RESEARCH PAPER

Integrative analysis of Propolis: phytochemical characterization, anticancer activity, and *in silico* molecular docking

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Abstract
The essential oil of propolis has gained significant interest in traditional medicine, specifically for its potential as a therapeutic agent for cancer disorders. The essential oil of Propolis obtained through hydro-distillation underwent analysis via a time-of-flight mass spectrometry in conjunction with a two-dimensional gas chromatography (GCxGC-TOF-MS) tool. Our study aimed to identify the chemical constituents of propolis, investigate its anti- cancer effects of propolis oil was evaluated for the human oral KB 3.1 cell line. In KB 3.1 cells, propolis oil induced apoptosis by stopping the cell cycle, raising ROS levels, depolarising the mitochondria, and boosting the expression of caspase-3, caspase-8, and caspase-9. The top major constituents are Heneicosane (23.886%), n-Hexadecanoic acid (16.65508%), Eicosane (3.5654%), cis-Vaccenic acid (3.0652%), 9,12-Octadecadienoic acid (Z,Z)-Benzyl Benzoate (2.04%), Dodecanoic acid (1.9665%), Undecanoic acid (3.653806%), 9 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-(1.856876%) Tetradecanoic acid (1.4671%), Decanal (1.3914%), Heptacosane (1.37767%), Terpineol (1.1439%), Decanal dimethyl acetal (1.41845%) , identified through GC-TOF analysis were retrieved from PubChem, docked by the molecular-docking process and tested for drug-likeness properties. The top major constituent were identified from docking, Methyl ester (-9.64), benzyl benzoate (-7.33), terpineol (-7.21), benzoic acid (-6.08), 9,12-octadecadienoic acid (Z,Z) (-6.48), tetradecanoic acid (-6.59), n-hexadecanoic acid (-5.16), 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z) (-5.59), heptacosane (-5.32), and decane (-5.29) exhibited the highest binding-affinity with p53. The present work with *in vitro* and *in silico* studies revealed that, Propolis could be a potential anti-cancer agent, thus necessitating further *in vivo* studies to develop promising therapeutic agents in the treatment of Oral cancer diseases.

Key words: Propolis, GC-TOF analysis, Oral cancer, Chemical compounds, *in silico*
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1. Introduction

The United States Department of Agriculture (USDA) defines propolis, commonly known as 'bee glue,' as "a gum that is gathered by bees from various plants." The color ranges from pale yellow to dark brown. The term propolis is derived from a Greek name in which 'pro' means 'police' and 'polis' means 'city', and one of its functions is to defend the entrance of beehives from invaders [1]. Raw propolis' specific composition may vary according on location and supplier. It has been stated that propolis have major ingredients and varies significantly according to their diverse botanical and geographical origins [2]. These elements include polyphenols (flavonoids, phenolic acids and their ester, phenolic aldehydes, alcohol and ketones), amino acids, steroids, coumarins, sesquiterpene quinones, and inorganic chemicals.

Because of the variety in their chemical compositions, it seems to be difficult to identify and standardize propolis profiles from various regions globally [3]. Bioactive chemicals have been identified as the cause of the Propolis sample's wide spectrum of pharmacological activity, including anti-inflammatory, antibacterial, antifungal, and antioxidant properties. Propolis has also been linked to immunomodulatory, hematostimulative, hepatoprotective, and cytotoxic effects [4,5].

Cancer is a growing global mortality issue, prompting the exploration of alternative anti-cancer strategies. Propolis, particularly Brazilian green propolis, presents potential as a pharmaceutical agent due to its compounds like artepillin C and caffeic acid phenethyl ester (CAPE) [6]. These substances exhibit significant anti-tumor properties, inducing apoptosis through various mechanisms including caspase activation and interaction with TRAIL (tumor necrosis factor-related apoptosis ligand inducer). CAPE enhances reactive oxygen species (ROS) generation and influences apoptotic pathways by modulating proteins like Bax and XIAP (X-linked inhibitor of apoptosis protein) [7]. Additionally, propolis components such as chrysin

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and quercetin contribute to apoptosis by disrupting mitochondrial functions and inhibiting essential cell cycle enzymes, respectively. These compounds demonstrate effectiveness even against drug-resistant colon cancer cells [8,9].

Propolis has good efficacy on dental health care. Oral pathogens are group of microorganisms that colonize the oral cavity, leading to conditions like periodontal diseases, caries, and infective endocarditis which can be treated by Propolis. Dental caries, a widespread infectious disease, is primarily caused by oral bacteria that attach to the salivary pellicle on tooth surfaces [10]. Initial colonizers include Streptococcus and Actinomyces species, with Streptococcus mutans being a key contributor to dental caries due to its ability to create an acidic environment from carbohydrate fermentation. This process results in the formation of biofilms (dental plaque) and tooth decay. Other acid-resistant bacteria such as S. sobrinus and S. sanguinis also contribute to cavity formation, indicating a polymicrobial origin of oral diseases. Propolis is the best natural product treated for dental plaque [11].

