GC-MS Analysis for Identification of Active Compounds in Propolis and Molecular Docking Studies of Selected Compounds against Chronic Hepatitis B Protein (Large Envelop Protein)

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J. Flora Priyadarshini, K. Sivakumari,
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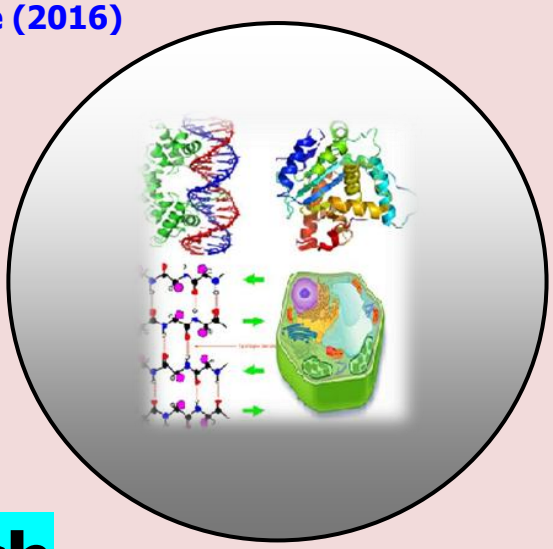

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K. Sivakumari
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RESEARCH PAPER

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GC-MS Analysis for Identification of Active Compounds in Propolis and Molecular Docking Studies of Selected Compounds against Chronic Hepatitis B Protein (Large Envelop Protein)

J. Flora Priyadarshini, K. Sivakumari, K. Ashok, P. Jayaprakash and S. Rajesh

Department of Zoology, Presidency College, Chennai-5, Tamil Nadu, India

ABSTRACT

The present study was aimed to identify the active compounds present in propolis by GC-MS analysis and analyzing the binding interaction of ligands viz., 1,2- Benzenedicarboxylic acid and Desmethyldeprenyl of two compounds present in propolis with the chronic hepatitis B (large envelop protein). GC-Ms analysis showed the presence of 13 volatile compounds in propolis methanolic extract with different RT, molecular weight and peak area. Docking studies revealed that there is a presence of binding site between these two ligands and the protein. Hydrogen bond formation was good in the two ligands, when docked with the protein. The result of Lipinski rule suggests the analyzed compound as best therapeutic drug. Docking study result proves the application of 1, 2- Benzenedicarboxylic acid and Desmethyldeprenyl compounds as potential and natural therapeutic agents to treat disease.

The results obtained from this study would be useful in both understanding the inhibitory mode as well as in rapidly and accurately predicting the activities of new inhibitors on the basis of docking scores. Analysis of propolis ligand binding interaction with the large envelop protein can be useful for new preventive and therapeutic drug for cancer.

Key words: Propolis, GC-MS, Molecular docking and large envelop protein.

INTRODUCTION

Propolis is a natural product that belongs to the great family of bee products. The word Propolis is a complex term originating from two ancient Greek words: *pro*- standing for "before or in defense" and *the polish meaning city*. Thus, in apiculture, its meaning refers to the harboring of the hive. Propolis is a sticky, resinous substance, collected from various floral sources that are transformed and used by honey bees to construct and maintain their hives by sealing holes in their honeycombs. It is also used for smoothing out the internal walls and shelter the entrance of the hive from intruders. Trends and development in Propolis research have been reviewed by Bankova (2005). Propolis is a traditional remedy in alternative medicine that has been used for centuries in Egypt, Greece, and other countries as well. Propolis possesses antimicrobial (Veiga *et al*., 2017), antioxidative (Veiga *et al*., 2017), anti-inflammatory (Wang *et al*., 2014), tuberculosis infection (Yildirim *et al*., 2004), lifestyle related disorders (Saeed *et al*., 2016), antiproliferative and proapoptotic activity (Demir *et al*., 2016), innate immune responses (Soltani *et al*., 2017), anti-leishmanial activity (Cuesta-Rubio *et al*., 2017),

multi-drug resistant microbial pathogens (Issam *et al.,* 2015), cytotoxic (Dos Santos *et al*., 2017), contact allergy (De Groot, 2013), anti-tumor activity (Bassani Silva *et al*., 2007), dermatophytosis in dog (Cruz Sánchez *et al.,* 2014), acute giardiasis (Abdel Fattah and Nada, 2007), anti-protozoans (Gressler *et al*., 2012) and anti-ulcer (Alfaris *et al*., 2009).

Currently, the use of synthetic chemicals to control Hepatitis B concerns related to human health. An alternative is the use natural products that possess good efficacy and are environmentally friendly. Among those chemicals, volatile compounds from propolis have been tested to assess their antiviral (Hepatitis B) properties as a valuable natural resource as opined by Al Naggara *et al*. (2016). In view of this, the main objective of the present investigation was to identify the natural antiviral compounds from propolis and to find its binding interaction against Hepatitis B protein (large envelope protein) by *in silico* molecular docking studies.

MATERIALS AND METHODS

Collection and Extraction of Propolis

Commercially available pure propolis powder (Stakich organic) was purchased from USA and 10 gram propolis was mixed with 100 ml of methanol and kept in kept in a orbital shaker for 3 days and the extract was filtered through a Whatman No. 1 filter paper and the filtrate was stored at 4ºC until further studies.

