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**Research Article**

# Coat protein of alfalfa mosaic alfamovirus (AMV) from Türkiye: genetic inference and *in silico* docking analysis for potential antiphytoviral purposes

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## Abstract

In 2021, a study was conducted in the Denizli region of Türkiye to investigate the phylogenetic relationship and presence of alfalfa mosaic alfamovirus (AMV) infecting pepper plants exhibiting viral disease symptoms. A total of 57 samples were collected, of which twenty-four tested positive for AMV with reverse transcriptase polymerase chain reaction (RT-PCR) assays. Samples from pepper plants displaying virus symptoms gave positive bands on the agarose gel, while healthy plants yielded negative results. One of the positive samples was randomly selected, cloned and sequenced. This sequence of the Denizli AMV isolate (753 bp) was recorded in the GenBank database with accession number OQ845956. The nucleotide sequence showed a high nucleotide consensus of 97%-99% compared with the nucleotide sequences of the same variants from different origins in GenBank. According to the phylogenetic tree generated through the Neighbour Joining (NJ) method, this AMV isolate belongs to the same group as Iranian isolates from various of hosts. Furthermore, *in silico* docking analysis of the coat protein (CP) of the AMV isolate and promising 12 essential oil compounds was performed to enable potential antiviral drug development. Docking study showed that eucalyptol, eugenol and carvacrol can make important contributions to the advancement of drug-based strategies for the managing of plant viruses by interacting with the virus coat protein of high binding energies, -5.3, -5.2 and -5.0 kcal mol$^{-1}$, respectively. Although the presence of AMV in Denizli province has been reported previously, this study reports the phylogenetic relationships and docking analysis of the new AMV isolate in pepper crops.

***Keywords:*** antiphytoviral compound; AMV; *in silico* modelling; molecular docking; phylogeny

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## Introduction

Pepper (*Capsicum annuum* L.) is the most commonly cultivated and economically important species of the *Capsicum* genus, which comprises 25 species and belongs to the Solanaceae family (Pernezny *et al.,* 2003). Rapid population growth is leading to increased demand for food resources (Cemeroğlu *et al.,* 2009). Due to the limited area available for crop production, it is necessary to protect against biotic and abiotic factors that limit the yield per unit area. Virus diseases are prominent biotic factors, because of their physical and chemical structure, size, mode of infection, ability to spread, ability to multiply and lack of effective control methods (Agrios, 1997; Yılmaz and Davis, 1984). It is estimated that more than 70 viruses, including potato Y potyvirus (PVY), tomato mosaic virus (ToMV), pepper mild mottle tobamovirus (PMMoV), alfalfa mosaic virus (AMV), tobacco mosaic tobamovirus (TMV), tomato spotted wilt virus (TSWV), and cucumber mosaic cucumovirus (CMV) cause annual losses ranging from 42% to 80% in pepper production (Çelik *et al*., 2012; Çelik *et al*., 2013; Kenyon *et al*., 2014; Kılıç *et al*., 2015; Colimba *et al*., 2016; Cho *et al*., 2021). AMV, a member of the family Bromoviridae and the genus Alfamovirus, possesses a genome comprised of three single-stranded positive-sense RNAs, designated RNA 1, RNA 2, and RNA 3. The first two RNAs encode the replicase subunits 1a and 2a, respectively. The movement protein is expressed by RNA 3, while the viral coat protein is expressed by subgenomic RNA 4 (Smit and Jaspars, 1982; Jaspars, 2018). AMV can be transmitted through non-persistent routes with a minimum of 70 distinct aphid species, and can persist in weeds and other alternative hosts, facilitating its spread to susceptible crops (He *et al*., 2010). AMV may also be transmitted by mechanical methods, through sap (Wintermantel and Natwick, 2012), and through seed (Hiruki and Hampton, 1990). The symptoms of AMV can vary from mild to severe depending on the plant species, virus virulence, age and size of the plant, and environmental conditions. The leaves infected with AMV can show various symptoms, including light and dark green mosaic patterns on leaves, stunted growth, reduced yield, yellowing, and necrotic lesions on various crops (Al-Shahwan *et al*., 1997; Zitikaitė and Samuitienė, 2008; Al-Abrahim, 2014).

