European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 10 , 2020

Lead Compound Analysis Of Frankincense Oil Into Cancer Cells With Molecular Docking

Adeeb M*[1], Neelam Tripathi[2], Sunil Kumar Shah[3]

[1]Ph.D. Scholar, Department of Biotechnology, Sri Satya Sai University of Technology & Medical Sciences, Madhya Pradesh, India.

[2]Professor, University Teaching Department, Sri Satya Sai University of Technology & Medical Sciences, Madhya Pradesh, India.

[3]Associate Professor. College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Madhya Pradesh, India.

Abstract

Oil was extracted from Frankincense (Boswellia Sacra) by soxhelet extraction method and various bioactive compounds were identified using gas chromatography (GC).These compounds can be developed as active pharmacophore. The present study involves extraction and identification of bioactive compounds and their invitro study on the interaction these compounds to target proteins. Various biological databases like Pubchem, Protein Data Bank and softwares like Argus Lab, Rasmol were used to retrieve and analyse the structural and molecular interactions of bioactive compounds from Frankinsense oil with receptor proteins. Target protein structure were retrieved from Protein data bank ligand structures were downloaded from Pubchem which was visualized using Rasmol Software. Protein ligand interaction was studied using Argus Lab software by docking simulations and various docking poses were analysed. The energy values of docking conformations were analyzed for obtaining best docking pose & score.

Keywords: Frankincense oil, Soxhelet extraction, Gaschromatography, Docking, Protein Data Bank, Rasmol, Argus Lab

Introduction

Frankincense resin has been considered throughout the ages to have a wealth of health supporting properties. The resins of Boswellia carteri and Boswellia serrata have been used for the treatment of rheumatoid arthritis and other inflammatory diseases such as Crohn's disease[1-2]

The anti-inflammatory activity has been attribute to the resin's ability in regulating immune cytokines production [3] and leukocyte infiltration. Boswellia serrata extract also exhibits anti-bacterial and anti-fungal activities [4]. Additionally, extracts from Boswellia species gum resins

might possess anti-cancer activities, based on their anti-proliferative and pro-apoptotic activities in rat astrocytoma cell lines [5] and in human leukemia cell lines [6], as well as their anti-carcinogenic activity in chemically induced mouse skin cancer models [7]. Clinically, extract from the resin reduces the peritumoral edema in glioblastoma patients and reverses multiple brain metastases in a breast cancer patient [8]. These results suggest that frankincense resin contains active ingredients that modulate important biological activities. Docking is the process by which two molecules fit together in 3D space [9]. In addition to docking, the atomicaffinity grid scan be visualized. This can help, for example, to guide organic synthetic chemists design better binders. Consider an active site on a usually large receptor molecule, and a ligand molecule, which could be small or large. The general question is how snugly the ligand fits into the active site. Quality of fit has a geometric and a chemical component. The geometric component measures how well the surface shapes complement each other as a handing love. Docking functions are believed to be the essential component of docking algorithms. The approach to study both molecular mechanics and statistical potentials are applied. Structure-Based Drug Design is based on a firm understanding of molecular recognition between active site groups and interacting molecules and is a strategy that has become an integral part of modern drug discovery. Due to the recent volume and pace at which the 3-D structures of protein targets and their co-crystals have been made available, coupled with advances in computation tools, Structure-Based Drug Design has become a tool for lead generation.

Materials and methods:

1.1 Extraction of essential oil from Frankinsense

100 g of Frankincense resin were taken in the thimble and kept in the siphon of the soxhelet apparatus and 250 ml of hexane solvent was taken in 500 ml round bottom flask which was placed in a heating mantle. The siphon is connected with reflux condenser to condense the vapours of hexane solvent to react with Frankinsense resin inside the thimble. The reaction temperature was set on the heating mantle based on the boiling point of solvent and kept for 4 hours. Oils separated from resin were collected back in the round bottom flask along with solvent. Later oil is separated from solvent using rotavapour equipment.(Figure 1).Separated oil was collected in test tubes for chemical profiling using gas chromatography.

