



Frankincense essential oil extraction and lead compound analysis into cancer cells using molecular docking

Adeeb M^{*1}, Sunil Kumar Shah², Sahab Ahmed³

¹Department of Biotechnology, Sri Satya Sai University of Technology and Medical Sciences, Madhya Pradesh, India

²Department of Pharmacy, Sri Satya Sai University of Medical Sciences & Technology, Madhya Pradesh, India

³Department of Botany, Sri Satya Sai University of Technology and Medical Sciences, Madhya Pradesh, India



Article History:

Received on: 15.09.2019

Revised on: 10.12.2019

Accepted on: 16.12.2019

Keywords:

Frankincense oil,
Soxhlet extraction,
Gas chromatography,
Docking,
Protein Data Bank,
Rasmol,
Argus Lab

ABSTRACT

Oil was extracted from Frankincense (*Boswellia Sacra*) by the soxhlet extraction method and various bioactive compounds were identified using gas chromatography (GC). These compounds can be developed as active pharmacophores. The present study involves the extraction and identification of bioactive compounds and their in-vitro study on the interaction of these compounds to target proteins. Frankincense oil collected from *Boswellia Sacra* species was subjected to Soxhlet extraction using Hexane solvent and essential oil (EO) was separated using vacuum distillation. Chemical profiling of essential oil was done using GC-MS. Various biological databases like PubChem, Protein Data Bank and software like Argus Lab, RasMol were used to retrieve and analyze the structural and molecular interactions of bioactive compounds from Frankincense oil with receptor proteins. The target protein structure was retrieved from Protein data bank ligand structures that 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 analyzed. The energy values of docking conformations were analyzed for obtaining the best docking pose & score.

*Corresponding Author

Name: Adeeb M

Phone:

Email: adeebmb@gmail.com

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i1.1939>

Production and Hosted by

IJRPS | www.ijrps.com

© 2020 | All rights reserved.

INTRODUCTION

Frankincense resin has been considered throughout the ages to have a wealth of health-supporting prop-

erties. 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 (Banno *et al.*, 2006; Langmead and Rampton, 2006). The anti-inflammatory activity has been attributed to the resin's ability to regulate immune cytokines production (Chevrier *et al.*, 2005) and leukocyte infiltration. *Boswellia serrata* extract also exhibits anti-bacterial and anti-fungal activities (El-Nagerabi *et al.*, 2013). 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 (Ni *et al.*, 2012) and in human leukemia cell lines (Suhail *et al.*, 2011), as well as their anti-carcinogenic activity in chemically induced mouse skin cancer models (Mostafa *et al.*,

2014). Clinically, extract from the resin reduces the peritumoral edema in glioblastoma patients and reverses multiple brain metastases in a breast cancer patient (Siddiqui, 2011).

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 (Gray *et al.*, 2003). In addition to docking, the atomic affinity grids can be visualized. This can help, for example, to guide organic synthetic chemists to 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 chemical component. The geometric component measures how well the surface shapes compliment each other like a hand in glove. 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

Extraction of essential oil from Frankincense

100 g of Frankincense resin was taken in the thimble and kept in the siphon of the soxhlet 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 a reflux condenser to condense the vapors of hexane solvent to react with Frankincense resin inside the thimble.

The reaction temperature was set on the heating mantle based on the boiling point of the 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 the solvent using rota vapor equipment (Figure 1). Separated oil was collected in test tubes for chemical profiling using gas chromatography.

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 0.25 μ m film thickness; with maximum temperature of 350°C) coupled to a Perkin Elmer Clarus 600C 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 autotune. 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, 9th edition).

Invitro studies on molecular interactions of a 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 with 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 retrieved from Protein Data Bank (PDB) and was visualized using RasMol software.

The protein-ligand complex of both receptors was visualized using Ligplot software. Argus lab software was used to study the molecular interaction of BA to the active site of both receptors and was evaluated using molecular dynamics and their binding affinities using free energy simulations (Shoichet *et al.*, 1992).

Statistical Analysis

19 independent experiments were performed in order to ensure 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 was considered at the confidence level of $P < 0.5$ and $P < 0.1$.