AMV has been detected in many plant species worldwide, not just in one region. It has been found in vegetable and forage crops, including alfalfa in Saudi Arabia (Al-Shahwan, 2002), soybean in China (Che *et al*., 2020), pea in Iran (Esfandiari *et al.,* 2005), natural tobacco, alfalfa and pepper plants in Türkiye (Arli-Sökmen *et al*., 2005; Erdem, 2010; Usta *et al*., 2020;), potatoes in Canada (Xu and Nie, 2006), tomatoes in Lithuania (Zitikaitė and Samuitienė, 2008), and in many other studies. AMV is infectious to weeds, spice and ornamental plants, and various fruit trees; it has been detected in basil in Egypt, ornamental plants (*Narcissus*) and *Campsis radicans* known as trumpet creeper in Iran, *Lavandula* × *Intermedia* in Croatia, eggplant in Egypt, and medicinal plants (*Gynostemma pentaphyllum*) in China (Vrandečić *et al*., 2013; Pourrahim and Farzadfar, 2015; Al-Shahwan *et al*., 2017; El-Attar *et al*., 2019; Song *et al*., 2019).

Essential oils, also known as volatile monoterpenes, sesquiterpenes, and phenylpropanoids, are aromatic plant molecules generating and accumulating in aromatic plant families such as Apiaceae, Myrataceae, Asteraceae, Lauraceae, Lamiaceae, and Rutaceae. They are particularly rich in terms of secondary metabolites. Hydrocarbons, ketones, aromatic and aliphatic chemicals, alcohols, phenols, aldehydes, esters, and acids are all chemical ingredients found in essential oils, which are principally extracted using steam or hydro-distillation (Božovic *et al*., 2017). A number of these chemicals have been studied for their purported effects on both mammalian and plant infections, and they have been shown to have insecticidal activities, antiparasitic, antimycotic, antiviral, and antimicrobial (Lamiri *et al*., 2001; Juglal *et al*., 2002; Moon *et al*., 2006; Michaelakis *et al*., 2007; Feriotto *et al*., 2018). The antiphytoviral activity of EOs is a relatively new area of study. Preliminary investigations have indicated that these phytometabolites are responsible for the immune reaction that occurs during viral infection. (Dunki´c *et al*., 2010; Vuko *et al*., 2012). Several investigations have demonstrated that essential oils rich in sesquiterpenes from different plant species can alleviate viral infections in host plants at close range (Dunkic *et al*., 2010).

Concerns about sustainability, human health, environmental protection, and social issues have led to a shift in focus away from chemical control measures that damage cultivated plant virus epidemics. Pesticides, which are contaminants in soil, air, and water, are widely believed to be a major contributor to the observed declines in terrestrial biodiversity (Jones, 2006; Assey *et al*., 2021). Due to the fact that they are readily accessible, inexpensive, biodegradable, have a variety of mechanisms of action, and are low in toxicity to organisms that are not the intended targets, plant-based extracts and natural substances have been characterized as promising instruments for the management of various crop pests and weeds (Lengai *et al*., 2020; Souto *et al*., 2021). Studies have shown that plant-based compounds have activity against phytoviruses (Emamzadeh-Yazdi *et al*., 2018; Song *et al*., 2021). In other regions of Türkiye, the presence of AMV on various plants has been documented (Morca *et al*., 2024); nevertheless, information regarding the molecular phylogeny of the pathogen that infects pepper plants in Denizli province, Türkiye, is limited. Therefore, this study is remarkable in terms of contributing to the literature on the existence and spread of AMV in Türkiye. Moreover, because essential oils found in plant extracts have antiviral properties against virus infection (Dunkic *et al.,* 2010), the aim of this study was to investigate the potential antiviral activity of essential oils by evaluating commercial antiviral against AMV with molecular docking approach revealing the interaction between the compound and the target. Specifically, it utilized *in silico* techniques to analyze the interaction between particular essential oil compounds and the coat protein of the AMV Denizli isolate.

## Materials and Methods

### *Plant material and RNA extraction*

In 2021, both symptomatic and non-symptomatic pepper plants were collected from pepper cultivation fields in Denizli province, Türkiye. The methodology outlined by Foissac *et al.* (2001) was utilized to extract total RNA from new leaf tissues.

### *Detection of AMV infection by RT-PCR*

Sense and antisense primers targeting the coat protein (CP) gene (S: 5′-TCCATCATGAGTTCTTCAC-3' and A: 5′-AGGACTTCATACCTTGACC-3′) were used to detect viral RNA. Viral RNA was reverse transcribed using random primers, and then subjected to polymerase chain reaction (PCR) using sense and antisense primers. PCR was performed using an Eppendorf Mastercycler (Germany) with a total of 25 µl of reaction volume under the same reaction mixture and temperature conditions as previously described by Güller *et al.* (2022). For the identification of pathogens identified during preliminary testing, an alfalfa AMV isolate was utilized as a positive control. A negative control was included in the study, using genomic RNA extracted from a healthy pepper plant. Tris-acetate-EDTA (TAE) buffer and 1% agarose gel were utilized in agarose gel electrophoresis to separate and visualize the PCR products that were generated. Following ethidium bromide staining of the gel, the products were evaluated using a UV transilluminator.

