GC-MS Analysis for Identification of Active Compounds in Propolis and Molecular Docking Studies of Selected Compounds against Apototic Proteins (Caspase-3, Caspase-9 and B-Actin)

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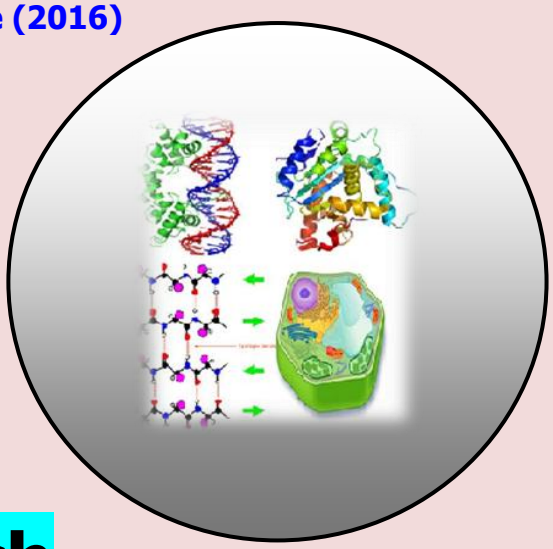

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RESEARCH PAPER

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GC-MS Analysis for Identification of Active Compounds in Propolis and Molecular Docking Studies of Selected Compounds against Apototic Proteins (Caspase-3, Caspase-9 and B-Actin)

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ABSTRACT

GC-MS analysis of propolis methanolic extract revealed thirteen active phytocompounds and the analysis of ligand binding interaction with the apoptotic proteins could be used as a tool to find propolis as a new preventive and therapeutic drug for cancer. 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. Hydrogen bond formation was good in seven ligands. The docking is also valid by the formation of hydrogen bond between them. The result of Lipinski rule suggests the analyzed compound as best therapeutic drug. Docking study result proves the application of seven active phytocompounds as potential and natural therapeutic agents to treat cancer.
Key words: Propolis, GC-MS, Molecular Docking and Apoptotic Proteins.

INTRODUCTION

Propolis is a natural product that is collected from bee hives. 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 shelters 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 (Falcao *et al*., 2013).

Propolis possess 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 (AbdelFattah and Nada, 2007), anti-protozoans (Gressler *et al*., 2012) and anti-ulcer (Alfaris *et al*., 2009).

Propolis is generally composed of 50% to 60% resins and balsams, 30% to 40% waxes, 5% to 10% essential oils, and 5% pollen grains and micronutrients, with small amounts of vitamins B1, B2, B6, C, and E (Park *et al.,* 2002). Currently, the use of synthetic chemicals to control cancer concerns related to human health. An alternative is the use natural products that possess good efficacy and are eco-friendly. Among those chemicals, volatile compounds from propolis have been tested to assess their anticancer properties as a valuable natural resource. The main objective of the present investigation was to identify the natural anticancer compounds from propolis and also to its *in silico* docking potential against apoptotic proteins.

MATERIALS AND METHODS

Collection and Extraction of Propolis

Commercially available pure propolis powder (Stakich organic) was purchased from USA and 10 g propolis was mixed with 100 ml of methanol and kept in an 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 the GC-MS analysis. Exactly, 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 (1 µl) 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 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 was 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, the structures of ligands (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) and [22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione) were drawn by using ChemSketch, a chemical structure drawing program for Windows. By using PubChem, chemical structure and molecular formula was retrieved and the 2D structure was converted to 3D with physicochemical properties to analyze and promote activity, Pfam was used for multiple sequence alignments and Hidden Markov Models covering many protein domains. 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 and for the molecular docking analysis AutoDock software was used. The docked complex was visualized by PyMol tool.

RESULTS AND DISCUSSION

GC-MS Analysis

The peaks of compounds and retention times present in methanolic extract of propolis were identified by GC-MS analysis 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 Fig. 9. The GC-MS showed the presence of 13 volatile compounds, which were identified as follows: 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) and [22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione (RT-28.27).

