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FLAVONOIDS FROM *ECLIPTA ALBA* INHIBIT HIV-1 REVERSE TRANSCRIPTASE: AN *IN VITRO* STUDY

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ABSTRACT

HIV-1 reverse transcriptase (RT) is a crucial enzyme in the replication cycle of the human immunodeficiency virus (HIV), making it a primary target for antiretroviral therapies. This study aimed to evaluate the inhibitory potential of *Eclipta alba*-derived flavonoids against HIV-1 RT through an in vitro enzyme inhibition assay. Flavonoids from *Eclipta alba* were investigated for their inhibitory potential against HIV-1 reverse transcriptase (RT) using an *in vitro enzyme* inhibition assay. Flavonoid-rich fractions were isolated through solvent partitioning and characterized using spectroscopic techniques. The inhibitory effects of the flavonoid extract were assessed at concentrations ranging from 10 to 200 µM and compared with the reference drug Efavirenz. Results demonstrated a dose-dependent increase in HIV-1 RT inhibition, with flavonoids exhibiting superior activity compared to Efavirenz at all tested concentrations. At 200 µM, the flavonoid fraction achieved 89.7% inhibition, significantly higher than the 76.8% inhibition observed with Efavirenz ($p < 0.05$). The enhanced antiviral efficacy may be attributed to synergistic interactions among the bioactive flavonoid compounds. These findings suggest that *Eclipta alba*-derived flavonoids could serve as promising candidates for HIV-1 therapy. Further studies are warranted to elucidate their mechanism of action, optimize bioavailability, and evaluate their in vivo potential for antiretroviral applications.

Key words: Flavonoid; *Eclipta alba*; Efavirenz; HIV-1 RT.

INTRODUCTION

The human immunodeficiency virus type 1 continues to pose a significant global health challenge, despite the advancements in antiretroviral therapies. The development of drug resistance and the adverse side effects associated with current treatments necessitate the continuous search for novel therapeutic agents (Venkanna et al., 2013; Mamidala et al., 2013). Among the various viral enzymes, HIV-1 reverse transcriptase remains a crucial target for therapeutic intervention due to its pivotal role in viral replication. This enzyme is responsible for converting the single-stranded viral RNA into double-stranded DNA, which is then integrated into the host cell's genome. Consequently, inhibiting RT can effectively halt viral replication and disease progression. Current RT inhibitors, including nucleoside/nucleotide RT inhibitors and non-nucleoside RT inhibitors, have demonstrated clinical efficacy but are often plagued by the emergence of drug-resistant viral strains. Therefore, the exploration of alternative sources for RT inhibitors, particularly from natural products, has gained considerable attention. Islatravir is a relatively new reverse

transcriptase translocation inhibitor (Manjula et al., 2013: Poojari et al., 2014).

Flavonoids, a diverse group of polyphenolic compounds found ubiquitously in plants, have garnered significant interest due to their diverse pharmacological activities, including antiviral properties. These compounds have demonstrated a wide range of biological effects, such as antioxidant, anti-inflammatory, and anticancer activities. Several studies have reported the potential of flavonoids as inhibitors of HIV-1 RT, highlighting their ability to interfere with different stages of the viral replication cycle. The mechanisms by which flavonoids inhibit HIV-1 RT are diverse and may involve direct binding to the enzyme's active site, allosteric modulation, or interference with the binding of substrates. Given the structural diversity and broad range of biological activities associated with flavonoids, they represent a promising source of novel antiviral agents. It has been reported that Eclipta prostrata extracts possess anti-HIV-1 integrase activities (Tewtrakul et al., 2007).

This study focuses on the in vitro evaluation of the HIV-1 RT inhibitory activity of flavonoids extracted from *Eclipta alba*, a plant traditionally used in various medicinal systems. *Eclipta alba* is a widely distributed plant species known for its diverse phytochemical constituents, including flavonoids, terpenoids, and coumarins. Previous investigations have suggested that extracts from *Eclipta alba* possess antiviral and immunomodulatory properties. Considering the current need for novel RT inhibitors and the medicinal properties of *Eclipta alba*, this study aims to isolate and identify flavonoid compounds from *Eclipta alba* and assess their potential to inhibit HIV-1 RT *in vitro*. The findings of this research could provide valuable insights into the development of new therapeutic strategies for the treatment of HIV-1 infection (Gujjeti et al., 2014; Paindla et al., 2014).

MATERIALS AND METHODS

Plant Material and Extraction

Dried aerial parts of *Eclipta alba* were procured from an authenticated source and powdered using a mechanical grinder. The powdered plant material (50 g) was subjected to extraction with 70% ethanol using a Soxhlet apparatus for 24 hours. The resulting extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further analysis.

Flavonoid Isolation

The crude extract was subjected to fractionation using liquid-liquid partitioning with solvents of increasing polarity (hexane, chloroform, ethyl acetate, and methanol). The flavonoid-rich fraction was identified using thin-layer chromatography (TLC) and purified via column chromatography on silica gel.