GC-MS Analysis

The methanolic extract of propolis was used for GC-MS analysis. 2 µl of the methanolic extract of the propolis was dissolved in HPLC grade methanol (≥99.9%) and subjected to GC and MS. JEOL GCMATE II GC-MS (Agilent Technologies 6890 N). The column (HP5) was fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min, at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1µl) was evaporated in a split less injector at 300°C. Run time was 22 min. The compounds were identified by gas chromatography coupled with mass spectrometry. The molecular weight and structure of the compounds of test materials were ascertained by the interpretation of mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. By making use of this the name, molecular weight and structure of the components of the test materials were ascertained.

In silico Molecular Docking

In silico molecular docking is the process in which the molecules fit together in 3D space. It is a key tool in structural biology and computer-aided drug design. In this study, two compounds which were identified by GC-Ms were selected to assess their interaction with large envelope protein. The sequence of Large envelope protein (Swissprot ID: P03140) was retrieved from Swissprot database. The three dimensional structure of large envelope protein (PDB ID: 1WZ4) was downloaded from PDB Database. The domain Major surface antigen from heptoadenovirus (region 30-395) is identified using PFam database. The Ligand compounds structure was drawn using ACD ChemSketch and converted in to PDB format using Open Babel. Structure Data Format (SDF) files were used for representing multiple chemical structure records and associated data fields, 3D-Structure visualization of protein was done by RASMOL. The 3D structure of large envelope protein was docked with two inhibitors viz., 1,2-Benzenedicarboxylic acid and Desmethyldeprenyl using AutoDock Software. The Docking results were analyzed using PyMol visualization tool.

RESULTS AND DISCUSSION

GC-MS Analysis

The peak of compounds and retention times present in methanolic extract of propolis was identified by GC-MS analysis and are reported in chromatogram (Fig. 1). The composition of the volatile compounds with retention time, molecular formula and molecular weight are presented in Fig. 2 to 9. The GC-MS showed the presence of 13 volatile compounds, which were identified as Desulphosinigrin (RT-12.17), Ethyl iso-allocholate (RT-14.27), 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]- (RT-16), Pentadecanoic acid, 14-methyl-, methyl ester (RT-17.23), 1-Monolinoleylglycerol trimethylsilyl ether (RT-17.62), 16-Octadecenoic acid, methyl ester (RT-19.07), Ethyl iso-allocholate (RT-25.35), [22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione (RT-28.27)).

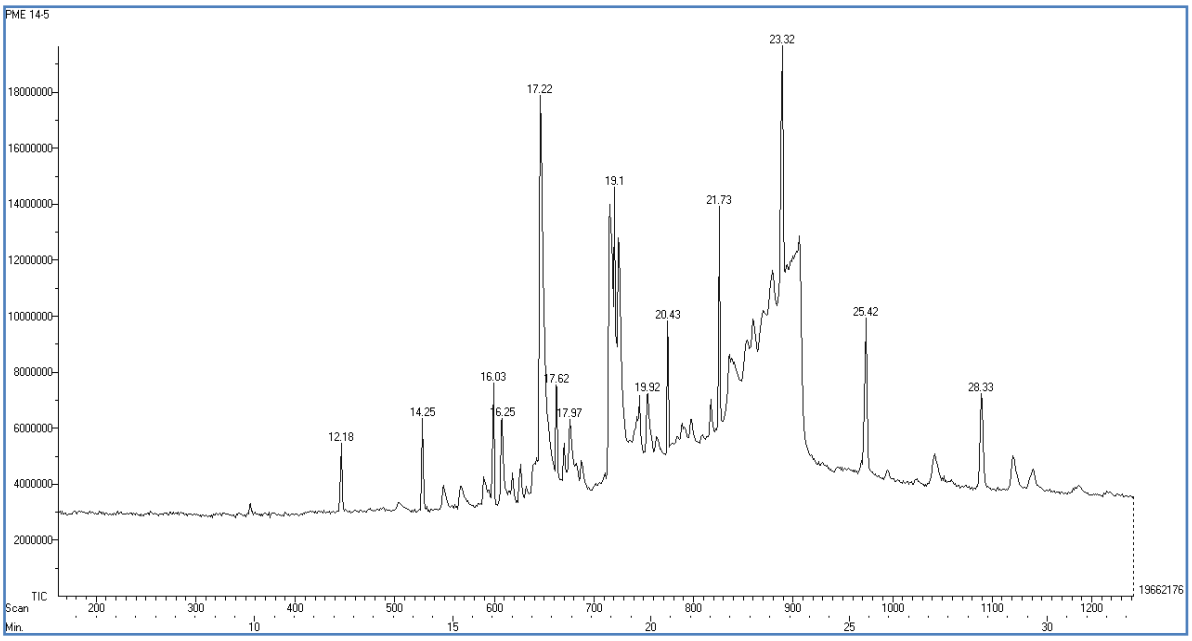

Figure 1. GC-MS Chromatogram of propolis methanolic extract.

