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Bioactive Compounds of Plant Essential Oils and Their Antiviral Properties: A Comprehensive Review

Ahmad Khalis Yahya[1], Noor Zarina Abd Wahab[1]* and Nazlina Ibrahim[2]
[1]School of Biomedicine, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, 21300 Kuala Nerus, Terengganu, Malaysia
[2]Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi 43600, Selangor, Malaysia
*Corresponding author (e-mail: zarinawahab@unisza.edu.my)

The utilization of essential oils, a group of biologically active natural compounds, has garnered significant attention due to their multifaceted health-promoting attributes. The potential health benefits of essential oils may be subject to modulation by the unique chemical composition inherent to each plant species. The lipophilic nature of essential oils enables them to readily traverse the viral membrane, leading to the subsequent disruption and rupture of the said membrane. Essential oils usually have bioactive compounds that can act on different parts of a virus, including the virus's entry into a cell, transcription, translation, and assembly. The purpose of this review is to characterize the biological and therapeutic properties of medicinal plants' essential oils towards various viral infections. Findings show that most previous research reported the antiviral properties of essential oils through various inhibition of viruses. The present discourse endeavors to elucidate the traditional utilization of a diverse array of 44 distinct botanical species, as documented in a comprehensive compilation of 33 scholarly investigations, in the context of mitigating a multitude of viral infections. The data collected for the current review were obtained through the PubMed database only. In conclusion, essential oils of every type of medicinal plants could be considered as natural therapeutic agents for the treatment of viral infections. Therefore, essential oils of these medicinal plants have a great potential to be developed for modern medicinal use.

Keywords: Essential oil; antiviral; bioactive compound; medicinal plant; natural product

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Numerous commodities derived from botanical sources exhibit compelling constituents, which hold promise for the exploration of bioactive compounds in the pursuit of novel antiviral agents [1]. The extraction of botanical specimens possessing medicinal properties has the potential to yield intriguing extracts, such as essential oils. The utilization of essential oils derived from botanical sources with medicinal properties has been deeply rooted in traditional practices as a therapeutic modality for addressing a wide range of ailments, particularly those of viral etiology. Notably, several essential oils have demonstrated significant *in vitro* antiviral properties against a range of pathogenic viruses, such as dengue virus serotype 2 (DENV-2), SARS-CoV2, and Zika virus, among others [2, 3]. The chemical constitution of essential oils is distinguished by the existence of a wide range of sesquiterpene and monoterpene hydrocarbons, alongside oxygenated compounds encompassing aldehydes, ketones, phenols, and an assortment of other constituents. The utilization of essential oils as a foundation for the advancement of antiviral agents has gained significant attention due to their demonstrated *in vitro* antiviral activity against various pathogenic human enveloped viruses. The antiviral properties of various medicinal plants, including *Cymbopogon citratus* [4], *Pelargonium graveolens* [4], *Melaleuca aternefolia* [5], *Salvia desoleana* [6], *Goniothalamus umbrosus* [7], *Catharanthus roseus* [8], and several others, have been substantiated through scientific investigations. The utilization of essential oils as a prominent reservoir of groundbreaking therapeutic agents for diverse infectious diseases has been extensively explored in numerous studies [9-11].

In recent years, there has been a notable surge in the scholarly attention directed towards the utilization of essential oils. The surge in fascination surrounding essential oils has been paralleled by an escalating apprehension regarding their safety implications, thus instigating a more concentrated and scrupulous scrutiny of these fragrant substances. A comprehensive investigation into the antiviral properties of essential oils derived from medicinal plants is deemed necessary, particularly in relation to their potential antiviral effects against DENV-2. The existing body of research on this subject remains limited, warranting further exploration to ascertain the inhibitory potential of these essential oils against this specific virus. Considering the burgeoning body of literature pertaining to essential oils, it has become

imperative to undertake a comprehensive evaluation of this subject matter. Such an assessment is crucial to prevent redundant future research endeavors pertaining to the antiviral properties of essential oils derived from diverse medicinal plants. This necessity arises from the potential oversight of extant studies, which may have been inadvertently neglected in previous reviews.

The significance of natural products as antiviral agents holds immense value. Derived from various sources such as plants, fungi, and marine organisms, these products contain bioactive compounds that possess antiviral properties. Exploring the potential of natural products in antiviral therapies offers several advantages. Natural products exhibit diverse chemical structures, enabling a wide range of mechanisms to combat viruses. They can hinder viral entry into host cells [7], impede viral replication [8], modulate the immune response [9], and target vital viral proteins and enzymes. The multi-target effects of natural products are particularly advantageous, as they can act on multiple stages of the viral lifecycle simultaneously. This could be explained by a previous study that the methanol extract derived from *Kyllinga nemoralis* roots exhibits the promising potential in altering the replication cycle of HSV-1 at various stages including immediate early, early, and late phases of the replication cycle [10], which could reduce the risk of viral resistance development and enhance the overall effectiveness of antiviral therapies.

Natural products have long been utilized in therapeutic research and treatment due to their potential effectiveness against various human diseases, as demonstrated by the antiviral activities of terpenoids and phenols derived from plants like *Andrographis paniculate* and *Cinchona officinalis* [11]. Terpenoids, including monoterpenes and sesquiterpenes, comprise around 90% of essential oils' chemical makeup, lending them their distinctive color, flavor, and aroma, and serving as crucial components for applications in industries such as cosmetics, pharmaceuticals, perfumes, and food [12]. In this review, relevant studies related to antiviral properties of various types of essential oils are focused, identified, and critically analyzed. Furthermore, analyses of bioactive compound research reveal that various types of essential oils have high-quality contents which can be used as excellent sources of antivirals.

