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In vitro Assessment of the Antiviral Activity of Medicinal Plant Extracts against Dengue Virus Serotype 2 (DENV-2)

Atta ur Rahman[1], Dr. Syed Anis Ali Jafri[2], Nasir Anwar[3], Syeda Okasha Javed[4]

1. *Department of Public Health, Alhamd Islamic University, Islamabad, Pakistan*
2. *Research Associate, Quality Enhancement Cell (QEC), Allama Iqbal Open University (AIOU), Islamabad. Pakistan*
3. *Assistant Professor, Department of Nursing, HBS College of Nursing, Islamabad, Pakistan*
4. *Plant Breeding and Molecular Genetics, University of Poonch, Rawalakot Pakistan*

* *Corresponding author: Syeda Okasha Javed (okashasyed@upr.edu.pk)*

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Abstract

Introduction: Dengue virus infection remains a significant global health challenge with limited antiviral treatment options.

Objective: To evaluate the antiviral potential of selected medicinal plant extracts against Dengue virus serotype 2 (DENV-2) through phytochemical screening and in vitro assays.

Methodology: This descriptive, in vitro experimental study was conducted over one year (August 2023–July 2024) at University of Poonch Rawalakot. Ten medicinal plants traditionally used for febrile and viral illnesses in Pakistan were selected and authenticated. Plant extracts were prepared using methanol, ethanol, and aqueous solvents and screened for key phytochemicals including flavonoids, alkaloids, tannins, saponins, and terpenoids. Cytotoxicity on Vero cells was assessed by MTT assay to determine CC_{50} values. Non-toxic concentrations of extracts were evaluated for antiviral activity against DENV-2 using Plaque Reduction or Virus Yield Reduction Assays. IC_{50} and Selectivity Index (SI) were calculated. Experiments were conducted in triplicate, and data analyzed using ANOVA with significance at $p < 0.05$ in SPSS version 25.0.

Results: All plants contained various bioactive compounds; Curcuma longa showed the richest phytochemical profile. Curcuma longa exhibited the highest safety (CC_{50} = 455 µg/mL) and antiviral efficacy (IC_{50} = 38.20 µg/mL) with an SI of 11.91 and 82.34% viral inhibition. Andrographis paniculata, Azadirachta indica, Nigella sativa, and Phyllanthus niruri also demonstrated significant antiviral activity with SI values above 6. Dose-dependent viral inhibition was confirmed for these top five extracts.

Conclusion: Selected medicinal plants, particularly Curcuma longa, show promising antiviral activity against DENV-2 and merit further investigation for therapeutic development.

Keywords: Dengue virus, medicinal plants, antiviral activity, phytochemicals, Curcuma longa, in vitro assay

Introduction

Dengue virus (DENV) infection continues to pose a significant public health burden in many parts of the world, particularly in tropical and subtropical regions [1]. The disease is transmitted primarily through the bite of infected Aedes aegypti and Aedes albopictus mosquitoes, with clinical outcomes ranging from mild dengue fever to severe complications such as dengue hemorrhagic fever and dengue shock syndrome [2,3]. According to the World Health Organization, dengue infects an estimated 100–400 million people each year, with a rising incidence due to urbanization, climate change, and inadequate vector control strategies [4].

Currently, there is no specific antiviral therapy approved for dengue. Management is largely supportive, focusing on fluid replacement and symptom control [5]. Although a vaccine (Dengvaxia) has been developed, its use is limited by safety concerns and restricted efficacy in seronegative individuals [6]. The lack of an effective, widely accessible antiviral treatment underscores the urgent need for alternative therapeutic approaches that can directly target the virus or modulate the host's immune response [7].

Medicinal plants have long been used in

traditional healing systems to treat viral infections [8]. These plants are rich sources of bioactive compounds, such as flavonoids, alkaloids, saponins, and tannins, which exhibit a broad spectrum of pharmacological activities, including antiviral effects [9]. Previous studies have reported that certain plant extracts can interfere with key stages of viral replication, inhibit viral entry, or enhance host antiviral defenses [10]. Their structural diversity and biological activity make them valuable candidates for developing novel antivirals against RNA viruses, including dengue [11].