HIV-1 Reverse Transcriptase Inhibition Assay

The inhibitory activity of the flavonoids against HIV-1 reverse transcriptase was evaluated using a colorimetric enzyme inhibition assay. Recombinant HIV-1 reverse transcriptase enzyme was incubated with varying concentrations of the flavonoid fraction (10–200 µM) in reaction buffer for 30 minutes at 37°C. The reaction was initiated by adding the appropriate substrate, and absorbance was recorded at 405 nm using a microplate reader. The percentage inhibition was calculated relative to a control reaction without flavonoids.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered significant

RESULTS AND DISCUSSION

HIV-1 Reverse Transcriptase Inhibition

The inhibitory activity of flavonoids isolated from *Eclipta alba* was assessed at various concentrations (10–200 µM) and compared with the reference drug, Efavirenz (10–200 µM). The results demonstrated a dose-dependent inhibition of HIV-1 RT, with the flavonoid fraction exhibiting a significantly higher inhibitory effect

at all tested concentrations compared to the reference drug.

At the lowest concentration (10 µM), the flavonoid fraction showed 23.4 ± 1.2% inhibition, slightly higher than Efavirenz (18.9 ± 1.0%). As the concentration increased, the inhibitory potential improved, reaching 89.7 ± 2.3% inhibition at 200 µM, whereas the reference drug exhibited 76.8 ± 2.1% inhibition at the same concentration.

Statistical analysis indicated that the flavonoid fraction's inhibition was significantly higher ($p < 0.05$) than the reference drug at all concentrations tested, suggesting enhanced efficacy in suppressing HIV-1 RT activity.

Dose-Dependent Inhibition Profile

A detailed comparison of flavonoid-mediated inhibition versus the reference drug is provided in Table 1 below:

Table 1: HIV-1 RT Inhibition by Flavonoids and Reference Drug

Concentration (µM)	Flavonoid Fraction (% Inhibition ± SD)	Reference Drug (% Inhibition ± SD)
10	23.4 ± 1.2	18.9 ± 1.0
25	38.7 ± 1.5	32.6 ± 1.3
50	56.2 ± 1.8	47.9 ± 1.6
100	72.5 ± 2.0	61.3 ± 1.9
200	89.7 ± 2.3	76.8 ± 2.1

The results clearly indicate that flavonoids from *Eclipta alba* exhibited superior inhibition of HIV-1 RT compared to the reference drug, demonstrating their potential as promising antiviral compounds.

The inhibitory activity of flavonoids from *Eclipta alba* against HIV-1 reverse transcriptase (RT) observed in this study aligns with previous reports demonstrating the antiviral potential of plant-derived polyphenols. Flavonoids are known to exhibit direct inhibitory effects on viral enzymes, particularly HIV-1 RT, through mechanisms such as enzyme binding and disruption of nucleotide incorporation (Wang et al., 2011). Earlier studies have highlighted the ability of flavonoids, including quercetin, apigenin, and luteolin, to hinder HIV replication by targeting essential viral proteins (Lopez-Garcia et al., 2013). Our results indicate that the flavonoid fraction exhibited significantly greater inhibition than the reference drug Efavirenz at all tested concentrations, suggesting a potential advantage in therapeutic applications. This enhanced efficacy may be attributed to synergistic interactions among flavonoid compounds, a phenomenon observed in polyphenolic extracts from medicinal plants (Yao et al., 2007). The dose-dependent inhibition profile further underscores the potency of *Eclipta alba* flavonoids and aligns with previous findings on natural RT inhibitors (Mukherjee et al., 2012).

The superior inhibition of HIV-1 RT by *Eclipta alba* flavonoids compared to Efavirenz raises interesting possibilities for antiviral drug development. Many flavonoids possess additional pharmacological properties, such as immunomodulation and antioxidant activity, which may contribute to their antiviral potential beyond direct enzymatic inhibition (Tsuchiya et al., 2009). Prior studies have demonstrated that plant-derived flavonoids can improve cellular defense mechanisms against viral infections by modulating cytokine responses and reducing oxidative stress (Chen et al., 2008). Additionally, the ability of flavonoids to bind viral enzymes and cellular receptors involved in HIV entry suggests a multi-targeted approach to antiviral intervention (Silva et al., 2010). Given the significant inhibition observed in our study, further research should focus on identifying the specific flavonoids responsible for this activity through bioassay-guided fractionation and computational docking studies. Moreover, structural optimization could enhance the bioavailability and stability of these compounds, making them viable candidates for novel antiretroviral therapeutics.

CONCLUSION

The findings of this study demonstrate that flavonoids extracted from *Eclipta alba* exhibit potent inhibitory activity against HIV-1 reverse transcriptase (RT), surpassing the efficacy of the reference drug Efavirenz. The dose-dependent inhibition observed suggests a strong therapeutic potential of these bioactive compounds, reinforcing previous reports on plant-derived flavonoids as antiviral agents. Given their promising efficacy, further studies should focus on elucidating their precise mechanism of action, optimizing their bioavailability, and evaluating their in vivo antiviral effects. Additionally, the multifaceted pharmacological properties of flavonoids, including immunomodulation and antioxidative benefits, may offer a broader advantage in HIV therapy beyond enzyme inhibition. These results pave the way for exploring *Eclipta alba* flavonoids as novel therapeutic candidates for HIV treatment, warranting comprehensive clinical investigations. Future research should integrate molecular docking, *in vivo* assays, and formulation strategies to enhance their applicability as potential alternatives or adjuncts to existing antiretroviral drugs.

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