METHODS OF REVIEW

A comprehensive review of the existing literature was undertaken to ascertain prior scholarly articles that elucidate the effectiveness of essential oils in the treatment of diverse viral infections. One of the prominent databases utilized for information retrieval through querying is PubMed. A comprehensive literature search was conducted by amalgamating the following set of keywords: "essential oil" AND "antiviral". Furthermore, an extensive review of the existing literature was conducted with the aim of identifying and delineating pertinent articles pertaining to the antiviral efficacy of essential oils against a range of viral infections. The scope of this comprehensive review encompasses a span of 47 years, commencing from the year 1977 and extending until the year 2023, thereby encapsulating a wide range of scholarly publications. The preliminary investigation yielded a total of 309 search results, indicating a substantial corpus of relevant information for further analysis and examination. The abstracts of the publications were meticulously examined to ascertain their relevance and suitability for the present study. Following the application of additional exclusion criteria, namely the exclusion of non-English manuscripts, manuscripts lacking full-text availability, studies employing a combination of multiple essential oils, studies involving the concomitant use of essential oils with medication, and studies with incomplete data, a total of 33 articles were retained for further analysis.

Table 1. Isolated compounds of essential oils from various plants with antiviral properties. The table is sorted in alphabetical order of the plant source.

Plant Source	Bioactive Compound	Antiviral Effects	Reference
Achillea millefolium	Kessanyl acetate Farnesol	Showed high binding energies against SARS-CoV-2 $3CL^{pro}$ by forming H-bonds with CYS 145 and HIS 41	[14]
Agathis robusta	α-Pinene Tricyclene α-terpineol Limonene Camphene trans-pinocarveol α-phellandren-8-ol β- pinene Borneol	*In vitro* activity exhibited against NRC-03-nhCoV with a significant selectivity index (17.5)	[15]
Artemisia mendozana	Camphor Artemisole	Inhibitory effect on DENV-2, JUNV, and HSV-1	[1]

	Artemisia alcohol Borneol		
Artemisia princeps var. *orientalis*	α-thujone	Showed strong inhibition on FCV-F9 and MNV-1	[16]
Baccharis dracunculifolia	Nerolidol Spathulenol β-pinene trans-caryophyllene	Inhibited PV1 replication in post-treatment	[17]
Buddleja cordobensis	Caryophylene oxide trans-caryophylene α-copaene	Inhibitory effect on DENV-2, JUNV, and HSV-1	[1]
Cinnamomum zeylanicum	Cinnamaldehyde	Exerted antiviral activity involving both virucidal effect and viral reproduction of SARS-CoV-2	[18]
Citrus bergamia	Limonene β-pinene	Strongly reduced IAV viral cytopathic effect without exerting any cytotoxicity	[19]
Citrus limon	Limonene β-pinene γ-terpinene Citral α-pinene β-thujene	Significantly decreased FduscCV viral titer of 0.75 $\log_{10}$ $TCID_{50}$/50 µL after 8 h at 302.0 µg/mL for as surrogates for *in vitro* evaluation of antivirals for NoV	[20]
Citrus limon	Limonene	Downregulated ACE2 expression of SARS-CoV-2 spike receptor-binding domain in epithelial cells	[21]
Cymbopogon citratus	Geranial Neral Myrcene	Inhibited RRV-renLuc in luciferase activity and residual infectivity titer	[4]
Eucalyptus caesia	1,8-cineole p-cymene γ-terpinene α-pinene terpinen-4-ol α-terpineol	Reduced virus titers by 57.9% for HSV-1 and 75.4% for HSV-2 at concentration of 0.03% Significant inhibitory effect on HSV-1 and HSV-2 plaque formation	[22]
Eucalyptus camaldulensis	γ-terpinene terpinen-4-ol 1,8-cineole	1/10 dilution of 100 µL of oil reduced Rotavirus strain Wa, Coxsackievirus B4, and HSV-1 plaque formation by 50%, 53.3%, and 90%, respectively	[22]
Eucalyptus globulus	1,8-cincole Limolene α--pinene	Significant inhibitory effect on HSV-1 plaque formation	[22]
Eucalyptus globulus	1,8-cineole	Reduced IAV viral infection by 78% with no cytotoxicity	[19]
Eucalyptus polybractea	1,8-cineole p-cymene terpinen-4-ol limonene α-terpineol	Exposure to the aerosol (15 s; 125 µg/L of air) achieved 100% inactivation of IFV-A in the air One day of exposure to oil vapor (saturated) reduced viral infection by IFV-A by 86%	[22]
Eupatorium arnottianum	Spathulenol Trans-caryophyllene Germacrene-D Bicyclogermacrene α-Humulene γ-Muurolene α-Cadinol cis-Cadin-4-en-7-ol Caryophyllene oxide	CC_{50} values of 396.7 ppm Inhibitory effect against DENV-2 and HSV-1 with IC_{50} values of 38.2 and 52.1 ppm, respectively	[23]