Given the challenges posed by DENV's high mutation rate and the need for safe, affordable, and effective antiviral agents, the investigation of medicinal plants offers a promising strategy for drug discovery. This approach also aligns with the global trend toward the use of natural, plant-based therapies that can be locally sourced and integrated into existing healthcare practices.

Research Objective

To investigate the antiviral activity of selected medicinal plant extracts against dengue virus through phytochemical screening and in vitro antiviral assays.

Materials and Methods

Study Design and Duration

This descriptive, in vitro experimental study was conducted over one year, from August 2023 to July 2024, at University of Poonch Rawalakot.

Selection of Medicinal Plants

Ten medicinal plants were selected for this study based on a combined rationale of ethnobotanical relevance and scientific evidence. These plants have been traditionally used across various regions of Pakistan for managing febrile and viral illnesses. Selection criteria included, frequent citation in ethnomedicinal literature or folk practices, accessibility in local herbal markets or home gardens, and prior reports in peer-reviewed literature supporting their antiviral or immunomodulatory activities [12]. Although a formal ethnobotanical survey was not conducted, the selection process was informed by a comprehensive review of traditional knowledge and published pharmacological data [13]. Each plant specimen was taxonomically authenticated by a qualified botanist to ensure accurate identification and reproducibility.

Preparation of Plant Extracts

The collected plant materials, including leaves, stems, or roots, were washed thoroughly, shade-dried, and ground into fine powder. Extraction was performed using methanol, ethanol, and/or aqueous solvents through maceration or Soxhlet extraction methods. The resulting extracts were filtered, concentrated using a rotary evaporator, and stored at 4°C until further use in antiviral assays.

Phytochemical Screening

Preliminary phytochemical analysis was conducted on all plant extracts to detect the presence of key bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and terpenoids. Standard qualitative tests—including ferric chloride for tannins, Dragendorff's reagent for alkaloids, and froth test for saponins—were employed to confirm these constituents.

Cell Lines and Virus

African green monkey kidney (Vero) cells were used for cytotoxicity and antiviral assays due to their susceptibility to dengue virus infection. A well-characterized strain of Dengue virus serotype 2 (DENV-2) was procured from a certified virology repository and utilized for the antiviral testing.

Cytotoxicity Assay

The cytotoxicity of each plant extract was assessed on Vero cells using the MTT assay to determine the 50% cytotoxic concentration (CC_{50}). This step ensured that subsequent antiviral testing was performed at non-toxic concentrations.

In Vitro Antiviral Assay

The antiviral activity of the non-toxic concentrations of all plant extracts was evaluated against DENV-2 using the Plaque Reduction Assay (PRA). This assay was uniformly applied to all ten plant samples to ensure consistency in viral inhibition assessment. Vero cells were infected with DENV-2 in the presence of varying concentrations of each extract, and the number of plaques formed was counted to calculate the percentage of viral inhibition. The 50% inhibitory concentration (IC_{50}) was determined to quantify the antiviral potency of each extract.

Experimental Replicates

All assays were conducted in triplicate to ensure reproducibility and accuracy of results. The mean values from these replicates were used for analysis.

Data Analysis

Results were expressed as mean ± standard deviation (SD). IC_{50} and Selectivity Index (SI), calculated as the ratio of CC_{50} to IC_{50}, were computed for each extract to evaluate safety and efficacy. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with a p-value of less than 0.05 considered statistically significant. Data analysis was performed using SPSS version 25.0 and GraphPad Prism software.

Schematic Workflow of the Experimental Methodology

Figure 1 illustrates the overall experimental workflow, from plant selection and extraction to phytochemical screening, cytotoxicity testing, antiviral assay, and data analysis. This schematic provides a concise visual summary of the study's methodology.

