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# Comprehensive Analysis of Anticancer Activities in *Melissa officinalis* Extract: Gas Chromatography Profiling, Biological Assessment, Network Pharmacology, and Molecular Docking

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**Abstract:** *Melissa officinalis*, an herb known for its bioactive compounds, was subjected to aqueous extraction, with the resultant extracts employed for treatment on A549 and BEAS-2B cell lines. Gas chromatography analysis of the aqueous extract revealed prominent constituents, including palmitic acid, stearic acid, linoleic acid, oleic acid, palmitoleic acid, and linolenic acid. Through MTT colorimetric assays, the $IC_{50}$ values for the aqueous extracts were determined as 32.84 and 58.60 for A549 and BEAS-2B cell lines, respectively, after 48 hours of exposure. Notably, the aqueous extracts exhibited significant growth inhibition on A549 and BEAS-2B cells, with notable effect at higher concentrations (100 µg/mL). To delve deeper, a protein-protein interaction network analysis was conducted to identify pivotal targets governing biological processes and pathways. Subsequently, network pharmacology was employed to delineate the pathways involved in studying anticancer properties in *Melissa officinalis* Extract. Molecular docking simulations were carried out to estimate the binding affinities between the extracts and the identified hub targets. The analysis pinpointed ALOX5, PTGES, and CYP2C19 as the most promising targets through protein-protein interaction assessment. KEGG pathway analysis corroborated the potential of these compounds to modulate cancer-associated pathways, including large- and small-cell lung cancer. Furthermore, molecular docking simulations underscored the high binding affinities of linolenic acid, oleic acid, and stearic acid against cancer-associated targets, suggesting a promising strategy for treating lung cancer.

**Keywords:** *Melissa officinalis*; anticancer; MTT; molecular docking; network pharmacology.

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## 1. Introduction

Herbs can be identified as organic plants' dried leaves or flowers and utilized as sources of antioxidants, antimicrobials, and anticancer agents. Various methods can extract effective compounds from these herbs [1]. Despite the growing utilization of synthetic compounds in the pharmaceutical processes, many developing countries still rely on medications derived

from natural sources. Medicinal plants exhibit diverse biological properties, rendering them pivotal in the prevention and treatment of different diseases. These plants serve as abundant sources of biologically active agents, offering potential raw materials for developing new semi-synthetic drugs [1,2].

The effective compounds obtained from medicinal plants often yield indirect or direct therapeutic impacts. Consequently, these plants have played a remarkable role in healthcare, encompassing treatment, disease prevention, and health promotion within human communities. Notably rich in secondary metabolites, medicinal herbs exert deep physiological impacts on mammalian tissues, influencing their function under health and disease conditions [3]. The extraction of medicinal plants involves processes aimed at isolating active compounds or secondary metabolites such as flavonoids, alkaloids, glycosides, steroids, saponins, and terpenes using appropriate solvents and specific procedures. Plant materials containing phenolic compounds and flavonoids are particularly recognized for their antioxidant properties and therapeutic applications in addressing specific diseases [4,5].

The separation of secondary metabolites can be achieved through techniques such as paper chromatography (PC), gas chromatography (GC), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Simultaneously, extraction methods, including Soxhlet extraction, maceration, decoction, infusion, superficial extraction, percolation, microwave-assisted extraction, and ultrasound-assisted extraction, are employed to isolate medicinal compounds. The choice of extraction method depends on factors such as the solvent's nature and temperature, in addition to the desired properties of the final products [6-8].

Since the inception of the nineteenth century, the isolation of active compounds from plants has marked a significant turning point. This progression was spurred by rapid advancements in chemistry. Subsequently, with the dawn of the twentieth century, producing synthetic compounds witnessed a substantial increase [9]. Among the well-known herbs, *Melissa officinalis*, commonly referred to as lemon balm, has been employed to treat diverse ailments for centuries. Belonging to the Lamiaceae family (Figure 1), this perennial herbaceous plant bears alternate names like bee balm, garden balm, melissa, and Melissengeist. Its natural habitat encompasses vast expanses, ranging from central and southern Europe to central Asia and Iran. Additionally, it is cultivated worldwide for its culinary attributes. With a rich history spanning over 2,000 years, this herb holds a significant place in traditional medicine.

The applications of *Melissa officinalis* are broad and varied, encompassing roles as a sedative, mild hypnotic agent, heart rate reducer, antibacterial, anti-inflammatory, and antioxidant. Furthermore, its potential therapeutic effects on cancer have been documented [3,10]. Recently, growing attention has been directed towards plant extracts as potential contenders for cancer treatment, primarily due to their inherent anticancer properties and the advantage of minimized adverse effects [11]. Scientific investigations have elucidated that *Melissa officinalis* L. harbors robust antioxidant and anti-inflammatory traits attributed to its flavonoids, carotenoids, and phenolic compounds. These attributes position it as a promising candidate for cancer prevention and treatment [12].

Diverse findings suggest that extracts derived from *Melissa officinalis* hold therapeutic potential across a spectrum of diseases and conditions, encompassing cancer, diabetes, bacterial and fungal infections, as well as neurodegenerative disorders [10]. Notably, polyphenols in these extracts impede cancer cell proliferation by inducing apoptosis. A recent study showcased the in vitro anti-proliferative efficacy of M. officinalis against three human

cancer cell lines, wherein the observed effect was coupled with diminished cell proliferation through both apoptosis and cell cycle arrest mechanisms. The anti-proliferative attribute of M. officinalis L. was attributed to its polyphenolic content [13].