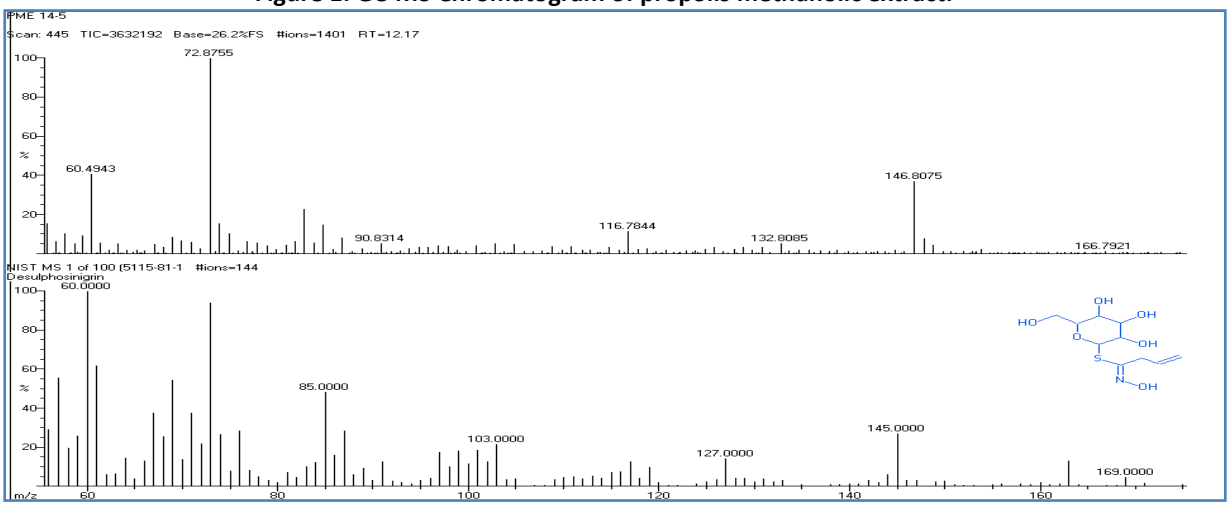

Figure 2. Mass spectra of Desulphosinigrin (RT-12.17).

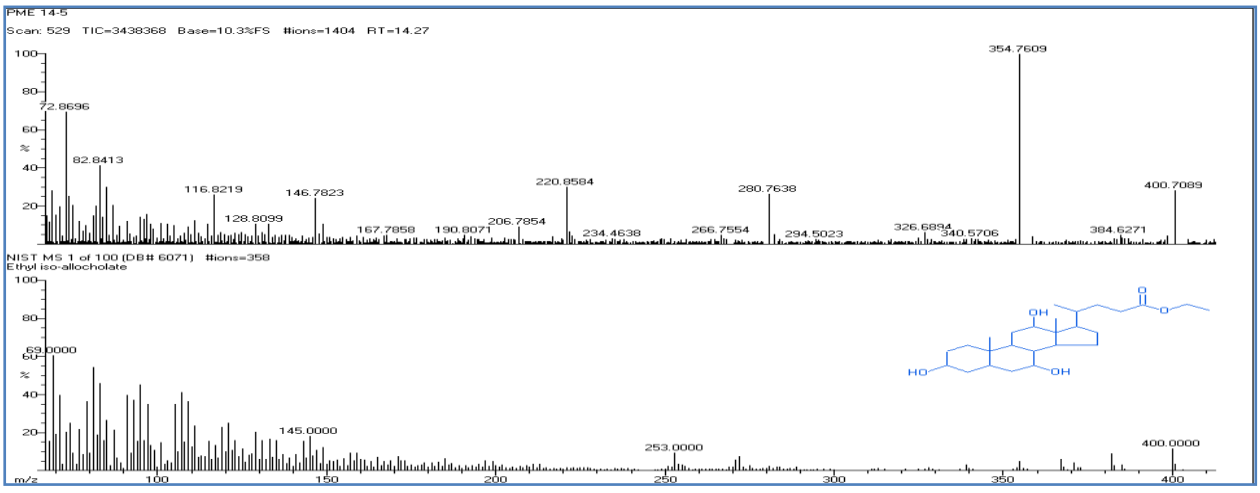

Figure 3. Mass spectra of Ethyl iso-allocholate (RT-14.27).

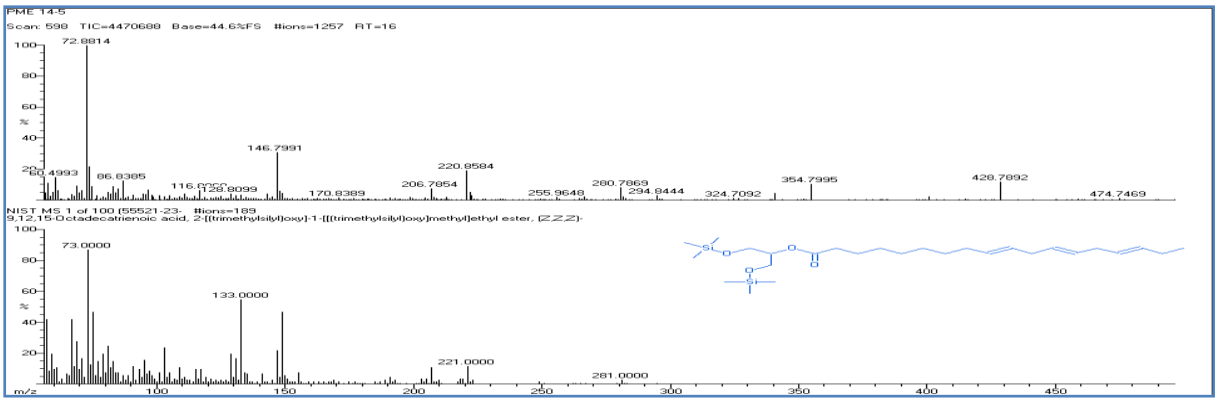

Figure 4. Mass spectra of 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]methyl]ethyl ester, [ZZZ]- (RT-16).