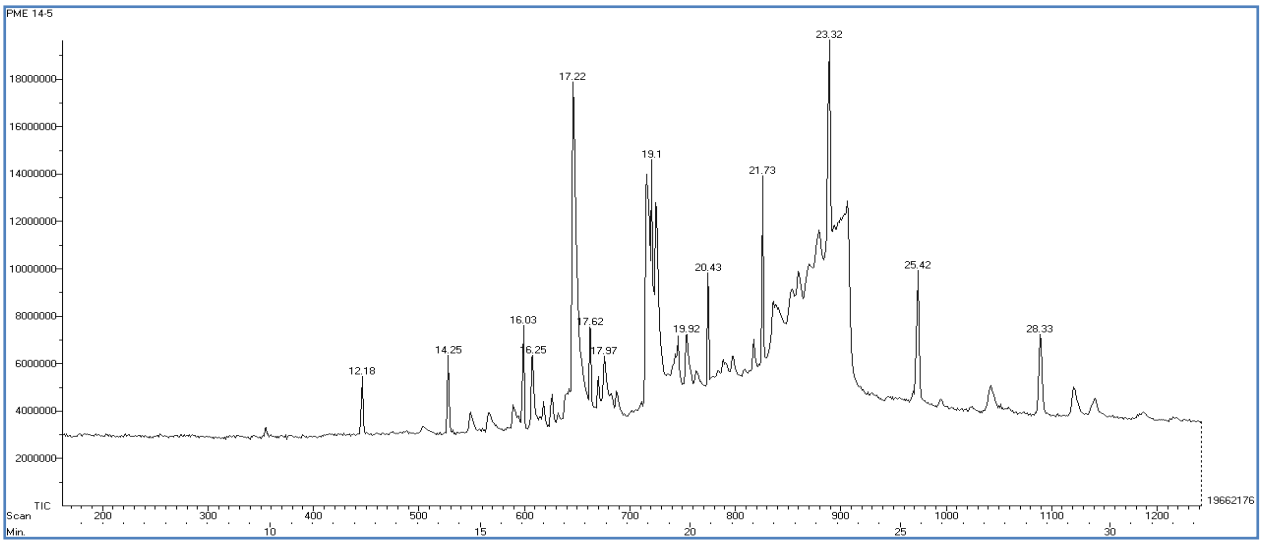

Fig. 1. GC-MS chromatogram of propolis methanolic extract.

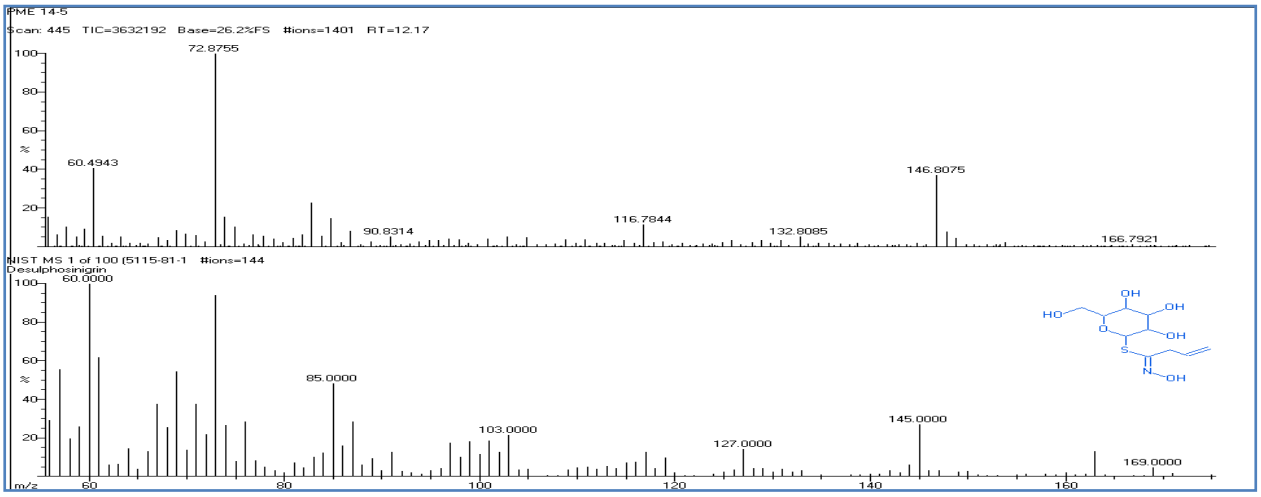

Fig. 2. Mass spectra of Desulphosinigrin (RT-12.17).