Eupatorium catarium	Limonene Piperitenone trans-Dihydrocarvone Camphor cis-Dihydrocarvone trans-caryophyllene Bicyclogermacrene	CC_{50} values of 483.5 ppm Inhibitory effect against DENV-2 and HSV-1 with IC_{50} values of 57.3 and 47.9 ppm, respectively	[23]
Galliardia megapotamica	β-pinene Z-β-ocimene α-pinene Limonene trans-caryophylene	Inhibitory effect on JUNV	[1]
Heterothalamus aleinus	β-pinene Sphatulenol Germacrene D	Inhibitory effect on JUNV	[1]
Hornstedtia bella	α-pinene β-pinene 1,8-cineole	Strongly reduced viral VV titer in cell-based assay at not cytotoxic concentration	[24]
Illicum verum	Trans-anethole Trans-caryophylene	Suppressed HSV-1 multiplication by >99%	[3]
Jungia polita	Caryophylene oxide Trans-caryophylene	Inhibitory effect on DENV-2	[1]
Juniperus oxycedrus ssp. *oxycedrus*	α-pinene β-myrcene	Antiviral activity against HSV-1 with an IC_{50} value of 200 mg/mL and SI of 5	[25]
Lantana grisebachiii (Seckt.) var. *grisebachii*	Bicyclogermacrene Germecrene-D Spathulenol Trans-Caryophyllene Piperitenone α–Copaene	Inhibitory effect against DENV-2 and HSV-1 with IC_{50} values of 21.1 and 26.1 ppm, respectively Selectivity indices > 23.7 and > 19.1 for DENV-2 and HSV-1, respectively	[23]
Laurus nobilis	β-ocimene 1,8-cineole α-pinene β-pinene	Exerted activity against SARS- CoV with an IC_{50} value of 120 mg/mL and SI of 4.16	[25]
Laurus nobilis	α-pinene β-pinene β-ocimene Eucalyptol	Exerted inhibitory activity against SARS-CoV replication (IC_{50} = 120 µg/mL)	[26]
Lippia alba	Geranial Geraniol Neral Trans-Caryophyllene	IC_{50} (SI): 78 ± 1.1 µg/mL (5.5) for DENV-1 IC_{50} (SI): 67 ± 1.2 µg/mL (6.4) for DENV-2	[2]
Lippia alba	Carvone Limonene	Direct DENV-1,2,3,4 inactivation before adsorption on host cell	[27]
Lippia graveolens	Carvacrol	Antiviral effects on HHV, BVDV, and HRSV	[28]
Lippia origanoides	Trans-Caryophyllene Thymol 1,8-Cineol p-cymene	IC_{50} (SI): 77 ± 1.1 µg/mL (6.6) for DENV-1 IC_{50} (SI): 75 ± 1.0 µg/mL (6.8) for DENV-2	[2]
Melaleuca alternifolia	Terpinen-4-ol	Inhibited entry of H1N1 into host cell	[5]
Melaleuca alternifolia	terpinen-4-ol terpinolene α-terpineol	Inhibitory effect on influenza virus A/PR8 replication	[29]

Melaleuca alternifolia	γ-terpinene	Strongly reduced IAV viral cytopathic effect without exerting any cytotoxicity	[19]
Melissa officinalis L.	Citral Caryophyllene-E Caryophyllene Oxide	Inhibited replication of AIV subtype H9N2 through different virus replication phases	[30]
Myristica fragrans	Sabinene α-pinene β-terpinene β-myrcene	92.5% reduction of the IBV viral titer and contributable virucidal activity in 1:15 dilution 94.38% reduction of the IBV viral titer and contributable virucidal activity in 1:30 dilution	[31]
Nigella sativa	Thymoqinone Dithymoqinone Thymohydroquinone	Anti-SARS-CoV-2 activity at non-cytotoxic nanomolar concentrations *in vitro* with a low selectivity index (CC_{50}/IC_{50} = 31.74/ 23.15 = 1.4)	[32]
Ocimum basilicum L.	Camphor 1,8-cineole	Showed high IC_{50} value (474.29 ± 8.65) towards BVDV	[33]
Pectis odorata	Limonene Neral Geranial	Inhibitory effect on DENV-2 and JUNV	[1]
Pelargonium graveolens	Citronellol Geraniol Citronellyl formate Linalool Isomenthone	Inhibited RRV-renLuc in luciferase activity and residual infectivity titer	[4]
Pelargonium graveolens	Citronellol	Downregulated ACE2 expression of SARS-CoV-2 spike receptor-binding domain in epithelial cells	[21]
Salvia dentata	terpinen-4-ol terpinolene α-terpineol	Showed very good H1N1 inhibition with 93% ± 1.3% and 94% ± 1.4% at 0.001% and 0.0001% concentration respectively	[34]
Salvia desoleana	Linalyl acetate Alpha terpinyl acetate Germacrene D	Inhibited both acyclovir sensitive and acyclovir resistant HSV-2 strains	[6]
Syzygium aromaticum	Eugenol β-caryophyllene α-copaene	High antiviral potential against HAV, with a selectivity index (SI) of 14.46	[35]
Thymus capitata	3-Methyl-4-isopropylphenol Camphor Linalool Eugenol Borneol	Viral CPE of BHV-1 significantly reduced, and the anti-BHV-1 activity showed dose-dependent response	[36]
Thymus schimperi	Geranylisobutanoate 3-octane	Exhibited strong binding to catalytic dyad of SARS-CoV-2 Mpro with acceptable ADMET profiles	[37]
Trachyspermum ammi	α-pinene p-cymene Limonene	80% JEV inhibition with concentration 0.5 mg/mL in pre-exposure treatment 40% JEV inhibition with concentration 0.5 mg/mL in post-exposure treatment	[38]
Trixis divaricata	Trans–caryophyllene Spathulenol β-Elemene Caryophyllene oxide	CC_{50} values of 159.7 ppm Inhibitory effect against DENV-2, JUNV, and HSV-1 with IC_{50} values of 56.5, 18.6 and 37.8 ppm, respectively	[23]

