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An Insight into the Antiviral Potential of African Medicinal Plants: *Prosopis africana* and Others Against Enteroviral Infections

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ABSTRACT

Increasing viral infections and rising drug resistance underscore the urgent need for new antiviral agents. This study evaluates the antiviral potential of extracts from Detarium microcarpum (stem bark), Prosopis africana (whole fruit, stem bark, and root bark), Parinari polyandra (stem bark), and Phyllanthus muellerianus (stem bark) against echoviruses E7 and E19. Plant materials were extracted via maceration using aqueous methanol (30:70) and assessed through cell culture-based assays. The virus titer ($TCID_{50}$) was determined to be 10^{-6} for both viruses, with 100 $TCID_{50}$ employed for antiviral screening. Cytotoxicity (CC_{50}) and maximum non-toxic concentrations (MNTC) were determined using the MTT assay. Among the tested extracts, Prosopis africana root (PAR) exhibited the strongest antiviral activity, with IC_{50} values of 0.073 µg/mL for E19 and 1.70 µg/mL for E7, coupled with high selectivity (SI = 90.96). Moderate activity was observed in the fruit extract of Prosopis africana (PAF) and the stem bark of Parinari polyandra (PPS), while Detarium microcarpum and Phyllanthus muellerianus showed limited or no efficacy. These findings reveal untapped antiviral potential in African medicinal flora and identify Prosopis africana as a promising candidate for further investigation and development of plant-based antiviral therapies.

Keywords: Prosopis africana, antiviral activity, echovirus, cell culture, cytotoxicity, root extracts.

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INTRODUCTION

The increasing incidence of viral infections and the growing resistance to current antiviral medications have made the quest for efficient antiviral treatments a top concern in contemporary medicine (Dodtale et al., 2024). Although synthetic antiviral drugs are essential for treating viral illnesses, their narrow range of action, possible adverse effects, and the development of resistance highlight the need for alternative therapies (Wang et al., 2024). With their varied chemical structures and modes of action, plant-derived chemicals have attracted a lot of interest for their antiviral potential in this setting (Davidova et al., 2024).

Numerous plant species that are often utilised in traditional African medicine have demonstrated encouraging antiviral qualities (Adeosun and Loots, 2024). These include the stem bark of Detarium microcarpum, the whole fruit, stem bark, and root bark of Prosopis africana, the stem bark of Parinari polyandre, and the stem bark of Phyllanthus muellerianus. They have been identified as having broad-spectrum bioactivity, which includes antioxidant, antimicrobial, and anti-inflammatory properties (Dogara, 2022, Sharifi-Rad et al., 2019, Brusotti et al., 2011). Bioactive substances such as flavonoids, alkaloids, terpenoids, and phenolic acids are abundant in these plants and are thought to have a role in their antiviral and other therapeutic qualities (Roy et al., 2022, Shahrajabian et al., 2022, Hilal et al., 2024).

Detarium microcarpum, which is widely distributed throughout West and Central Africa, has long been utilised for its antibacterial, anti-inflammatory, and wound-healing qualities (Iful, 2008, Salawu et al., 2021). The stem bark of this plant may have antiviral properties, especially against enveloped viruses, according to preliminary evidence (Musarra-Pizzo et al., 2021, Olugbuyiro et al., 2009, Dahiru et al., 2023). According to an earlier report, Prosopis africana, a drought-resistant tree indigenous to sub-Saharan Africa, has also been used to treat several illnesses, such as gastrointestinal and respiratory infections (Ravhuhali et al., 2021). Though some data suggests that its fruit and bark extracts may be able to prevent viral replication, research on its antiviral activity is still in its early stages (Naz et al., 2024, Famutimi and Adewale, 2021).

The plant Parinari polyandre, sometimes referred to as the African olive, has a long ethnobotanical history, especially in the treatment of illnesses and fevers (Patrice et al., 2024). Few research has been done on its stem bark's antiviral qualities, but its strong antibacterial and anti-inflammatory qualities point to a potential medicinal use (Ali et al., 2022, Mawire et al., 2021).

The herb Phyllanthus muellerianus, which has been used for a long time to treat viral infections and liver illnesses, especially hepatitis B, has garnered attention due to its antiviral qualities (Siddiqui et al., 2017). According to several research, lignans and flavonoids, which are especially abundant in the stem bark of this plant, exhibit antiviral properties (Roy et al., 2022, Chen et al., 2023a).