### *Sequencing and cladistic analysis*

The PCR amplicons (approximately 1 ml fraction) were gel-purified and then inserted into pGEM-T Easy Vector (Promega, Madison, USA). The obtained recombinant plasmids were transformed into competent bacteria (*E. coli* strain JM109) via an electric current (80 V at 45 m), and the resulting recombinant plasmids were purified and sequenced via next-generation sequencing (NGS).

Based on the resulting complete CP gene sequence, phylogenetic relationships between the present AMV and other global strains in the National Centre for Biotechnology Information (NCBI) database were evaluated using MEGA4 and CLC Main Workbench 6.6.1 software. Phylogenetic inference and multiple alignments were performed using 1000 bootstrap replicates and Clustal W options, respectively. A Turkish barley yellow dwarf virus isolate (KC900900) was selected as an outgroup to root the phylogenetic tree.

*Computational docking analysis*

Structural analysis of AMV CP

Three-dimensional protein structures aid in the design of investigations such as site-directed mutagenesis, disease-related mutation studies and structure-based inhibitor design by revealing the molecular basis of protein activity. Swiss-Model was used to model the 3D structure of the AMV Denizli 67 coat protein receptor (Waterhouse *et al.,* 2018). The Swiss-Model server automates the comparison of 3D protein structures from sequences. After selecting a template using BLAST and HHbits, a raw model is generated from a rigid fragment assembly. The evaluation of the predicted structure of the coat protein of the AMV Denizli 67 isolate was performed using the SAVES v5.0 server.

*Docking analysis*

For the determination of the molecular mechanism of action of phytocompounds in connection to viral protein targets, molecular docking research on essential oils was carried out *in silico* with the help of the Auto Dock Vina software (http://vina.scripps.edu/). The term "*in silico* analysis" relates to the practice of conducting scientific research using computer simulations, modeling, and analysis, as opposed to empirical experiments (Korkmaz *et al*., 2022). This methodology is used to save costs, optimize the use of time, and sometimes in situations where experimentation is challenging or dangerous has the potential to provide a valid alternative (Cairns *et al*., 2016; Usta *et al*., 2023, Demirel, 2021).

*Target protein retrieval and Ligand processing*

The three-dimensional structures of the CP of AMV Denizli 67 isolates were obtained from *https://swissmodel.expasy.org/*, and its pdb. The data used for docking is shown in Figure 3.

The three-dimensional structures of the main chemical constituents of essential oils were obtained through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). These structures were used in docking experiments and served as ligands. The ligands were energy minimized using the UCSF Chimera software. "Gasteiger's methods were used to optimize the ligand(s), with the net charge set to zero, and the optimization procedure was performed using the UCSF Chimera package (version 1.16). As a result, the optimized ligands were saved in "mol2 format" for the molecular docking studies.

*Receptor docking with ligands*

The selected chemicals were subjected to docking analysis against a specific target protein of the AMV Denizli 67 isolate using the UCSF Chimera program (version 1.16). This tool uses a gradient optimization approach throughout its local optimization phase for the detection of the ranking of the ligands based on the empirical binding free energy ($\Delta G$ in kcal $mol^{-1}$) function. Following the completion of the docking technique, a thorough investigation and comparison of the docked sites of the target proteins and ligands was carried out using UCSF Chimera (version 1.16).

## Results

*Characteristics of AMV- infected pepper plants*

Pepper samples were collected throughout the study, consisting of 30 samples with virus symptoms and 27 samples without symptoms. The peppers exhibited irregular mosaic patterns on the leaves (typically light green or yellow in color), general discoloration or chlorosis, leaf deformation and curling, and stunted growth and development (Figure 1). All 57 pepper samples were analyzed with RT-PCR, of which 24 were AMV positive and the rest were not. PCR amplification targeting the coat protein gene using specific primers resulted in a 750-bp DNA product on an agarose gel. No positive fragments were obtained from RNA extracted from healthy pepper plants.