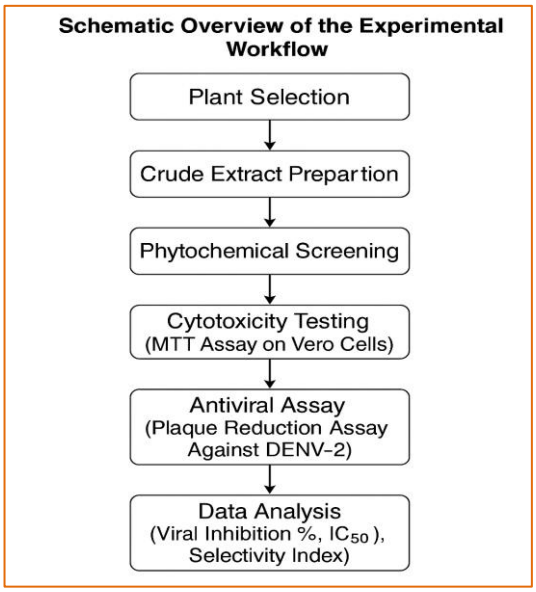

Figure 1: Schematic Workflow of the Experimental Methodology

Ethical Considerations

Since this study was laboratory-based and involved no human or animal subjects, ethical approval was obtained from The University of Poonch Rawalakot, Pakistan to ensure compliance with safety protocols related to the handling and disposal of infectious agents and chemical reagents.

Results

Table 1 presents the phytochemical profile of ten medicinal plants traditionally used for viral and febrile illnesses. Flavonoids were detected in all plants except Allium sativum, while alkaloids were present in 7 out of 10 species. Tannins appeared in 7 plants, saponins in 7, and terpenoids in 7, highlighting the diverse range of bioactive compounds available. Notably, Curcuma longa contained all tested phytochemicals, indicating its rich phytochemical composition potentially linked to its antiviral activity.

Table 1: Phytochemical Constituents of Selected Medicinal Plant Extracts

Plant No.	Scientific Name	Flavonoids	Alkaloids	Tannins	Saponins	Terpenoids
1	*Azadirachta indica*	+	+	+	+	-
2	*Ocimum sanctum*	+	-	+	+	+
3	*Zingiber officinale*	+	+	-	-	+
4	*Moringa oleifera*	+	-	+	+	+
5	*Nigella sativa*	+	+	-	+	+
6	*Allium sativum*	-	+	+	-	+
7	*Carica papaya*	+	-	+	-	-
8	*Curcuma longa*	+	+	+	+	+
9	*Phyllanthus niruri*	+	+	+	+	-
10	*Andrographis paniculata*	+	+	-	+	+

(+) = Present, (–) = Absent

Table 2 shows the cytotoxicity (CC_{50}), antiviral efficacy (IC_{50}), selectivity index (SI), and percentage viral inhibition of the plant extracts. *Curcuma longa* demonstrated the highest cytotoxic safety (CC_{50} = 455.00 µg/mL) and strongest antiviral effect with an IC_{50} of 38.20 µg/mL, yielding a high SI of 11.91 and 82.34% viral inhibition. *Andrographis paniculata* and *Azadirachta indica* also exhibited potent antiviral activity with SI values of 8.90 and 8.19, respectively. Extracts like *Carica papaya* showed lower antiviral activity and selectivity, indicating varying efficacy among the plants tested.

Table 3 summarizes the top five medicinal plants with the most promising antiviral properties. *Curcuma longa* ranked first with an 82.34% viral inhibition, the lowest IC_{50} (38.20 µg/mL), and highest SI (11.91), suggesting the best safety and efficacy profile. This was followed by *Andrographis paniculata* (77.38% inhibition, SI 8.90), *Azadirachta indica* (76.83%, SI 8.19), *Nigella sativa* (74.12%, SI 7.76), and *Phyllanthus niruri* (68.92%, SI 6.41). These plants represent potential candidates for further antiviral drug development.