The precise processes by which these plants produce their antiviral effects are still not well understood, despite their historical use and growing scientific interest. The purpose of this paper is to evaluate the antiviral activity of Phyllanthus muellerianus, Prosopis africana, Parinari polyandre, and Detarium microcarpum, with an emphasis on their chemical components and possible modes of action. By assessing the existing literature, we want to offer a thorough grasp of these plants' antiviral capabilities,

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opening the door for further study and the creation of plant-based antiviral treatments (Anand et al., 2019, Niharika et al., 2024).

METHODS

Plant Collection:

Prosopis africana (whole fruit, stem bark, and root bark), Detarium microcarpum (stem bark), Parinari polyandre (stem bark), and Phyllanthus muellerianus (stem bark) were gathered at the University of Ilorin's main campus in Kwara State, Nigeria. The plants were authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, with voucher numbers UILH/002/1272 for D. microcarpum, UILH/001/163 for P. africana, UILH/001/582/2021 for P. polyandre, and UILH/001/774/2021 for P. muellerianus. The use of plant voucher specimens for proper authentication follows standard procedures for ensuring the accuracy and reproducibility of ethnobotanical research (Applequist and Miller, 2013).

Extraction Procedure:

The plant materials were subjected to air-drying at ambient temperature (34°C) and subsequently ground to a fine powder using an electric grinder. A total of 200 grams of each powdered plant material was extracted using aqueous-methanol (30:70) through a maceration process conducted at room temperature (32°C) for 72 hours, following the method described by Ali et al. (2015). The resulting extracts were filtered, and the residual plant material (marc) was further extracted with redistilled methanol until the filtrate became colourless. The combined methanol extracts were concentrated under reduced pressure using a rotary evaporator (Büchi, Switzerland) to yield crude extracts, which were stored at 4°C for subsequent analysis. The extract yield was determined based on the following calculation:

$$\% \text{ yield of extract} = \frac{\text{Weight of Extract}}{\text{Weight of Plant Material}} \text{ X } 100\%$$

Viruses and Cell Line:

Two echovirus serotypes, E7 and E19, sourced from stool isolates by the Enterovirus Research Group, Department of Virology, University of Ibadan, Nigeria, were employed in this study. The viruses were stored at −70°C until required. Human rhabdomyosarcoma (RD) cells, procured from the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, were cultured in Eagle's Minimum Essential Medium (MEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS), 100 units/mL of penicillin, 100 µg/mL of streptomycin, 2 mM L-glutamine, 0.07% NaHCO3, 1% non-essential amino acids, and a vitamin solution. The cells were maintained at 37°C in a humidified incubator with 5% CO2. For both cytotoxicity and antiviral assays, the serum concentration in the culture medium was reduced to 2% FBS (maintenance medium) (Ogbole et al., 2021a).

Preparation of Stock Solutions for Plant Extracts:

Stock solutions of each plant extract (10 mg/mL) were prepared by dissolving 10 mg of the crude extract in 1 mL of dimethyl sulfoxide (DMSO). These stock solutions were stored at 4°C. For antiviral assays, the stock solutions were further diluted with a maintenance medium to achieve a final concentration of 1000 µg/mL (Ogbole et al., 2021b).

Tissue Culture Infective Dose (TCID50):

Virus titration was carried out to determine the TCID50, a measure of the virus concentration required to induce cytopathic effects (CPE) in

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RD cells. In brief, 100 µL of echovirus E7 was added to a T25 flask containing RD cells, followed by a 72-hour incubation at 37°C. After incubation, the virus stock was prepared, and 100 µL of RD cell suspension (1×10^6 cells/mL) was seeded into a 96-well microplate. A series of ten-fold dilutions of the virus stock was prepared, and 100 µL of each dilution was inoculated into the wells. The plates were incubated at 37°C, and daily CPE assessments were performed until cell death was observed in the control wells. The TCID50 value was calculated using the Spearman-Karber method (Lei et al., 2021). This procedure was replicated for echovirus E19, as described earlier (Lei et al., 2021).

Maximum Non-Toxic Concentration (MNTC)

The MNTC of the plant extracts was determined using the MTT assay (Ogbole et al., 2018), a well-established method for evaluating cell viability. RD cells were seeded into a 96-well plate and cultured for 24 hours. Subsequently, the cells were treated with six ten-fold serial dilutions (1000 to 0.01 µg/mL) of each plant extract in a maintenance medium. After 72 hours of incubation at 37°C, the old medium was replaced, and 25 µL of MTT solution (2 mg/mL) was added to each well. The plates were incubated for an additional 2 hours at 37°C, after which the MTT solution was removed, and 75 µL of DMSO was added to dissolve the purple formazan crystals. The optical density was measured at 490 nm using a microplate reader (Multiscan 347, MTX Lab). The CC50 (50% cytotoxic concentration) was determined by non-linear regression analysis using GraphPad Prism 5 software (Ogbole et al., 2017).