**Figure 1.** Symptoms of pepper plants naturally infected with alfalfa mosaic virus (AMV) from Denizli region of Türkiye

*Molecular characterization of the Denizli-AMV isolate*

In the present study, among 24 positive AMVs, a random isolate yielding a quality DNA band was cloned and sequenced with NGS for characterization and molecular phylogeny. The sequence obtained (753 nt in size) was denominated as Denizli 67 and registered in the gene bank under accession number OQ845956. Using the Basic Local Alignment Search Tool (BLAST) at NCBI, the AMV-Denizli CP gene sequence was found to have a similarity of 97.61% to 99.20% to other sequences worldwide. The highest and lowest similarity scores were observed for the Iranian (KM655875) and Chinese (OL521619) isolates. The complete DNA sequence of the AMV Denizli CP gene was aligned with 26 sequences, excluding the outgroup and the current sequence, and the phylogenetic tree was generated with the NJ approach with 1000 bootstrap replicates. AMV genome nucleotide data of various sizes from Türkiye are available at NCBI, but only complete CP gene nucleotide sequences were used for phylogenetic inference in this study. Complete gene-based phylogenetic analyses divided AMV isolates into four major groups (Figure 2).

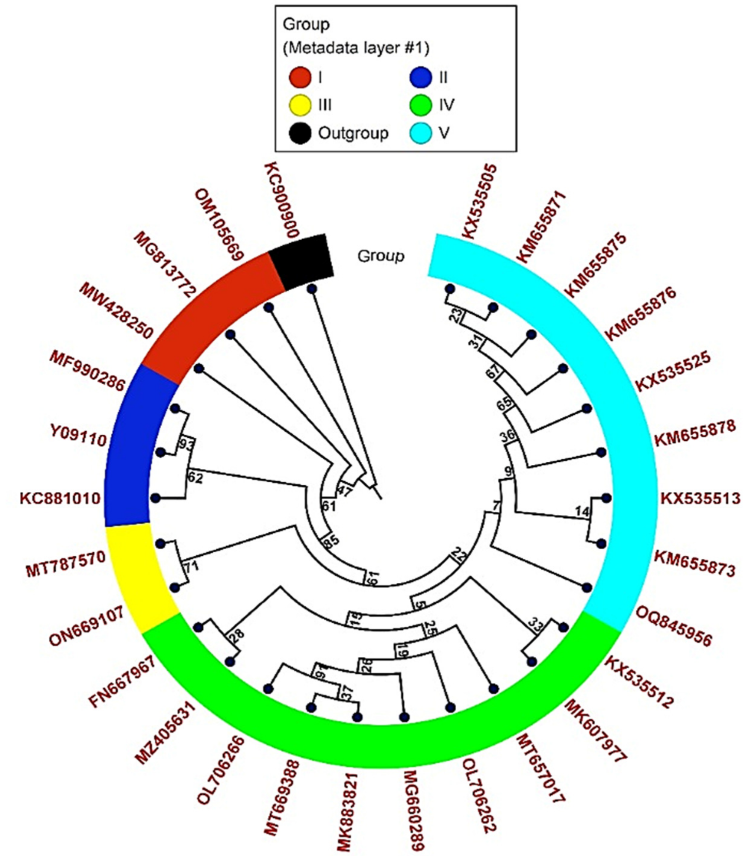


**Figure 2.** Phylogenetic dendrogram created by employing NJ, including the Denizli AMV isolate and other isolates from around the world
The sequence of the barley yellow dwarf virus sequence (KC900900) was used as an outgroup. Bootstrap values are given for each branch of the dendrogram.

The phylogenetic tree grouped the Turkish Denizli isolate (OQ845956) from pepper plants with Iranian isolates from different plant families, including alfalfa (*Medicago sativa*), pepper (*Capsicum annuum*), hot pepper (*Capsicum frutescens*), cowpea (*Vigna unguiculata*), and black pepper (*Solanum nigrum*) (turquoise colour).

*Molecular interaction of EOs with the AMV CP*

In the current study, homology modelling was performed using a bioinformatics tool to predict the 3D structure of the AMV CP protein of the Denizli 67 isolate. Molecular function and the identification of potential active sites can be better understood using 3D structures. To better understand the function of a protein from its 3D structure, computational homology modelling is a useful method. For the CP protein of the AMV Denizli 67 isolate, the values calculated by the Swiss online modeller for QMean, Cß. All atoms, Solvation, Torsion, Template and Sequence identity are listed in Table 1.

**Table 1.** Swiss-Model server structure verification scores for the AMV Denizli 67 isolate coat protein (CP)

| The Parameter's value was calculated using the Swiss Modeller | | | | | | |
|---|---|---|---|---|---|---|
| QMean | Cß | All atoms | Solvation | Torsion | Template | Sequence identity |
| -3,13 | -2.50 | -2.31 | -3.14 | -1.59 | 7epp.1.A CP | 94.12% |

Figure 3 shows the predicted protein structures of AMV CP. The model structure developed using the Swiss Model showed that 85.2% of the residues were within the most favourable region of the Ramachandran plot. As a result, the projected model of the AMV CP protein from the Denizli 67 isolate was found to meet all the requirements for a high-quality protein as defined by PROCHECK (Wu *et al.,* 2009). According to the evaluation conducted, the ab initio model provided by homology modelling of template 7epp.1.A exhibited the highest level of reliability and suitability for conducting molecular docking studies. It was therefore selected for the docking analysis.