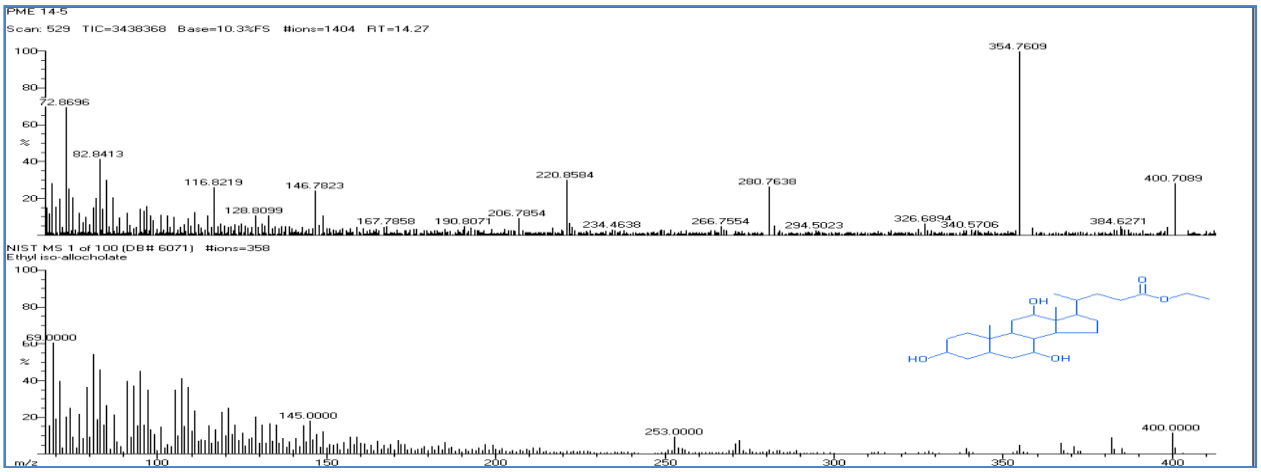

Fig. 3. Mass spectra of Ethyl iso-allocholate (RT-14.27).

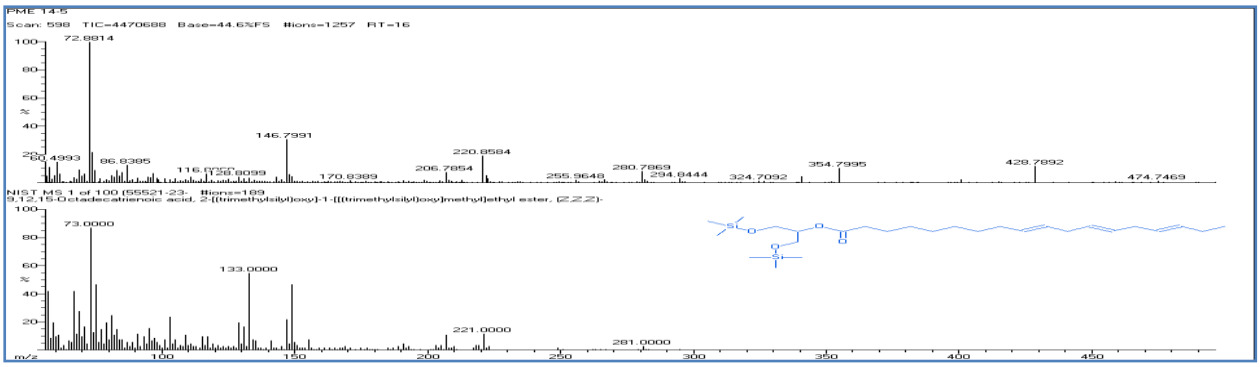

Figure 4. Mass spectra of 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ] (RT-16)

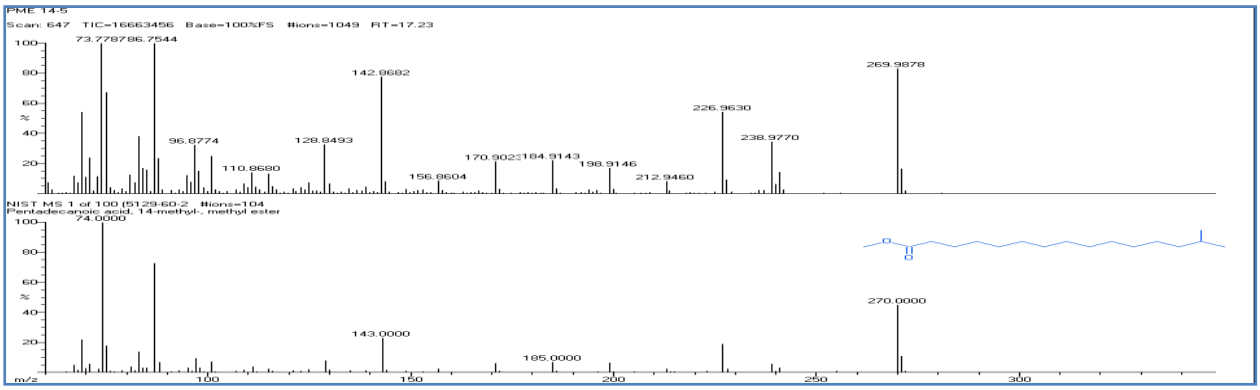

Figure 5. Mass spectra of Pentadecanoic acid (RT-17.23)