Frankincense Resin Soxhelet Extraction Separation by Rotavapour Oil sample

Figure 1. Soxhlet extraction of essential oil from frankincense resin

1.2 Chemical profiling of heavy oil by gas chromatography coupled with mass spectrometry (GC– MS)

GC MS analysis was done for the extracted oil on a Perkin Elmer Clarus 600 GC system with RTx capillary column(30m x 0.25mm inner diameter 0.25µm film thickness; with maxium temperature of 350°C) coupled to a Perkin Elmer Clarus600C MS.Ultra high purity helium(99.999%) was used as carrier gas at constant flow rate of 1.0 mL/min.The injection, transfer line and ion source temperatures were 270°C, 240°C and 240°C, respectively. The ionizing energy was 70 eV .Electron multiplier voltage was obtained from auto tune. All data were obtained by collecting the full-scan mass spectra within the scan range 40–550 amu. The injected sample volume was 1 mL with a split ratio of 50:1. The oven temperature program was 60°C and accelerated at a rate of 3°C min to a final temperature of 240°C
The unknown compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9thedition).

1.3 Invitro studies on molecular interactions of bioactive component of Frankincense oil with potential cancer receptor sites using Bioinformatics and Argus lab software

Boswellic acid(BA) component of frankincense oil which is considered to be a promising pharmacophore was selected to study its interaction various receptor sites.BA structure was retrieved from pubchem and visualized using RASMOL software. Structures of Human 5 Lipoxygenase receptor (PDB id: 3V92) and Human Cyclin Dependent Kinase 2 receptor (PDB id: 1Gii) were retrived from Protein Data Bank (PDB) and was visualized using RASMOL software. Protein ligand complex of both receptors were visualized using Ligplot software. Argus lab software was used to study the molecular interaction of BA to active site of both receptors and was evaluated using molecular dynamics and their binding affinities using free energy simulations.

1.4 Statistical Analysis

19 independent experiments were performed in order to ensure the reproducibility. Each experiment consisted of three factors and two responses. Experimental data were evaluated by ANOVA quadratic model. Significant difference between each set of data were considered at the confidence level of $P < 0.5$ and $P < 0.1$.

Results

2.1 Chemical profiling of frankincense derived heavy oil by GC MS

36 constituents were identified from frankincense heavy oil. The results of GC–MS analyses showed that a major portion of heavy oil were composed of terpenes (Table 1). Themain constituents were DELTA.3-Careneα-pinene (85.99%),δ-3-Carene (2.50%),D-Limonene(2.33%), β-Pinene (1.90%) and camphene (1.65 %).This observation was clearly evidenced from GC chromatogram (Figure 2)

Table 1 : Chemical composition of heavy oil derived from frankincense

Name	Rt (min)	Area	%
Beta-Myrcene	4.52	66352.35	0.09

.alpha.-Thujene	4.68	133096.1	0.19
alpha-Pinene	4.83	61485972	85.99
Camphene	5.14	1182193	1.65
alpha-Phellandrene	5.7	678001	0.95
.beta.-Pinene	5.78	1356002	1.90
beta-Phellandrene	6.49	555105.9	0.78
.DELTA.3-Carene	6.62	1850353	2.59
D-Limonene	7.14	1664696	2.33
Myrtenyl acetate	9.41	395192	0.55
Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)-	10.66	459129.8	0.64
Isobornyl acetate	14.99	137465.5	0.19
alpha-Cubebene	17.8	21043.73	0.03
.beta.-Bourbonene	18.06	127630.3	0.18
beta-Elemene	18.3	574730.4	0.80
trans-Caryophyllene	19.11	186969.7	0.26
.alpha.-Humulene	20.15	45689.67	0.06
Germacrene D	20.98	27159.1	0.04
(+)-Aromadendrene	21.13	147283.3	0.21
.alpha.-Selinene	21.42	62461.6	0.09
.delta.-Cadinene	22.22	55292.09	0.08

Figure 2. GC chromatogram of frankincense resin derived heavy oil

2.2 Molecular docking of BA to active sites using Argus Lab Software

Boswellic acid structure was retived from pubchem and was docked with Human Cyclin Dependent Kinase 2 receptor (Figure 3) and 5 Lipoxygenase receptor (Figure 4) using Argus lab software. The best pose was analysed based on the least binding energy conformations (e value).It was found that for when BA was docked Human Cyclin Dependent Kinase 2 receptor the e value was -4.84562 kcal/mol and e value for BA docked with 5 lipooxygenase was 4.82704 kcal/mol. The receptors sites are composed of amino acids which are hydrophobic. This property contributes for the active interaction of ligand (BA) with the receptors.