Turnera diffusa	Aristolochene Dehydrofukinone Valencene β-Selinene Trans-caryophyllene p-cymene	IC_{50} (SI): 54 ± 1.1 μg/mL (7.7) for DENV-1 IC_{50} (SI): 29 ± 1.1 μg/mL (14.3) for DENV-2	[2]
Vanillosmopsis arborea (Gardner) Baker	(-)-α-bisabolol	Non-cytotoxic to Vero cells and inhibited DENV in all assays (pre, post- and virucidal treatment), except for pretreatment against DENV-3	[39]
Zanthoxylum acanthopodium DC.	Terpinen-4-ol	Significant antiviral activity against DENV with an IC_{50} value of 13 μg/mL	[40]

RESULTS AND DISCUSSION

The physicochemical properties of essential oils encompass a diverse array of characteristics, which notably include their antiviral, antibacterial, antifungal, and various other attributes. The term "essential oil" refers to intricate combinations of volatile and lipophilic secondary metabolites that are extracted from medicinal plants. These metabolites include sesquiterpenes, phenylpropanoids, monoterpenes, and various others, and they play a crucial role in determining both the fragrance and biological characteristics of the plant in question [13]. Table 1 presents an overview of the bioactive compounds identified in the essential oils derived from diverse medicinal plants, as reported in various scientific investigations. A comprehensive examination of the phytoconstituents present in the essential oil of the chosen plant has been the primary focus of previous investigations, preceding the exploration of its antiviral properties.

The analysis of various studies reveals that essential oils contain a diverse range of bioactive compounds, including terpenes, phenols, alcohols, and ketones, which contribute to their antiviral properties. These compounds exhibit different mechanisms of action against viruses, such as direct inactivation, inhibition of viral replication, and modulation of host immune responses. The multi-faceted approach of essential oils in targeting various stages of the viral life cycle suggests their potential as effective antiviral agents. For example, Ross River Virus (RRV) was studied with the usage of *C. citratus* and *P. graveolens* essential oils that showed significant reduction of the RRV viral replication and infectivity as they were applied early after and during viral adsorption [4]. The results showed that the essential oil of *C. citratus* demonstrated significant reductions in luciferase activity and residual infectivity titer when used in pretreatment and co-treatment approaches. The most pronounced inhibitory effect was observed in the co-treatment group, where luciferase activity and virus residual infectivity titer were decreased by approximately 50% compared to the non-treated group ($p < 0.01$ and $p < 0.05$, respectively) [4]. Significant reductions in virus activity were observed with the application of *P. graveolens* essential oil, regardless of the treatment approach. The lowest level of luciferase activity was recorded in the pre-treatment group (34%), while the highest level (67%) was observed in the post-treatment group at 6 hours post-infection (ANOVA one-way test for trend slope = 13.2, $p < 0.001$) [4]. However, the reduction in virus residual infectivity titer remained consistently significant ($p < 0.05$ to 0.01) compared to the non-treated control, without showing a clear pattern across different treatment timings ($p > 0.058$, Kruskal-Wallis test). The maximum reduction in infectivity titer was observed in the co-treatment and post-treatment groups at 4 hours post-infection (less than 20%). Table 1 shows the summary of 33 studies on the antiviral properties of various medicinal plants based on their bioactive compounds. After comprehensive search on databases, the most common and the most reported viral model investigated in selected studies are DENV, CoV, IFV, and HSV. In this review, these viral models will be further discussed.

Essential Oils in Treating DENV(DENV)

There is a lot of bioactive compounds reported in Table 1 which showed a significant inhibitory effect against all serotypes of DENV. A total of 6 studies have discovered that 14 distinct essential oils demonstrate antiviral effects against DENV. These essential oils have shown a promising potential in inhibiting the replication and transmission of the virus, presenting potential therapeutic options for DENV infection management. Plaque-forming assays used in the studies are the major techniques in determining anti-DENV activity of potential compounds which is used to quantify the viral titer. The method employs plaque formation to determine the quantity of infectious particles (virions) of DENV. This assay measures the number of infected host cells and infectious DENV virions by observing plaque formation resulting from cell lysis caused by the DENV infection.

Based on Table 1, the most bioactive compound that has been reported to have DENV antiviral activities is trans-caryophyllene. Trans-caryophyllene is a naturally occurring bicyclic sesquiterpene, classified as a terpene, which is abundantly found in various plants. It is notably present in essential oils derived from medicinal plants such as *Buddleja cordobensis* [1], *Jungia polita* [1], *Lantana grisebachiii* (Seckt.) var. *grisebachii* [23], *Trixis divaricate* [23], *Lippia origanoides* [2], and others. With a chemical formula of $C_{15}H_{24}$ and a molecular weight of 204.36 g/mol, its name "trans" signifies the spatial arrangement of double bonds within its molecular structure [41]. This compound is widely distributed in nature and is known for its aromatic and therapeutic properties. Trans-caryophyllene might have the potential to disrupt the replication of DENV, leading to a reduction in the production of new viral particles. This interference may occur through various means, such as targeting essential viral enzymes responsible for viral genetic material synthesis (RNA) or new virus assembly. It also could prevent DENV from attaching to host cells by interfering with specific viral proteins or receptors on the cell surface, or it might interact with cellular components or membranes, potentially influencing the synthesis and stability of viral RNA, which is vital for the virus's replication process. Moreover, trans-caryophyllene might possess the ability to interact with the viral envelope that can lead to the disruption of DENV and hinder its ability to infect host cells.