Cancer, characterized by aberrant cell proliferation due to genetic mutations, comprises more than 277 distinct types. Progress in bioinformatics, molecular genetic research, and molecular techniques have profoundly impacted early diagnosis, drug response prediction, and appropriate treatment strategies [14]. Numerous studies have indicated the potential of network pharmacology to unravel the precise mechanisms underlying the activities of medicinal substances, particularly in the context of cancer. Molecular docking, facilitating the simulation of interactions between receptors and drugs, is instrumental in the design of drugs. These technologies have garnered considerable interest within the realm of drug development [15-18].

Against this backdrop, we investigated the impact of M. officinalis extract on human bronchial epithelial (BEAS-2B) and lung cancer (A549) cells. Furthermore, this study harnessed network pharmacology to delve into the potential constituents, prospective targets, and protective mechanisms implicated in employing *M. officinalis* extracts for lung cancer treatment. A molecular docking approach was also employed to gain insights into the molecular interactions of the compounds with anxiolytic and antidepressant receptors.

**Figure 1.** Aerial Parts of *Melissa officinalis L* [19].

## 2. Materials and Methods

### *2.1. Extraction and characterization of the Melissa officinalis essential oil.*

To extract and characterize *Melissa officinalis* essential oil, 10 grams of dried fresh leaves were ground to a fine consistency. The hydro-distillation process was carried out using a Clevenger apparatus over a duration of 4 hours, employing 400 milliliters of distilled water. The resulting extracted oils were meticulously collected and subsequently stored in a dark environment at a low temperature [20]. An appropriate quantity of the *Melissa officinalis* extract was subjected to analysis using gas chromatography (Master Fast gas chromatography, DANI, Italy, 2010).

*2.2. Cell culture.*

For this study, two human tumor cell lines were employed: A549 (human lung cancer) and BEAS-2B (bronchial epithelial). The cells were cultured within a 5% $CO_2$ atmosphere at a temperature of 37°C, utilizing DMEM media supplemented with streptomycin (100 µg/ml), penicillin (100 units/ ml), and 10% heat-inactivated fetal bovine serum, all maintained under humid conditions. The cells were seeded twice a week with 100 µg/ml streptomycin, 5% (v/v) $CO_2$ atmosphere at 37°C. Cultivation was conducted within 96-well flat micro-titer plates, with cell densities ranging from 104 to 106 cells per well. Following seeding, the cells were incubated for 24 hours within a 5% $CO_2$ atmosphere at 37°C [21].

*2.3. Cytotoxicity assessment.*

The extract's anticancer potential was assessed using the MTT assay. Following the removal of media from each well, a phosphate-buffered saline wash was performed, and varying concentrations (0, 25, 50, and 100 µg/mL) of the extract were introduced to the cells. The treated cells were then incubated at 37°C within a 5% $CO_2$ incubator for durations of 24 and 48 hours. Subsequently, MTT solution (20 µL) was administered to each well and maintained under the same incubation conditions for a duration of 4 hours in a dark environment. The resultant MTT solution underwent conversion into formazan crystals, which were then dissolved in 20 µL of dimethyl sulfoxide (DMSO) and incubated for an additional 4 hours at 37°C in darkness. Absorbance measurements were taken at 570 nm for the treated cells in each well after 24-hour and 48-hour incubation periods, and the $IC_{50}$ values were calculated using sigmoidal concentration-response curve fitting models (Sigmaplot software).

*2.4. Protein-ligand docking.*

Molecular docking investigations were conducted employing the Autodock Vina program [22]. Prior to docking, the protein and ligands underwent preparation using ADP tools. Grid parameters were established at 60x60x60 in the x, y, and z dimensions, with a grid point spacing of 0.375 Å for each configuration. All docking results were subjected to comprehensive analysis, and diverse structures were visualized utilizing BIOVIA (Biovia, 2017).

*2.5. Mechanism analysis via network pharmacology.*

Predicted targets associated with oleic acid, linoleic acid, palmitic acid, linolenic acid, stearic acid, and palmitoleic acid were acquired through Swiss Target Prediction (http://www.swisstargetprediction.ch/). The protein-protein interaction database STRING v11.0 (https://string-db.org/) was employed to establish protein-protein interactions and create a visual representation. Furthermore, KEGG pathways were analyzed utilizing the DAVID database [23].

## 3. Results and Discussion

*3.1. Characterization of the Melissa officinalis essential oil.*

Gas chromatography was employed to characterize the chemical composition of the base oil extracted from *Melissa officinalis* L. The chromatogram in Figure 2 vividly presents the obtained chemical profile. The analysis revealed the presence of six primary compounds

within the M. officinalis L. extract through GC analysis. These compounds were identified as oleic acid, palmitoleic acid, palmitic acid, linolenic acid, linoleic acid, and stearic acid. The magnitude of the chromatogram peak for each component is directly proportional to the corresponding compound's quantity. Moreover, the distinct retention times of the components upon elution from the column serve as a valuable feature for accurate identification. The specific retention times and corresponding peak areas are systematically documented in Table 1.

**Table 1.** Retention time and peak area of each component.

| Components | Retention Time (min) | Area (mV/s) |
|---|---|---|
| Palmitic acid | 6.827 | 54.823 |
| Palmitoleic acid | 13.607 | 72.959 |
| Stearic acid | 14.573 | 9.201 |
| Oleic acid | 16.193 | 71.281 |
| Linoleic acid | 20.26 | 81.536 |
| Linolenic acid | 21.877 | 15.847 |