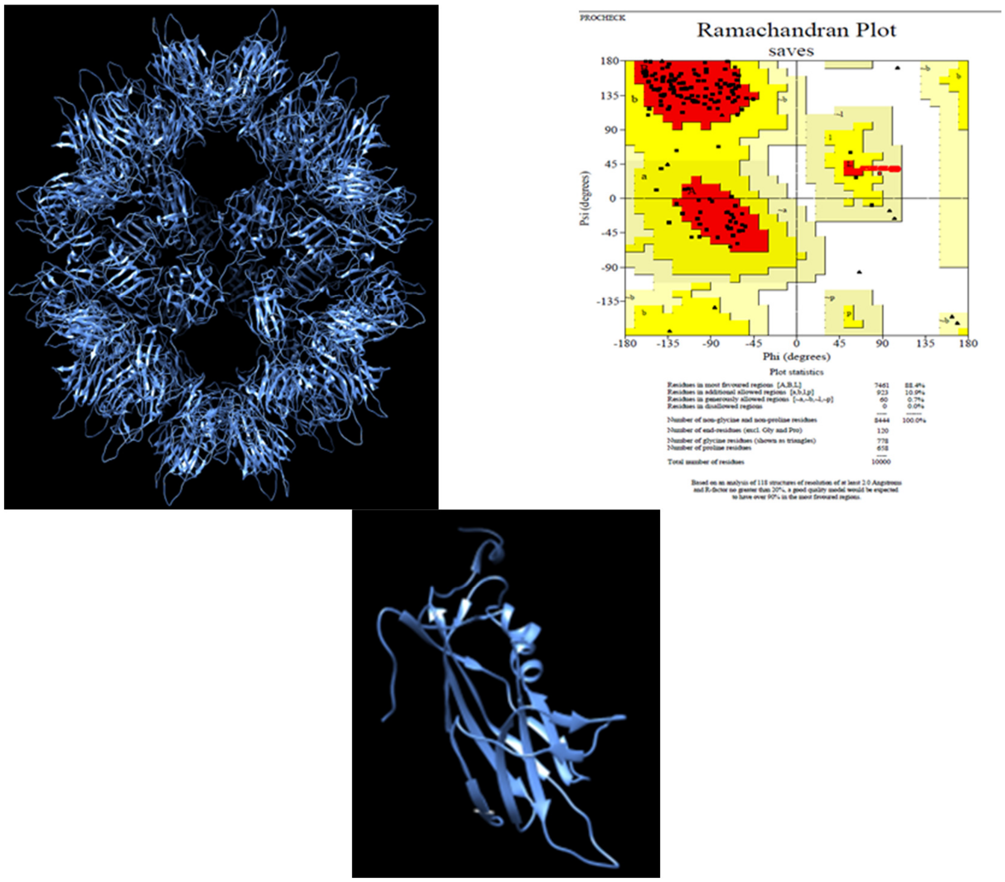


**Figure 3.** Homology model and Ramachandran plot of the coat protein of AMV obtained from the Swiss Model

In the light of all this information, we conducted a molecular docking study to investigate the molecular mechanism of action of the main chemical constituents of essential oils as antiphytovirals, as shown in Table 1. Using the UCSF Chimera program (version 1.16), we were able to dock molecules and determine the binding energy between them.

We also docked them to AMV CP using two different flavones and a commercial chemical to compare the *in silico* antiphytoviral potential of the essential oil components. Both epigallocatechin and quercetin exhibited rather high levels of binding energies (-6.6 and -6.5 kcal $mol^{-1}$, respectively). Among the chemical constituents of essential oils, eucalyptol exhibited the highest binding affinity interaction with Ile3 at -5.3 kcal $mol^{-1}$. This was followed by eugenol at -5.2 kcal $mol^{-1}$, carvacrol at -5.0 kcal $mol^{-1}$, camphor at -4.9 kcal $mol^{-1}$, thymol and alpha-terpineol at -4.8 kcal $mol^{-1}$, borneol at -4.6 kcal $mol^{-1}$, and geraniol at -4.5 kcal $mol^{-1}$, as shown in Figures 4-15 (Supplementary file), and summarized in Table 2.