Molecular docking was powerful computational approaches that help predict and analyze the interactions between bioactive phytoconstituents and biological targets associated with specific bioactivities. These techniques allow researchers to understand the binding affinity, stability, and possible mechanisms of action of natural compounds at the molecular level. In the case of bee propolis, such *in silico* approaches can provide insights into how its phytochemical constituents interact with proteins related to microbial infections and cancer progression [12,13]. However, studies that directly compare *in vitro* biological activity with *in silico* molecular modeling results for propolis remain limited. Comparative analyses integrating experimental assays with computational validation have been reported for several other natural products, demonstrating that combining these approaches can strengthen the understanding of their therapeutic potential.

2. Materials and methods

2.1 Collection of Propolis Samples and Oil Extraction:

Propolis samples were collected from 10 Agro-climatic areas in Eastern India such as East & South East Coastal Plain, North Eastern Coastal Plain, North Eastern Ghat, Mid Central Table Land, Western Central Table Land, Eastern Ghat High Land, North Central Plateau, South Eastern Ghat, North Western Plateau, and Western Undulating Zone, during late summer (June to October 2021). Each sample was washed with distilled water and air-dried at room temperature.

The Soxhlet apparatus efficiently extracted 14–15% of propolis oil at 30°C using Ethanol as the solvent, chosen for its low toxicity and high efficacy. The method utilizes an extract percentage of 18-19% in oil extraction to achieve a 1:5 ratio of propolis to solvent. After filtering the mixture to eliminate solid particles, the solvent is evaporated, resulting in an oil extract. The oils were stored in the refrigerator, and the propolis oil was dissolved in DMSO (dimethyl sulfoxide), at a concentration of 0.019 g/mL for testing.

2.2 GCxGC-TOFMS analysis

Propolis oil was stored at 40°C for analysis using gas chromatography combined with time-of-flight mass spectrometry (GC x GC-TOF) via a LECO Pegasus 4D system, which includes an Agilent A gas chromatography instrument, an automatic liquid sampler, a secondary oven, and a cold jet modulator from Zoex. The mass spectrometer features a 30 m × 0.25 mm I.D. column with 0.25 µm film thickness, alongside a secondary 2 m × 0.25 mm I.D. column, both sourced from Restek Corporation. The sample was analyzed using GCxGC with a split ratio of 1:700 and helium as the carrier gas at 1 mL/min. The mass spectrometer operated in electron impact mode with a detector voltage of 1620 V, ionization voltage of 70 eV, and a mass scan range of 30 to 350 m/z. The transfer line and mass spectrometer were set at 250°C. The primary oven temperature profile included an initial 50°C for one minute, a rise to 230°C for five minutes, and a final 260°C for one minute at a rate of 15 °C min−1. The secondary oven was maintained 10°C above the primary oven with a modulation time of five seconds. Using TOF-MS, spectra were recorded at 100 per second, processed with Chroma TOF software version 3.34, and compounds identified via contour plots and comparison to the internal NIST library.

2.3 Anti-cancer assays:

2.3.1 Cell culture

Cell culture involved KB cell lines (KB, KB 3.1, KB CHR 8.5) obtained from the National Centre for Cell Science, Pune, India. The cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution, maintained at 5% CO_2, 18–20% O_2, and 37°C, with sub-culturing conducted every 2 days.

2.3.2 MTT assay

The study evaluated cell cytotoxicity using the MTT assay on KB, KB 3.1, and KB CHR 8.5 cells exposed to different concentrations of propolis oil (6.25, 12.5, 25, 50, 100 µg/ml) for 24 hours. Post-treatment, cells were incubated with MTT reagent, formazan crystals were solubilized with DMSO, and absorbance at 570 nm was measured to determine the IC50 value and assess cell viability, calculated using the formula % cell viability = [Mean abs of treated cells / Mean abs of Untreated cells] × 100 [14].

2.3.3 Apoptosis assay

FITC-labelled annexin V was employed, with modifications, to assess cell death through flow cytometry. Cells were plated at a density of 5 × 10^5 cells/2 ml and incubated at 37°C for 24 hours before treatment with propolis oil (6 µg/ml). Following treatment, cells were rinsed with PBS, stained with 5 µl Annexin V-fluorescein isothiocyanate and 10 µl PI in 100 µl buffer, and incubated at 37°C for 15 minutes. The

cell death rate was evaluated using a BD FACS Calibur flow cytometer [15].

2.3.4 Apoptotic Protein Expression Assay

It investigates the roles of Caspase 3, Caspase 7, and Caspase 8 in apoptosis within human oral cancer cell lines. Following a 24-hour propolis oil treatment, cells were washed with PBS, trypsinized, incubated at 37°C, collected with culture media, centrifuged, and treated with ethanol and PBS rinses before the addition of FITC antibodies and another PBS rinse [16].

2.3.5 Cell cycle assay

The study explored the cell cycle phase distribution in KB 3.1 cells treated with propolis oil at an IC^{50} of 6 µg/ml for 48 hours. Post-treatment, cells were collected, rinsed with cold PBS, fixed in 70% ethanol, and then resuspended in RNAase A and PBS with propidium iodide for flow cytometer analysis [17].

2.3.6 ROS Expression Assay

D2CFH-DA was used to measure intracellular ROS levels in KB 3.1 cells following a modified protocol. Cells were cultured for 24 hours at 37°C and treated with an IC^{50} concentration of propolis oil (6 µg/ml) for one day. After treatment, the media was removed, and cells were rinsed with PBS, then suspended in H2DCFDA for 30 minutes. ROS production was evaluated using a Carl Zeiss LSM 880 microscope to measure DCF intensity [18].

