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Research Article

In-vitro Antioxidant Potential of Linolenyl Alcohol Isolated from *Cayratia trifolia* L.

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ABSTRACT

Cayratia trifolia, a plant rich in bioactive compounds, has been known for its diverse pharmacological properties, including antioxidant activity. In this study, we focus on linolenyl alcohol, a compound isolated from *C. trifolia*, and investigate its antioxidant potential through *in-vitro* analysis. Linolenyl alcohol was extracted from *C. trifolia* leaves using standard extraction techniques and characterized using spectroscopic methods. The antioxidant activity of linolenyl alcohol was evaluated using well-established antioxidant assays. The results explored the significant antioxidant activity of linolenyl alcohol, with dose-dependent scavenging of free radicals observed in all assays. Overall, our findings highlight the antioxidant potential of linolenic alcohol from *C. trifolia*, suggesting its utility as a natural antioxidant agent in pharmaceutical and nutraceutical applications. Further investigations are warranted to explore its therapeutic benefits and potential clinical applications.

Introduction

Over the past few years, there has been a growing fascination with natural antioxidants drawn from botanical sources owing to their potential health benefits and reduced adverse effects in contrast to synthetic antioxidants.[1] Among these natural sources, *Cayratia trifolia*, a member of the Vitaceae family, has gained attention for its rich repertoire of bioactive compounds, including polyphenols, flavonoids, and terpenoids, which are known to possess antioxidant properties.[2] Linolenyl alcohol, a compound isolated from *C. trifolia*, has emerged as a promising candidate for further exploration due to its structural features and potential antioxidant activity.[3]

The escalating prevalence of oxidative stress-related diseases, including cardiovascular disorders, neurodegenerative diseases, and cancer, has spurred intense research into identifying novel antioxidant compounds capable of neutralizing harmful free radicals and mitigating oxidative damage.[4] Oxidative stress happens due to an imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant mechanisms, leading to damage at the cellular level, inflammation, and, ultimately, disease progression. The crucial function of antioxidants in preserving redox stability involves scavenging ROS and shielding against oxidative damage to biomolecules such as lipids, proteins, and DNA.[5]

Natural antioxidants derived from plants offer a sustainable and eco-friendly alternative to synthetic antioxidants commonly used in food, pharmaceutical, and cosmetic industries. These phytochemicals possess diverse chemical structures and mechanisms of action, making them attractive candidates for the development of antioxidant-based therapeutics and functional foods.[6] *C. trifolia*, commonly known as bush grape or fox grape, is a climbing vine found in tropical and subtropical regions,

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particularly in Asia and Australia.[7] Traditionally, various parts of the *C. trifolia* plant, including leaves, stems, and fruits, have been employed for their therapeutic benefits, which encompass anti-inflammatory, antimicrobial, and antioxidant effects.[8]

Linolenyl alcohol, a naturally occurring unsaturated alcohol found in *C. trifolia*, has drawn attention due to its potential antioxidant activity. However, despite its structural similarity to other known antioxidants, such as vitamin E and flavonoids, the antioxidant potential of linolenyl alcohol remains largely unexplored. Understanding the antioxidant properties of linolenyl alcohol could provide valuable insights into its mechanism of action and therapeutic potential in combating oxidative stress-related diseases.[9]

The current study seeks to explore the antioxidant capabilities of linolenyl alcohol isolated from *C. trifolia* extract through comprehensive *in-vitro* analysis. We hypothesize that linolenyl alcohol exhibits notable antioxidant efficacy demonstrated by its capacity to neutralize free radicals and prevent oxidative harm in various biochemical assays. To test this hypothesis, we employed a series of well-established antioxidant assays to evaluate the antioxidant capacity of linolenyl alcohol. Additionally, molecular docking studies were conducted to elucidate the potential mechanisms underlying the antioxidant action of linolenyl alcohol at the molecular level.

The outcomes of this investigation hold significant inferences in the context of developing natural antioxidant-based therapeutics and functional foods aimed at combating oxidative stress-related disorders. By elucidating the antioxidant potential of linolenyl alcohol from *C. trifolia,* this research adds to the expanding body of evidence endorsing the therapeutic efficacy of natural antioxidants in promoting human health and well-being. Moreover, the identification of linolenyl alcohol as a potent antioxidant compound may inspire further investigations into its pharmacological properties, including its efficacy in preclinical and clinical settings.