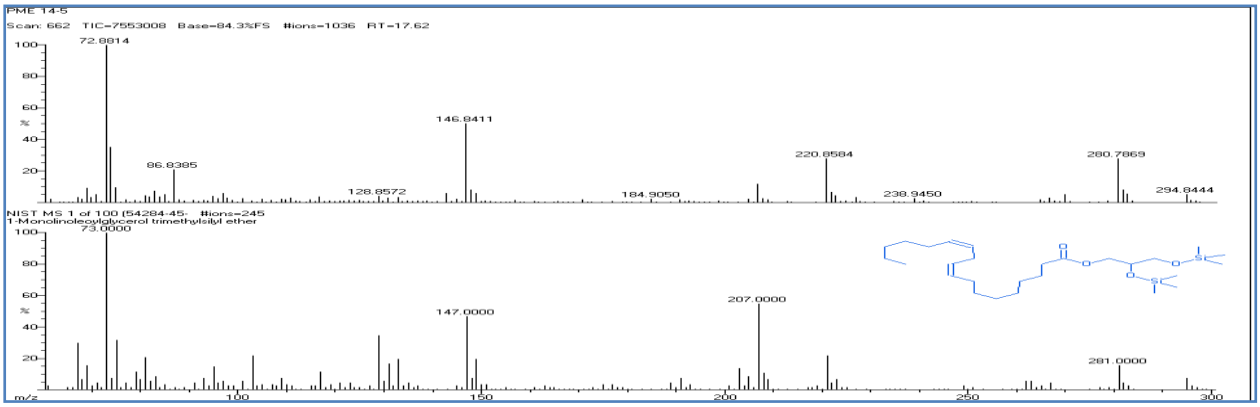

Figure 6. Mass spectra of 1-Monolinoleylglycerol trimethylsilyl ether (RT-17.62)

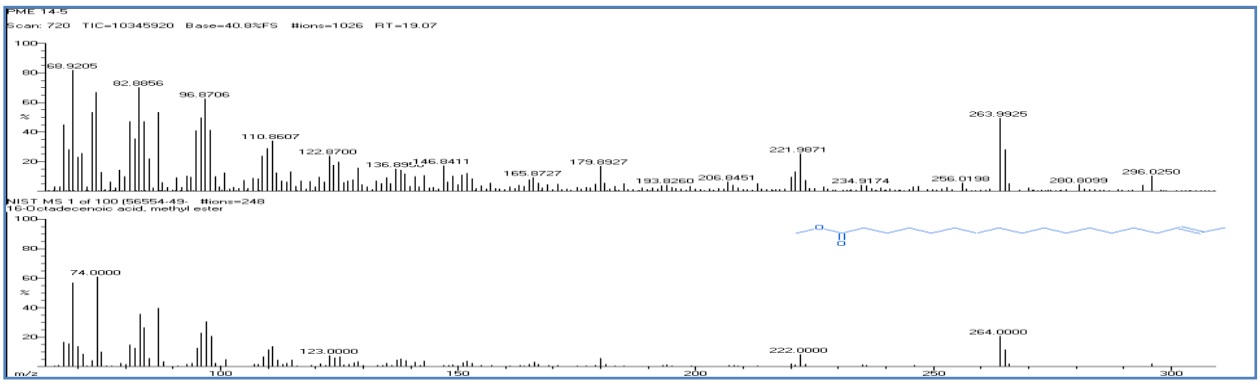

Figure 7. Mass spectra of 16-Octadecenoic acid, methyl ester (RT-19.07).