2.3.7 JC-1 staining assay

KB 3.1 cells were incubated for 24 hours in a CO_2 incubator, treated with 6 µg/ml of propolis oil at an IC^{50} value, rinsed with PBS, and analyzed microscopically after staining with JC-1 dye and Hoechst 33342 solution.

2.3.8 EtBr staining study

Fluorescence microscopy was utilized to examine the morphology of KB 3.1 cells, cultured at a concentration of 2 × 10^5 cells/2 ml in a CO_2 incubator for one day. Following treatment with the IC^{50} concentration of propolis oil (6 µg/ml) for 24 hours, the cells were washed with PBS, trypsinized, stained for ten minutes with acridine orange and ethidium bromide, and analyzed using a Carl Zeiss LSM 880 fluorescence microscope [19].

2.4 Pre-Processing of Ligand

Major bioactive chemicals identified during GC x GC-TOF analysis were obtained from 10 Agro-climatic areas in Eastern India. The ligands were energy minimized and converted to PDBQT format with AutoDock Vina, adjusting tautomers, stereoisomers, and protonation states for docking preparation.

2.5 Pre-Processing of Protein

The RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) was used to obtain the crystal structure of the target protein, p53 (PDB ID: 3NRZ). P53 is a tumor suppressor protein that activates genes for DNA repair, cell cycle arrest, or apoptosis in response to cellular stress. It prevents the proliferation of damaged cells, maintaining genomic stability and inhibiting cancer development. Using the AutoDock Vina tool, steps were taken to prepare the proteins, such as getting rid of water molecules, adding hydrogen atoms, assigning bond ordering, and minimising energy.

The binding sites of target proteins were identified using the PrankWeb server (https://prankweb.cz/), and the findings were subsequently verified by co-crystallizing ligand locations. Molecular docking analyses were used to identify the active site residues within a grid box.

2.7 *In silico* prediction of ADME and toxicity profiles

The evaluation of absorption, distribution, metabolism, and excretion (ADME) properties along with drug-likeness assessment for selected compounds involved characteristics related to these processes. Drug-likeness attributes were assessed using the SwissADME web server.
(http://www.swissadme.ch/). The drug likeness criteria involved adherence to Lipinski's rule of 5, the Egan rule, and Veber's rule. Potential toxicity of the phytochemical constituents was assessed using the ProTox-II webserver (https://tox-new.charite.de/ protox_II/) [20,21,22].

2.8 Molecular Docking

Molecular docking analysis was carried out using AutoDock Vina to examine the binding affinity and interaction patterns of selected ligands within the active sites of target proteins. The chemical structures of compounds identified from bee propolis were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Docking simulations were performed to evaluate the interaction between propolis-derived compounds and the tumor suppressor protein p53 (PDB ID: 1AIE). The major compounds analyzed included methyl ester, benzyl benzoate, terpineol, benzoic acid, 9,12-octadecadienoic acid (Z,Z), tetradecanoic acid, n-hexadecanoic acid, 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z), heptacosane, and decane.
In addition, docking studies were conducted with the tumor suppressor protein p53 (PDB ID: 1AIE). The three-dimensional crystal structures of the target proteins were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/). All ligand molecules used in the study were retrieved from the PubChem database and prepared for docking prior to analysis [23].

3 Statistical Analysis

Statistical visualisation was carried out using R Studio, and the findings were shown as a bar plot with standard deviation error bars.

4. Results and Discussion

4.1 GC-TOF

The hydro-distillation technique has been used in this investigation to extract essential oil of Bee Propolis. The volatile components in propolis oil were characterized using GCxGC-TOFMS analysis, focusing on peaks

identified by Chroma TOF software with a S/N ratio > 100. The analysis produced hundreds of peaks, while mass spectral identification relied on a library search with similarity and reverse factors above 750 and 800, respectively. Peaks with lower values were classified as unknown.

In this study, 182 components, comprising 81.39% of the total essential oil were identified using GC-TOF analysis. The top 32 compounds were taken for docking further, representing 81.39% of propolis oil, shown in **Table 1**. According to a study, the oil yield of bee propolis was 0.2 % (v/w). The top major constituents are Heneicosane (23.886%), n-Hexadecanoic acid (16.65508%), Eicosane (3.5654%), cis-Vaccenic acid (3.0652%), 9,12-Octadecadienoic acid (Z,Z)-Benzyl Benzoate (2.04%), Dodecanoic acid (1.9665%), Undecanoic acid (3.653806%), 9 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (1.856876%) Tetradecanoic acid (1.4671%), Decanal (1.3914%), Heptacosane (1.37767%), Terpineol (1.1439%), Decanal dimethyl acetal (1.41845%) , identified through GC-TOF analysis were retrieved from PubChem, docked by the molecular-docking process and tested for drug-likeness properties. The top major constituent were identified from docking, Methyl ester (-9.64), benzyl benzoate (-7.33), terpineol (-7.21), benzoic acid (-6.08), 9,12-octadecadienoic acid (Z,Z) (-6.48), tetradecanoic acid (-6.59), n-hexadecanoic acid (-5.16), 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z) (-5.59), heptacosane (-5.32), and decane (-5.29) exhibited the highest binding-affinity with p53 depicted in **Table 2**. Additionally, propolis may have anti-cancer effects, primarily through its major constituent, Methyl ester (-9.64), which shows significant binding energy in molecular docking studies targeting NF-κB pathways.