Materials And Methods

Isolation of Linolenyl Alcohol

In our previous studies, the natural bioactive compound linolenyl alcohol was extracted from *C. trifolia* using established extraction techniques. This purified linolenyl alcohol was utilized for subsequent *in-vitro* antioxidant assays.[10]

DPPH (2,2-diphenyl-1-picrylhydrazyl) Scavenging Activity

The DPPH scavenging activity of linolenyl alcohol was determined as per Blois's method (1958). A solution of 100 µM DPPH in ethanol was blended with varying concentrations (3.12–100 µg/mL) of Cmd^{-1} and incubated at ambient temperature for a duration of 30 minutes. Subsequently, the reduction measured in absorbance at 517 nm relative to an ethanol blank was gauged. Percentage reduction of the DPPH radical, indicative of Cmd^{-1}'s scavenging capacity, was computed. Each assay was carried out in triplicate.[11]

Nitric Oxide (NO) Scavenging Activity

Assessment of nitric oxide scavenging potential followed Garratt's protocol (1964). Sodium nitroprusside (SNP) served to generate nitric oxide, and the reaction mixture, comprising SNP, phosphate buffer, and various Cmd^{-1} concentrations, was incubated at ambient temperature. Post-incubation, the absorbance of the resulting chromophore was assessed at 540 nm. Triplicate analyses were performed for each test.[12]

Hydroxyl Radical Scavenging Potential

Evaluation of the hydroxyl radical scavenging potential of Cmd^{-1} was conducted per the method proposed by Elizabeth and Rao (1990), with minor adaptations. A reaction mixture containing 2-deoxy-2-ribose, KH2PO4-KOH buffer, FeCl3, EDTA, H2O2, ascorbic acid, and varying Cmd^{-1} concentrations was incubated at 37°C. Following incubation, the absorbance was measured at 532 nm using an appropriate blank. Reduced absorbance signified the hydroxyl scavenging activity of Cmd^{-1}. Each trial was performed in triplicate.[13]

Reducing Power Assay

Cmd^{-1}'s reducing power was determined following Oyaizu's technique (1986). Different Cmd^{-1} concentrations were mixed with phosphate buffer and potassium hexacyanoferrate and incubated at 50°C. Subsequent measurement of absorbance at 700 nm served as the gauge for measuring reducing power, with higher absorbance indicating greater potential. Triplicate analyses were conducted for each assay.[14]

FRAP Reducing Assay

The FRAP assay, per Chakraborthy *et al.*'s recommendation (2010), was employed. A FRAP reagent comprising TPTZ, HCl, FeCl3, and acetate buffer was mixed with varied Cmd^{-1} concentrations. After incubation, absorbance at 595 nm was measured, with the formation of a blue complex indicating a reduction of ferric ions to ferrous ions. All analyses were performed in triplicate.[15]

These assays collectively shed light on the antioxidant potential of linolenyl alcohol from *C. trifolia*, providing valuable insights for its potential applications in pharmacological and nutraceutical industries.

Results And Discussion

The investigation into the antioxidant potential of linolenyl alcohol (Cmd^{-1}) extracted from *C. trifolia* revealed

promising results across a spectrum of *in-vitro* assays. Through comprehensive analyses, we gained insights into the efficacy of Cmd^{-1} as a natural antioxidant compound.

In the DPPH scavenging activity assay, Cmd-1 displayed (Fig. 1) notable efficacy with IC_{50} values of 19.84 ± 0.23 µg/mL, comparable to the standard (09.56 ± 0.14 µg/mL). A dose-dependent reduction in absorbance at 517 nm was observed, indicating Cmd^{-1}'s ability to scavenge DPPH radicals. The percentage reduction of DPPH radicals increased with rising concentrations of Cmd^{-1}. These results highlight Cmd^{-1}'s robust free radical scavenging capacity, suggesting its potential in counteracting oxidative stress-induced damage.[16]

In the nitric oxide scavenging activity assay, Cmd^{-1} exhibited (Fig. 2) a notable IC_{50} value (28.63 ± 0.16 µg/mL), demonstrating significant efficacy when compared to the standard (22.57 ± 0.08 µg/mL). Cmd^{-1} exhibited significant scavenging activity against nitric oxide radicals induced by sodium nitroprusside. The decrease in absorbance at 540 nm indicated Cmd^{-1}'s inhibitory effect on nitric oxide production, suggesting its potential therapeutic application in conditions linked to oxidative stress.[17]