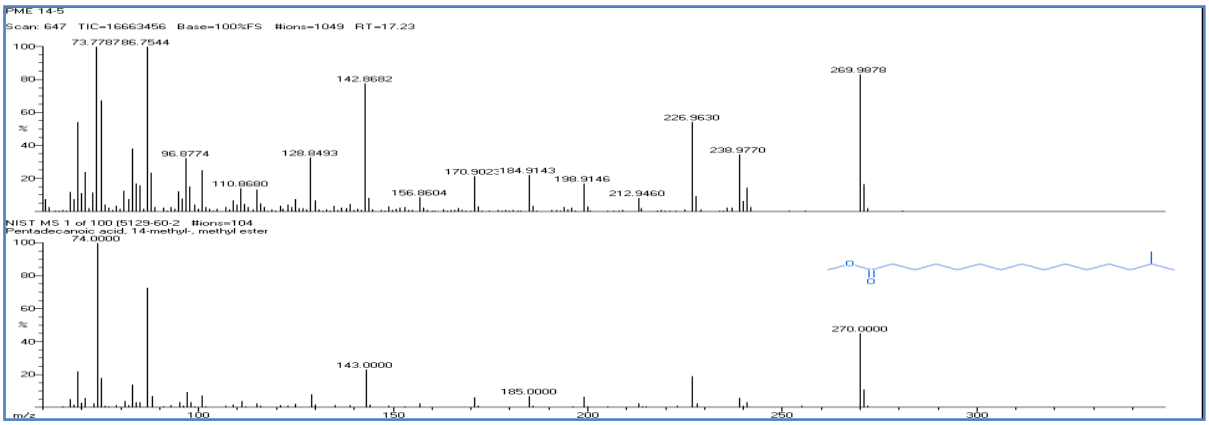

Figure 5. Mass spectra of Pentadecanoic acid (RT-17.23).

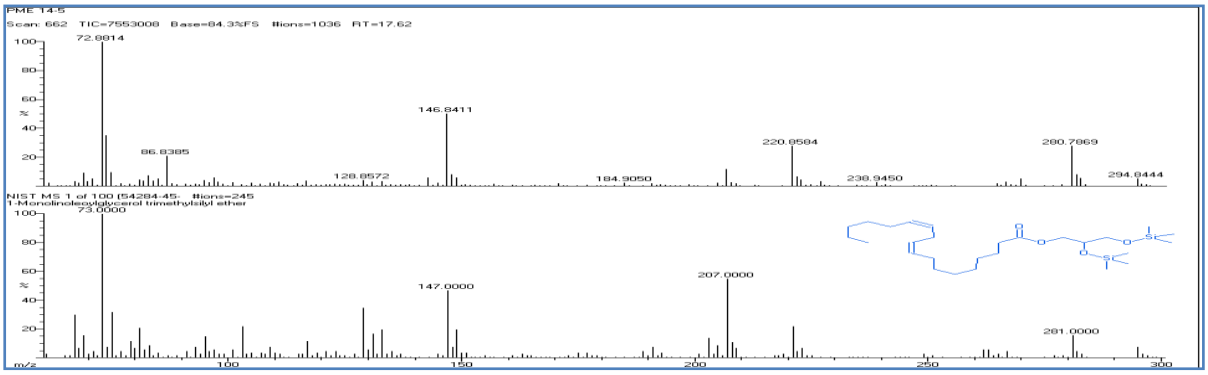

Figure 6. Mass spectra of 1-Monolinoleylglycerol trimethylsilyl ether (RT-17.62).

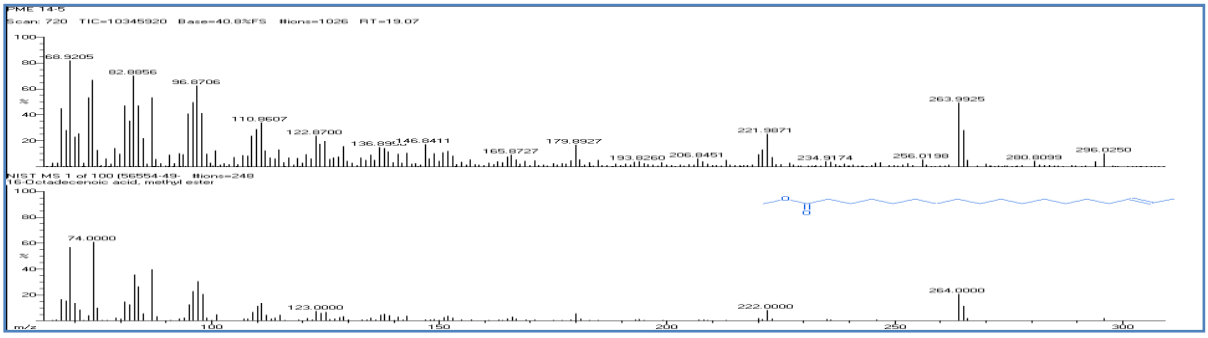

Figure 7. Mass spectra of 16-Octadecenoic acid, methyl ester (Rt-19.07).