4.2 Anti-cancer

Cytotoxicity study shows propolis oil has IC_{50} values of 40.99µg/ml for KB cells, 33.52µg/ml for KB 3.1 cells, and 63.18µg/ml for KB CHR 8.5 cells, indicating significant anticancer potential, especially against human oral cancer cells (KB 3.1). A bar graph shows IC_{50} values for Propolis in a R program shown in **Figure 1**, exhibiting effects on the KB, KB 3.1, and KB CHR 8.5 cell lines, as well as a cytotoxicity investigation of Propolis against the KB 3.1 cell line using the MTT test. This study examines Propolis oil's impact on apoptosis in KB-3.1 human oral cancer cells, utilizing Acridine Orange and Ethidium Bromide dual staining observed through fluorescence microscopy. Results indicate that treated cells exhibit enhanced late apoptotic features like membrane blebbing and chromatin condensation in a dose-dependent manner, particularly at concentrations of 25 µg/ml and 50 µg/ml after 24 hours of incubation. The study explores the apoptotic effects of propolis oil on the KB 3.1 cell line using Annexin V/PI dual staining. Two concentrations (25 µg and 50 µg) were tested against a control. Results, analyzed via BD FACSCalibur and Cell Quest Pro Software, show significant apoptosis and necrosis in treated cells compared to controls. Propolis oil effectively suppressed KB 3.1 cell lines after 24 hours, with 25µg/ml and 50µg/ml doses tested. Flow cytometry results showed a higher percentage of cells arrested in the G2/M phase in treated cells compared to untreated ones, indicating successful cell cycle arrest. Mitochondrial membrane potential studies show that propolis oil improves mitochondrial integrity in KB 3.1 cells in a dose-dependent manner, indicating its potential anti-cancer properties against human oral cancer cells and therapeutic cytotoxic potential for human-derived diseases. Propolis oil treatment increases DCF expression in KB3.1 cells in a dose-dependent manner, with 27.92% and 55.20% expression at 25µg/ml and 50µg/ml, respectively. This suggests its anti-cancer potential through an oxidative stress-induced apoptotic mechanism, supported by H2DCFDA staining and flow cytometry analysis.

Caspase3, Caspase7, and Caspase8 expression study shows that propolis oil induces significant apoptotic effects on KB 3.1 cells in a dose-dependent manner at concentrations of 25µg/ml and 50µg/ml after 24 hours. The results suggest that propolis oil enhances apoptotic protein expression and may have therapeutic potential in oral cancer via caspase-3 mediated apoptosis.

Major components α-pinene and β-pinene accounted for up to 77% of profiles from samples across China, Uruguay, Estonia, and Brazil. Some Indian propolis had volatile profiles akin to high-quality Brazilian propolis, rich in α- and β-pinene. Further analysis of Brazilian Green propolis via GC–MS and ESI–MS revealed 24 chemicals, including significant amounts of sesquiterpenes like viridiflorene and β-caryophyllene, alongside linalool and farnesol, although quantitative values were absent [24,25].

Propolis oil exhibits significant cytotoxic effects against human oral cancer cell lines, evidenced by IC_{50} values of 40.99 µg/ml, 33.52 µg/ml, and 63.18 µg/ml for KB, KB 3.1, and KB CHR 8.5, respectively. The study reinforces propolis's anticancer properties and explores apoptosis effects in KB-3.1 cells through dual staining techniques, particularly using the Annexin-V FITC/PI assay [26]. This assay detects apoptotic cells and differentiates between live and damaged cells. Mitochondrial membrane potential evaluated with JC-1 dye indicates intrinsic apoptosis, while reactive oxygen species (ROS) assessment via H2DCFDA highlights their potential to damage cells. Propolis oil triggers apoptosis by activating initiator caspases in oral cancer cells, underscoring its therapeutic potential [27,28].

4.3 Protein Binding Pocket:

Prank Web(https://prankweb.cz/), a machine learning based method was used for the prediction of plausible binding pockets of p53 protein shown in **Figure 2**. Binding site denotes the distribution of nearby amino acid residues in active pocket and act as catalytic residues Binding sites represent specific regions of a protein responsible for the interaction with small molecules shown in **Figure 3**. The crystal structure derived from complete models of p53 was analyzed to

predict binding sites using the PrankWeb server [29,30]. The protein binding pocket shown in **Figure 4.**

4.4 In silico ADME and toxicity assessment of ligands

Investigating the physicochemical characteristics of selected phytocompounds for drug development, we conducted ADME studies and applied Lipinski's, Egan's, and Veber's rules to assess drug likeness. The criteria for drug-like properties include a molecular weight below 500, a topological surface area less than 140, fewer than 5 hydrogen bond acceptors, fewer than 5 hydrogen bond donors, and fewer than 10 rotatable bonds. All 10 selected compounds met Lipinski's, Egan's, and Veber's rules, demonstrating favorable drug-like, lead-like, and pharmacokinetic properties displayed in **Table 3**. Toxicity assessments via the ProTox-II web server suggested that none of the compounds are mutagenic, carcinogenic, immunotoxic, or hepatotoxic, with LD50 values exceeding 2000 mg/kg, thus supporting their potential as safe anti-inflammatory medications depicted in **Table 4** [31,32,33].