Figure 2 illustrates the dose-dependent antiviral effects of the top five plant extracts at varying concentrations. All extracts showed increasing viral inhibition with higher doses, with *Curcuma longa* consistently showing superior inhibition, ranging from 35.20% at 10 µg/mL to 82.34% at 100 µg/mL. Similarly, *Andrographis paniculata* increased from 30.15% to 77.38%, while *Azadirachta indica*, *Nigella sativa*, and *Phyllanthus niruri* demonstrated comparable dose-dependent antiviral activity, confirming their potent inhibitory effects on DENV-2 replication. Values represent mean % viral inhibition at indicated concentrations.

Table 2: Cytotoxicity and Antiviral Activity of Medicinal Plant Extracts Against DENV-2

Plant No.	Scientific Name	CC_{50} (µg/mL)	IC_{50} (µg/mL)	Selectivity Index (SI)	Antiviral Activity
1	*Azadirachta indica*	345.20	42.15	8.19	76.83%
2	*Ocimum sanctum*	298.10	58.75	5.07	64.28%
3	*Zingiber officinale*	410.55	91.30	4.50	59.72%

Continue...

4	*Moringa oleifera*	370.40	70.25	5.27	61.87%
5	*Nigella sativa*	282.70	36.45	7.76	74.12%
6	*Allium sativum*	325.65	89.50	3.64	56.45%
7	*Carica papaya*	392.40	112.80	3.48	53.27%
8	*Curcuma longa*	455.00	38.20	11.91	82.34%
9	*Phyllanthus niruri*	305.30	47.60	6.41	68.92%
10	*Andrographis paniculata*	360.75	40.50	8.90	77.38%

CC_{50}: 50% Cytotoxic Concentration. IC_{50}: 50% Inhibitory Concentration. SI (CC_{50} / IC_{50}): Higher SI = safer and more effective antiviral. Antiviral Activity (%): Reduction in viral replication compared to control.

Table 3: Summary of Top 5 Plant Extracts Based on Antiviral Potential

Rank	Plant Name	Antiviral Activity (%)	IC_{50} (µg/mL)	SI
1	*Curcuma longa*	82.34%	38.20	11.91
2	*Andrographis paniculata*	77.38%	40.50	8.90
3	*Azadirachta indica*	76.83%	42.15	8.19
4	*Nigella sativa*	74.12%	36.45	7.76
5	*Phyllanthus niruri*	68.92%	47.60	6.41