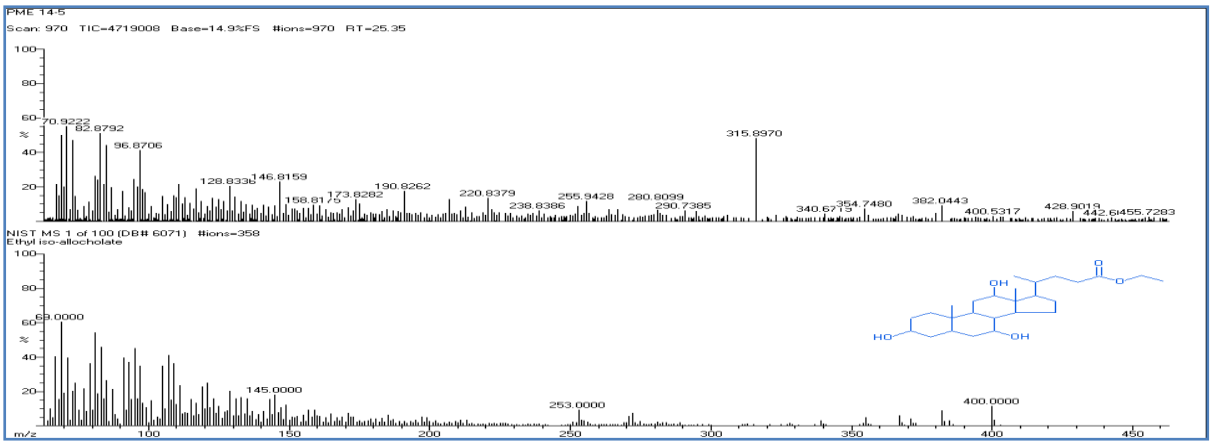

Figure 8. Mass spectra of Ethyl iso-allocholate (RT-25.35).

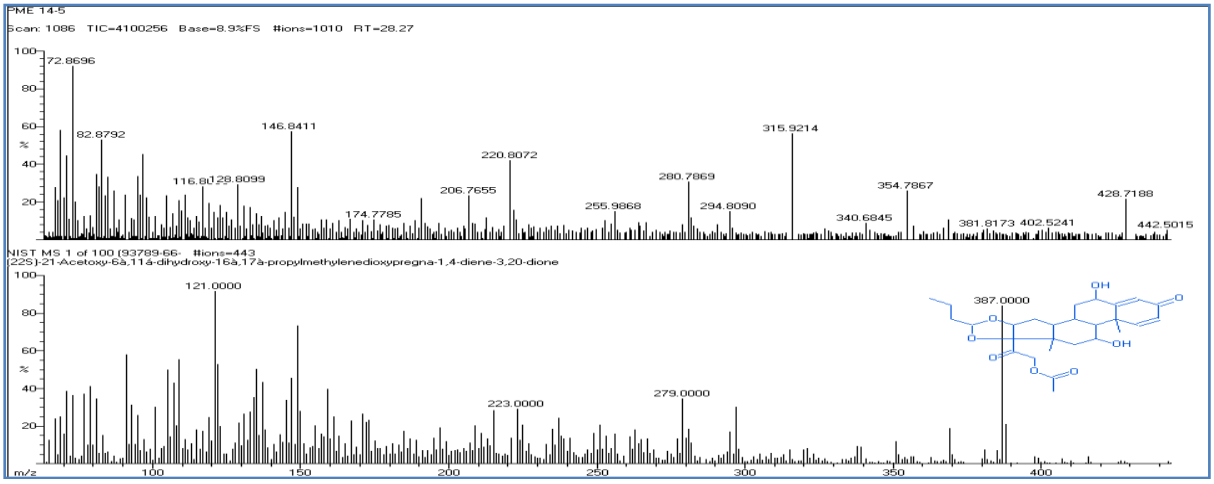

Figure 9. Mass spectra of [22S]-21-Acetoxy- 6a, 11a -dihydroxy-16a, 17a-propylmethylenedioxy pregna-1, 4-diene-3,20-dione (RT-28.27).

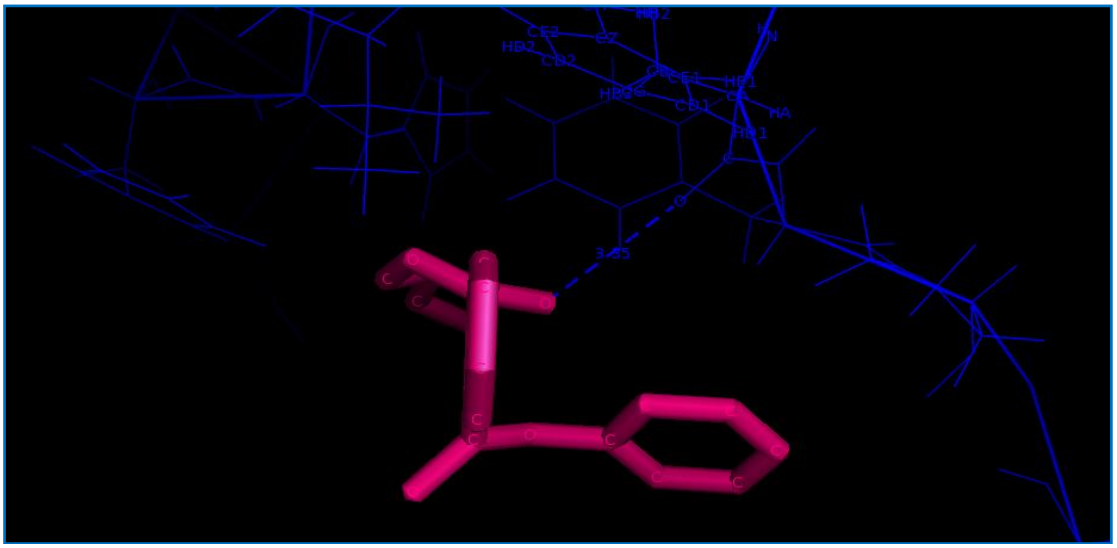

Figure 10. Final docked structure of large envelope protein with 1,2-Benzenedicarboxylic acid. Pictorial representation of docked complex using PYMOL tool where, protein is in blue color, 1,2-Benzenedicarboxylic acid in pink color and the H-Bond is indicated by blue color dots.