RESULTS AND DISCUSSION

Chemical profiling of frankincense derived heavy oil by GC MS.

36 constituents were identified from heavy frankincense oil. The results of GC-MS analyses showed

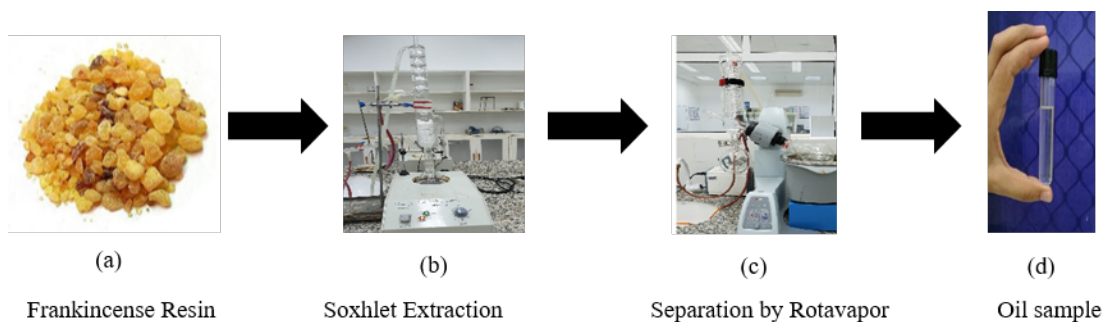


Figure 1: Soxhlet extraction of essential oil from frankincense resin

- (a) Frankincense resin was crushed to powder
 (b) Soxhlet extraction was done extraction of oil
 (c) Essential oil separated from solvents using Rotavapor
 (d) Essential Oil collected in tubes for chemical profiling.

that a major portion of heavy oil was composed of terpenes (Table 1). The main constituents were DELTA.3-Carene α -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 by GC chromatogram (Figure 2).



Figure 2: GC chromatogram of frankincense resin derived heavy oil

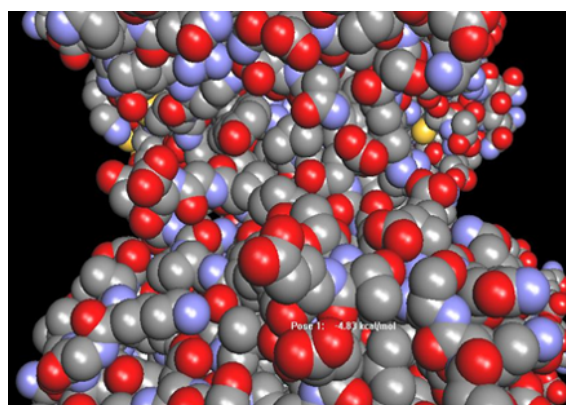


Figure 4: 3V92 and BA Argus dock result Pose 1

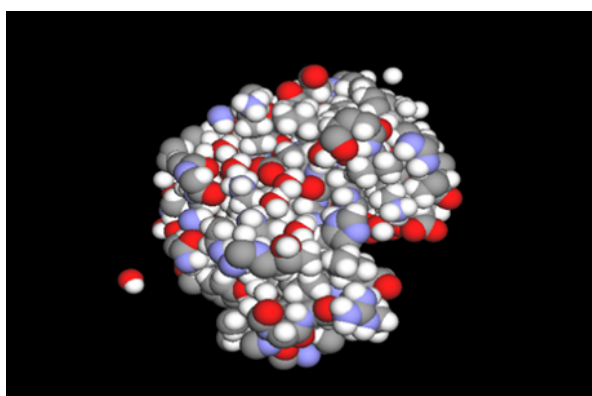


Figure 3: 1Gii and BA Argus dock result Pose 1

Molecular docking of BA to active sites using Argus Lab Software

Boswellic acid structure was retrieved from Pub-Chem and was docked with the Human Cyclin-Dependent Kinase 2 receptor (Figure 3) and 5 Lipoxygenase receptors (Figure 4) using Argus lab software. The best pose was analyzed 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 lipoxygenases was 4.82704 kcal/mol. The receptors sites are composed of amino acids that are hydrophobic.

