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Linking chemistry to activity: Antiviral effects and GC–MS phytochemical profile of *Smilax zeylanica Linn*

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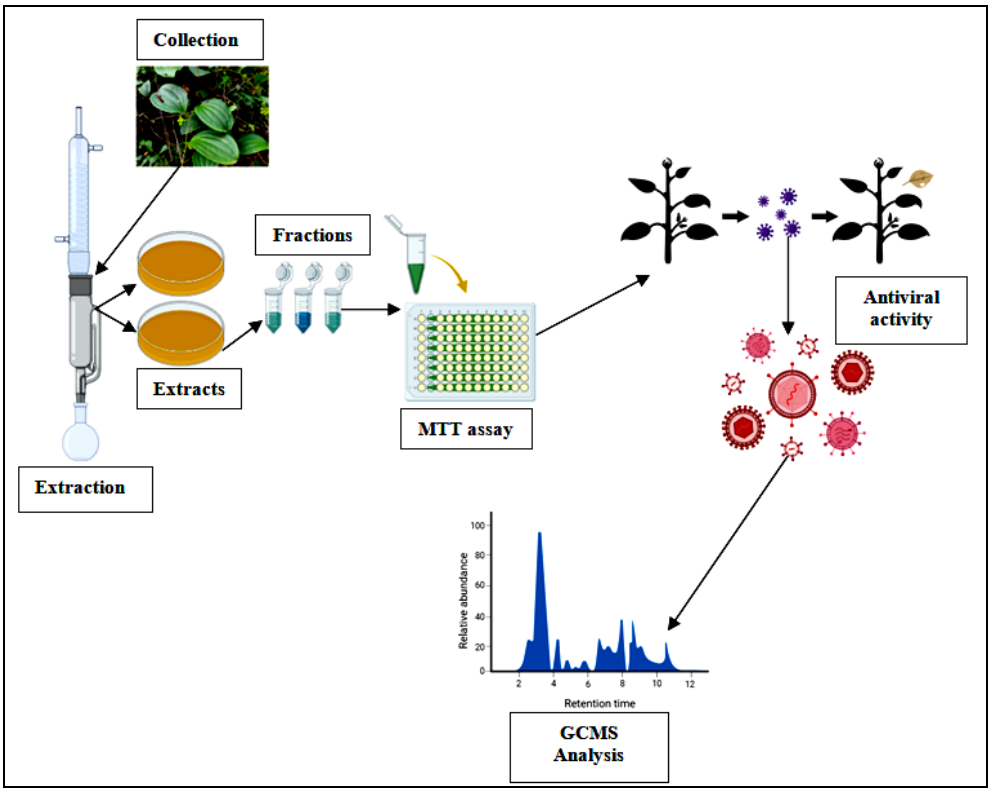
Abstract
Viral infections are among the most prevalent global health threats, with emerging strains capable of disrupting normal life. Current antiviral drugs are often expensive, have significant side effects, and can promote secondary infections, creating an urgent need for safer, plant-based alternatives. *Smilax zeylanica Linn*, a medicinal plant widely used in Indian traditional medicine, was investigated for its antiviral potential. *Smilax zeylanica Linn* leaves were extracted using aqueous and ethanol solvents, the ethanol extract exhibited higher antiviral activity and was fractionated into petroleum ether, chloroform, and ethyl acetate fractions. Cytotoxicity and antiviral activity were evaluated on lentivirus-infected Vero cells using the MTT assay. The chloroform fraction demonstrated the most notable activity. GC–MS analysis revealed a rich profile of bioactive compounds, including phenolics, terpenoids, and fatty acid derivatives. These findings suggest *Smilax zeylanica Linn* as a promising candidate for developing novel, plant-derived antiviral agents, warranting further phytochemical isolation and mechanistic studies.

Keywords: *Smilax zeylanica*, Antiviral activity, medicinal plants, GC–MS analysis, phytochemicals, lentivirus

1. Introduction
Viral infections remain a major health concern despite the limited effectiveness of antiviral drugs, such as interferon and ribavirin, in controlled settings. Many viral infections lack effective treatments because of limited antiviral options and the emergence of drug-resistant strains in genetically diverse environments [1]. Viral diseases have serious global impacts. Influenza causes over 3 million severe cases and 500,000 deaths annually, with limited vaccine effectiveness. Noroviruses infect up to 21 million people in the U.S. each year, causing hundreds of deaths. The Zika virus, with no treatment, threatens pregnant women due to the risk of fetal microcephaly. Ebola, with a fatality rate of 25–90%, remains a major threat, as it is resistant to standard disinfection and lacks a cure [2]. The recurrence of such infections is driven by weak initial immune responses and the complex nature of the viruses.
Medicinal plants have long been used in traditional systems to treat various illnesses, including infections, owing to their rich pharmacological properties [3]. Approximately 40% of current drugs are plant-derived, highlighting their value in antiviral drug development due to their low cost, good bioavailability, and fewer side effects. The WHO defines traditional medicine as culturally rooted practices based on knowledge and experience. Despite over 350,000 known medicinal plant species, only 2% have been scientifically studied for their active compounds [4]. The growing populations, drug resistance, high treatment costs, and side effects of synthetic drugs have renewed interest in plant-based remedies. The WHO continues to support research on discovering new plant-based therapeutics.
Smilax zeylanica Linn (*S. zeylanica)*, a dioecious shrub from the Smilacaceae family, belongs to the therapeutically important and second-largest genus *Smilax* [5]. In traditional Indian medicine, such as Ayurveda and Unani, *S. zeylanica* is used to treat venereal diseases, rheumatism, sores, swellings, abscesses, and dysentery [6]. The genus *Smilax* is known for the bioactive compound sarsaparilla, or chopacheeni in Ayurveda, which is most abundant in *Smilax china* but is also found in other *Smilax* species [7, 8]. Recent studies have highlighted the medicinal potential of *S. zeylanica*, revealing its various therapeutic properties. Leaf extracts exhibit anthelmintic, analgesic, cytoprotective, hypoglycemic, hypotensive, antioxidant, and antifungal activities. Roots and rhizomes exhibit antihypertensive and antisyphilitic effects and

are used as diuretics, stimulants, and for treating rheumatism [9, 10]. Traditionally valued in Indian folk medicine, especially among the Kerala and Theni tribes, the plant is used to treat skin disorders, blood dysentery, gynecological issues such as leucorrhea, and arthritis [11, 12].