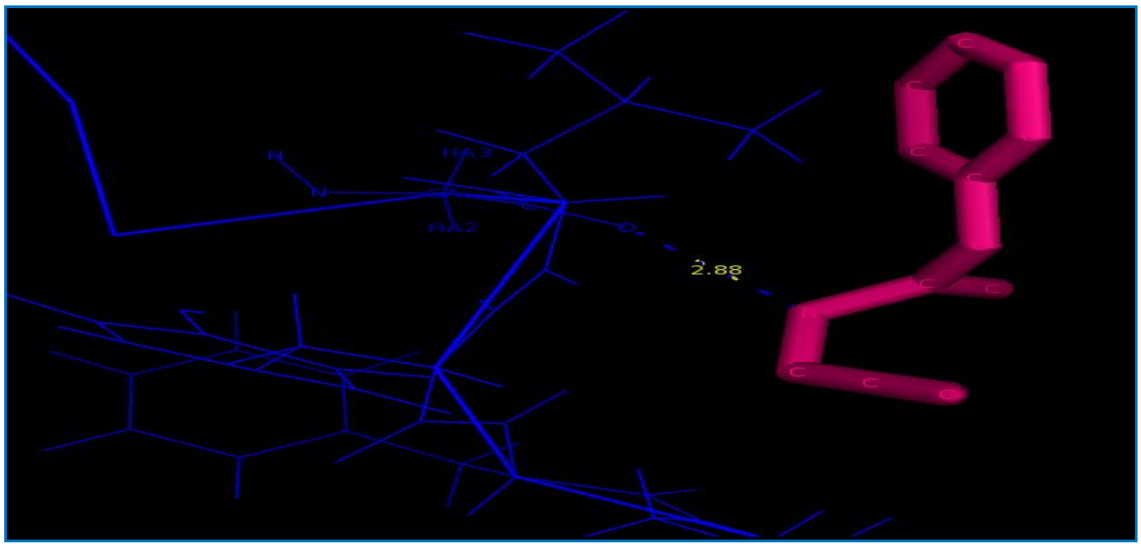

Figure 11. Pictorial representation of docked complex using PYMOL tool where, protein is in blue color, Desmethyldeprenyl acid in pink color and the H-Bond is indicated by blue color dots

Table 1. Molecular docking of 1, 2-Benzenedicarboxylic acid and Desmethyldeprenyl acid against large envelope protein.

Name of the protein	Name of the compound	Distance	Docking score K Cal/Mol	H-Bond
Large envelop protein	1,2-Benzenedicarboxylic acid	3.35	-1.63	1
	Desmethyldeprenyl acid	2.88	-1.83	1

The GC-MS showed the presence of 13 volatile compounds, which were identified as Desulphosinigrin (RT-12.17), Ethyl iso-allocholate (RT-14.27), 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]- (RT-16), Pentadecanoic acid, 14-methyl-, methyl ester (RT-17.23), 1-Monolinoleylglycerol trimethylsilyl ether (RT-17.62), 16-Octadecenoic acid, methyl ester (RT-19.07), Ethyl iso-allocholate (RT-25.35), [22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione (RT-28.27)). Similar observations were also reported by El Hady and Hegazi (2000) in GC-MS profiling of East Nile Delta Propolis and Jordan *et al*. (2002) in aqueous essence and fruit juice of *Passiflora edulis* by GC-MS and GC/O profiling. The GC-MS as a simple method and the chromatographic and mass spectral characteristics of the diterpenes identified are important and useful tools for rapid chemical characterization of this propolis type. It has potential also as an instrument for revealing its plant sources. Further research, aimed to find out these sources, including observation of bee behavior, is needed. Detailed studies of the biological activity of this propolis type are in progress.

Propolis has a long history of medicinal use, since the time of Aristotle. It has many medicinal uses today, although its effectiveness has only been shown for a couple of them. Propolis is also used as an anti-viral, anti-bacterial, antiparasitic, anti-human immunodeficiency virus, anti-HIV, anti-tumor, anti-hypertension, anti-inflammatory, antiseptic, analgesic, and anti-cardiovascular disease. By using GC-MS the various chemical compound has been identified. From that two chemical compounds namely (Desmethyldeprenyl and 1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester) have been selected for the docking studies based on the medicinal properties *i.e.,* antimicrobial and antifouling activity. On the other hand probably the bioactive property of propolis probably is related to flavonoid follow this regard, several papers supported our finding concerning to antimicrobial property of flavonoid, tanin, and steroid (Markham *et al.,* 1996; Vassya *et al*., 2000; Silvia *et al.,* 2013) Many studies have analysed the propolis compounds using GC-MS (Murat 2002; Park *et al.* 2002) and HPLC methods (Medana *et al.* 2008) since the propolis compounds has varied polarity.

The large envelope protein exists in two topological conformations, one which is termed 'external' or Le-HBsAg and the other 'internal' or Li-HBsAg.

In its external conformation the protein attaches the virus to cell receptors and thereby initiating infection. This interaction determines the species specificity and liver tropism. This attachment induces virion internalization predominantly through caveolin-mediated endocytosis. The large envelope protein also assumes fusion between virion membrane and endosomal membrane (Probable). In its internal conformation the protein plays a role in virion morphogenesis and mediates the contact with the nucleo-capsid like a matrix protein.