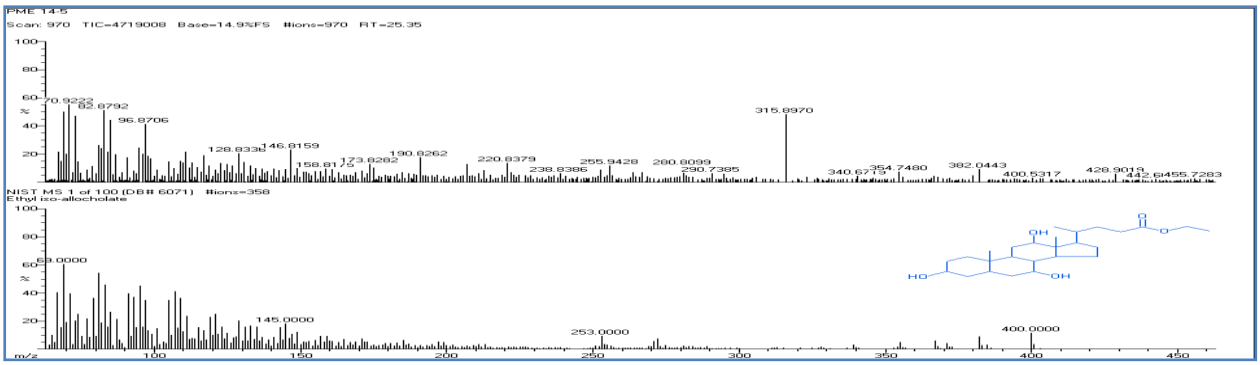

Figure 8. Mass spectra of Ethyl iso-allocholate (RT-25.35).

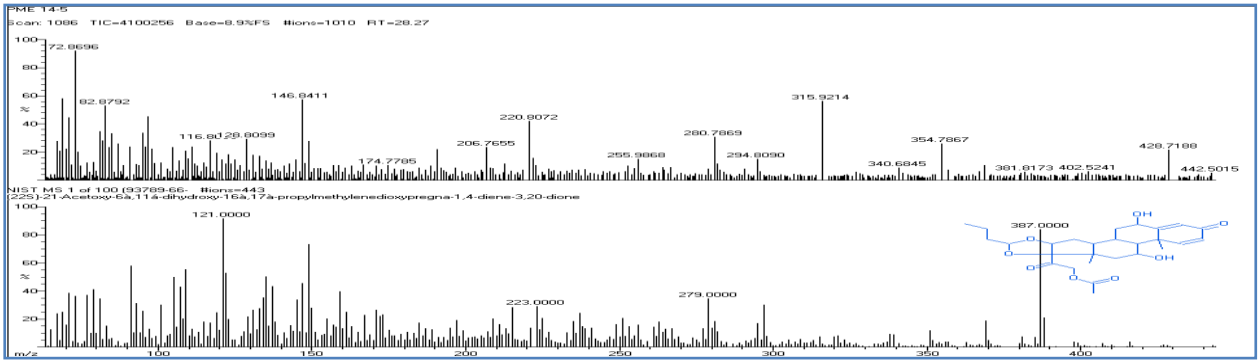

Figure 9. Mass spectra of [22S]-21-Acetoxy-6a, 11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione (RT-28.27)

Table 1. Molecular docking of identified seven compounds against Caspase-3 protein.

Name of the protein	Name of the compound	Docking score Kcal/mol.	Distance	H-Bond
Caspase-3	Desulphosinigrin	3388	3.16	1
	Ethyl iso-allocholate	4722	1.97	4
	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]-	4666	2.91	4
	, Pentadecanoic acid, 14-methyl-, methyl ester	4032	3.14	1
	1-Monolinoleylglycerol trimethylsilyl ether	6172	3.35	1
	16-Octadecenoic acid, methyl ester	**No interaction**		
	[22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione	4118	3.14	4

***In silico* Molecular Docking**

Propolis has a long history of medicinal use, 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, anti-parasitic, anti-human immunodeficiency virus, anti-tumour, anti-hypertension, anti-inflammatory, antiseptic, analgesic, and anti-cardiovascular drug.

By using GC-MS the various chemical compound has been identified. From that seven chemical compounds namely (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) and [22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione) have been selected for the docking studies based on the medicinal properties.