**Table 2.** Ligands' CID number, molecular weight, and Auto Dock Vina score virtually screened against the AMV CP protein

| No | Compound | Compound CID | MW | Kcal mol$^{-1}$ | AAR | H bind number |
|---|---|---|---|---|---|---|
| **1** | Carvacrol | 10364 | 150.22 | -5.0 | Ile72- | 1 |
| **2** | Oct-1-En-2-Ol | 15372159 | 128.21 | -3.3 | Leu26, Val28 | 2 |
| **3** | Alpha-terpineol | 17100 | 154.25 | -4.8 | Val28 | 1 |
| **4** | Camphor | 2537 | 152.23 | -4.9 | Ile3 | 1 |
| **5** | Eucalyptol | 2758 | 154.25 | -5.3 | Ile3 | 1 |
| **6** | Borneol | 6552009 | 154.25 | -4.6 | Val28 | 1 |
| **7** | Thymol | 6989 | 150.22 | -4.8 | Ile3 | 1 |
| **8** | Eugenol | 3314 | 164.2 | -5.2 | Ile3 | 1 |
| **9** | Geraniol | 637566 | 154.25 | -4.5 | Ala77 | 1 |
| **10** | Myrcene | 31253 | 136.23 | 0 | - | 0 |
| **11** | D-Limonene | 440917 | 136.23 | 0 | - | 0 |
| **12** | Calamenene | 6429077 | 202.33 | 0 | - | 0 |
| **13** | Ribavirin | 37542 | 244.2 | -4.6 | Lys137 | 1 |
| **14** | Epigallocatechin | 72277 | 306.27 | -6.5 | Val28 | 1 |
| **15** | Quercetin | 5280343 | 302.23 | -6.6 | Ser21, Ala77 | 3 |

* MW: molecular weight (g/mol), AAR: amino acid residue interacting with ligand and its distance

Oct-1-EN-2-OL from the essential oil components displayed the highest vina score of -3.3 kcal mol$^{-1}$. In addition, the docking results revealed that the three compounds (myrcene, d-limonene and calamenene) did not interact with AMV CP. While flavones have the highest vina score among the compounds docked to AMV CP, the commercial compound ribavirin has a lower vina score than carvacrol, camphor, eucalyptol, alpha-terpineol, eugenol, and thymol.

## Discussion

AMV has a high potential to spread over large geographical areas and hosts. The ability of AMV to infect multiple host species is due to its broad range of aphid vectors and its ability to adapt to different plant environments. Alfalfa mosaic virus (AMV) infects numerous plants, such as alfalfa (*Medicago sativa*), clover (*Trifolium* spp.), bean (*Phaseolus* spp.), pea (*Pisum sativum*), tobacco (*Nicotiana tabacum*), carrot, parsley, and tomato (*Solanum lycopersicum*) (Campbell and Melugin, 1971; Kvíčala, 1975; Esfandiari *et al.*, 2005; Trucco *et al.*, 2021). During the course of our surveys, virus characteristic symptoms including mosaic patterns of different sizes and colours, leaf anomalies and stunting were noticed on the virus-infected leaves in pepper plants. These symptoms were consistent with the reports of Demir (2005), Buzkan *et al.* (2006), Keleş-Öztürk (2017), Keleş-Öztürk and Baloğlu (2020).

The detection of AMV in plant tissues is mainly based on molecular and serological techniques. Literature screening indicates that ELISA tests have been widely used for detecting the presence of AMV in various plant species. In Hakkari province, a study was conducted to investigate 184 tomato and pepper plants exhibiting viral symptoms of AMV infection using DAS-ELISA. The diagnosis of AMV was confirmed in two tomato samples (Akdura and Kılıç, 2022). In another study using DAS-ELISA, single and mixed infections of three viruses, including AMV, were detected in bean plants in Muğla province. The infection rate for AMV was found to be quite low, at 3.57% (Kılıç *et al.*, 2013). The presence of seven viral agents was investigated in potato production fields in Hatay province using DAS-ELISA. PVY infection was detected in potato samples at a rate of 49.5%, followed by PLRV and AMV infections at 5.4% (Carpar and Sertkaya, 2016). Furthermore, the presence of AMV was serologically detected in pepper plants in Gaziantep, Şanlıurfa, and Kahramanmaraş