S. zeylanica contains key phytochemicals like spirostanol triglycoside (dioscin) and steroidal saponin glycosides such as diosgenin, smilagenin, and sarsasapogenin. It also contains flavonoids, tannins, and phenolic acids, which contribute to its antioxidant, antimicrobial, antipyretic, and cytotoxic properties [13, 14]. Chromatographic analyses confirmed the presence of compounds such as hydroxyl-tyrosol and squalene. Steroidal saponins in *Smilax* are classified into isopirostane, furostane, pregame, and cholestane. Diosgenin, dioscin, and smilagenin belong to the isopirostane group, while sarsasapogenin is of the spirostane type [15, 16, 17]. These bioactive compounds are believed to drive the pharmacological effects of the plants. This study was conducted to explore the antiviral activity of *Smilax zeylanica Linn*, as no prior research has focused on this aspect. GC-MS analysis was performed using different solvent extracts.



2. Materials and Methods

2.1 Collection and authentication of *Smilax zeylanica Linn* plant material

The leaves of the plant were collected between September and February from the Gudalur region of The Nilgiris district, Tamil Nadu, India, and submitted to the School of Life Sciences, JSSAHR, The Nilgiris (Reference No: JSS1110) for authentication. Table1 contains vernacular names for this plant.

Table 1: Vernacular names of different *Smilax* species

S.no	Vernacular names
1.	*Smilax ceylanica Oken*
2.	*Smilax collina Kunth*
3.	*Smilax elliptica Desv*
4.	*Smilax hohenackeri Kunth*
5.	*Smilax indica Burm f*
6.	*Smilax ovalifolia var.nervulosa A.DC*
7.	*Smilax villandia Bunch- Ham.ex Royle*
8.	*Smilax zeylanica var. penangensis A.DC*
9.	*Smilax zollingeri Kunth*

2.2 Extraction of plant leaves

Freshly collected *S. zeylanica* leaves were thoroughly washed with clean water and shade-dried at ambient temperatures. The dried leaves were then finely powdered. The powder was macerated in ethanol at room temperature for 3 days. After extraction, the mixture was filtered through Whatman No.1 filter paper, and the remaining residue (marc) was subjected to a second extraction with distilled water under the same conditions. The combined filtrates were concentrated using a Soxhlet apparatus, followed by rotary evaporation for 15 min. The semi-solid residue obtained was freeze-dried (lyophilized) to yield a crude extract. For further analysis, the crude extract was sequentially partitioned using solvents of increasing polarity—petroleum ether, ethyl acetate and chloroform following the method described by Elgorashi & van Staden 2004 [18].

2.3 Phytochemical screening

Crude extracts were subjected to qualitative phytochemical screening using conventional processes and methodologies [19].

2.4 Cells

The Vero (African green monkey kidney cell line) was purchased from NCCS, Pune, India. The cells were maintained in DMEM high glucose media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO2, 18-20% O2 at 370 C temperature in the CO2 incubator and sub-cultured for every two days. The lentiviral plasmid pFUGW was obtained from Addgene, which was deposited by David Baltimore (Addgene plasmid #14883) [20, 21].

2.5 Cytotoxicity assay

The cytotoxicity of *S. zeylanica* leaf extracts on Vero cells was determined using the MTT assay. Without the test agent, seed a 200l cell suspension was seeded in a 96-well plate at the desired cell density (20,000 cells per well). The cells were allowed to grow for approximately 24 h. The test agents were added at suitable concentrations (1, 3, 10, 30, and 100 g/ml) diluted in the culture medium. The plate was incubated at 37 °C for 24 h in a 5% CO2 environment. The plates were removed from the incubator after incubation, the media were discarded, and MTT reagent was added to a final concentration of 0.5 mg/mL. To avoid light exposure, the plates were wrapped in aluminum foil. The plates were returned to the incubator for 3 h. (Note: Incubation times differed between cell lines. While making comparisons, keep the incubation period constant within one experiment. After removing the MTT reagent, add 100l of solubilization solution (DMSO) was added. Gentle shaking in a gyratory shaker improves dissolution. In some cases, particularly in dense cultures, pipetting up and down may be necessary to completely dissolve the MTT formazan crystals. Using a spectrophotometer or an ELISA reader, measure the absorbance at 570 nm [22].

% Cell viability is calculated using below formula:

% Cell viability = [Mean abs of treated cells/Mean abs of Untreated cells] x 100

2.6 Antiviral assay

2.6.1 MTT assay in lentivirus-infected Vero cell lines

The antiviral activity of the plant components was tested using the MTT assay on lentivirus-infected Vero cell lines, the procedure as described before [22].

2.7 GC-MS analysis

Gas chromatography–mass spectrometry (GC–MS) analysis was performed on the methanol fraction of the ethanol extract of *S. zeylanica* leaves, which exhibited notable antiviral activity in the plaque reduction assay. The analysis was performed using a Perkin Elmer Clarus 680 GC system coupled with a Clarus 600 mass spectrometer operating in electron ionization (EI) mode. Chromatographic separation was achieved using a fused silica capillary column (Elite-5MS, 5% biphenyl, and 95% dimethylpolysiloxane; 30 m × 0.25 mm internal diameter × 0.25 µm film thickness). High-purity helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. A 1 µL aliquot of the sample was injected in split mode with a split ratio of 10:1, and the injector temperature was maintained at 260 °C. The oven temperature was initially set at 60 °C for 2 min, followed by a temperature ramp of 10 °C per minute up to 300 °C, and held at 300 °C for 6 min, resulting in a total run time of 32 min. The mass spectrometer operated in electron-impact mode at 70 eV. The transfer line and ion source temperatures were maintained at 240 °C. A solvent delay of 2 min was applied to prevent detector saturation. Mass spectra were acquired over a scan range of 50–600 Da with a scan time of 0.2 s and a scan interval of 0.1 s. Compound identification was performed by comparing the obtained mass spectra with those available in the NIST library database using TurboMass software version 5.4.2 [23].