Compounds exhibiting a proclivity for selectively targeting the nascent phases of viral engagement with host cells have the potential to function as a preliminary line of defense against pathogenic invasion. The present investigation postulates the plausible occurrence of inhibitory effects exerted by the tested samples in the virucidal assay, targeting either the viral receptors or the virus itself. The study examines the efficacy of essential oils in a subsequent treatment assay, shedding light on its potential to hinder the progression of viral replication at advanced stages, specifically protein transcription. The extant body of research has predominantly centered its attention on the screening of diverse compounds, as evidenced by numerous studies conducted to date. However, it is imperative to conduct additional research to thoroughly investigate the anti-DENV potential exhibited by these compounds and gain a comprehensive understanding of the fundamental mechanisms that contribute to their efficacy. The study aims to investigate the potential inhibitory effects of certain compounds on viral genomes and essential viral enzymes associated with encoding processes. The primary objective is to gain a comprehensive understanding of the mechanisms by which these compounds may impede the replication and propagation of DENV. By elucidating the specific candidates that exhibit promising antiviral properties, this research endeavors to contribute to the development of effective therapeutic interventions for DENV infection.

Various scientific endeavors have been undertaken by diligent scholars and researchers to explore diverse methodologies in the relentless pursuit of countering the pernicious DENV through the precise targeting of distinct phases within its intricate replication cycle. Direct-acting antiviral agents, commonly referred to as antivirals, engage in direct molecular interactions with viral proteins, thereby manifesting their potent antiviral properties (Figure 1) [42]. The present methodology exhibits greater potential in contrast to host-directed antiviral agents due to its ability to selectively target viral proteins, thereby potentially providing a wide range of antiviral efficacy while minimizing toxic effects. One of the primary obstacles encountered in the utilization of direct-acting antiviral agents pertains to the potential emergence of drug resistance [43]. Within the realm of DENV proteins, the E protein has garnered considerable attention due to its role as a structural protein. Conversely, the non-structural proteins NS3 and NS5 have emerged as prominent subjects of investigation within the scientific community [44]. The potential inhibition of DENV non-structural proteins hold promise in impeding various critical stages after infection, encompassing viral replication, assembly, maturation, and polyprotein cleavage. The proteins under consideration herein are of utmost significance in the intricate workings of DENV replication apparatus, thereby rendering them highly desirable targets for the advancement of antiviral therapeutics. Proteins NS3 and NS5 garnered considerable attention in the scientific community and are widely recognized for their pivotal roles in the replication of DENV owing to their enzymatic activities [45].

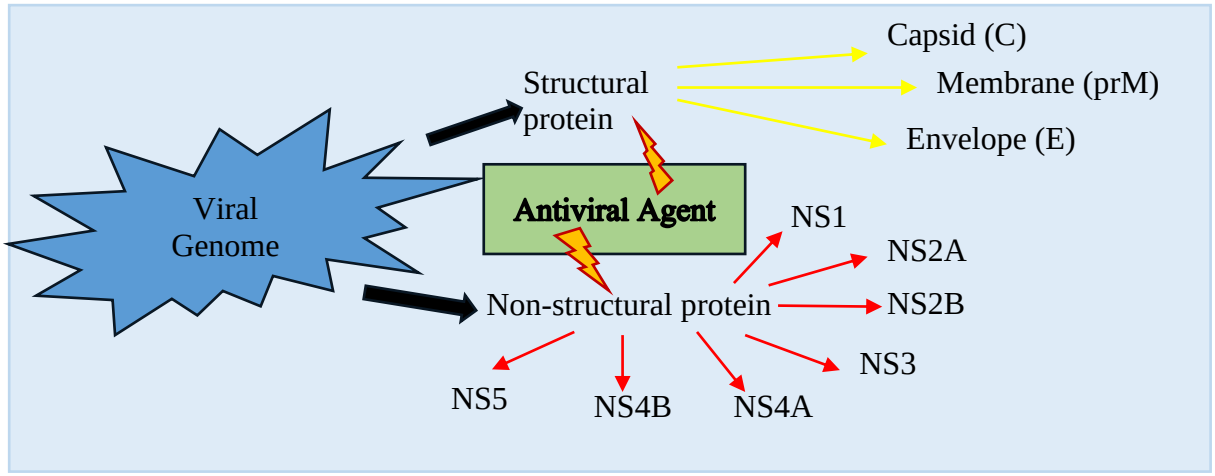

Figure 1. Diagrammatic representation of possible inhibitory sites towards DENV.

Essential Oils in Treating SARS-CoV

Numerous bioactive compounds, as delineated in Table 1, have been documented to exhibit a substantial inhibitory impact against the formidable coronavirus (CoV) pathogen. A comprehensive body of research comprising eight independent studies has collectively elucidated the antiviral properties exhibited by seven unique essential oils against the CoV pathogen. A multitude of botanical remedies have exhibited considerable promise in the therapeutic management of nascent pandemics through the inhibition of the interaction between severe acute respiratory syndrome coronavirus (SARS-CoV) and its cellular receptor, angiotensin-converting enzyme 2 (ACE-2) [21]. Elevated ACE-2 activity and expression have been linked to the regulation of inflammatory cytokine levels, particularly interleukin-1 and high-mobility group box (HMGB1). A comprehensive investigation has been undertaken to explore the botanical specimens belonging to various taxonomic families, with a specific focus on the extraction and analysis of essential oils derived from a total of seven distinct plant species. The findings derived from a meticulous examination of eight separate scholarly inquiries have shed light on the remarkable efficacy exhibited by these botanical extracts in the context of combating the ongoing global pandemic.