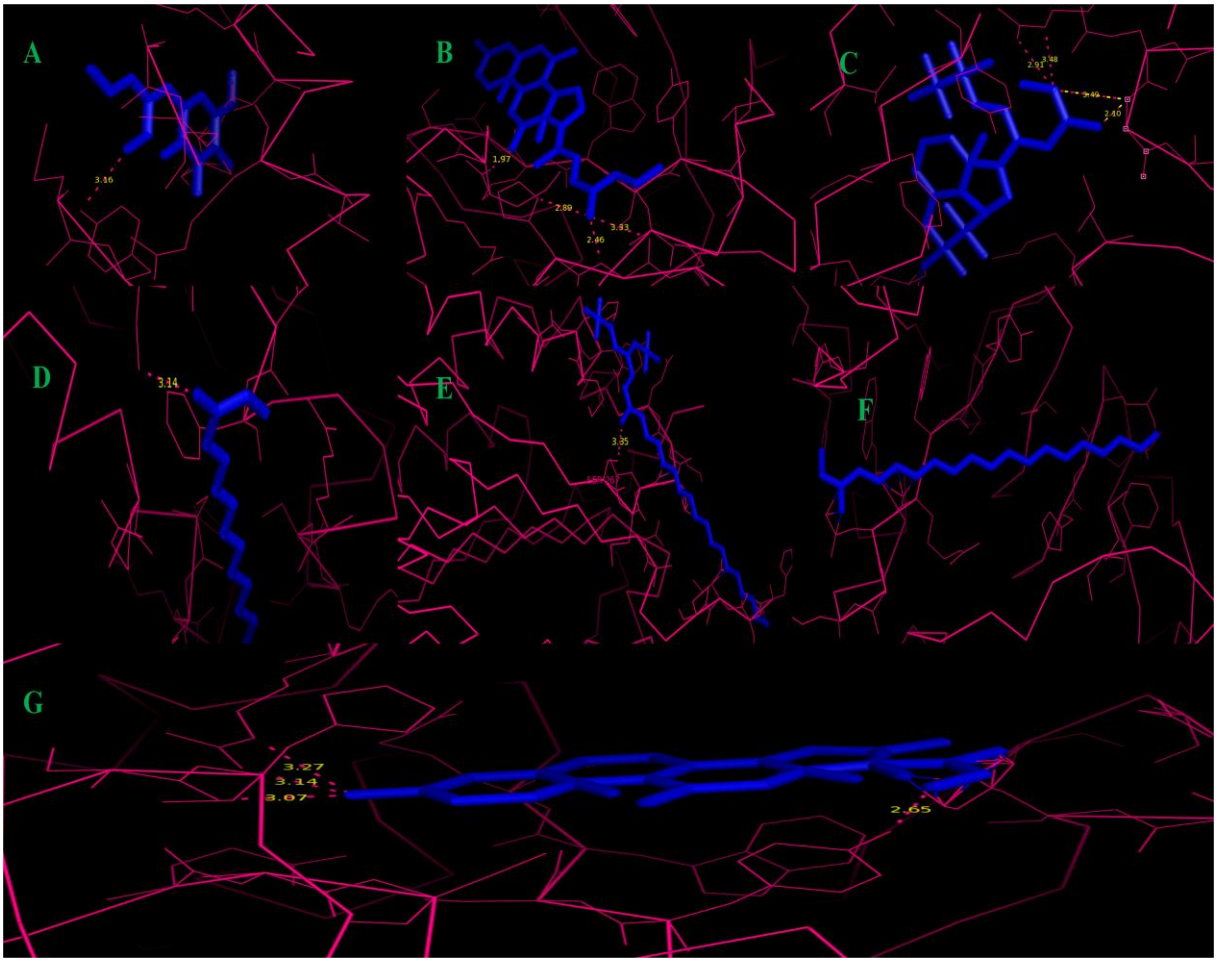

Figure 10. Final Docked structure of Caspase-3 protein with Desulphosinigrin (RT-12.17), Ethyl iso-allocholate (RT-14.27), 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]- (RT-16), Pentadecanoic acid, 14-methyl-, methyl ester (RT-17.23), 1-Monolinoleylglycerol trimethylsilyl ether (RT-17.62), 16-Octadecenoic acid, methyl ester (RT-19.07) and [22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione. 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.

1-Monolinoleylglycerol trimethylsilyl ether docked with Caspase-3 showed the docking score of 6172 kcal/mol and formed 1 Hydrogen bond whereas, the remaining six compounds showed least docking scores as shown in the Table 1. Likewise, among seven compounds docked with Caspase-9 and β- Actin higher scores were recorded only in the Ethyl iso-allocholate (4744 kcal/mol) and 1-Monolinoleylglycerol trimethylsilyl ether (7042 kcal/mol) as shown in the Table 2 and 3. This results shows that there is a presence of binding site between these three proteins and seven ligands. The docking is also valid by the formation of hydrogen bond between them. The result of Lipinski rule suggests the analyzed compound as best therapeutic drug. The *in silico* docking studies proves the application of these seven compounds present in propolis as potential and natural therapeutic agent to treat cancer. It is interesting to know that docking studies of seven compounds present in propolis against apoptotic proteins has not carried out and this is the first report that is recorded.

Similar type of studies with fucoidan compound was perpormed by Ashok and Sivakumari (2015) [19], Quercetin compound was performed by Muthukala *et al*. (2015) [20], Manimaran *et al*. (2015) [21] have also done similar type of study with Resveratrol compound.