(Buzkan *et al.*, 2006), *Amaranthus retroflexus*, pepper, *Malva sylvestris*, *Solanum nigrum*, and *S. oleraceus* in Hatay (Sertkaya and Özdağ, 2017; Sertkaya *et al.*, 2017), tobacco in Samsun (Erdem, 2010), and pepino in Uşak (Özdemir and Erilmez, 2012). AMV-infected pepper plants were reported in Samsun by Arli-Sökmen *et al.* (2005), in Adana by Çetinkıran and Baloğlu (2011), and in Kahramanmaraş by Demir (2005). In addition, the presence of AMV in Türkiye was molecularly shown with RT-PCR in parsley in Konya (Morca *et al.*, 2022), potato in Tokat (Topkaya, 2022), and alfalfa in Bingöl and Van (Usta and Güller, 2020; Güller *et al.*, 2022). AMV was also reported in the provinces of Manisa, Balıkesir, and Burdur from different plant origins (Özdemir *et al.*, 2011; Özdemir *et al.*, 2011; Kılıç and Yardımcı, 2015). Therefore, it can be concluded that the infection of AMV in both cultivated and wild plants is extensively spread at a national level. This is likely because the virus can propagate to potential host plants through various transmission pathways such as cell sap, seeds, or aphid vectors (Wintermantel and Natwick, 2012).

Through the use of phylogenetic analysis based on the AMV coat protein of AMV isolates from different regions of the world, several studies have reported on genetic relationships between AMV isolates as well as the differentiation between AMV subgroups (Bergua *et al.*, 2014; Bandjo-Oreshkovikj *et al.*, 2017; Trucco *et al.*, 2022; Wang *et al.*, 2023). Although numerous studies have been conducted on AMV in various regions of Türkiye, few of them have investigated the phylogenetic relationships of this pathogen. According to the literature review, Fidan (1992) was the first to report AMV infection in alfalfa plants displaying virus-like symptoms using test plants in the Denizli region, Türkiye. On the other hand, Özdemir and Erilmez (2007) reported the detection of AMV in lettuce and pepper plants using serological methods. In the current study, most of the virus symptomatic samples (30/24) gave positive results on PCR tests, while others were negative. The absence of the virus in some symptomatic samples is likely associated with the presence of other pepper viruses such as tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), pepper mild mottle virus (PMMoV) and potato virus Y (PVY), which can cause similar symptoms (Pazarlar *et al.*, 2013; Petrov, 2014; Karavina *et al.*, 2021).

The coat protein gene from a positive sample was PCR-amplified and subsequently cloned. Following this, the full nucleotide sequence was established and phylogenetic relationships were determined. In the resulting phylogenetic tree, 4 clusters were formed using AMV isolates from varied ecogeographical regions. Notably, the Turkish Denizli AMV isolate clustered with Iranian isolates from the Asia region, regardless of plant species. Geographically, the fact that Türkiye and Iran are neighbors may have paved the way for the emergence of this grouping due to the transport of viral isolates through seedling and seed exchange, although mostly by aphid transmission (Fegla *et al.*, 2000; Çulal-Kılıç and Yardımcı, 2015). In a previous study, Parrella *et al.* (2010) classified AMV isolates into two categories, designated as group I and group II. Following that, in the research that Parrella *et al.* (2011) carried out, group II was divided even further into subgroups known as IIA and IIB. Later on, Stanković *et al.* (2014) extended this classification by dividing AMV isolates into four or more distinct groups based on sequence data from the CP gene region. Different main groups of AMV isolates have been identified by (Bergua *et al.*, 2014; Trucco *et al.*, 2014; Abdalla *et al.*, 2015; Wang *et al.*, 2023) phylogenetic analysis of the complete viral coat protein gene. Usta and Güller (2020) analyzed the molecular phylogeny of the partial CP gene sequence of Van AMV isolates using sequences available in Genbank. The phylogenetic tree divided AMV isolates worldwide into 2 main groups, with Van isolates showing high phylogenetic similarity with isolates from the USA, Brazil and Puglia. In addition, the partial CP gene of Bingöl AMV isolates (MW962977 and MW962976) from alfalfa was found to contain 700 bp nucleotides. The phylogenetic tree related these isolates to other Turkish isolates from Van and Iğdır provinces with 3 major groups (Güller *et al.*, 2022). Topkaya (2022) reported the presence of AMV by PCR amplification based on the CP gene from potato plants in the Tokat province, Türkiye. The two sequences (700 bp) obtained showed phylogenetic affinity in subgroup I, consisting of isolates from Iran, Canada, Türkiye, Korea, and Serbia. These phylogenetic relationships indicate that Turkish AMV isolates are highly divergent and mostly related to Asian isolates, but also to American and European isolates. Genetic variation in AMV isolates may be due to recombination, specialization to different geographical regions or plant species, evolutionary pressure, or natural mutation due to errors in genetic material or environmental conditions (Moradi and Mehrvar, 2019). Although the literature indicates that AMV isolates are categorized into many categories, our research classified AMV isolates into 5 main groups based on phylogenetic analysis.