Limonene is one of the highlighted bioactive compounds that has potential antiviral properties against SARS-CoV. It is notably present in essential oils derived from *Agathis robusta* [15] and *Citrus limon* [21]. With a chemical formula of $C_{10}H_{16}$ and a molecular weight of 136.23 g/mol, its name "trans" signifies the spatial arrangement of double bonds within its molecular structure [46]. It is a colorless liquid classified as monoterpene composed of two isoprene units, exists in two optically active forms: L-limonene with a pine and turpentine aroma and D-limonene with a pleasing orange scent, while D,L-limonene is a mixture of the two isomers which share identical chemical properties except for their mirror-image molecular structures [46]. Limonene has shown strong antiviral effects against SARS-CoV, primarily achieved by inhibiting viral replication and targeting ACE-2, a key receptor involved in the infection process [21]. It also could downregulate ACE-2 expression in epithelial cells, effectively preventing CoV virus entry and reducing the likelihood of viral infection. Other highlighted bioactive compounds that have potential antiviral properties against CoV are α-pinene and β-pinene. These compounds are reported to be found in *Agathis robusta* [15] and *Laurus nobilis* [25, 26].

There are different studies on medicinal plants with antiviral properties towards CoV. Two bioactive compounds, kessanyl acetate and farnesol, derived from the essential oil of *A. millefolium* demonstrated potent inhibitory effects against the viral target [14]. Kessanyl acetate exhibited interactions with the viral target through the formation of three hydrogen bonds. These hydrogen bonds are established with two key residues, Ser 144 and Cys 145, located in domain II of the target. Cys 145 plays a crucial role as a nucleophile in the initial step of the hydrolysis reaction within the catalytic region of the enzyme, while His 41 acts as a base catalyst. On the other hand, farnesol formed hydrogen bonds with Leu 141 and Ser 144. The essential oil derived from *C. zeylanicum* exhibited a notable antiviral activity, as indicated by its IC_{50} value for SARS-CoV-2 of 15.16 mg/mL [18]. This oil exerts its influence on viral propagation through two distinct mechanisms. Firstly, it displays a virucidal effect, effectively inactivating the virus. Secondly, it interferes with viral replication when applied at various concentrations. Among the natural products with potential antiviral properties against coronaviruses, *C. limon* and *P. graveolens* have also been identified. In a previous study, it was observed that essential oils of these species exhibited a dose-dependent reduction in ACE2 activity in HT-29 cells, which is a significant factor in CoV infection [21]. To validate this effect, the protein expression levels of ACE2 were assessed using immunoblotting. Consistent with the ACE-2 ELISA assay, a notable decrease in

ACE-2 protein levels was observed. The bioactive compounds derived from plants also could exhibit antiviral effects by targeting key proteases, namely papain-like protease (PL^{pro}) and $3CL^{pro}$, which play essential roles in the viral life cycle. The inhibition of these proteases by various medicinal plants hinders viral replication, thereby highlighting their potential in combating viral infections. The coronavirus relies heavily on a key protease known as $3CL^{pro}$, which plays a critical role in the cleavage of viral polyproteins at multiple sites. This enzymatic activity leads to the generation of various viral proteins that are necessary for viral replication [47].

Figure 2 presents a comprehensive depiction of the potential receptors and proteins found within the severe acute respiratory syndrome coronavirus (SARS-CoV) that may be subject to modulation by medicinal plants and their phytoconstituents. Furthermore, it is worth noting that the type II trans-membrane serine protease 2 (TMPRSS2) represents an additional viable candidate for therapeutic intervention. The protease exhibits the remarkable capability of cleaving the receptor ACE-2, which is crucial for the entry of coronaviruses into host cells. This enzymatic activity not only enhances the infectivity of the virus but also initiates the activation of the viral spike protein, leading to the fusion of the viral membrane with the host cell membrane [48]. It is imperative to underscore that the investigations conducted thus far have not employed *in vivo* models. To evaluate the potential efficacy of extracts in combating the coronavirus, a range of animal models have been employed, including non-human primates, hamsters, mice, and ferrets.