Antiviral Assay

Cytopathic Effect Inhibition: The antiviral activity of the plant extracts was evaluated by measuring the inhibition of virus-induced cytopathic effects (CPE) in RD cells, as described by Ogbole et al. (2018) (Ogbole et al., 2018). RD cells were seeded into a 96-well microplate and incubated for 24 hours to form a monolayer. Ten-fold serial dilutions (50 µL) of the plant extracts, derived from the maximum non-toxic concentration (MNTC), were added to the wells, followed by 50 µL of a virus suspension containing 100 TCID50 of either echovirus E7 or E19. The negative control wells contained only RD cells, while the positive control wells contained RD cells infected with the virus. The plates were incubated at 37°C in a 5% CO2 incubator for 72 hours. At the end of the incubation period, the MTT assay was performed to measure cell viability. The 50% inhibitory concentration (IC50) was determined based on optical density readings. The IC50 value represents the concentration of the extract that reduced the CPE by 50% compared to the virus control group, as previously outlined by Schmidtke et al. (2021) (Schmidtke et al., 2001).

Data Analysis: The cytotoxic concentration (CC50) and the inhibitory concentration (IC50) were calculated using non-linear regression analysis via GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA). The selectivity index (SI) for each active extract was calculated by dividing CC50 by IC50, indicating the safety margin for the extract (Dahdooh et al., 2024)

RESULTS

A total of 16 health facilities (two hospitals and The extraction yields of the six plant materials were evaluated and are summarized in Table 1. The yields ranged from 6% to 17%, with Detarium microcarpum stem bark (DMS)

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producing the highest yield at 17%. This was followed by Prosopis africana stem (PAS) at 16% and P. africana root (PAR) at 14.5%, indicating these plant materials contain substantial quantities of extractable compounds. In contrast, P. africana fruit (PAF) and Phyllanthus muellerianus stem bark (PMS) exhibited the lowest extraction yields, each at 6%, while Parinari polyandre stem bark (PPS) showed a moderate yield of 8%.

The cytotoxic and antiviral properties of the plant extracts were assessed using Maximum Non-Toxic Concentration (MNTC), 50% Cytotoxic Concentration (CC50), 50% Inhibitory Concentration (IC50) against Echovirus 7 (E7) and Echovirus 19 (E19), and the Selectivity Index (SI) as shown in Table 2. Most extracts displayed an MNTC of 10 µg/mL, except for PAF, PPS, and DMS, which exhibited a much lower MNTC of 0.1 µg/mL, suggesting higher inherent cytotoxicity. The CC50 values varied significantly across the extracts, with P. muellerianus stem bark (PMS) having the highest value (35.73 µg/mL), indicating lower toxicity, while PAF showed the lowest CC50 (1.84 µg/mL), reflecting higher cytotoxicity. Among the tested extracts, only Prosopis africana root (PAR) showed activity against Echovirus 7, with an IC50 of 1.7 µg/mL and a Selectivity Index (SI) of 3.91. None of the other extracts demonstrated significant activity against E7. For Echovirus 19, Prosopis africana fruit (PAF) exhibited remarkable antiviral activity with an IC50 of 0.004 µg/mL and an exceptionally high SI of 460, indicating strong selectivity and potential for therapeutic application. Prosopis africana root (PAR) and Prosopis africana stem (PAS) also showed moderate activity against E19, with SIs of 90.96 and 52.19, respectively. Other extracts, such as PPS and DMS, were either not active or not tested against E19

DISCUSSIONS

The extracts from Detarium microcarpum (stem bark), Prosopis africana (whole fruit, stem bark, and root bark), Parinari polyandre (stem bark), and Phyllanthus muellerianus (stem bark) were tested for their antiviral efficacy against echoviruses E7 and E19. The antiviral activity of the six extracts varied significantly, according to the findings of our screening, with Prosopis africana root (PAR) showing the most antiviral activity.

This study aimed to evaluate the antiviral activity of plant extracts from Detarium microcarpum (stem bark), Prosopis africana (whole fruit, stem bark, and root bark), Parinari polyandre (stem bark), and Phyllanthus muellerianus (stem bark) against echoviruses E7 and E19. The results of our screening indicated significant variability in the antiviral potency of the six extracts, with Prosopis africana root (PAR) exhibiting the highest antiviral activity.