4.5 Molecular Docking:

Molecular docking is an essential computational method used to predict how small molecules bind to proteins. This study evaluated the interaction of specific compounds with a target enzyme, revealing that several compounds displayed significant binding affinities, indicating potential as effective inhibitors. The binding modes of these compounds align with that of the standard ligand, suggesting a similar mechanism of action, supported by interactions with key catalytic residues. While the results indicate promising therapeutic potential, further validation through molecular dynamics simulations and experimental studies is necessary to confirm their efficacy [34,35]. Docking Result protein p53 bind with ligand Methyl ester, shown in **Figure 5.**

5. Conclusion

The anti-cancer activity of Propolis was investigated through in vitro and *in silico* studies. GC-TOF analysis identified compounds in its essential oil, demonstrating its bioactive elements' inhibitory effects on the p53 protein via computational methods. The methyl ester (-9.64) derived from Propolis emerged as the primary compound with p53 inhibiting potential. This study marks the preliminary identification of bioactive compounds with high binding affinity to p53 permitting ligands to move around inside the binding interface. This computational work is the first investigation to determine, methyl ester contains bioactive chemicals that have a greater affinity for binding to p53. It suggesting a need for further *in vivo* validation to explore their potential as anti-cancer drugs for human cancers.

Declarations

Clinical trial number: Not applicable.

Ethics approval and consent to participate: Not applicable

Consent For publications: Not applicable

Availability of data and materials: Not applicable.

Conflict of Interest

The authors have no affiliation with any organization with a direct or indirect conflict of interest in the subject matter discussed in the manuscript. Financial contributions and any potential conflict of interest must be clearly acknowledged under the heading 'Conflict of Interest'.

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Authors Contributions:

SB conceptualizes the methodology. GN performs the data collection, validation, preparation, and analysis. RB supported the scientific discussions. AS, DK, and AK supported the validation and preparation of the final manuscript. All the authors contribute to the final manuscript.

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List of figures:

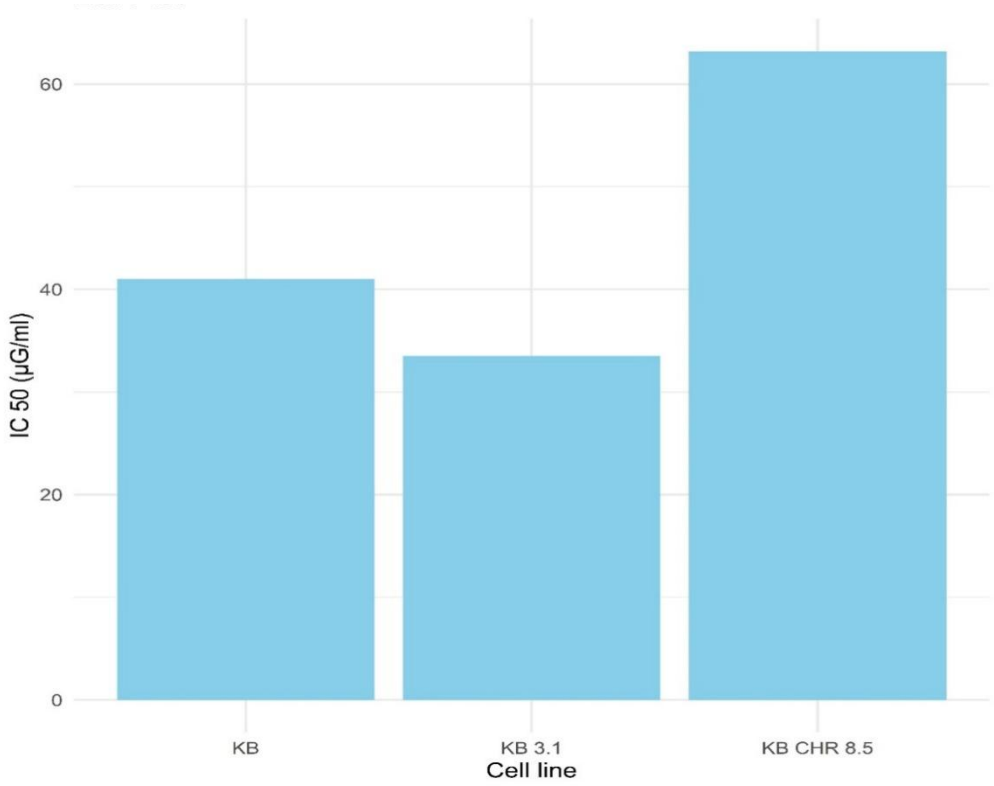

Figure: 1- Bar graph illustrates IC50 concentrations for Propolis in a R program, showing effects on KB, KB 3.1, and KB CHR 8.5 cell lines, alongside a cytotoxicity study of Propolis against the KB 3.1 cell line using the MTT assay.