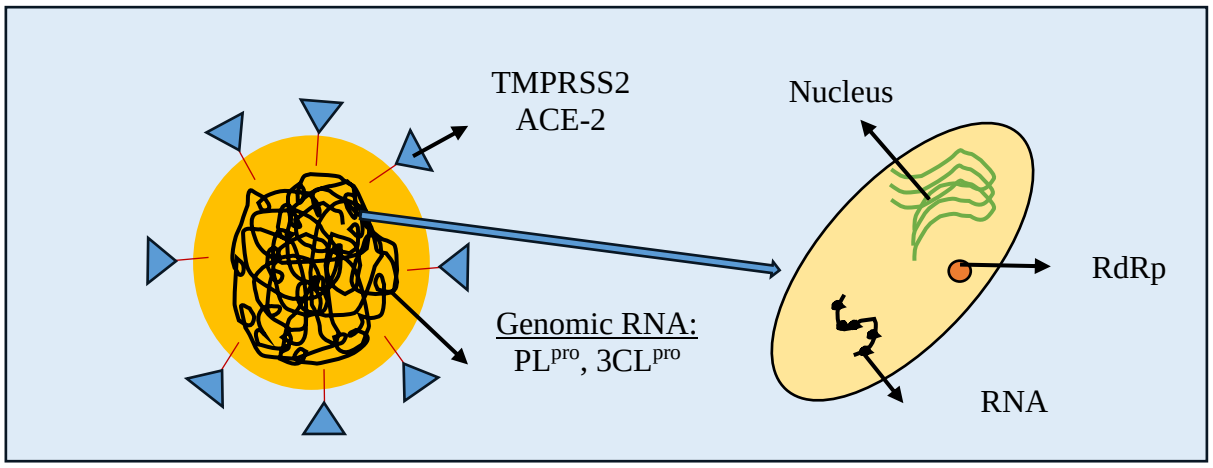

Figure 2. Schematic diagram of possible inhibitory sites for SARS-CoV that could be modulated by natural products: ACE-2, angiotensin-converting enzyme 2; $3CL^{pro}$, 3C-like protease; PL^{pro}, papain-like protease; RdRp, RNA-dependant RNA polymerase; TMPRSS2, transmembrane serine protease 2.

These models have been selected due to their ability to closely replicate the pathological and clinical features observed in humans afflicted with SARS-CoV infection. Prospective investigations may employ these animal models to assess the efficacy of botanical remedies or isolated bioactive agents against SARS-CoV-2, thus making a valuable contribution to the advancement of antiviral therapeutic interventions.

Essential Oils in Treating Influenza Virus (IFV)

The study on the antiviral properties of medicinal plants towards influenza virus (IFV) has been performed in numerous research [19, 22, 29, 30, 34]. The assessment of viral hemagglutinin protein expression within infected cell monolayers unveiled a noteworthy reduction in hemagglutinin levels among cells that were infected with IFV A Puerto Rico 8/H1N1 virus (PR8) and concurrently treated with *Melaleuca alternifolia* essential oil, commonly known as tea tree oil, in comparison to PR8-infected cells that were left untreated [19]. In a separate investigation, it has been ascertained that the essential oil derived from *M. alternifolia* possesses inhibitory properties against the replication of Influenza A virus subtype A/PR8 (IFV A A/PR8). This inhibitory effect can be attributed to the presence of bioactive constituents such as terpinen-4-ol, terpinolene, and α-terpineol in the essential oil [29]. The investigation further determined the ID_{50} value, which represents the concentration at which 50% inhibition of viral replication is achieved, to be 0.0006% (v/v). Remarkably, this value is significantly lower than the CD_{50} value of 0.025% (v/v), indicating a potent antiviral activity of *M. alternifolia* essential oil at concentrations below the threshold that would cause cytotoxicity [29].

The exploration of the antiviral properties of *Eucalyptus polybractea* essential oil against IFV-A primarily focused on *in vitro* experimentation, wherein the essential oil's ability to inactivate IFV-A was examined. The study employed a plaque reduction assay, utilizing MDCK cells to assess the direct binding of the essential oil to unbound free floating

virus particles, shedding light on the potential medicinal applications of *E. polybractea* essential oil in combating IFV-A [22]. The investigation yielded a compelling evidence showcasing the efficacy of active diffusion of pure essential oil through the utilization of a nebulizer for a duration of 15 seconds. This process led to the attainment of an oil concentration of 125 µg/L within the test chamber. Remarkably, this concentration was found to effectively neutralize IFV-A present in the surrounding air, rendering it inactive. The study investigated the efficacy of saturated oil vapor as a potential method for viral inactivation. The results indicate that despite a slightly reduced effectiveness compared to other techniques, the exposure of viral particles to saturated oil vapor for a duration of one day resulted in a substantial viral inactivation rate of 86%. Based on the findings elucidated in this investigation, it can be deduced that the utilization of both aerosol and *E. polybractea* oil vapor manifests auspicious prospects as innate antiviral agents for the explicit objective of disinfection.

A dedicated investigation was undertaken to assess the antiviral efficacy of the essential oil derived from *S. dentata* against H1N1 IFV-A [34]. The results obtained from this investigation demonstrated highly promising outcomes, indicating that the essential oil exhibits a robust inhibitory effect on the H1N1 virus. Notably, at concentrations of 0.001% and 0.0001%, the essential oil displayed inhibition rates of 93% ± 1.3% and 94% ± 1.4%, respectively. These compelling findings strongly suggest that the essential oil derived from *S. dentata* possesses potent antiviral properties, particularly targeting the H1N1 virus. A previous investigation conducted on the essential oil of *M. officinalis* demonstrated its antiviral properties against Avian Influenza Virus (AIV) by targeting various stages of the viral replication cycle [30]. The findings of this study shed light on the potential therapeutic applications of *M. officinalis* essential oil in combating AIV infections. The study elucidated the impact of the essential oil on cell surface proteins, hypothesizing that it may exert its effects through the modulation of cellular receptor binding by concealing the cell surface. Significantly, it was observed that the application of *M. officinalis* essential oil on MDCK cells prior to the inoculation of IFV-A, during the pre-infection stage, resulted in a noteworthy decrease in the quantity of virus genome copies. This reduction was determined through meticulous analysis using real-time PCR methodology.