3. Results

3.1 Qualitative phytochemical analysis

When compared to the aqueous extract, the ethanol extracts showed the presence of more phytochemicals in qualitative analysis as shown Table 2**.**

Table 2: Qualitative analysis of ethanol and aqueous extract

S. No.	Phytochemical Test	Ethanol Extract	Aqueous Extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Proteins	-	-
4	Amino acids	-	-
5	Glycosides	+	+
6	Steroids	+	+
7	Anthraquinones	-	-
8	Flavonoids	+	+
9	Tannins and Phenols	+	+
10	Triterpenoids	+	-
11	Saponins	+	-
12	Fats and Fixed oils	+	-

3.2 Cytotoxicity assay

The MTT assay findings showed that the extracts were non-toxic to Vero cells at the maximum concentration of 100µg/ml displayed in Figure 1 and 2. Cisplatin at 10M/ml was used as the standard control. According to the findings, both extracts exhibited non-toxic qualities and greater percentage cell viability values, indicating their potential as promising non-toxic agents.

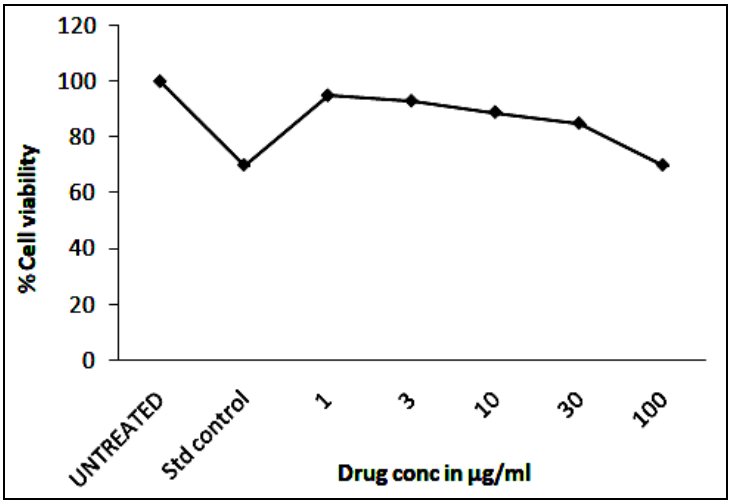


Fig 1: % Cell viability of ethanol extract against Vero cells

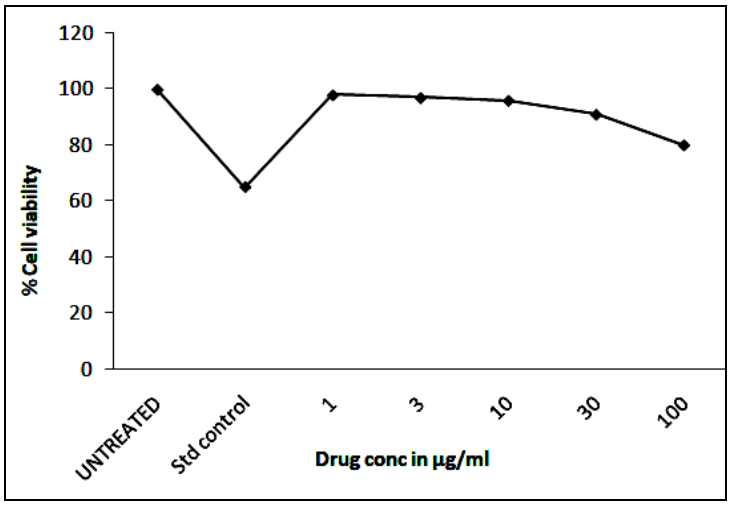
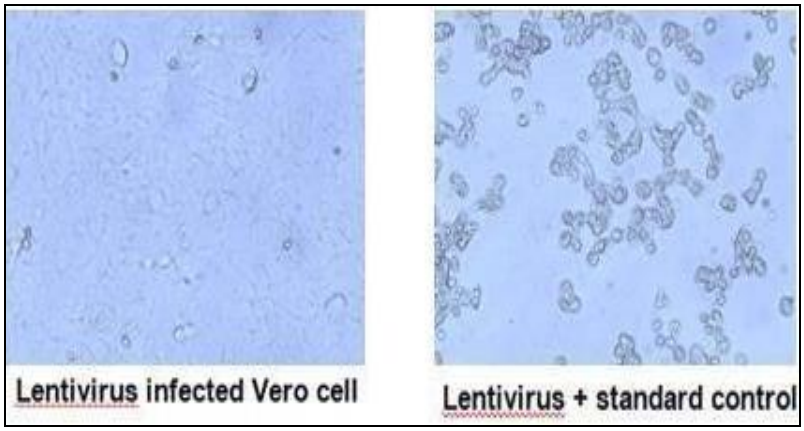


Fig 2: % Cell viability of aqueous extract against Vero cells

3.3 Antiviral activity

3.3.1 MTT assay on lentivirus-infected Vero cell lines of crude extracts

In the experiment findings of MTT assay on lentivirus-infected Vero cell lines, the aqueous extract exhibited moderate cytotoxicity, with an IC50 value of 92µg/ml, whereas the ethanol extract exhibited higher cytotoxicity, with an IC50 value of 54µg/ml, both at the highest concentration of 100µg/ml. Furthermore, the IC50 values for the chloroform, and ethylacetate fractions of ethanol extract on lentivirus-infected Vero cells were 91.63µg/ml, and 86.64µg/ml, respectively. In contrast, the pet ether, fraction exhibited significant cytotoxicity, with an IC50 value of 41.19µg/ml at the highest concentration of 100µg/ml. The standard control in this study was cisplatin at 10M/ml. The outcomes are illustrated in with pictures Figure 3, 4, 5, 6, and 7