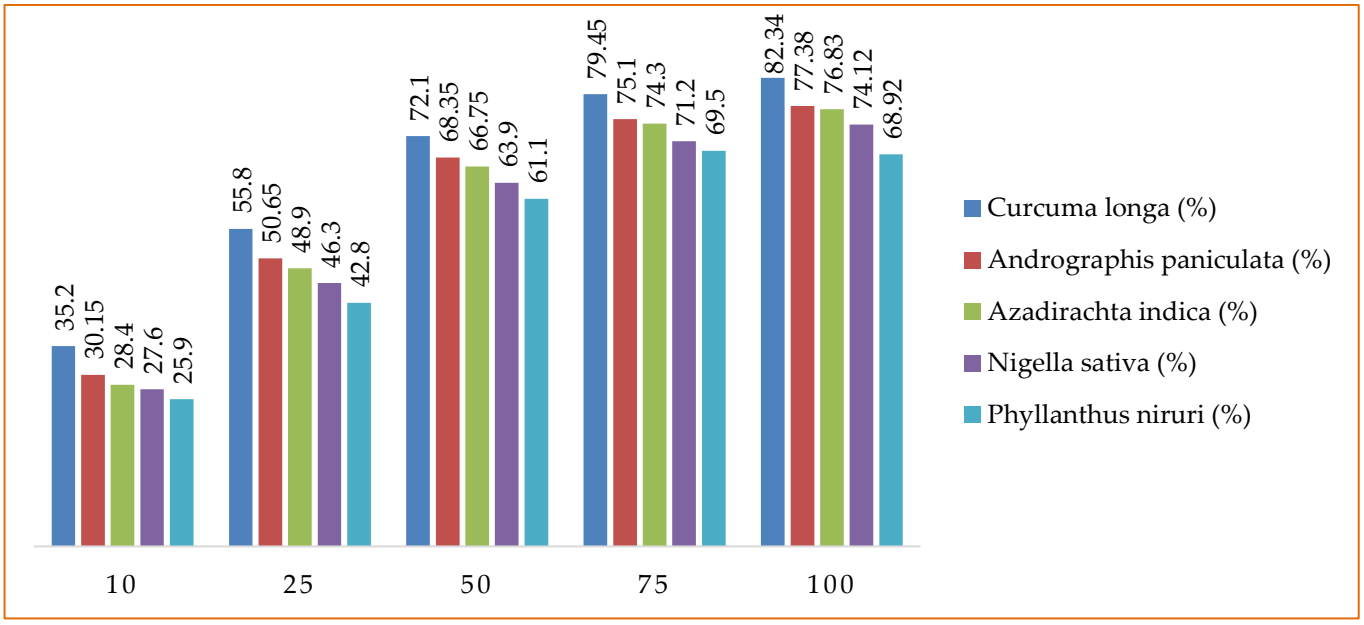

Figure 2: Dose-dependent antiviral effects of the top five plant extracts at varying concentrations.

Discussion

The current study aimed to evaluate the *in vitro* antiviral activity of selected medicinal plant extracts against DENV-2, with particular focus on their phytochemical profiles and safety-to-efficacy ratios. Among the ten tested medicinal plants, *Curcuma longa* exhibited the most potent antiviral activity, with 82.34% viral inhibition, an IC_{50} of 38.20 µg/mL, and the highest selectivity index (SI) of 11.91. These results are in line with earlier studies which demonstrated that curcumin the major active compound in *Curcuma longa*—inhibits dengue viral replication by interfering with envelope fusion and viral RNA synthesis [14]. The high SI observed suggests that *Curcuma longa* holds significant promise as a safe and effective antiviral agent.

Andrographis paniculata ranked second in efficacy (77.38% inhibition, IC_{50} = 40.50 µg/mL, SI = 8.90), corroborating prior reports that identified andrographolide as a potent inhibitor of DENV protease activity [15]. Similarly, Azadirachta indica showed 76.83% inhibition (IC_{50} = 42.15 µg/mL, SI = 8.19), consistent with previous findings highlighting the role of neem-derived limonoids in suppressing dengue virus replication [16]. These consistent findings further validate the antiviral properties of these traditional remedies.

Nigella sativa also demonstrated notable antiviral effects (74.12% inhibition, IC_{50} = 36.45 µg/mL, SI = 7.76). This is supported by earlier research showing that thymoquinone, its principal bioactive component, modulates host immune responses and reduces viral gene expression [17]. Likewise, *Phyllanthus niruri* (68.92% inhibition, SI = 6.41) aligns with evidence that its phytoconstituents inhibit viral polymerase activity in related flaviviruses, suggesting potential cross-flavivirus efficacy [18].

The phytochemical screening indicated the presence of flavonoids, alkaloids, tannins, saponins, and terpenoids in most bioactive plants. Notably, *Curcuma longa* contained all five tested

phytochemical groups, which may explain its superior antiviral activity. This is consistent with prior studies suggesting that combinations of phytochemicals can exert synergistic effects by targeting multiple stages of the viral lifecycle, such as viral entry, replication, protein synthesis, and host immune modulation [19]. For instance, flavonoids and terpenoids have been reported to inhibit viral proteases and polymerases, while saponins and alkaloids may enhance cell membrane permeability or immune responses. Such synergism could enhance overall antiviral potency and reduce the risk of viral resistance development.