It is interesting to know that *in silico* molecular docking studies of compound present in propolis methanolic extract against large envelop protein has not carried out and this is the first report that is recorded. *In silico* molecular docking study revealed that the interaction of 1, 2-Benzenedicarboxylic acid and Desmethyldeprenyl with large envelop protein formed 1 hydrogen bond whereas the interaction of Desmethyldeprenyl with large envelop protein showed higher docking score (-1.83 KCal/Mol) than the 1,2-Benzenedicarboxylic acid with the docking score of (-1.63 KCal/Mol) as shown in Table 1.,This result clearly indicates that there is binding site between the protein and ligands. The docking is also valid by the formation of one hydrogen bond between them. The result of Lipinski rule suggests the analyzed compound as best therapeutic drugs. Similar type of studies were performed with fucoidan compound against HepG-2 cell line proteins by Ashok and Sivakumari (2015), kappa-carrragenan present in *Kappaphycus alvarezii* against InhA enzyme by Mayakrishnan *et al*. (2015), quercetin compound against HeLa cell line proteins by Muthukala *et al*. (2015), resveratrol compound against KB cell line proteins by Manimaran *et al*. (2015), stearic acid against transferrin and plasminogen proteins present on HepG-2 cells present in *Cardiospermum halcacabum* by Rajesh *et al*. (2016) and rutin compound against apoptotic proteins (Tumor Necrosis Factor, Caspase-3, NF-Kappa-B, P53,Collagenase, Nitric oxide synthase and Cytochrome C).

According to Lipinski *et al*. (2001) the ligand should have good absorption with a log p value *i.e.,* partition coefficient below 5, molecular weight lower than 500 daltons and 10 H_2 bond acceptors *i.e.,* N_2 and H_2 atoms and based on these parameters the ligands can attain a drug-likness and also used to predict wheather a chemical or isolated compound possess a pharmacological or biological activity as an orally active drug in humans or not. In similar way our ligands also obeyed the rules of Lipinski to attain the nature of drug-likness.

CONCLUSION

This is the first report of *in silico* molecular docking studies of compound present in propolis methanolic extract against large envelop protein has not carried out and in future these compounds can be used as a potential and natural therapeutic agents to treat liver cancer.

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REFERENCES

Bankova, V. (2005). Recent trends and important developments in propolis research. *Evidence Based Complementary and Alternative Medicine*. **2:** 29-32.

Veiga, RS., De Mendonça, S., Mendes, PB., Paulino, N., Mimica, M.J., Lagareiro Netto, A.A., Lira, IS., López, BGC., Negrão, V., Marcucci, MC. and **Artepillin, C. (2017).** Phenolic compounds responsible for antimicrobial and antioxidant activity of green propolis and *Baccharis dracunculifolia* DC*. J. Appl. Microbiol*. **122:** 911-920.

Wang, K., Zhang, J., Ping, S., Ma, Q., Chen, X., Xuan, HZ., Shi, J., Zhang, C. and **Hu, F. (2014).** Anti-inflammatory effects of ethanol extracts of chinese propolis and buds from poplar (*Populus canadensis*). *J. Ethnopharmacol*. **155:** 300-311.

Yildirim, Z., Hacievliyagil, S., Kutlu, NO., Aydin, NE., Kurkcuoglu, M., Iraze, M. and **Durmazf, R. (2004).** Effect of water extract of Turkish propolis on tuberculosis infection in guinea-pigs. *Pharmacol. Res*. **49:** 287-292.

Saeed, F., Ahmad, R.S., Arshad, M.U., Niaz, B., Batool, R., Naz, R. and **Ansar Rasul Suleria, H. (2016).** Propolis to curb lifestyle related disorders: An overview. *Int. J. Food Prop*. **19:** 420-437.

Demir, S., Aliyazicioglu, Y., Turan, I.; Misir, S., Mentese, A., Yaman, SO. and **Deger, O. (2016).** Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. *Nutr. Cancer*. **68:** 165-172.

Soltani, EK., Cerezuela, R., Charef, N., Mezaache-Aichour, S., Esteban, MA. and **Zerroug, MM. (2017).** Algerian propolis extracts: Chemical composition, bactericidal activity and in vitro effects on gilthead seabream innate immune responses. *Fish Shellfish Immunol*. **62:** 57-67.

Cuesta-Rubio, O., Fernández, MC., Hernández, IM., Jaramillo, CGJ., González, VH., Porto, RMDO., Delange, DM., Fidalgo, LM., Piccinelli, AL. and **Campone, L. (2017).** Chemical profile and anti-leishmanial activity of three Ecuadorian propolis samples from Quito, Guayaquil and Cotacachi regions. *Fitoterapia* **120:** 177-183.

Issam, A.A., Zimmermann, S., Reichling, J. and **Wink, M. (2015).** Pharmacological synergism of bee venom and melittin with antibiotics and plant secondary metabolites against multi-drug resistant microbial pathogens. *Phytomedicine*. **22:** 245-255.

Dos Santos, L., Hochheim, S., Boeder, AM., Kroger, A., Tomazzoli, MM., Dal Pai Neto, R. and **de Cordova, C.M.** (2017). Chemical characterization, antioxidant, cytotoxic and antibacterial activity of propolis extracts and isolated compounds from the Brazilian stingless bees *Melipona quadrifasciata* and *Tetragonisca angustula*. *J. Apic. Res*. **56:** 543-558.