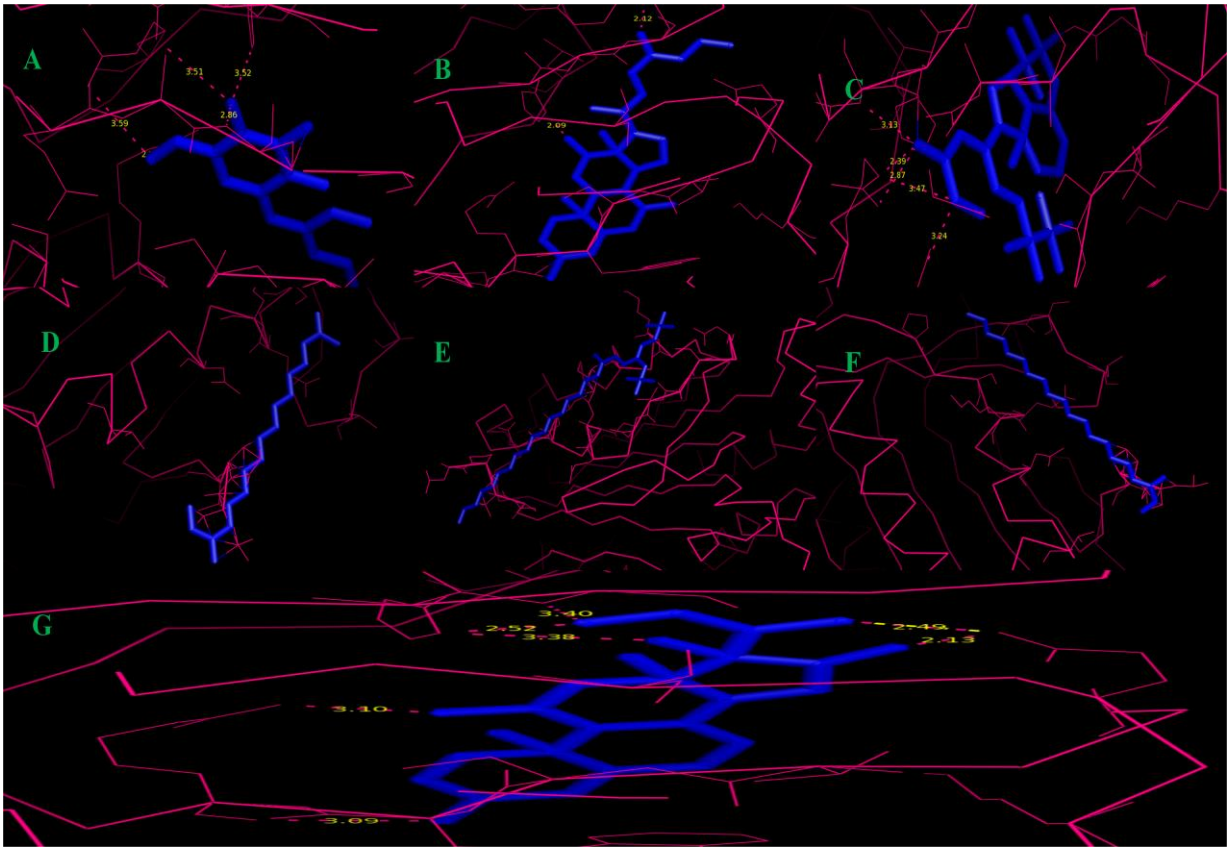

Figure 11. Final Docked structure of Caspase-9 protein with Desulphosinigrin (RT-12.17), Ethyl iso-allocholate (RT-14.27), 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]- (RT-16), Pentadecanoic acid, 14-methyl-, methyl ester (RT-17.23), 1-Monolinoleylglycerol trimethylsilyl ether (RT-17.62), 16-Octadecenoic acid, methyl ester (RT-19.07) and [22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione. 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.

Table 2. Molecular docking of identified seven compounds against Caspase-9 protein.

Name of the protein	Name of the compound	Docking score	Distance	H-Bond
Caspase-9	Desulphosinigrin	3374	3.52	5
	Ethyl iso-allocholate	4744	2.12	2
	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]-	4742	3.13	5
	, Pentadecanoic acid, 14-methyl-, methyl ester	**No interaction**		
	1-Monolinoleylglycerol trimethylsilyl ether	**No interaction**		
	16-Octadecenoic acid, methyl ester	**No interaction**		
	[22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione	4156	3.40	7