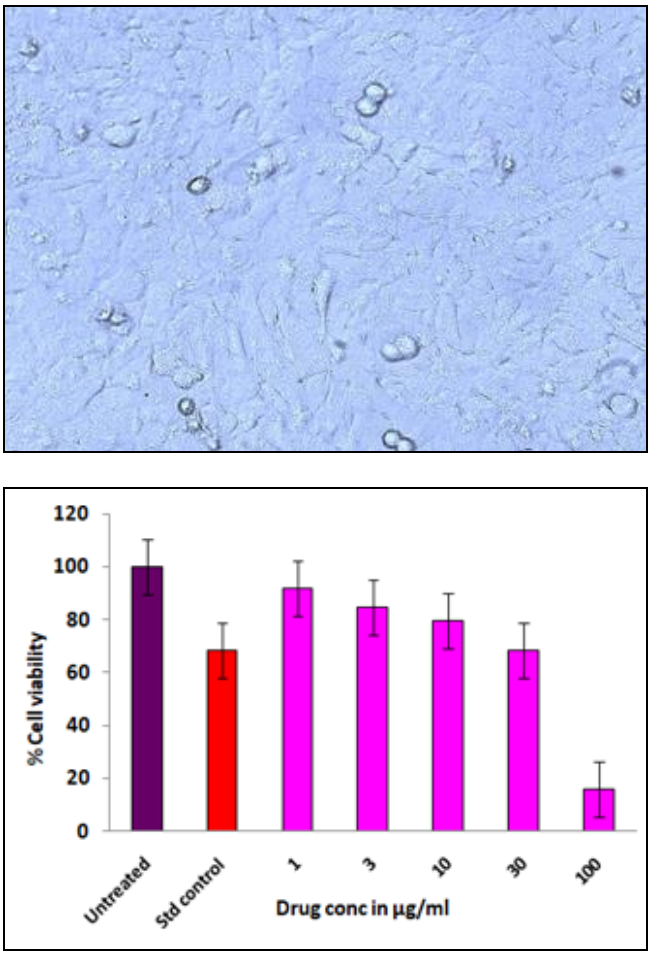


Fig 3: %Cell viability of ethanol extract against lentivirus infected Vero cells

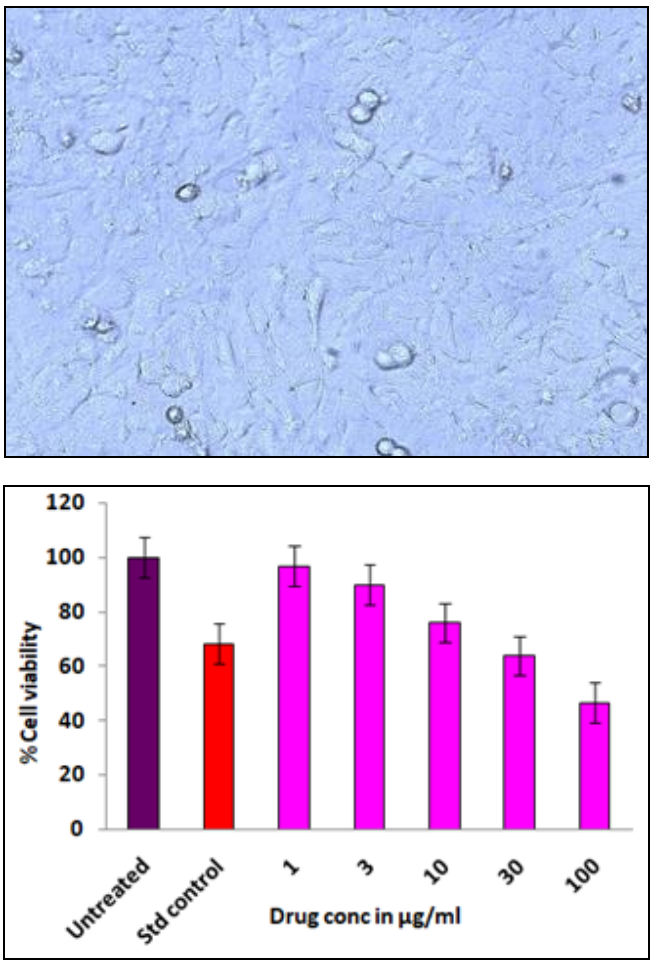


Fig 4: % Cell viability of aqueous extract against lentivirus infected Vero cells

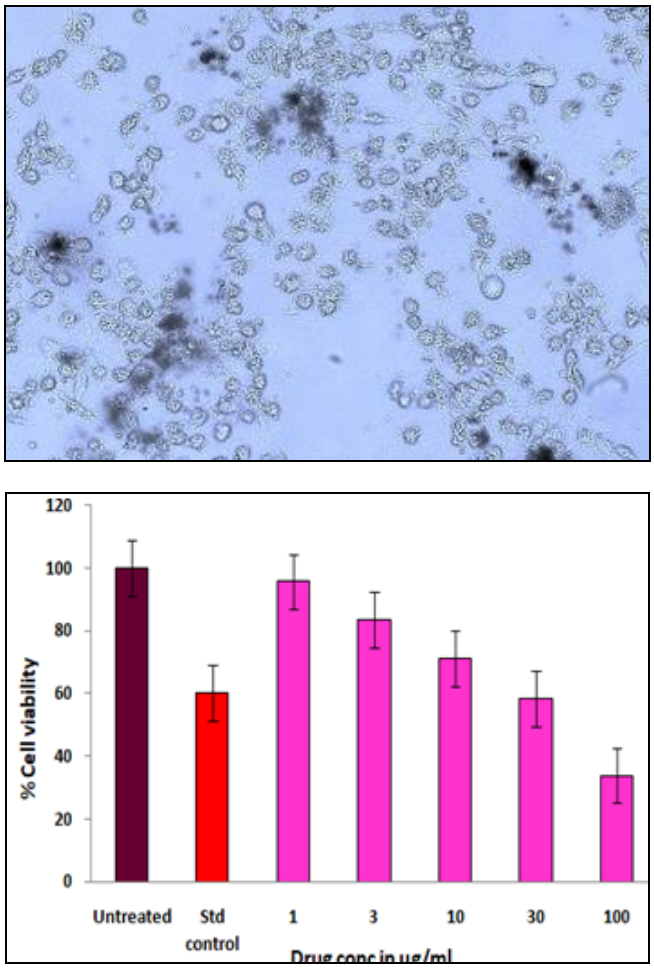


Fig 5: % Cell viability of pet ether fraction against lentivirus infected Vero cells