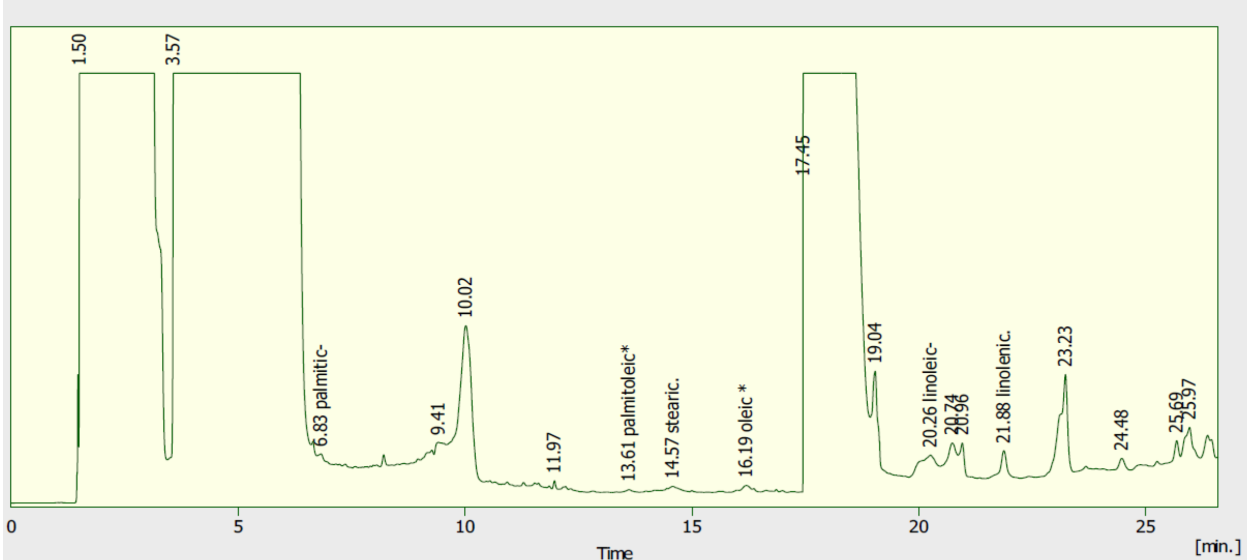


**Figure 2.** Chromatogram of *Melissa officinalis* essential oil.

**3.2. MTT cytotoxicity assessment.**

Evaluating aqueous extracts from *Melissa officinalis* as potential anticancer agents was conducted utilizing the MTT colorimetric assay. This assay capitalizes on the conversion of 3-(4,5-dimethyl-2-thiazolyl)bromide-2,5-diphenyl-2H-tetrazolium (MTT) from its yellow form to purple formazan, catalyzed by mitochondrial dehydrogenases present within viable cells subsequent to apoptosis [24].

Both A549 and BEAS-2B cells were cultured in suitable media and subjected to treatment with varying concentrations (0, 25, 50, and 100) µg/mL of the aqueous extracts within an incubator for durations of 24 and 48 h. Alternatively, the MTT solution was introduced to the media and then incubated for 4 hours in a dark environment, after which the optical density was measured at 570 nm. The resultant impact of varying aqueous extract concentrations on the viability of A549 and BEAS-2B cells was depicted graphically in Figures 3 and 4.

The viability of cells in their initial state was quantified through optical densities (ODs), which were standardized at 100% when the aqueous extract concentration was 0 µg/mL. This quantification was in accordance with the equation described as follows [25]:

$$Percentage\ of\ viability = \frac{OD\ of\ test}{OD\ of\ control} \times 100\% \qquad (1)$$

The fluctuation in optical density (OD) values is contingent upon several factors, including the cell count within each well, the composition of the culture media, the concentration of the aqueous extracts, the metabolic activity of cells, the cytotoxicity of MTT reagents, and the duration of the incubation period for formazan crystal formation [26].

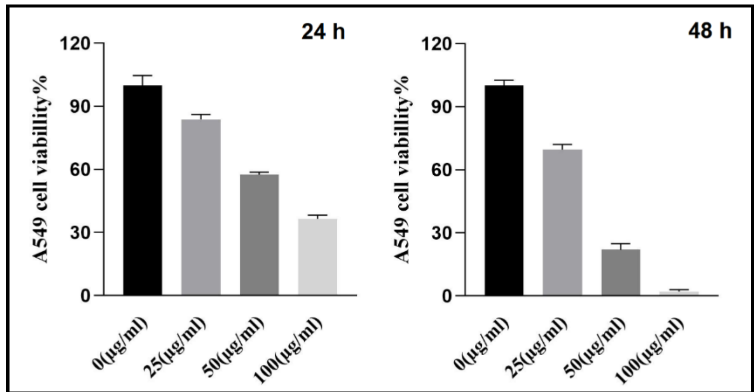


**Figure 3.** Impact of extracts and their concentrations on A549 cell viability.

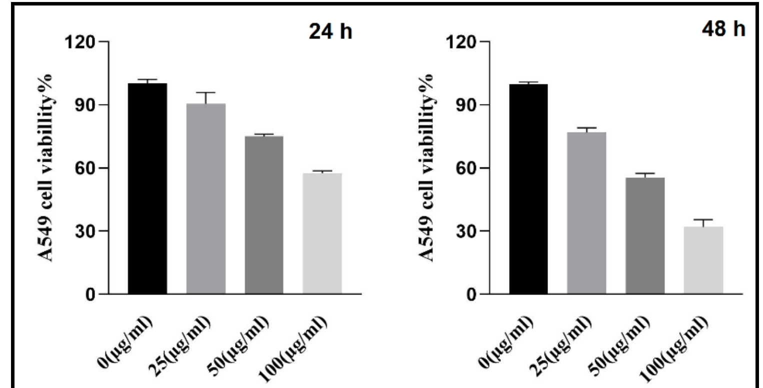


**Figure 4.** Impact of extracts and their concentrations on BEAS-2B cell viability.

**Table 2.** Values of $IC_{50}$ for extracts solution.

| Cell Lines | $IC_{50}$ | |
|---|---|---|
| | **24 h** | **48 h** |
| A549 | 66.16 | 32.84 |
| BEAS-2B | 124 | 58.60 |