In contrast, plants like *Carica papaya* and *Allium sativum*, although rich in certain phytochemicals, exhibited relatively lower antiviral activity (53.27% and 56.45% inhibition, respectively), suggesting that not all phytochemicals equally contribute to antiviral efficacy or that their active constituents may require further refinement for activity.

Study Strengths and Limitations

This study's strengths include the comprehensive evaluation of ten medicinal plants with ethnobotanical relevance, combined with rigorous phytochemical screening and standardized in vitro antiviral assays using well-characterized DENV-2 and Vero cell lines. Conducting experiments in triplicate enhanced the reliability and reproducibility of results, while the calculation of selectivity indices provided insight into the therapeutic safety margin of each extract. However, limitations exist such as the exclusive use of in vitro methods, which may not fully replicate in vivo dynamics, including metabolism and immune system interactions. The study also focused on a single dengue serotype (DENV-2), limiting the generalizability to other serotypes. Moreover, the use of Vero cells—non-human primate kidney epithelial cells—limits the translational relevance of findings, as they do not fully represent human cellular and immune responses. Additionally, the convenience sampling of plants and variability in extraction solvents may influence consistency and bioactive compound yield. Further in vivo studies and mechanistic investigations are needed to validate these findings and explore clinical relevance.

Conclusion

The study identified *Curcuma longa*, *Andrographis paniculata*, *Azadirachta indica*, *Nigella sativa*, and *Phyllanthus niruri* as promising antiviral agents against dengue virus, with *Curcuma longa* exhibiting the strongest activity and safety profile. These findings support the potential of traditionally used medicinal plants as sources of novel antiviral compounds and provide a scientific basis for further drug development efforts targeting dengue infection.

Authors' Contributions

Atta ur Rahman: Conceptualization, experimental design, laboratory work, data collection, and drafting of the manuscript. Approved the final version and is accountable for all aspects of the work.

Dr. Syed Anis Ali Jafri: Data analysis, interpretation of results, and critical revision of the manuscript for important intellectual content. Approved the final version and is accountable for the integrity of the work.

Dr. Nasir Anwar: Supervision of experimental protocols, methodological input, and assistance in result interpretation. Contributed to manuscript revision, approved the final version, and agrees to be accountable for all aspects of the work.

Syeda Okasha Javed: Preparation and extraction of plant samples, contribution to molecular analysis, and support in data acquisition. Reviewed and approved the final version and agrees to be accountable for all aspects of the work.

References

1. Yang X, Quam MB, Zhang T, Sang S. Global burden for dengue and the evolving pattern in the past 30 years. Journal of travel medicine. 2021 Dec;28(8):taab146. https://doi.org/10.1093/jtm/taab146.

2. Zerfu B, Kassa T, Legesse M. Epidemiology, biology, pathogenesis, clinical manifestations, and diagnosis of dengue virus infection, and its trend in Ethiopia: a comprehensive literature review. Tropical Medicine and Health. 2023 Feb 24;51(1):11. https://doi.org/10.1186/s41182-023-00504-0

3. Chawla P, Yadav A, Chawla V. Clinical implications and treatment of dengue. Asian Pacific journal of tropical medicine. 2014 Mar 1;7(3):169-78. https://doi.org/10.1016/S1995-7645(14)60016-X.

4. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clinical epidemiology. 2013 Aug 20:299-309. https://doi.org/10.2147/CLEP.S34440.

5. Tayal, A., Kabra, S.K. & Lodha, R. Management of Dengue: An Updated Review. Indian J Pediatr 90, 168–177 (2023). https://doi.org/10.1007/s12098-022-04394-8

6. Tully D, Griffiths CL. Dengvaxia: The world's first vaccine for prevention of secondary dengue. Therapeutic Advances in Vaccines and Immunotherapy. 2021 May;9:25151355211015839. https://doi.org/10.1177/25151355211015839.