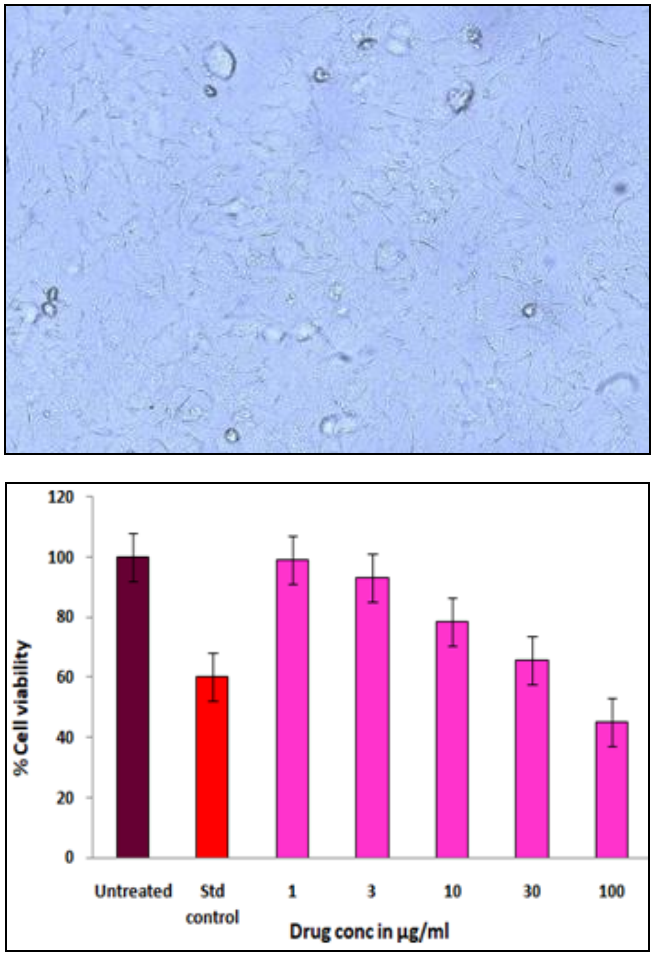


Fig 6: %Cell viability of chloroform fraction against lentivirus infected Vero cells

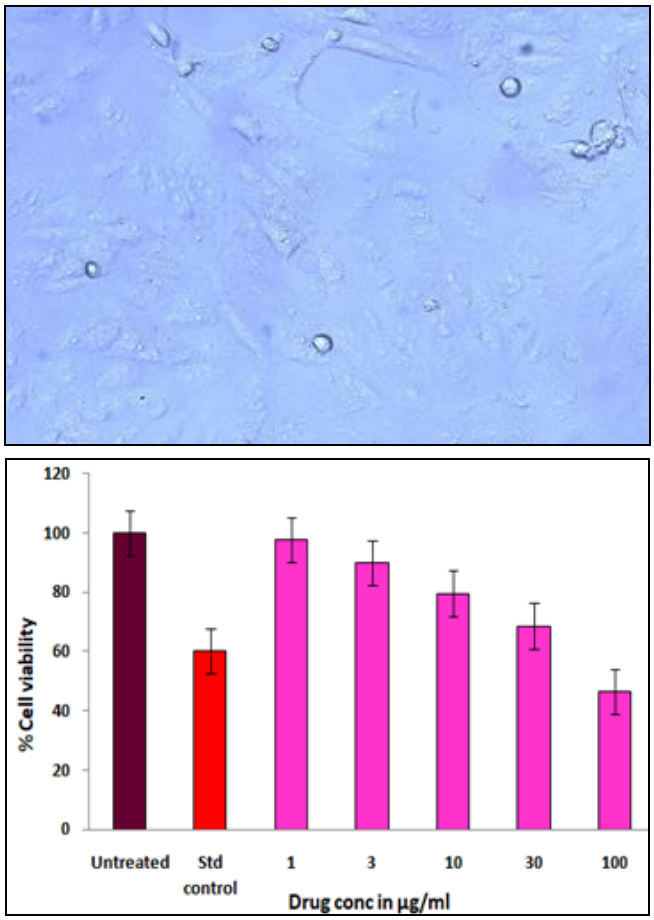


Figure 7: % Cell viability of ethyl acetate fraction against lentivirus infected Vero cells

3.4 GCMS characterization of ethanol fractions

The ethanol extract fractions that showed effective antiviral activity were further subjected to GC-MS analysis.

3.4.1 Petroleum ether fraction

GC-MS analysis of the petroleum ether fraction from *S. zeylanica* leaves revealed the presence of 14 bioactive compounds. These included 2,4-di-tert-butylphenol; neophytadiene; dibutyl phthalate; benzenepropanoic acid, 3,5-bis(1,1-dimethyl ester); hexadecanoic acid, ethyl ester; *n*-nonadecanol-1; 2-hexadecen-1-ol, 3,7,11,15-tetramethyl; ethyl oleate; octacosanol; 1-heptacosanol; heptacosyl heptafluorobutyrate; 1-hexacosanol; and phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite. The compound 2-hexadecen-1-ol, 3,7,11,15-tetramethyl and phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite were the most abundant, contributing 15.47% and 15.28% of the total peak area, respectively. Detailed chromatographic data, including retention times and peak areas, are presented in Table 3 and Figure 8.