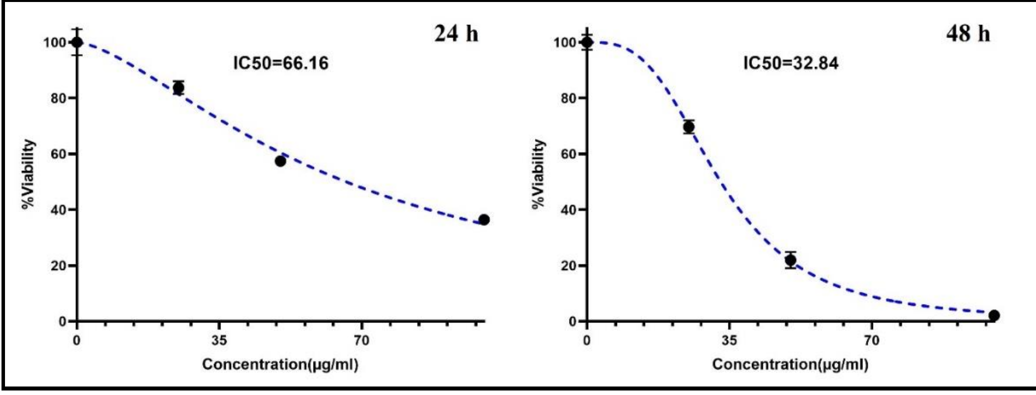


**Figure 5.** Correlation between IC50 Profile and the impact of aqueous extract concentration on A549 cells.

The MTT assay serves as the predominant method for determining the half-maximal inhibitory concentration ($IC_{50}$). Through this assay, the inhibitory activity of aqueous extracts

becomes evident by elucidating the $IC_{50}$ values, which signify the concentration of extracts required to inhibit 50% of the cells' activity [27]. These $IC_{50}$ values have been compiled in Table 2. The determination of $IC_{50}$ is a fundamental approach for assessing cell viability in the presence of cytotoxic agents within cell cultures. Notably, as cell viability decreases over different time intervals (24 and 48 hours), the corresponding $IC_{50}$ values exhibit a reduction, as depicted in Figures 5 and 6.

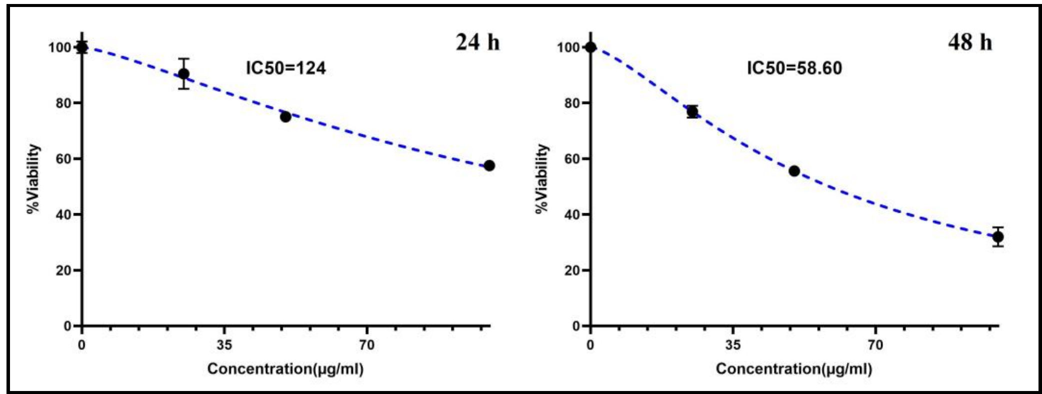


**Figure 6.** Correlation between $IC_{50}$ profile and the impact of aqueous extract concentration on BEAS-2B cells.

The findings from the study demonstrated a notable inhibition of A549 and BEAS-2B cell proliferation by the aqueous extracts derived from *Melissa officinalis*. Notably, an escalation in cellular apoptosis was observed as the concentrations of the aqueous extracts increased. It is evident that at a higher concentration (100 µg/mL), the viability of cells was impacted, which could be attributed to the induction of autophagy rather than mere inhibition of cell proliferation [10].