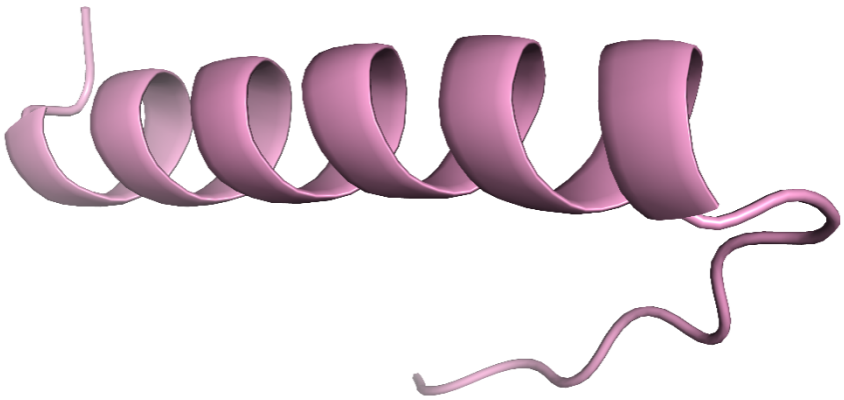

Figure: 2- The 3D p53 protein orientation in the structural view of pink colour

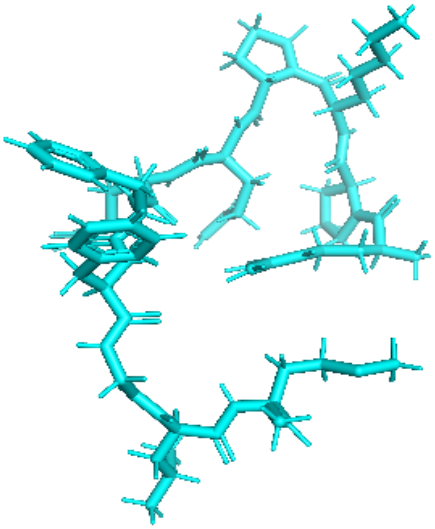

Figure: 3 – Ligand (Methyl ester) cyan colour snapshots

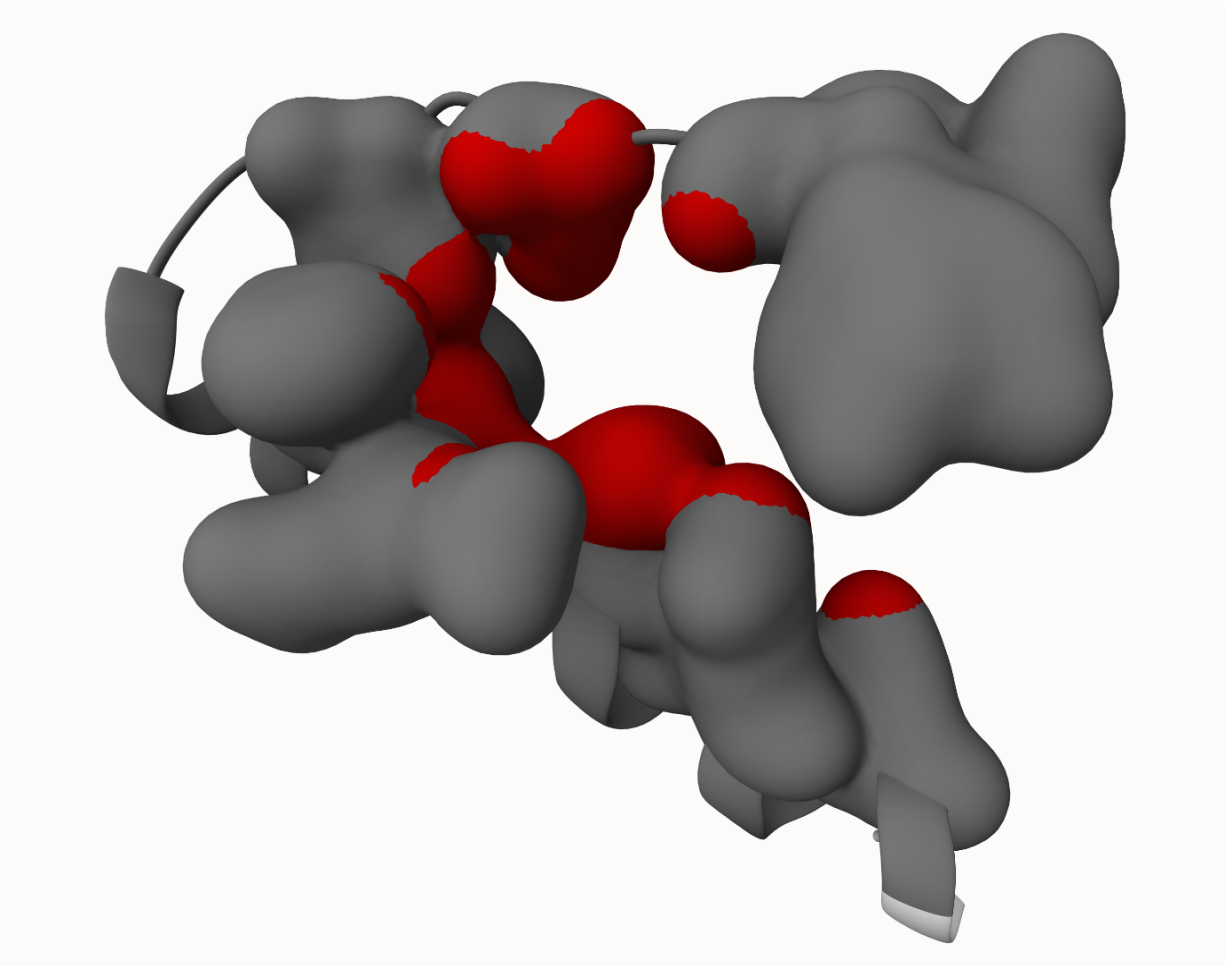

Figure:4- The binding of ligands to the active sites of target proteins, providing valuable insights into the interaction between the ligands and the protein receptors