7. Ganguly G, Sahoo T, Ghosh S, Gupta A, Nayak A, Mojamel SK, Sahabuddin M. Emerging

Interventions & Therapies For Effective Dengue Management. vaccine.;4:5. DOI: 10.13040/IJPSR.0975-8232.IJP.12(2).37-57.

8. Abd Kadir SL, Yaakob H, Mohamed Zulkifli R. Potential anti-dengue medicinal plants: a review. Journal of natural medicines. 2013 Oct;67:677-89. https://doi.org/10.1007/s11418-013-0767-y.

9. Akwayamai JJ, Ahmed MU, Tirah G, Samuel K, Usman WA, Bulus H. A review of medicinal plants with anti-dengue virus activity. European Journal of Pharmaceutical Research. 2022;2(2):1-1. DOI :10.24018/pharma.2022.2.2.20

10. Loaiza-Cano V, Monsalve-Escudero LM, Filho CD, Martinez-Gutierrez M, Sousa DP. Antiviral role of phenolic compounds against dengue virus: A review. Biomolecules. 2020 Dec 24;11(1):11. https://doi.org/10.3390/biom11010011.

11. Lee MF, Wu YS, Poh CL. Molecular mechanisms of antiviral agents against dengue virus. Viruses. 2023 Mar 8;15(3):705. https://doi.org/10.3390/v15030705.

12. Kaushik S, Kaushik S, Sharma V, Yadav J. Antiviral and therapeutic uses of medicinal plants and their derivatives against dengue viruses. Pharmacognosy Reviews. 2018 Jul 1;12(24). https://phcogrev.com/sites/default/files/PhcogRev_2018_12_24_177.pdf.

13. Dhiman M, Sharma L, Dadhich A, Dhawan P, Sharma MM. Traditional knowledge to contemporary medication in the treatment of infectious disease dengue: a review. Frontiers in Pharmacology. 2022 Mar 14;13:750494. https://doi.org/10.3389/fphar.2022.750494

14. Ichsyani M, Ridhanya A, Risanti M, Desti H, Ceria R, Putri DH, Sudiro TM, Dewi BE. Antiviral effects of Curcuma longa L. against dengue virus in vitro and in vivo. InIOP Conference Series: Earth and Environmental Science 2017 Dec 1 (Vol. 101, No. 1, p. 012005). IOP Publishing. DOI 10.1088/1755-1315/101/1/012005.

15. Ali-Seyed M, Vijayaraghavan K. Dengue virus infections and anti-dengue virus activities of Andrographis paniculata. Asian Pacific Journal of Tropical Medicine. 2020 Feb 1;13(2):49-55. DOI: 10.4103/1995-7645.275412.

16. Dwivedi VD, Bharadwaj S, Afroz S, Khan N, Ansari MA, Yadava U, Tripathi RC, Tripathi IP, Mishra SK, Kang SG. Anti-dengue infectivity evaluation of bioflavonoid from Azadirachta indica by dengue virus serine protease inhibition. Journal of Biomolecular Structure and Dynamics. 2021 Mar 4;39(4):1417-30. https://doi.org/10.1080/07391102.2020.1734485

17. Maideen NM, Hadda TB, Almalki FA, Laarousi H, Soliman SS, Kawsar SM. Black seeds (Nigella sativa) for the management of Dengue viral disease: POM analyses for the identification of new antiviral pharmacophore sites. https://doi.org/10.21203/rs.3.rs-3163496/v1.

18. Martins IC, Ricardo RC, Santos NC. Dengue, West Nile, and Zika Viruses: potential novel antiviral biologics drugs currently at discovery and preclinical development stages. Pharmaceutics. 2022 Nov 20;14(11):2535. https://doi.org/10.3390/pharmaceutics14112535.

19. Ghildiyal R, Prakash V, Chaudhary VK, Gupta V, Gabrani R. Phytochemicals as antiviral agents: recent updates. Plant-derived bioactives: production, properties and therapeutic applications. 2020:279-95. https://doi.org/10.1007/978-981-15-1761-7_12