Plant virus infections result in significant economic losses in the global agricultural sector (Lecoq and Katis, 2014; Coşkan *et al*., 2022; Morca *et al*., 2022; Topkaya *et al*., 2023). The primary focus of defensive strategies in plant virus control is on preventive measures, including the utilization of sanitarily certified propagating material and insect vector management (Fereres and Raccah, 2015; Golino *et al*., 2017). Unlike bacteria and fungi, there are currently no recuperative treatments available for virus control in plants, whether they are derived from natural or synthetic compounds (Rubio *et al*., 2020). Studies have revealed the antiphytoviral activity of EOs (Zhao *et al*., 2017; Raveau *et al*., 2020) and many compounds, both natural and manufactured, have been employed to control plant virus infections. The measuring of localized lesions is the primary method used in research on tobacco mosaic virus (TMV). Some studies have explored the impact of various essential oils (EOs) on the development of local symptoms in the experimental host, *Nicotiana glutinosa* (Bishop, 1995; Lu, 2013). Their findings indicated that treatments using EOs derived from lemon, lemongrass, *Melaleuca alternifolia*, ginger, artemisia, tea tree, and tangerine peel, successfully prevented the development of symptoms. In addition, Dunkic *et al*. (2010) found that the use of essential oil decreased the amount of local lesions in infected plants with the tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV). They found that the application of thymol alone was 33.2% effective against CMV infection and 34.3% effective against TMV infection.

The antifungal and antibacterial effects of essential oils against fungi and bacteria that infect both humans and plants have been demonstrated by many studies. Additionally, new studies have revealed the antiphytoviral activities of essential oils against plant viruses, even though numerous studies have already shown their antiviral activities against viruses that infect humans and animals (Kalemba and Kunicka, 2003; Bozkurt *et al*., 2020; Ma and Yao, 2020; Wani *et al*., 2021; Parikh *et al*., 2021; Taglienti *et al*., 2022). There is not any study that discusses the docking technique of essential oils against plant viruses, despite the fact that several studies in the scientific literature demonstrate the antiviral potential of essential oils against viruses that infect humans using an *in silico* docking approach. Essential oils have been shown to be effective against plant viruses, according to the docking findings of this research. Findings from in vivo studies of such components are necessary to support docking results in addition to docking studies. Many researchers have investigated the antiviral properties of commercial chemicals such as ribavirin as well as plant-derived compounds against different viruses and have demonstrated the higher antiviral potential of natural components compared to synthetic chemicals (Zhao *et al*., 2015; Gan *et al*., 2017; Lu *et al*., 2019). Based on results of this study, in which the interaction of AMV envelope protein and essential oils was investigated for the first time with in silico docking, some of the essential oil compounds exhibited higher binding affinity than the commercial chemical ribavirin but lower binding affinity than the two different flavonoids used in the study and these results are in agreement with the results reported from previous studies (Iobbi *et al*., 2022; Mohan Kumar *et al*., 2022).

AMV has a high degree of infectivity across many plant species belonging to distinct families, hence presenting an elevated threat to agricultural products (Xu and Nie, 2006). Insect-mediated virus transmission is the main cause of new infections in host crops in neighboring fields. To prevent the spread of the virus and to minimize future crop losses, it is necessary to implement appropriate control programs, eradicate weeds that its natural epidemiological reservoirs, and raise awareness among farmers about the use of clean seeds and planting materials. This novel work contributes to the identification of new antiviral drugs in the fight against AMV and sheds light on virus-antiviral interactions.

## Conclusions

The presence of AMV in pepper growing areas in the Denizli region of Türkiye was determined using molecular tools. The nucleotide sequence information of the complete CP gene of the pathogen was deposited in GenBank, and phylogenetic relationships and docking analyses were reported for the first time in this study. In recent years, there has been a significant increase in the number of research that has focused on exploring essential oils (EOs) as a potential source for the development of novel antivirals. In particular, the relative efficacy against pathogens, multiple modes of action, and comparatively low toxicity to animals and humans have been highlighted as positive characteristics of this compound. Our computational approach led to the discovery of potent inhibitors of AMV CP. Molecular docking was used to compare the phytochemical library with the coat protein to find the most potent lead compounds. In addition, eucalyptol, eugenol and carvacrol, three of the most promising ligand molecules among essential oil compounds, were found to bind to the active site of the target protein. Further *in silico* cross-docking and in vitro research, including enzymatic assays, is required to verify the computational results, as this work is based solely on computational mining.

## Authors' Contributions

MU and AG: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. SD, GK and AK: Methodology, Performing experiments, Data curation, Formal analysis, in silico analysis, Software, Writing-original draft, Writing – review & editing.

All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.


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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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