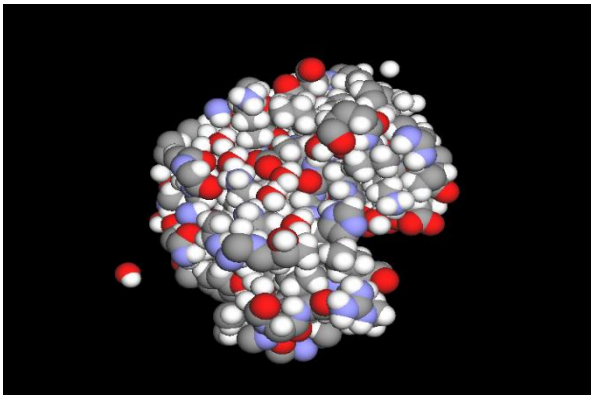


Figure 3: 1Gii and BA Argus dock result Pose 1

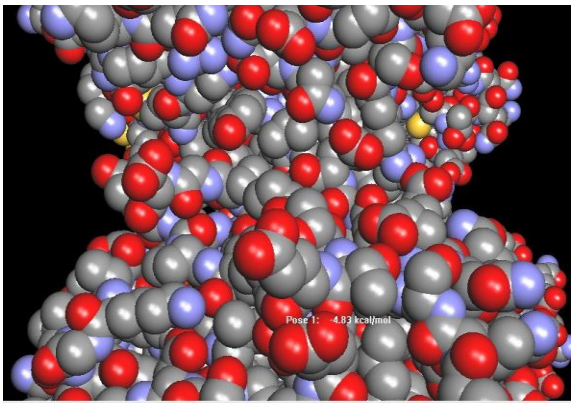


Figure 4: 3V92 and BA Argus dock result Pose 1

2.3 Statistical analysis of extraction process using Response Surface Methodology (RSM)

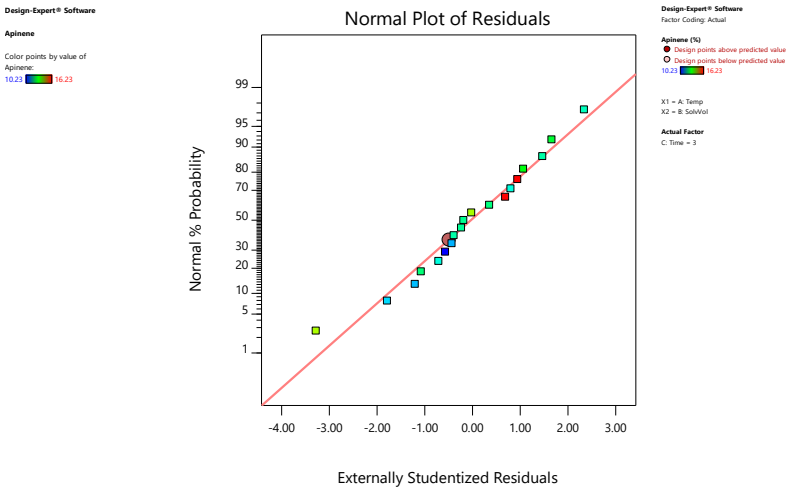
ANOVA for Quadratic model

Response 1: Apinene

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	39.53	9	4.39	10.13	0.0010	significant
A-Temp	7.26	1	7.26	16.74	0.0027	
B-SolvVol	0.0314	1	0.0314	0.0723	0.7941	
C-Time	6.13	1	6.13	14.13	0.0045	
AB	0.1953	1	0.1953	0.4503	0.5190	
AC	6.94	1	6.94	15.99	0.0031	
BC	1.58	1	1.58	3.63	0.0891	
A^2	3.57	1	3.57	8.23	0.0185	
B^2	2.48	1	2.48	5.72	0.0404	
C^2	17.10	1	17.10	39.43	0.0001	
Residual	3.90	9	0.4338			
Lack of Fit	2.57	5	0.5132	1.53	0.3496	not significant