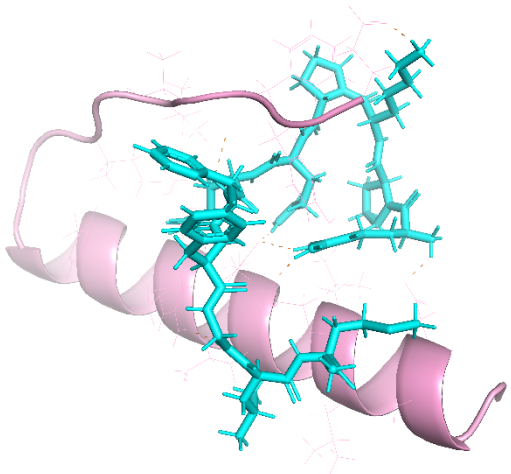

Figure: 5- Docking result protein ligand bind with Methyl ester

List of Tables:

Table: 1- Major compounds identified from GC-TOF analysis

SL No.	Name	Formula	Area %	Area	R.T. (s)	Class

1	Henicosane	C21H44	23.886	71901424	2545, 2.950	Saturated hydrocarbons
2	Palmitic acid	C16H32O2	16.65508	29226405	1901.5, 3.270	Fatty Acyls
3	icosane	C20H42	3.5654	10732706	1907, 3.140	Saturated hydrocarbons
4	(Z)-11-Octadecenoic acid	C18H34O2	3.0652	9226891	2264.5, 2.730	Fatty Acyls
5	Linolic acid	C18H32O2	2.5358	7633422	2094, 3.460	Fatty Acyls
6	Benzoic acid	C14H12O2	2.04	6140795	2088.5, 3.620	Benzene and substituted derivatives
7	Lauric acid	C12H24O2	1.9665	5919696	1665, 0.370	Fatty Acyls
8	Undecylic acid	C11H22O2	3.653806	5435744	1384.5, 3.230	Fatty Acyls
9	methyl ester	C19H32O2	1.856876	5407680	2121.5, 3.210	Fatty Acyls
10	Myristic acid	C14H28O2	1.4671	4416423	2094, 3.830	Fatty Acyls
11	n-Decanal	C10H20O	1.3914	4188550	1648.5, 3.230	Organooxygen compounds
12	n-Heptacosane	C27H56	1.37767	3563513	834.5, 3.160	Saturated hydrocarbons
13	Decane	C12H26O2	1.41845	3143706	1109.5, 2.790	Saturated hydrocarbons
14	2-Methylnonadecane	C20H42	1.03099	3021930	2649.5, 2.500	Saturated hydrocarbons
15	Decanoic acid	C10H20O2	1.470359	2777009	1093, 3.200	Fatty Acyls
16	n-Nonanal	C9H18O	0.87539	2635132	658.5, 3.140	Organooxygen compounds
17	L-α-Pinene	C10H16	0.82952	2497044	812.5, 3.680	Prenol lipids
18	dodecyl ester	C15H28O2	0.70944	2135598	2682.5, 1.870	Fatty Acyls
19	Phenol	C8H9ClO	0.69503	2092220	1120.5, 4.830	Phenols
20	Nonoic acid	C9H18O2	0.744708	2076058	933.5, 3.150	Fatty Acyls
21	Lauraldehyde	C14H30O2	1.910649	2049804	950, 2.780	Organooxygen compounds
22	Methyl tridecanoate	C14H28O2	0.7596454	1822601	1852, 3.050	Fatty Acyls
23	2-Methyloctadecane	C19H40	1.14723	1750703	2385.5, 3.200	Saturated hydrocarbons
24	Oleic acid	C18H34O2	0.56568	1702821	2127, 3.220	Fatty Acyls
25	Nonadec-1-ene	C19H38	0.56262	1693614	2033.5, 3.110	Unsaturated hydrocarbons
26	n-Dodecanal	C12H24O	0.55197	1661579	1159, 3.160	Organooxygen compounds
27	5-Methyl-3-hexanol	C7H16O	0.51396	1547137	1060, 3.410	Organooxygen compounds
28	Docos-1-ene	C22H44	0.78183	1538913	2231.5, 2.760	Unsaturated hydrocarbons
29	1-Pentadecanal	C15H30O	1.6386707	1497999	1593.5, 3.130	Fatty Acyls

30	Salicylic acid	C12H38O5Si6	0.845704	1415825	1802.5, 1.880	Benzene and substituted derivatives
31	benzyl ester	C14H12O3	0.45329	1364507	1791.5, 0.240	Benzene and substituted derivatives
32	Myristaldehyde	C14H28O	0.42478	1278679	1456, 3.140	Fatty Acyls