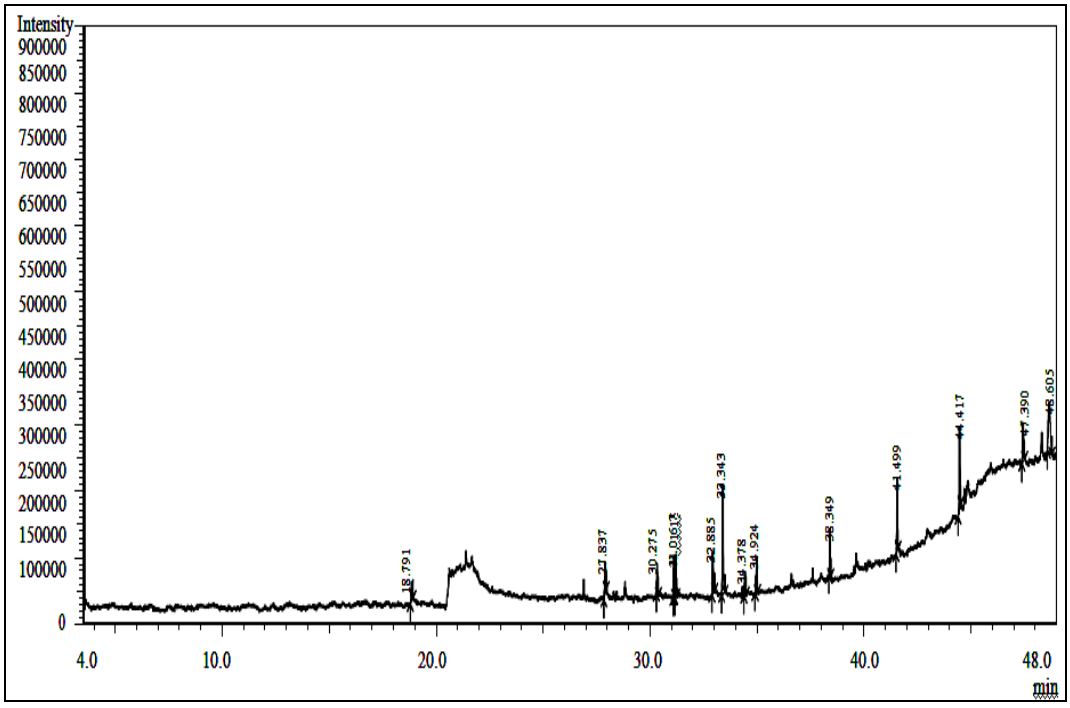
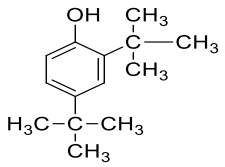
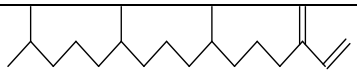
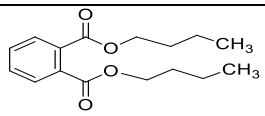
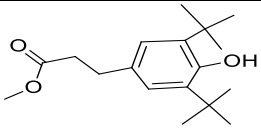
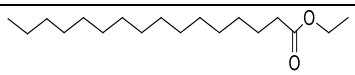
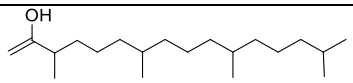
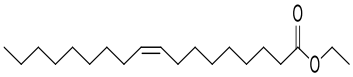
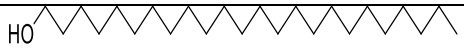
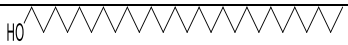
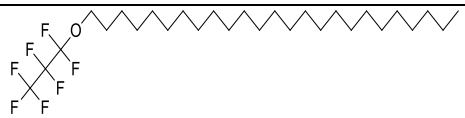
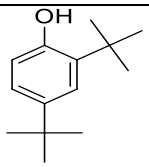


Fig 8: GCMS analysis of petroleum ether fraction

Table 3: Compounds identified in the petroleum ether fraction by GC-MS

Peak#	R. Time	Area	Area %	Name	Structure
1	18.791	105515	3.34	2,4-di-tert-butylphenol	
2	27.837	153734	4.87	Neophytadiene	
3	30.275	120423	3.82	dibutyl phthalate	
4	31.067	168370	5.34	benzenepropanoic acid, 3,5-bis(1,1-dimethyl ester)	
5	31.119	244277	7.74	hexadecanoic acid, ethyl ester	
6	32.885	173937	5.51	n-nonadecanol-1	
7	33.343	487928	15.47	2-hexadecen-1-ol, 3,7,11,15-tetramethyl	
8	34.378	107970	3.42	ethyl oleate	
9	34.924	162847	5.16	Octacosanol	
10	38.349	165315	5.24	1-heptacosanol	
11	41.499	270517	8.57	Octacosanol	
12	44.417	320002	10.14	heptacosyl heptafluorobutyrate	
13	47.390	191972	6.08	1-hexacosanol	
14	48.605	482053	15.28	phenol, 2,4-bis(1,1-dimethylethyl)-, phosphate	

3.4.2 Ethyl acetate fraction

GC-MS analysis of the ethyl acetate fraction of *S. zeylanica* leaves revealed the presence of nine bioactive compounds. These included 2,4-di-tert-butylphenol; diethyl phthalate; 1,2-benzenedicarboxylic acid, diester; neophytadiene; dibutyl phthalate; hexadecanoic acid, ethyl ester; behenic alcohol; 2-hexadecen-1-ol, 3,7,11,15-tetramethyl; and ethyl oleate. Among these, diethyl phthalate (19.75%) and 2-hexadecen-1-ol, 3,7,11,15-tetramethyl (17.13%) were the most abundant constituents. The detailed chromatographic profile, including retention times and peak areas, is provided in Table 4 and Figure 9.

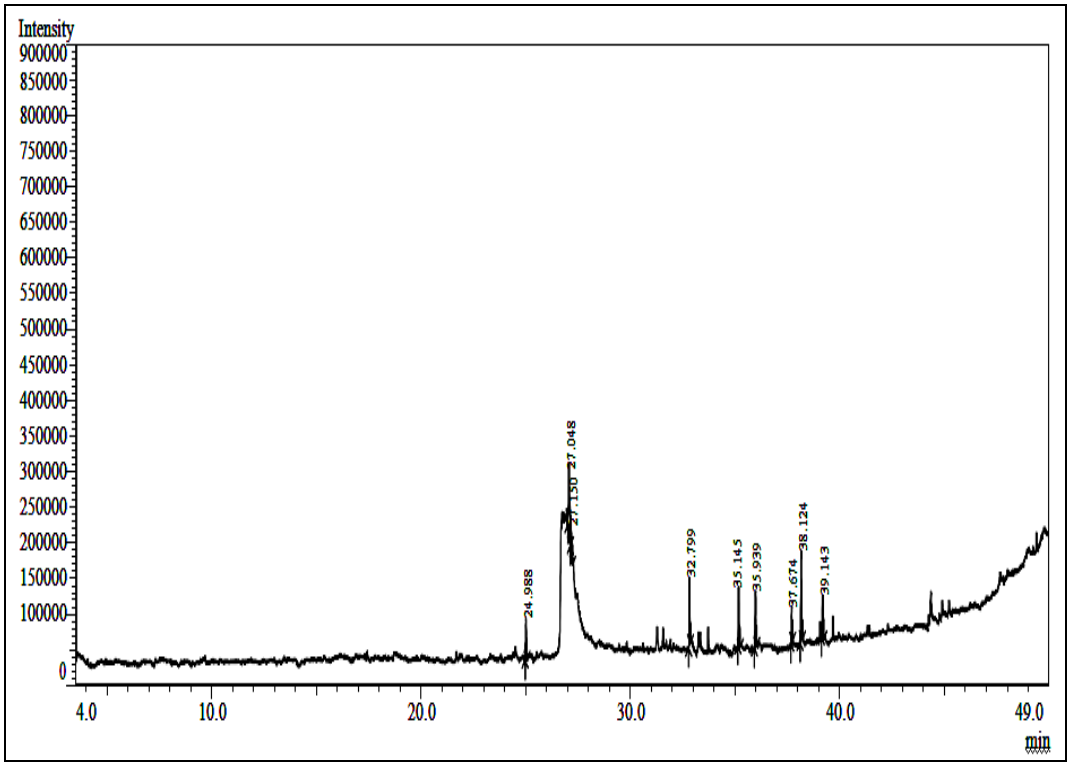
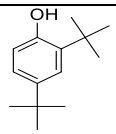
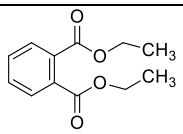
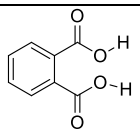
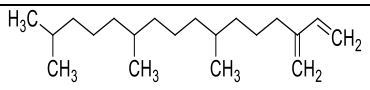
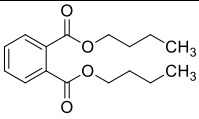
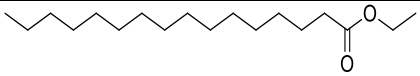
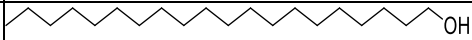
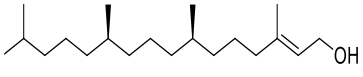
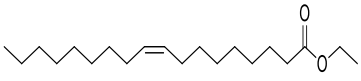