Pure Error	1.34	4	0.3345			
Cor Total	43.43	18				

Factor coding is **Coded**. Sum of squares is **Type III – Partial** The **Model F-value** of 10.13 implies the model is significant. There is only a 0.10% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case A, C, AC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The **Lack of Fit F-value** of 1.53 implies the Lack of Fit is not significant relative to the pure error. There is a 34.96% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good which means model to fit.

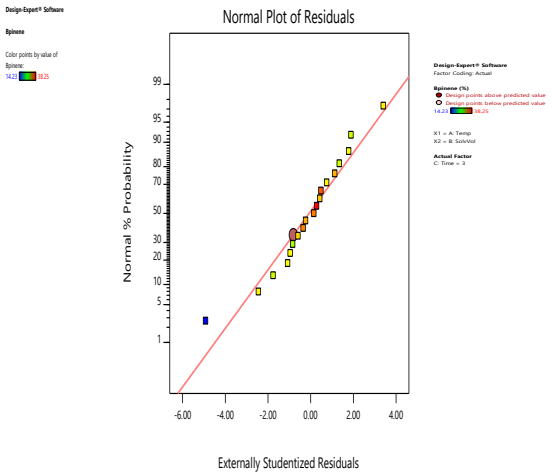


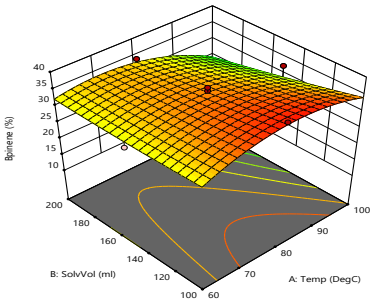
Response 2: Bpinene

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	338.72	9	37.64	4.55	0.0170	significant
A-Temp	21.70	1	21.70	2.62	0.1398	
B-SolvVol	59.73	1	59.73	7.22	0.0249	
C-Time	41.66	1	41.66	5.04	0.0515	
AB	45.36	1	45.36	5.48	0.0439	
AC	26.68	1	26.68	3.23	0.1061	
BC	33.99	1	33.99	4.11	0.0733	
A^2	52.23	1	52.23	6.31	0.0332	
B^2	0.2675	1	0.2675	0.0323	0.8613	
C^2	2.58	1	2.58	0.3122	0.5900	
Residual	74.44	9	8.27			

Lack of Fit	64.57	5	12.91	5.24	0.0669	not significant
Pure Error	9.87	4	2.47			
Cor Total	413.16	18				

Factor coding is **Coded**. Sum of squares is **Type III – Partial**. The **Model F-value** of 4.55 implies the model is significant. There is only a 1.70% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case B, AB, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The **Lack of Fit F-value** of 5.24 implies there is a 6.69% chance that a Lack of Fit F-value this large could occur due to noise.





Final Equation in Terms of Coded Factors

Apinene	=
+12.50	
+0.8520	A
-0.0560	B
-0.7830	C
+0.1563	AB
-0.9312	AC
-0.4437	BC
-1.14	A^2
-0.9533	B^2
+2.50	C^2

Bpinene	=
+34.89	
-1.47	A
-2.44	B
+2.04	C
-2.38	AB
+1.83	AC
+2.06	BC
-4.37	A^2
+0.3129	B^2
-0.9721	C^2

Discussion

Essential oil was extracted from frankinsense resin soxhlet extraction method by setting the dependent factors namely temperature, solvent volume and time.Chemical profiling was done by GCMS analysis. A-pinene was found to be major constituent in heavy oil. Molecular docking of active compound with target receptors like Human Cyclin Dependent Kinase 2 receptor and 5 Lipoxygenase receptor using Argus lab software and binding confirmations was analysed based least binding energy values. Statistical analysis using ANOVA was done to find the model for extraction using design expert software and response surface methodology (RSM).