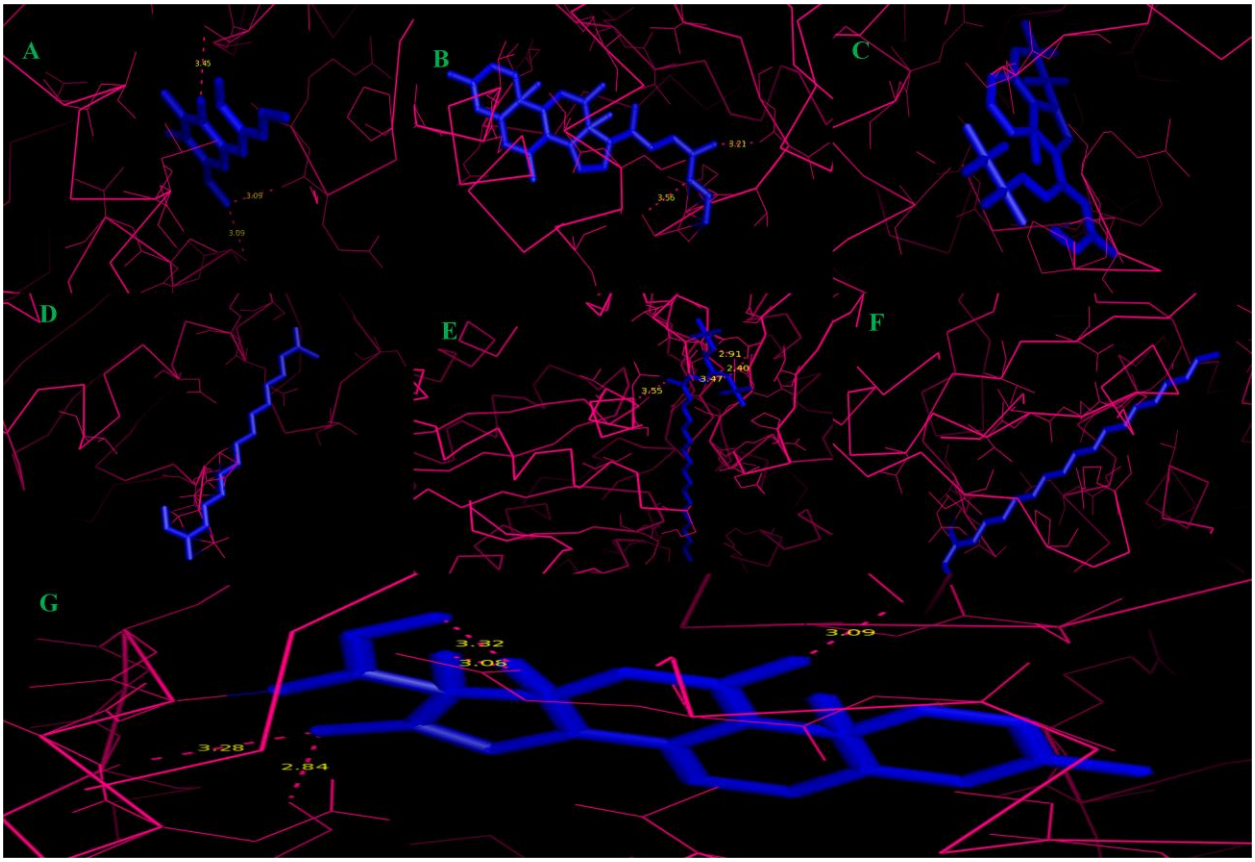

Figure 12. Final Docked structure of β-Actin protein with Desulphosinigrin (RT-12.17), Ethyl iso-allocholate (RT-14.27), 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]- (RT-16), Pentadecanoic acid, 14-methyl-, methyl ester (RT-17.23), 1-Monolinoleylglycerol trimethylsilyl ether (RT-17.62), 16-Octadecenoic acid, methyl ester (RT-19.07 and [22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione. 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.

Table 3. Molecular docking of identified seven compounds against β-Actin protein.

Name of the protein	Name of the compound	Docking score	Distance	H-Bond
β- Actin	Desulphosinigrin	3878	3.45	3
	Ethyl iso-allocholate	5552	3.41	2
	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]-	**No interaction**		
	, Pentadecanoic acid, 14-methyl-, methyl ester	**No interaction**		
	1-Monolinoleylglycerol trimethylsilyl ether	7042	2.91	4
	16-Octadecenoic acid, methyl ester	No interaction		
	[22S]-21-Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione	4832	3.09	5

According to Lipinski *et al*. (2001) [22] 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 whether 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. Similar observation were observed by Chandrika *et al*. (2016) [23] in Hespertin and Naringenin polyphenolic compounds against HER2 tyrosine kinase inhibitors.

CONCLUSION

This is the first report of identification of 13 active phytocompounds in pure propolis by GC-MS studies and also assessing it's *in silico* docking potential against apoptotic proteins.

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