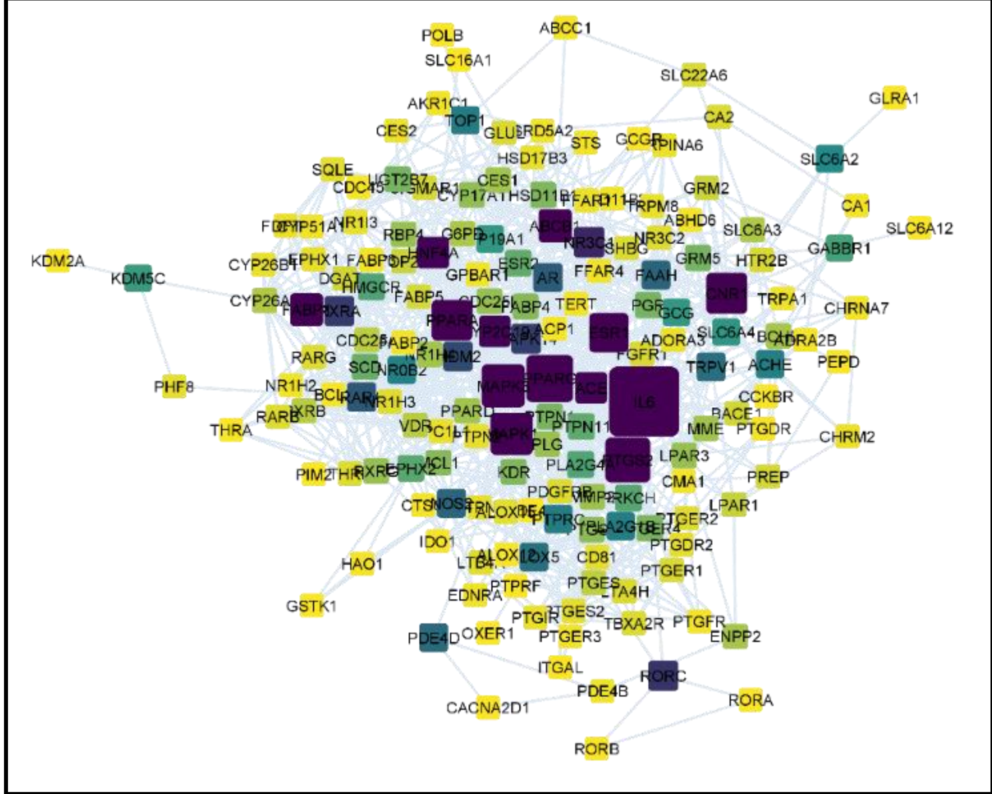


**Figure 7.** Predicted hub proteins of protein-protein interactions.

*3.3. Network pharmacology analysis.*

In this phase of the study, we utilized the Swiss Target Prediction [28] web platform to amass a list of anticipated target genes (100) associated with linolenic acid, linoleic acid, oleic acid, stearic acid, palmitoleic acid, and palmitic acid. Through the utilization of Cytoscape 3.9 [29], we predicted hub genes, leading to the creation of the network visualization presented in Figure 7. Notably, ACE, NR3C1, HNF4A, ALOX12, PTGES, CYP2C19, MDM2, ALOX5, PTGS1, RARA, RXRG, RXRB, PLA2G1B, MMP2, MCL1, VDR, ALOX15, PLG, RARG, RARB, and THRB were identified as hub genes.

In Figure 8, a Venn diagram showcases the common targets shared by linolenic acid, linoleic acid, oleic acid, palmitoleic acid, palmitic acid, and stearic acid. The main targets shared across these compounds are PGR, ALOX5, PTGES, SCD, PTGER4, and AR CYP2C19. Subsequently, by intersecting the shared targets of the extracted compounds with the top 22 hub genes (ALOX5, PTGES, and CYP2C19), we identified the most promising targets, as demonstrated in Figure 9.

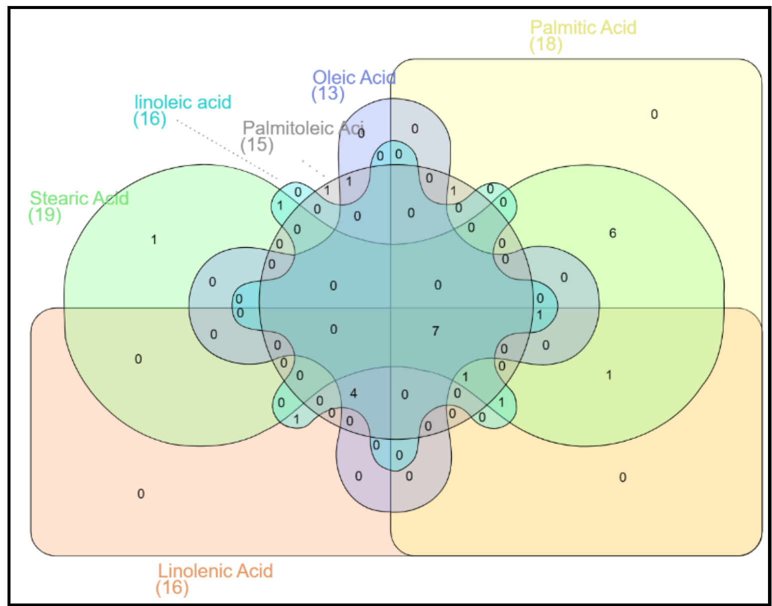


**Figure 8.** Top predicted compound-protein interactions.

**Figure 9.** The intersection of A (top predicted compound-protein interactions) and B (hub genes).

*3.4. KEGG enrichment analysis.*

The KEGG enrichment analysis yielded a total of 15 significant signaling pathways ($P < 0.05$) subsequent to the investigation of common targets of linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid, showcasing their potential as potent anticancer agents [29].

Notably, the anticancer mechanisms of these effective compounds were observed to be mediated through a diverse array of pathways. The implicated pathways encompassed Arachidonic acid metabolism, Serotonergic synapse, Thyroid hormone signaling pathway, Pathways in cancer, Chemical carcinogenesis - receptor activation, Linoleic acid metabolism, Non-small cell lung cancer, Transcriptional misregulation in cancer, PPAR signaling pathway, Neuroactive ligand-receptor interaction, Small cell lung cancer, Parathyroid hormone synthesis, secretion and action, Th17 cell differentiation, and AMPK signaling pathway [30]. These findings underscore the involvement of linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid in several pivotal cell signaling pathways, as illustrated in Figure 10.