This property contributes to the active interaction of ligand (BA) with the receptors (Ballante and Marshall, 2016). Essential oil was extracted from the frankincense 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 a major constituent in heavy oil. Molecular docking of the active compound with target receptors like Human Cyclin-Dependent Kinase 2 receptor and 5 Lipoxygenase receptor (Kar et al., 2018) using Argus lab software and binding confirmations was analyzed based least binding energy values.

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-methyl ethyl)-, (1 α ,2 α ,5 α)- | 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-Element | 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 |

CONCLUSION

Anticancer drugs from plant sources play a key role in drug discovery against various types 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 developed into pharmacophore and further administered for clinical trials.

ACKNOWLEDGEMENT

We would like to thank the Faculty of Pharmacy, 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

- Ballante, F., Marshall, G. R. 2016. An Automated Strategy for Binding-Pose Selection and Docking Assessment in Structure-Based Drug Design. *Journal of Chemical Information and Modeling*, 56(1):54-72.
- Banno, N., Akihisa, T., Yasukawa, K., Tokuda, H., Tabata, K., Nakamura, Y., Suzuki, T. 2006. Anti-inflammatory activities of the triterpene acids from the resin of *Boswellia carteri*. *Journal of Ethnopharmacology*, 107(2):249-253.
- Chevrier, M. R., Ryan, A. E., Lee, D. Y., Zhongze, M., Wu-Yan, Z., Via, C. S. 2005. *Boswellia carterii* extract inhibits TH1 cytokines and promotes TH2 cytokines in vitro. *Clinical and Diagnostic Laboratory Immunology*, 12:575-580.
- El-Nagerabi, S. A. F., Elshafie, A. E., Alkhanjari, S. S., Al-Bahry, S. N., Elamin, M. R. 2013. Biological activities of *Boswellia sacra* extract on the growth and aflatoxins secretion of two aflatoxigenic species of *Aspergillus* species. *Food Control*, 34(2):763-769.
- Gray, J. J., Moughon, S., Wang, C., Schueler-Furman, O., Kuhlman, B., Rohl, C. A., Baker, D. 2003. Protein-Protein Docking with Simultaneous Optimization of Rigid-body Displacement and Side-chain Conformations. *Journal of Molecular Biology*, 331(1):670-673.
- Kar, I., Majumder, H. K., Chattopadhyaya, R. 2018. Extracts of Seven Indian Plants Inhibit Human Topoisomerase I and Partially Inhibit Human Topoisomerase II. *Molecular Enzymology and Drug Targets*, 3(1).

- Langmead, L., Rampton, D. S. 2006. Review article: complementary and alternative therapies for inflammatory bowel disease. *Alimentary Pharmacology and Therapeutics*, 23(3):341–349.
- Mostafa, M., Sabra, D. S., Al-Masoudi, R., M, L. 2014. The Effect of Using Frankincense (*Boswellia sacra*) Chewing Gum on the Microbial Contents of Buccal/Oral Cavity, Taif, KSA. . *IOSR Journal of Dental and Medical Sciences*, 13(4):77–82.
- Ni, X., Suhail, M. M., Yang, Q., Cao, A., Fung, K. M., Postier, R. G., Lin, H. K. 2012. Frankincense essential oil prepared from hydrodistillation of *Boswellia sacra* gum resins induces human pancreatic cancer cell death in cultures and in a murine xenograft model. *BMC Complementary and Alternative Medicine*, 12(1):1212–1212.
- Shoichet, B. K., Kuntz, I. D., Bodian, D. L. 1992. Molecular docking using shape descriptors. *Journal of Computational Chemistry*, 13(3):380–397.
- Siddiqui, M. Z. 2011. *Boswellia serrata*, a potential antiinflammatory agent: An overview. *Indian Journal of Pharmaceutical Sciences*, 73(3):255–61.
- Suhail, M. M., Wu, W., Cao, A., Mondalek, F. G., Fung, K. M., Shih, P. T., Lin, H. K. 2011. *Boswellia sacra* essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells. *BMC Complementary and Alternative Medicine*, 11(1):129–129.