Fig 9: GCMS analysis of ethyl acetate fraction

Table 4: Compounds identified in the ethyl acetate fraction by GC-MS

Peak#	R. Time	Area	Area %	Name	Structure
1	24.988	161955	8.06	2,4-di-tert-butylphenol	
2	27.048	396893	19.75	diethyl phthalate	
3	27.150	136635	6.80	1,2-benzenedicarboxylic acid,	
4	32.799	236150	11.75	Neophytadiene	
5	35.145	231048	11.50	dibutyl phthalate	
6	35.939	210622	10.48	hexadecanoic acid, ethyl ester	
7	37.674	117913	5.87	behenic alcohol	
8	38.124	344214	17.13	2-hexadecen-1-ol, 3,7,11,15-tetramethyl ester	
9	39.143	173929	8.66	ethyl oleate	
		2009359	100.00		

3.4.3 Chloroform fraction

GC-MS analysis of the chloroform fraction of *S. zeylanica* leaves revealed the presence of 24 bioactive compounds. These included 1-dodecanol; tetradecane; biphenyl; 1-tetradecanol; 2,4-di-tert-butylphenol; 1-nonadecene; hexadecane; eicosane; neophytadiene; dibutyl phthalate; phytol isomer; octacosanol; dotriacontane; O,O′-biphenol, 4,4′,6,6′-tetra-tert-butyl; 1-heptacosanol; tetrapentacontane; 1-hexacosanol; and γ-sitosterol. Among these, 2,4-di-tert-butylphenol (15.31%) and γ-sitosterol (12.27%) were the major constituents. Detailed chromatographic data, including retention times and peak areas, are presented in Table 5 and Figure 10.

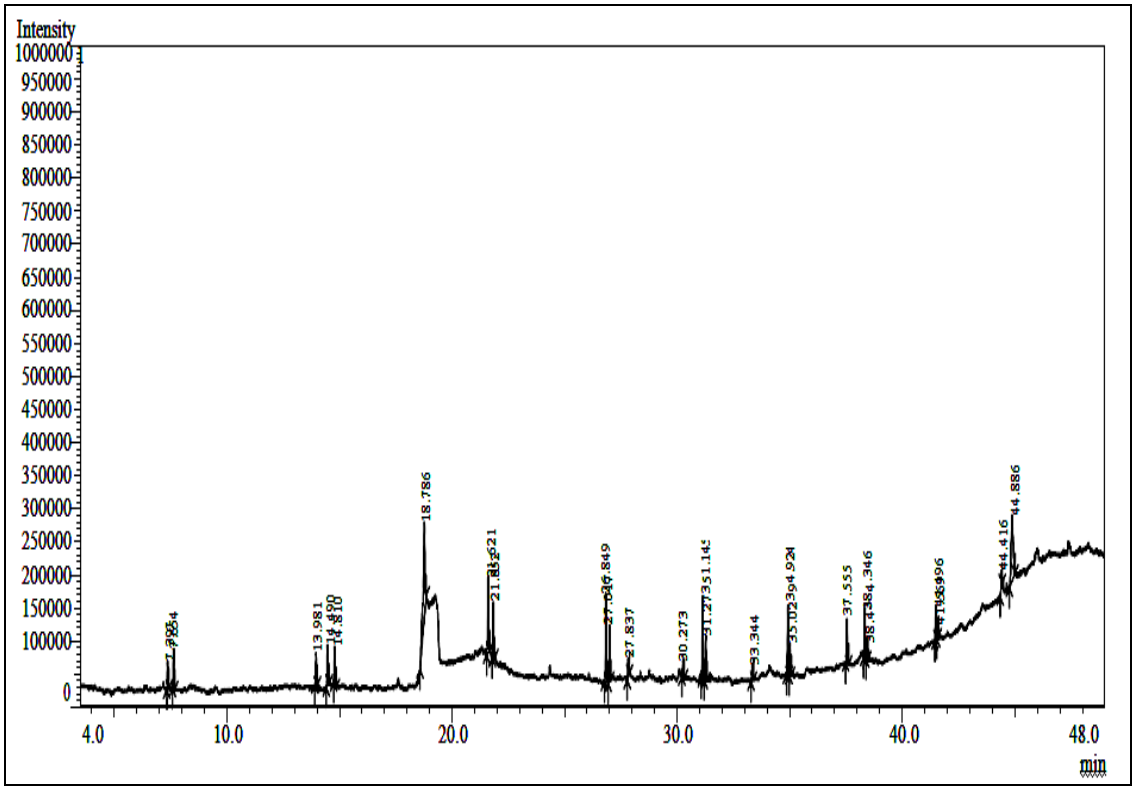
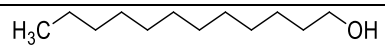
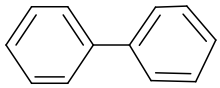
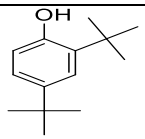
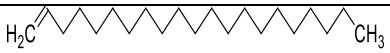
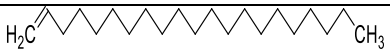
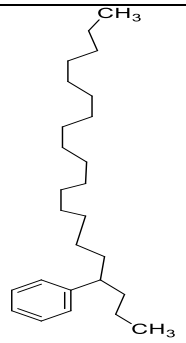
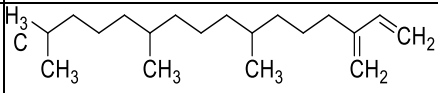
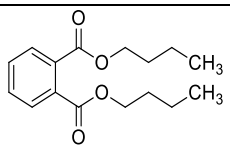
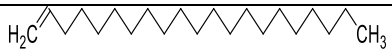
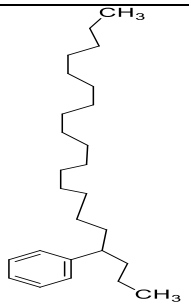
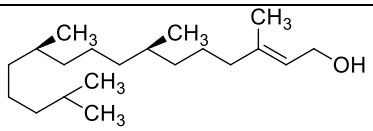
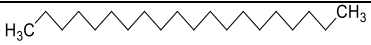
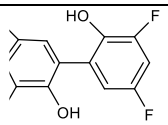
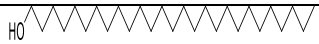
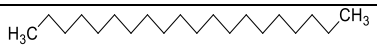
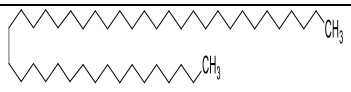
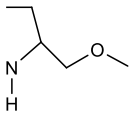
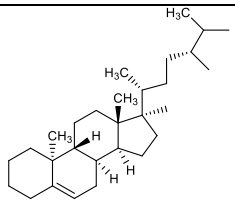