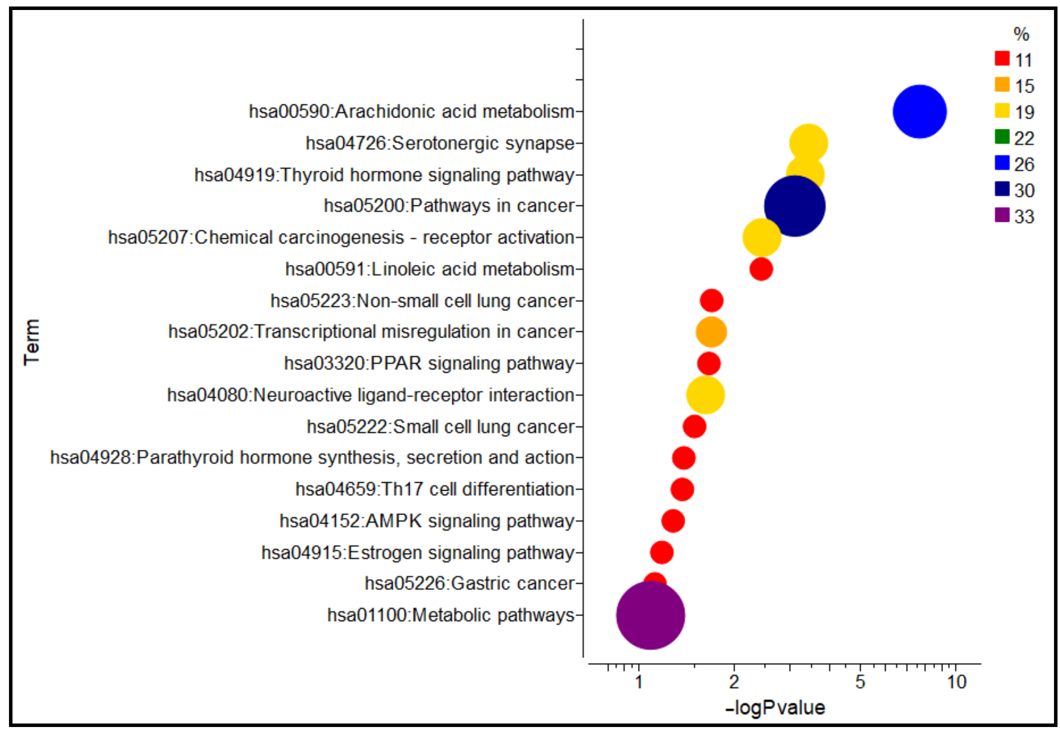


**Figure 10.** KEGG pathway analysis of the 22 core targets of linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid against cancer: bubble diagram.

*3.5. Molecular docking.*

Molecular docking is a computational technique used to predict the binding affinity and interactions between small molecules (compounds in the extract) and protein targets associated with cancer. The docking analysis of linolenic acid, linoleic acid, stearic acid, palmitoleic acid, palmitic acid, and oleic acid was performed with ALOX5, PTGES, and CYP2C19 as the primary targets for their potential anticancer activity [31]. The intricate details of docking energies and RMSD values are comprehensively outlined in Table 3. Upon evaluation, it was discerned that linolenic acid exhibited the highest docking score and a remarkable affinity for the active site of ALOX5 (PDB: 3V99), accompanied by notably negative binding energy. This

robust interaction is illustrated in Figure 11 A, showcasing hydrogen bond interactions and hydrophobic residue interactions between linolenic acid and ALOX5.

