is no conflict of interest among the researchers who have contributed in this work.

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