De Groot, AC. (2013). Propolis: A review of properties, applications, chemical composition, contact allergy, and other adverse effects. *Dermatitis.* **24:** 263-282.

Bassani Silva, S., Sforcin, JM. and **Amaral, AS. (2007).** Propolis effect *in vitro* on canine transmissible venereal tumor cells. *Revista Portuguesa de Ciências Veterinárias*.**102(563-564):**261-265.

Cruz Sánchez, TA., Estrada García, PA. and **López Zamora, CI. (2014).** Use of propolis for topical treatment of dermatophytosis in dog. *Open Journal of Veterinary Medicine*. **4(10):** 239-245.

Abdel Fattah, NS. and **Nada, OH. (2007).** Effect of propolis versus metronidazole and their combined use in treatment of acute experimental giardiasis. *J Egypt Soc Parasitol*. **37(2):** 691-710.

Gressler, LT., Da Silva, AS. and **Machado, G. (2012).** Susceptibility of *Trypanosoma evansito* propolis extract *in vitro* and in experimentally infected rats. *Res Vet Sci.* **93(3):**1314-1317.

Al Naggara, Y., Tanc, Y., Rutherfordc, C., Connore, W. and **Griebele, P. (2016).** Effects of treatments with Apivar and Hmovar on V. destructor populations, virus infections and indoor winter survival of Canadian honey bee (*Apis mellifera* L.) colonies. *J Apic Res*. **54:** 548-554.

El Hady, FKA. and **Hegazi, AG. (2000).** Egyptian propolis: 2. Chemical composition, antiviral and antimicrobial activities of East Nile Delta propolis. *Extraction*. **57:** 386-394.

Jordan, MJ., Goodner, KL. and **Shaw, PE. (2002).** Characterization of the aromatic profile in aqueous essence and fruit juice of yellow passion fruit (*Passiflora edulis*) by GC-MS and GC/O. *J. Agri. and Food Chem*., **50(6):** 1523-1528.

Markham, K., Mitchell, K., Wilkins, A., Daldy , J. and **lu. Y. (1996).** HPLC and GC-MS identification of the major organic constituents in New Zealand propolis. *Phytochemistry.* **12:** 205-211.

Vassya, S., Solangel , D., Cast, R. and **Mari, C. (2000).** Review article. Propolis: recent advances in chemistry and plant origin. *Apidologie*. **10:** 313-15.

Silvia, H., Marcos, J., Evandro, W., Izabel, Y. and **Dioneia. C. (2013).** Antimicrobial activity of flavonoids and sreroids isolated from two Chromolaena species. *Brazilian journal of pharmaceutical scinces*. **2:** 12-16.

Murat, K., Serdar, K. and **Semra, K. (2002).** GC-MS analysis of propolis samples from two different regions of Turkey. *Zeitschrift fur Naturforschung C-Journal of Biosciences*. **57(9-10):** 905-909.

Park, YK., Alencar, SM. and **Aguiar, CL. (2002).** Botanical origin and chemical composition of Brazilian propolis. *Journal of Agricultural and Food Chemistry*. **50(9):** 2502-2506.

Medana, C., Carbone, F., Aigotti, R., Appendino, G. and **Baiocchi, C. (2008).** Selective analysis of phenolic compounds in propolis by HPLC-MS/MS. *Phytochemical Analysis*. **19(1):** 32-39.

Ashok, K. and **Sivakumari, K. (2015).** *In silico* docking of fucoidan compound against the selective proteins of HepG-2 cell line. *Int. J. Chem. Pharm. Sci.,* **6(4):** 976-979.

Mayakrishnan, A., Sivakumari, K., Ashok, K., Prabhu Srinivasan. and **Vanaja Kumari. (2015).** Antimycobacterial activity of *Kappaphycus alvarezii* against *Mycobacterium tuberculosis* and *in silico* molecular docking of kappa-carrageenan against InhA enzyme. *Int. J. Drug Res. and Tech*., **5(1):** 35-46.

Muthukala, B., Sivakumari, K. and **Ashok, K. (2015).** *In silico* docking of Qucertin compound against the HeLa cell line proteins. *Int. J. Curr. Pharm. Res.,* **7(1):**13-16.

Manimaran, M., Sivakumari, K. and **Ashok, K. (2015).** Molecular docking studies of resveratrol against the human oral cancer cell line proteins (KB cells). *Int. J. Curr. Adv. Res.,* **4:** 2319-2325.

Rajesh, S., Sivakumari, K. and **Ashok, K**. (2016). *In silico* docking of selected compound from *Cardiospermum halicacabum* Linn. leaf against human hepatocellular carcinoma (HepG-2) cell line. *Int. J Comput Bioinfo In Silico Model.,* **5(2):** 780-786.

Jayameena, P., Sivakumari, K., Ashok, K. and **Rajesh, S. (2018).** *In silico* molecular docking studies of Rutin compound against apoptotic proteins (Tumor Necrosis Factor, Caspase-3, NF-Kappa-B, P53,Collagenase, Nitric oxide synthase and Cytochrome C). *J. Cancer Research and Treatment*. **6(2):** 28-33.

Lipinski, CA., Lombardo, F., Dominy, BW. and **Feeney, PJ. (2001).** Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev*., **46(3):** 3-26.

Corresponding author: Dr. K. Ashok, Department of Zoology, Presidency College, Chennai-5, Tamil Nadu, India.
Email: ashok1984biotech@gmail.com dr.sivakumari@rediffmail.com

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