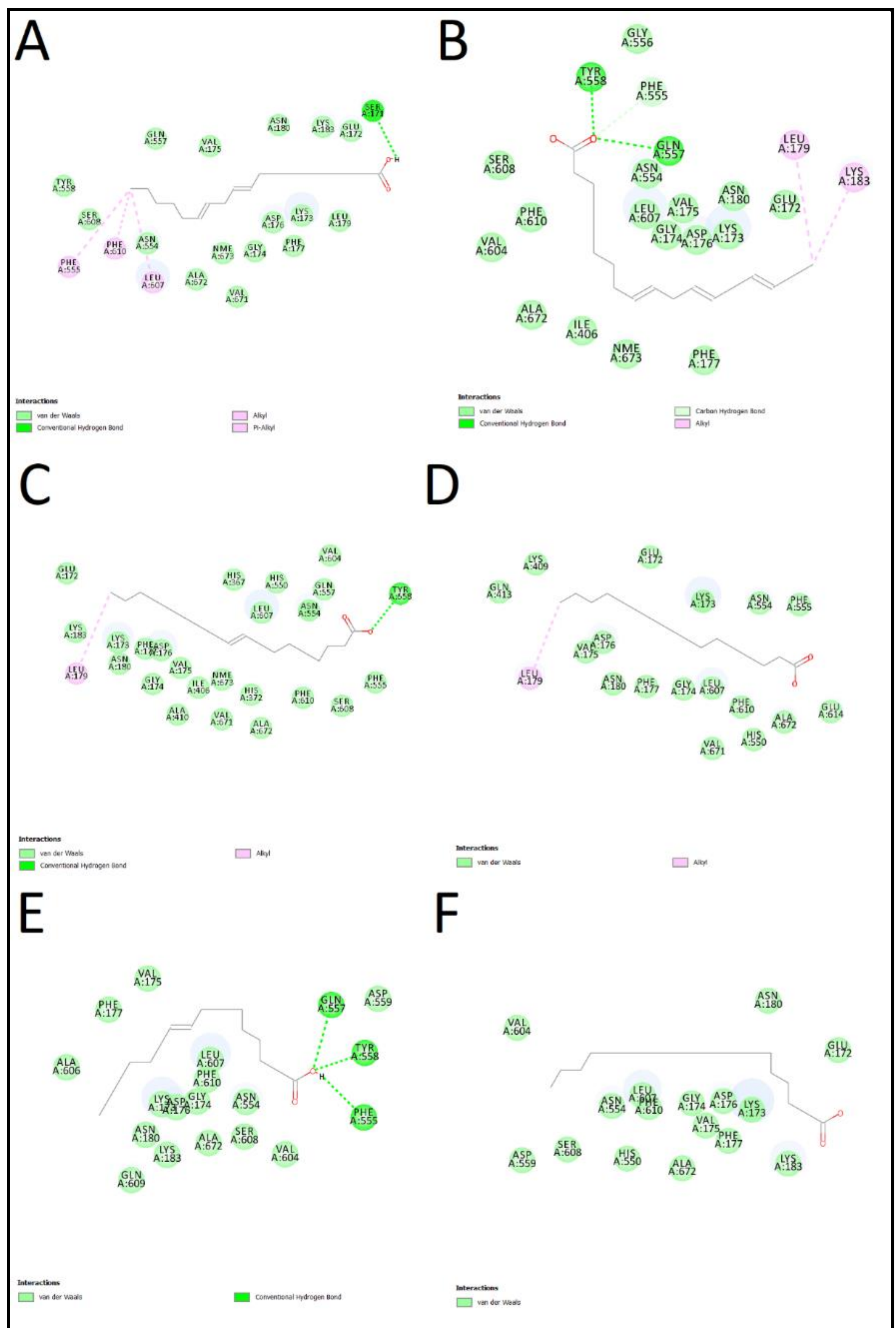


**Figure 11.** Interaction of linoleic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid with ALX5 (PDB: 3V99).

In the case of the next target, PTGES (PDB: 4AL1), stearic acid displayed the most substantial negative binding energy, indicating a strong binding affinity with PTGES. The 2D representation of stearic acid's interaction with PTGES is depicted in Figure 12 F. Furthermore, in the docking assessments involving CYP2C19 (PDB: 4gqs), oleic acid emerged as the top

scorer with the highest docking score. This interaction's visualization and oleic acid's interactions with the residues of CYP2C19 are presented in Figure 13 C. This analysis can identify the potential mechanisms of action and specific molecular targets for the anticancer effects of the extract.