Table: 2- Docking results of compounds from Propolis with p53 protein

Sl no.	Compounds with PubChem CID	Docking Score
1	Methyl ester_CID_123764	-9.64
2	Benzyl_Benzoate_CID_2345	-7.33
3	Terpineol_3D_CID_17100	-7.21
4	Benzoic Acid_CID_243	-6.08
5	9,12-Octadecadienoic acid (Z,Z)-_CID_5365200	-6.48
6	Tetradecanoic acid_CID_2756016	-6.59
7	n-Hexadecanoic acid_CID_628589	-5.16
8	9_12_15-Octadecatrienoic_acid_methyl_ester_Z_Z_Z_COMPOUND_CID_5319706	-5.59
9	Heptacosane_CID_11636	-5.32
10	Decane_CID_15600	-5.29

Table: 3- SwissADME-derived drug-likeness evaluation of Propolis derived compounds meeting Lipinski's Rule of Five, Veber's, and Egan's criteria

Sl no.	Compounds	Lipinski	Veber	Egan	Abbott bioavailability score
1	Methyl ester_CID_123764	Yes	Yes	Yes	0.55
2	Benzyl_Benzoate_CID_2345	Yes	Yes	Yes	0.55
3	Terpineol_3D_CID_17100	Yes	Yes	Yes	0.55
4	Benzoic Acid_CID_243	Yes	Yes	Yes	0.55
5	9,12-Octadecadienoic acid (Z,Z)-_CID_5365200	Yes	Yes	Yes	0.55
6	Tetradecanoic acid_CID_2756016	Yes	Yes	Yes	0.55
7	n-Hexadecanoic acid_CID_628589	Yes	Yes	Yes	0.55
8	9_12_15-Octadecatrienoic_acid_methyl_ester_Z_Z_Z_COMPOUND_CID_5319706	Yes	Yes	Yes	0.55
9	Heptacosane_CID_11636	Yes	Yes	Yes	0.55
10	Decane_CID_15600	Yes	Yes	Yes	0.55

Table: 4- Toxicity assessment of compounds predicted by ProTox-II

Sl no.	Compounds	Classification	Target	Prediction	Probability	Class
1	Methyl ester_CID_123764	Organ toxicity	Hepatotoxic	Nonfunctional	0.82	**5**
		Organ toxicity	Carcinogenic	Functional	0.73	
		Toxicity end points	Immunotoxic	Nonfunctional	0.71	
		Toxicity end points	Mutagenic	Nonfunctional	0.69	
2	Benzyl_Benzoate_CID_2345	Toxicity end points	Cytotoxic	Nonfunctional	0.81	5
		Toxicity end points	Hepatotoxic	Functional	0.82	
		Toxicity end points	Mutagenic	Nonfunctional	0.73	
		Organ toxicity	Carcinogenic	Functional	0.74	
3	Terpineol_3D_CID_17100	Toxicity end points	Immunotoxic	Nonfunctional	0.71	4
		Organ toxicity	Carcinogenic	Nonfunctional	0.78	
		Toxicity end points	Hepatotoxic	Functional	0.82	
		Organ toxicity	Cytotoxic	Nonfunctional	0.65	
4	Benzoic Acid_CID_243	Toxicity end points	Carcinogenic	Functional	0.59	3

		Organ toxicity	Immunotoxic	Nonfunctional	0.84	
		Organ toxicity	Hepatotoxic	Nonfunctional	0.80	
		Toxicity end points	Mutagenic	Functional	0.71	
5	9,12-Octadecadienoic acid (Z,Z)-_CID_5365200	Toxicity end points	Cytotoxic	Nonfunctional	0.83	6
		Toxicity end points	Carcinogenic	Nonfunctional	0.87	
		Toxicity end points	Hepatotoxic	Nonfunctional	0.73	
		Organ toxicity	Immunotoxic	Functional	0.74	
6	Tetradecanoic acid_CID_2756016	Toxicity end points	Cytotoxic	Nonfunctional	0.79	4
		Toxicity end points	Immunotoxic	Nonfunctional	0.63	
		Organ toxicity	Mutagenic	Functional	0.62	
		Organ toxicity	Hepatotoxic	Nonfunctional	0.87	
7	n-Hexadecanoic acid_CID_628589	Organ toxicity	Cytotoxic	Functional	0.76	5
		Organ toxicity	Carcinogenic	Nonfunctional	0.71	
		Toxicity end points	Mutagenic	Nonfunctional	0.86	
		Toxicity end points	Hepatotoxic	Functional	0.79	
8	9_12_15-Octadecatrienoic_acid_methyl_ester_Z_Z_Z_COMPOUND_CID_5319706	Organ toxicity	Cytotoxic	Functional	0.75	5
		Toxicity end points	Hepatotoxic	Nonfunctional	0.83	
		Toxicity end points	Mutagenic	Nonfunctional	0.76	
		Organ toxicity	Carcinogenic	Nonfunctional	0.72	
9	Heptacosane_CID_11636	Toxicity end points	Mutagenic	Nonfunctional	0.58	3
		Organ toxicity	Immunotoxic	Nonfunctional	0.81	
		Organ toxicity	Cytotoxic	Nonfunctional	0.83	
		Toxicity end points	Hepatotoxic	Functional	0.72	
10	Decane_CID_15600	Organ toxicity	Carcinogenic	Nonfunctional	0.74	3
		Toxicity end points	Hepatotoxic	Nonfunctional	0.73	
		Toxicity end points	Mutagenic	Functional	0.85	
		Organ toxicity	Cytotoxic	Nonfunctional	0.83	