The root extract of Prosopis africana demonstrated potent antiviral activity, especially against E19, with an IC50 of 0.073 µg/mL. This activity was notably selective, with a selectivity index (SI) of 90.96, suggesting that it could be a promising candidate for antiviral development. Previous studies have highlighted the bioactivity of Prosopis africana, particularly its antimicrobial and antiviral properties (Sharifi-Rad et al., 2019, Doughari and Saa-Aondo, 2021). These findings are consistent with the observed antiviral activity against enteroviruses, suggesting that the root extract may contain bioactive compounds such as flavonoids, saponins, and tannins, which are known for their antiviral potential (John, 2024).

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The significant antiviral activity against both echovirus serotypes (E7 and E19) supports the hypothesis that Prosopis africana root may contain compounds capable of interfering with viral replication or attachment to host cells. Studies have shown that polyphenolic compounds, particularly flavonoids, play a crucial role in inhibiting viral entry and replication (Montenegro-Landívar et al., 2021, Chen et al., 2023b). The strong activity of PAR, particularly against E19, could be attributed to the presence of these compounds.

Detarium microcarpum (DMS), Parinari polyandre (PPS), and Phyllanthus muellerianus (PMS) exhibited varying degrees of antiviral activity, but none of them achieved the same level of inhibition as PAR. DMS, PAS, and PAF showed minimal antiviral activity against both viruses and in some cases, no inhibition was observed at the maximum non-toxic concentrations (MNTC). The differences in activity may reflect the diverse chemical compositions of these plants. Detarium microcarpum has been reported to contain phenolic compounds and alkaloids, which are generally known for antimicrobial activities but may require higher concentrations for antiviral effects (Dogara, 2022). Parinari polyandre, on the other hand, is less studied, and while its stem bark showed moderate antiviral effects, further investigation into its bioactive constituents is needed.

Interestingly, Phyllanthus muellerianus did not show any antiviral activity against echoviruses, although its hepatoprotective properties have been documented (여상구, 2014, Lim et al., 2021). This lack of activity against echoviruses may suggest that its bioactive compounds do not interact effectively with the viral targets of echoviruses, or the concentration of active components in the stem bark may not be sufficient to inhibit viral replication at the tested concentrations.

The cytotoxicity results (CC50 values) for the extracts were in line with the antiviral findings, with PAR exhibiting the highest selectivity index (SI) for E19, demonstrating its potential as an antiviral agent with minimal toxicity to the host cells. SI values greater than 1.0 indicate that an extract selectively inhibits viral replication while preserving cell viability, which is crucial for the development of therapeutics. Prosopis africana root, with an SI of 90.96, emerged as a strong candidate for further research in antiviral drug development. In contrast, the other extracts with lower SI values, such as PAS and PPS, require further optimization and evaluation.

The results also reinforce the need for establishing the cytotoxic threshold of plant extracts before their clinical use, as seen with extracts like Parinari polyandre stem bark (PPS), which did not exhibit antiviral activity despite being non-toxic at the tested concentrations. Understanding the dose-response relationship and toxicity profiles is crucial in determining safe and effective dosages for potential drug development (Emilien et al., 2000).

The antiviral activity of these plant extracts may be attributed to several mechanisms of action, including inhibition of viral entry, replication, or assembly. Previous studies on plant-derived antiviral agents have suggested that polyphenols and flavonoids can inhibit viral attachment and entry into host cells by interacting with viral surface proteins or cellular receptors (Chojnacka et al., 2021). For instance, flavonoids are known to disrupt viral membrane integrity, which may explain the observed antiviral activity of Prosopis

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africana root. Future studies should focus on identifying the specific bioactive compounds responsible for these effects and elucidating their molecular mechanisms. Moreover, the screening of additional plant extracts from the same genera and families as those used in this study could uncover new potential antiviral candidates. For example, Parinari polyandre has shown antimicrobial and antioxidant activities (Patrice et al., 2024), and further investigation could identify compounds with broader antiviral potential. Additionally, further testing of Detarium microcarpum and Phyllanthus muellerianus on other viral strains and enteroviruses would provide more comprehensive data on their antiviral spectra.

CONCLUSION

The study demonstrates that plant extracts from Prosopis africana root exhibit significant antiviral activity against echoviruses E7 and E19, with high selectivity, making it a promising candidate for antiviral drug development. In comparison, Detarium microcarpum, Parinari polyandre, and Phyllanthus muellerianus showed varying degrees of antiviral activity, but they require further investigation. The results emphasize the potential of African medicinal plants as sources of bioactive compounds with antiviral properties and highlight the need for more focused research into the isolation and characterization of these compounds. Future research should focus on identifying the active phytochemicals in Prosopis africana root and other promising extracts, investigating their mechanisms of action, and optimizing their antiviral activity. This will pave the way for the development of novel, plant-based antiviral agents to combat enteroviral infections and other viral diseases.

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