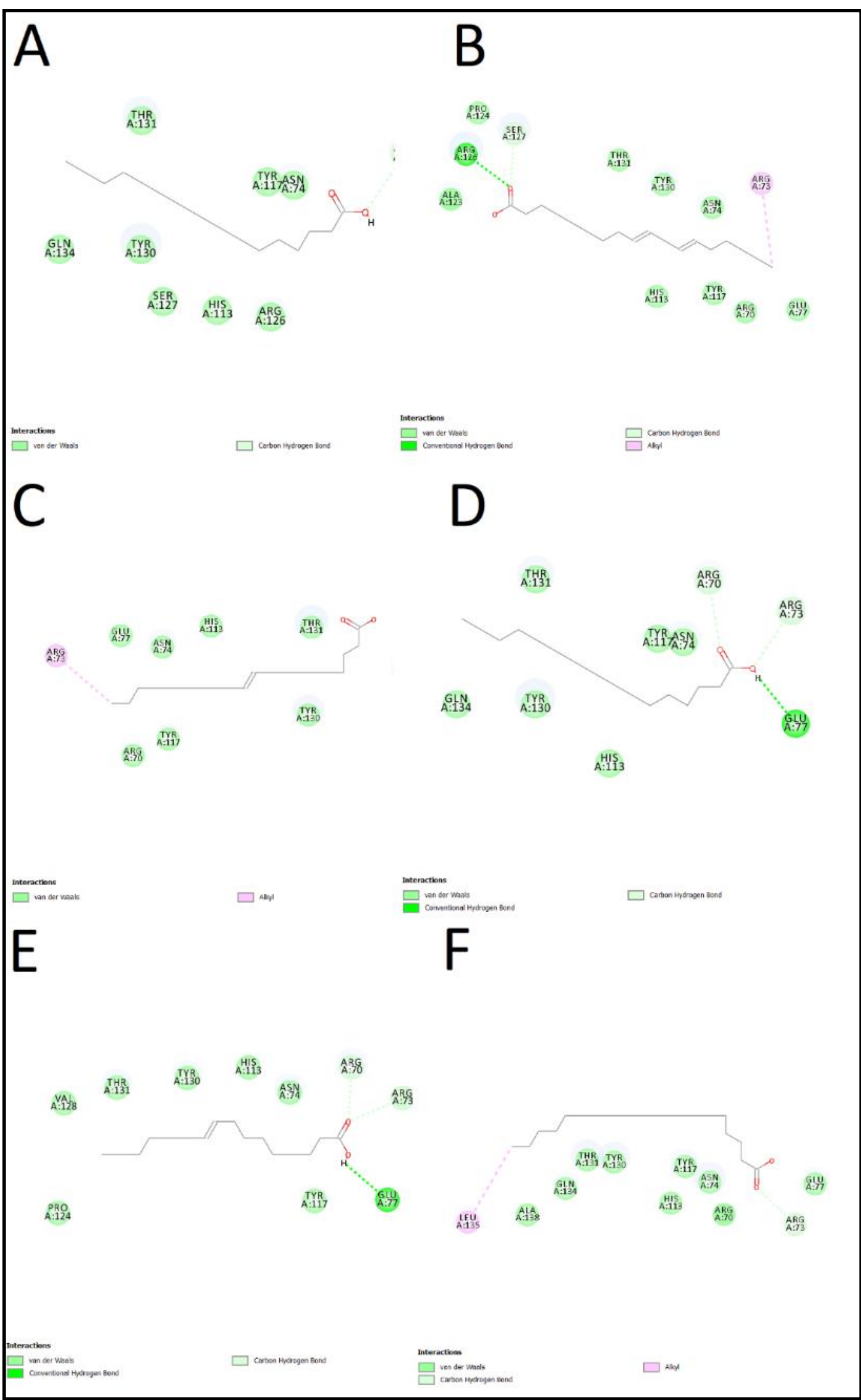


**Figure 12.** Interactions of linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid with PTGES (PDB: 4AL1).

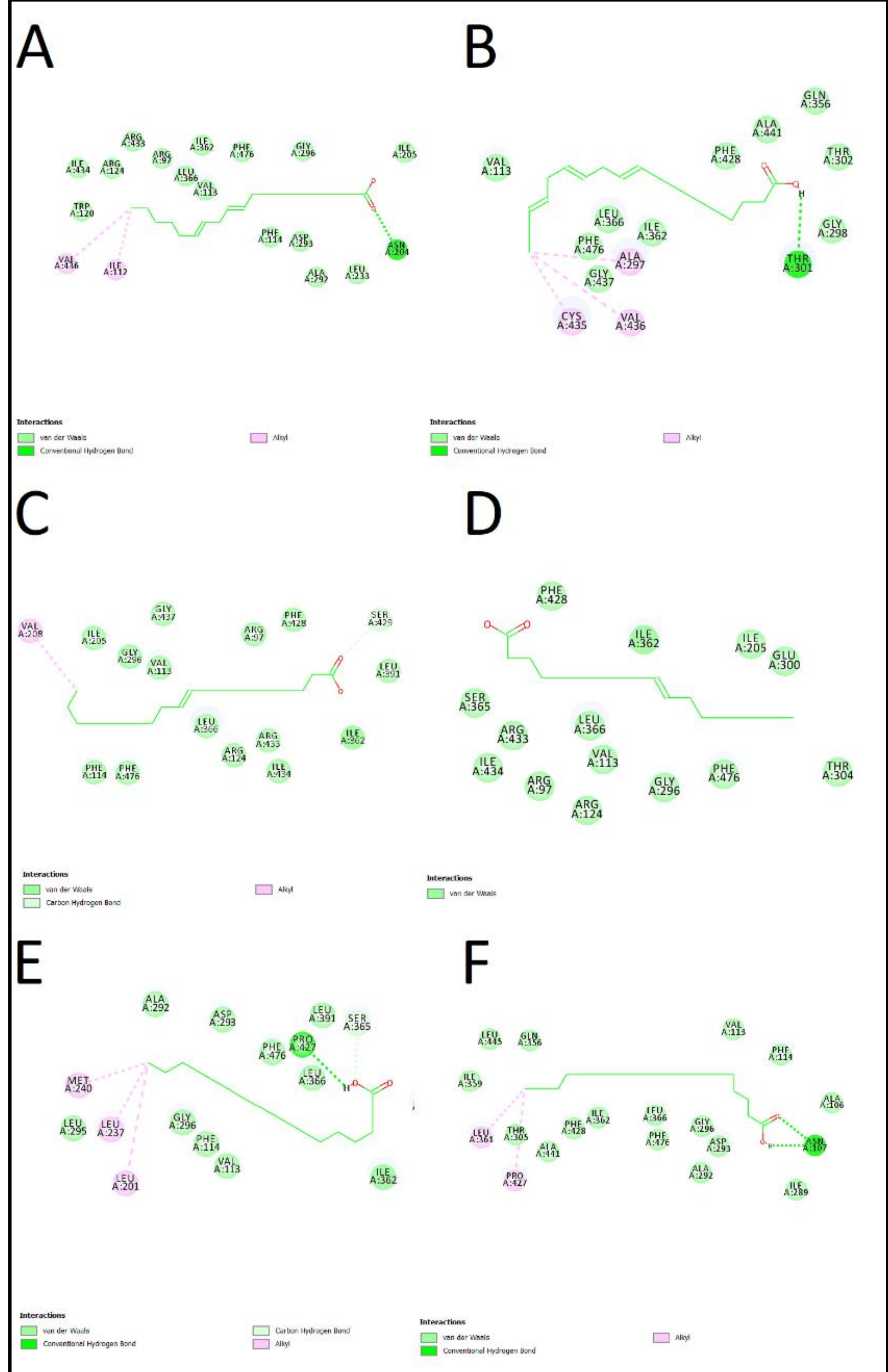


**Figure 13.** Interactions of linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid with CYP2C19 (PDB: 4gqs).

Combining network-based pharmacology [1] and molecular docking [2] allows for a comprehensive understanding of the anticancer potential of *Melissa officinalis* extract, from its chemical composition to its effects on biological systems and potential mechanisms of action.

Such studies contribute to the growing knowledge of natural compounds [1] as potential cancer therapeutics.

**Table 3.** Docking results of linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid with proteins 4AL1, 4gqs, and 3V99.

| Compound name | Docking score (kcal/mol) | RMSD (Å) | Docking score (kcal/mol) | RMSD (Å) | Docking score (kcal/mol) | RMSD (Å) |
|---|---|---|---|---|---|---|
| | 4AL1 | | 4gqs | | 3V99 | |
| linolenic acid | -5.36 | 3.13 | -7.36 | 2.08 | -7.79 | 2.52 |
| linoleic acid | -5.38 | 3.10 | -7.33 | 2.60 | -7.08 | 1.90 |
| oleic acid | -5.54 | 1.41 | -8.01 | 2.29 | -7.49 | 1.68 |
| stearic acid | -5.60 | 1.53 | -7.86 | 1.63 | -7.35 | 1.82 |
| palmitoleic Acid | -5.36 | 1.51 | -6.95 | 1.50 | -7.23 | 1.61 |
| palmitic acid | -5.07 | 1.06 | -7.08 | 1.25 | -6.96 | 1.18 |

## 4. Conclusions

Applying aqueous extracts from *Melissa officinalis* leaves to A549 and BEAS-2B cell lines revealed varying optical densities in the culture media treated with MTT reagent, reflecting the cells' viability. The primary compounds found in these extracts, namely palmitoleic acid, palmitic acid, oleic acid, stearic acid, linolenic acid, and linoleic acid, demonstrated their potential to reduce cell viability as concentrations increased. This effect could be attributed to autophagy induction rather than mere inhibition of cell proliferation. Furthermore, the analysis of protein-protein interactions highlighted ALOX5, PTGES, and CYP2C19 as the most promising candidates for regulating cancer pathways, particularly those associated with non-small and small-cell lung cancer. In molecular docking, linolenic acid exhibited the highest binding affinity to ALOX5, oleic acid displayed the highest binding affinity to CYP2C19, and stearic acid showcased the highest binding affinity to PTGES. These findings collectively suggest that a combination of these extracts could potentially wield considerable efficacy in treating lung cancer.

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## Conflicts of Interest

The authors declare no conflict of interest.

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