Fig 10: GCMS analysis of chloroform fraction

Table 5: Compounds identified in the chloroform fraction by GC-MS

Peak#	R. Time	Area	Area %	Name	Structure
1	7.397	106981	2.05	1-dodecanol	
2	7.654	161492	3.09	tetradecane	
3	13.981	161486	3.09	Biphenyl	
4	14.490	195766	3.75	1-tetradecanol	
5	14.810	167145	3.20	tetradecane	
6	18.786	798791	15.31	2,4-di-tert-butylphenol	
7	21.621	337220	6.46	1-nonadecene	
8	21.852	228397	4.38	hexadecane	
9	26.849	353539	6.77	1-nonadecene	
10	27.017	223799	4.29	eicosane	

					
11	27.837	86351	1.65	neophytadiene	
12	30.273	75923	1.45	dibutyl phthalate	
13	31.145	321227	6.16	1-nonadecene	
14	31.275	191955	3.68	Eicosane	
15	33.344	81339	1.56	phytol isomer	
16	34.924	269352	5.16	octacosanol	
17	35.029	120104	2.30	dotriacontane	
18	37.555	181554	3.48	o o'-biphenol, 4,4',6,6'-tetra-t-butyl ester	
19	38.346	197436	3.78	1-heptacosanol	
20	38.434	80429	1.54	dotriacontane	
21	41.496	132096	2.53	octacosanol	
22	41.569	43871	0.84	tetrapentacontane	
23	44.416	62177	1.19	1-hexacosanol	

24	44.886	640098	 12.27	.gamma.-Sitosterol	
			5218528	100.00	

4. Discussion

Several *Smilax* species have been reported to exhibit antiviral activity against various viruses [24]. This is the first study to demonstrate the antiviral potential of *S. zeylanica*. Previously, this plant has shown antibacterial and antifungal activities [25]. Based on these findings and considering the limitations of current antiviral drugs and the urgent need for effective antiviral therapies, especially in the post-COVID-19 era [26], *S. zeylanica* was evaluated for its antiviral activity. In this study, a lentiviral plasmid was used to evaluate antiviral activity because of its non-pathogenic nature and ability to efficiently deliver and stably integrate genetic material into a wide variety of cell types [27]. Two types of solvents, aqueous and ethanol, were used because of their polarity and because most pharmacological studies commonly employ these solvents to extract bioactive compounds. The leaves of *S. zeylanica* have been reported to exhibit antibacterial, antifungal, pesticidal, anticonvulsant, cytotoxic, anticancer, antidiabetic, and hepatoprotective activities [6]. Leaves have also been traditionally used to treat gynecological disorders and alleviate weakness during menstruation [7]. Qualitative phytochemical analysis revealed the presence of phytochemicals in both extracts; however, the ethanol extract contained a greater variety of phytochemicals than the aqueous extract. Previous studies have also reported the presence of various phytochemicals in ethanol extracts, including flavonoids, tannins, phenols, glycosides, carbohydrates, alkaloids, steroids, saponins, triterpenoids, anthraquinones, fats, and fixed oils [8]. As the plant is edible and has been traditionally used for various treatments, it was found to be non-toxic to cell lines in the cytotoxicity study. In the MTT assay, which assessed antiviral activity based on the effect of the extract on lentivirus-infected Vero cells, the ethanol extract exhibited higher antiviral activity. The ethanol extract, which exhibited higher cytotoxicity, was further fractionated using four solvents of increasing polarity.

Further, the fractions were analyzed for the presence of various phytochemicals using GC-MS analysis. All three fractions showed the presence of phytochemicals: the petroleum ether fraction contained 14 compounds, the ethyl acetate fraction had 9 compounds, and the chloroform fraction revealed 24 compounds. Moreover, the fractions were evaluated for their antiviral activity using the MTT assay, in which the petroleum ether fraction exhibited higher cytotoxicity compared to the other fractions. Moreover, the fractions were evaluated for their antiviral activity using the MTT assay, in which the petroleum ether fraction exhibited higher cytotoxicity compared to the other fractions. Thus, the plant demonstrates promising antiviral activity, indicating its potential for future studies.

5. Conclusion

S. zeylanica exhibits a wide range of ethno botanical uses and therapeutic properties, supported by both traditional knowledge and modern scientific studies. Rich in diverse phytochemicals, the plant shows strong potential for future therapeutic applications. However, its overexploitation highlights the urgent need for conservation and further research into its bioactive compounds.

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Conflict of interest

No conflict of interest.

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