There are various potential receptors and proteins within IFV that could be inhibited by bioactive compounds of essential oils such as haemagglutinin (HA) binding site [49], neuraminidase (NA) active site [50], M2 ion channel [51], nucleoprotein (NP) binding site [52], and others. Bioactive compounds have the potential to inhibit the HA binding site of IFV through various strategies. Some compounds act as competitive inhibitors, resembling host cell receptors and compete for binding to HA, thereby preventing viral attachment to host cells. Additionally, certain bioactive compounds bind to alternative sites on the HA protein, leading to conformational changes that hinder effective receptor binding. Other inhibitory mechanisms involve disrupting glycosylation processes, targeting pH-dependent conformational changes during viral entry, or stabilizing the trimeric form of HA to hinder viral fusion with host cells. Bioactive compounds also hinder IFV NA activity through diverse methods. They competitively bind to the active site, blocking viral particle release, and some compounds mimic the substrate, impeding its cleavage. Others induce conformational changes, reducing NA's catalytic function and disrupt NA oligomerization and assembly, inhibiting its activity. However, developing these compounds is intricate, and continuous monitoring is necessary due to the influenza virus's high mutation rate.

Essential Oils in Treating Herpes Simplex Virus (HSV)

Essential oils exert their effects against herpes viruses through multiple mechanisms, including direct virucidal activity, inhibition of viral replication, and modulation of the immune response. There are also a lot of previous studies on medicinal plants with antiviral properties towards HSV. A range of antiviral medications, including acyclovir (ACV), are commonly prescribed for the treatment of HSV-1 infection, serving as the first-line antiviral therapy, but instances of ACV resistance in HSV cases have been documented, prompting researchers to explore alternative options derived from plant extracts as potential antiviral agents [53]. The essential oil derived from *S. desoleana* exhibited an EC_{50} value of 23.72 µg/mL against HSV-2, while the CC_{50} value was determined to be 1577 µg/mL, resulting in a selectivity index of 66.48 [6]. These findings indicate that the antiviral activity against HSV-2 is not attributed to cytotoxic effects, ensuring the specificity of the oil's antiviral effect. Star anise essential oil demonstrated potent antiviral activity against HSV-1 in viral suspension tests, leading to a remarkable reduction of plaque formation by more than 99% at concentrations that were noncytotoxic [3]. These findings indicate that the antiviral activity of star anise essential oil is attributed to its direct interaction with free virus particles. Furthermore, pre-treating cells with the oil did not affect viral infectivity or intracellular replication. Natural products also could inhibit the attachment of HSV-1 to host cells by interfering with the glycoprotein receptors on the cell membrane, thereby preventing the attachment of HSV-1 to the cell surface prior to viral replication [53]. Essential oils exhibit direct virucidal activity against herpes viruses, allowing them to directly inactivate or destroy the viruses. This virucidal activity can be attributed to specific bioactive components present in essential oils, such as terpenes and phenols, which interfere with the viruses' attachment, entry, and replication

processes. By directly targeting the viruses, essential oils effectively hinder the initiation and spread of the infection.

Essential oils possess the ability to inhibit the replication of herpes viruses by disrupting crucial steps in their replication cycle. HSV-1 gene expression follows a sequential pattern during viral replication, consisting of immediate early, early, and late genes. These genes play distinct roles in the replication process. Among them, U_L27, an immediate early gene, encodes Glycoprotein B, a vital protein involved in viral entry by facilitating attachment [54, 55]. U_L30, an early gene, is responsible for encoding DNA polymerase, essential for viral replication. U_L54, another early gene, influences the functions of other early and late genes, including the attachment gene gC [56]. Additionally, U_L54 encodes DNA polymerase catalytic enzymes, crucial proteins in the viral lytic cycle. The expression of these genes follows a specific order, with immediate early genes being transcribed and translated first, followed by early genes, and eventually, late genes. To ensure accurate gene expression analysis, a housekeeping gene like Ribosomal Protein L32 (RPL32) is often employed as a reference [10, 57]. Understanding the temporal regulations and functions of these genes provides valuable insights into the complex mechanisms underlying HSV-1 replication and aids in the development of targeted antiviral approaches.

Studies have elucidated the intricate mechanisms of action that synergistically contribute to the potent antiviral effects exhibited by essential oils against herpes viruses. The confluence of direct virucidal activity, viral replication inhibition, and immune response modulation synergistically contributes to the comprehensive antiviral efficacy and symptomatic relief observed in the context of herpes infections. Further investigation is imperative to acquire a more exhaustive comprehension of the precise bioactive constituents present in essential oils and their optimal concentrations for the efficacious management of herpes infections. However, it is important to note that the intricate and diverse mechanisms of action exhibited by essential oils offer considerable potential for the advancement of alternative or supplementary therapeutic approaches targeting herpes viruses.

CONCLUSION

In this comprehensive review, an in-depth analysis is presented, elucidating the intricate nature of bioactive compounds present in essential oils. The focus of this examination revolves around the therapeutic properties of these compounds, specifically in relation to their impact on viruses. By synthesizing a vast array of scientific literature, this review aims to provide a holistic understanding of the subject matter. The antiviral properties of essential oils have been extensively demonstrated, showcasing their ability to effectively hinder the replication and infectivity of various viral strains. The antiviral potential of essential oils is attributed to the presence of bioactive constituents, which exert their effects through diverse mechanisms. These mechanisms include the inhibition of viral entry, the suppression of viral replication, and the modulation of the host immune response. It is pertinent to acknowledge that most of the conducted studies have predominantly centered their attention on *in vitro* investigations, thereby necessitating additional research endeavors to assess the effectiveness of essential oils in real-world and clinical contexts. Furthermore, it is imperative to focus endeavors on the standardization of essential oil compositions, the identification of distinct bioactive compounds accountable for their antiviral properties, and the elucidation of the fundamental mechanisms governing their mode of action. By conducting an in-depth exploration of these domains, one can acquire invaluable discernments and conceivably exploit the remedial advantages of essential oils as efficacious antiviral interventions.

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