Conclusion

Anticancer drug from plant sources play a key role in drug discovery against various type of cancer conditions. The present study includes bioactive compound extraction and its molecular interactions with various biological receptors using molecular docking software. These studies conclude bioactive compounds can be develoloped into pharmaphore and further administerd for clinical trials.t

Acknowledgment:

We would like to thank Faculty of Science, Sri Satya University of Medical Sciences and Technology for carrying out the research work**.**

Conflict of interest:

We declare that there is no conflict of interest

References:

1. Banno N, Akihisa T, Yasukawa K, Tokuda H, Tabata K, Nakamura Y, Nishimura R, Kimura Y, Suzuki T. Anti-inflammatory activities of the triterpene acids from the resin of Boswellia carteri. J Ethnopharmacol. 2006;107:249–253.
2. Langmead L, Rampton DS. Review article: complementary and alternative therapies for inflammatory bowel disease. Aliment Pharmacol Ther. 2006;23:341–349.
3. Chevrier MR, Ryan AE, Lee DY, Zhongze M, Wu-Yan Z, Via CS. Boswellia carterii extract inhibits TH1 cytokines and promotes TH2 cytokines in vitro. Clin Diag Lab Immunol. 2005;12:575–580.
4. S.A.F. El-Nagerabi, A.E. Elshafie, S.S. AlKhanjari, S.N. Al-Bahry, M.R. ElaminBiological activities of Boswellia sacra extracts on the growth and aflatoxins secretion of two aflatoxigenic species of Aspergillus speciesFood Control, 34 (2) (2013), pp. 763-769
5. Ni X, Suhail MM, Yang Q, Cao A, Fung KM, Postier RG, frankincense essential oil prepared from hydrodistillation of Bos-wellia sacra gum resins induces human pancreatic cancer cell death in cultures and in a xenograft murine model. BMC Complement Altern Med 2012; 12: 253.
6. Suhail MM, Wu W, Cao A, Mondalek FG, Fung KM, Shih PT,et al. Boswellia sacra essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured humanbreast cancer cells. BMC Complement Altern Med 2011; 11: 129.

7. Sabra SM, Al-Masoudi LMR. The effect of using frankincense (Boswellia sacra) chewing gum on the microbial contents of buccal/oral cavity, Taif, KSA. IOSR J Dent Med Sci 2014; 13(4):77-82.
8. Siddiqui MZ. Boswellia serrate, a potential antiinflammatory agent: an overview. Ind J Pharm Sci 2011; 73(3): 255-61.
9. Gray, J. J., Moughon, S., Wang, C., Schueler-Furman, O., Kuhlman, B., Rohl, C. A., Baker, D., Protein-protein docking with simultaneous optimization of rigid-body displacement and side-chain conformations. Journal of Molecular Biology 331: (1) 281-99 Aug 1 2003
10. R. Salomon-Ferrer, D.A. Case, R.C. Walker. An overview of the Amber biomolecular simulation package. WIREs Comput. Mol. Sci. 3, 198-210 (2013)
11. Shoichet BK, Kuntz ID, Bodian DL (2004). "Molecular docking using shape descriptors". Journal of Computational Chemistry. 13 (3): 380–397.
12. Ballante F, Marshall GR (Jan 2016). "An Automated Strategy for Binding-Pose Selection and Docking Assessment in Structure-Based Drug Design". Journal of Chemical Information and Modeling 2016, 56 (1), pp 54–72
13. LEADS-PEP: A Benchmark Data Set for Assessment of Peptide Docking Performance Alexander Sebastian Hauser and Björn Windshügel Journal of Chemical Information and Modeling 2016 56 (1), 188-200
14. Indrani Kar (2017) Extracts of Seven Indian Plants Inhibit Human Topoisomerase I and Partially Inhibit Human Topoisomerase II. Mol Enz Drug Tar 3:1
15. Miteva MA, Violas S, Montes M, Gomez D, Tuffery P, et al. (2006) FAF- Drugs: free ADME/tox filtering of compound collections. Nucleic Acids Res 34: W738-W744.