The hydroxyl radical scavenging activity assay provided additional insights into the antioxidant capabilities of Cmd^{-1} (Fig. 3), demonstrating dose-dependent scavenging of hydroxyl radicals. In this study, Cmd^{-1} demonstrated a noteworthy IC_{50} value of 20.37 ± 0.13 µg/mL, whereas the reference compound exhibited an IC_{50} value of 14.54 ± 0.06 µg/mL. The reduction in absorbance at 532 nm indicated Cmd-1's capability to efficiently counteract hydroxyl radicals, consequently shielding biomolecules from oxidative harm. This underscores the potential therapeutic application of Cmd^{-1} in combating oxidative stress-related conditions and highlights its efficacy as a natural antioxidant agent.[18]

Additionally, the reducing power assay revealed the remarkable reducing capability of Cmd^{-1}, as evidenced by the increasing absorbance at 700 nm with rising concentrations of Cmd^{-1}. At a concentration of 100 µg/mL, the Cmd^{-1} exhibited the highest absorbance values at 0.84 ± 0.01, while the reference drug showed absorbance values of 0.86 ± 0.03, as depicted in Fig. 4. This capacity to donate electrons and reduce ferric ions underscores Cmd^{-1}'s antioxidant effects, further supporting its potential therapeutic utility in combating oxidative stress-related conditions. These results highlight the promising role of Cmd^{-1} as a natural antioxidant agent and suggest its potential applications in preventive and therapeutic interventions against oxidative damage.[19]

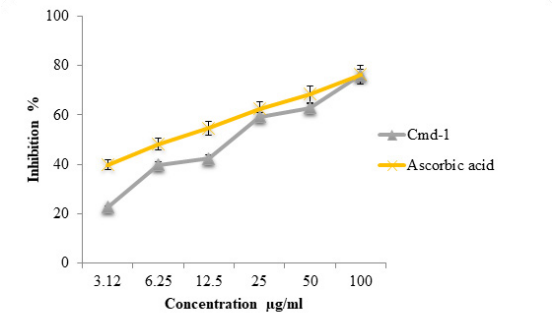

Fig. 1: DPPH scavenging activity assay

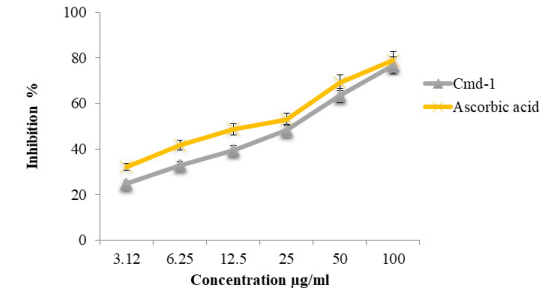

Fig. 2: Nitric oxide scavenging activity assay

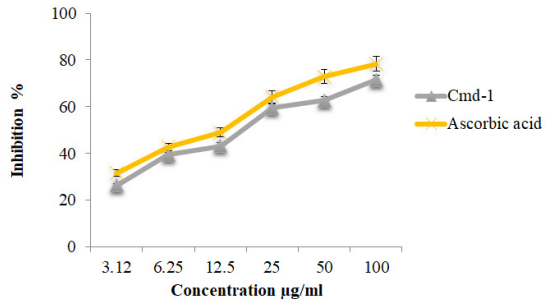

Fig. 3: Hydroxyl radical scavenging activity assay

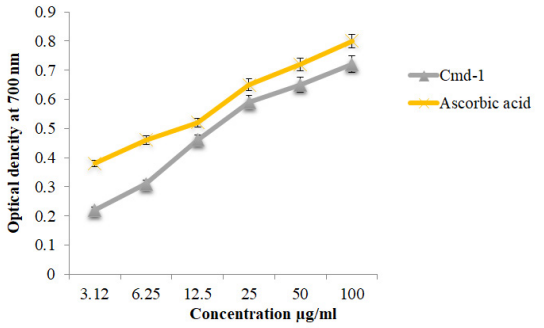

Figure 4: Reducing power assay

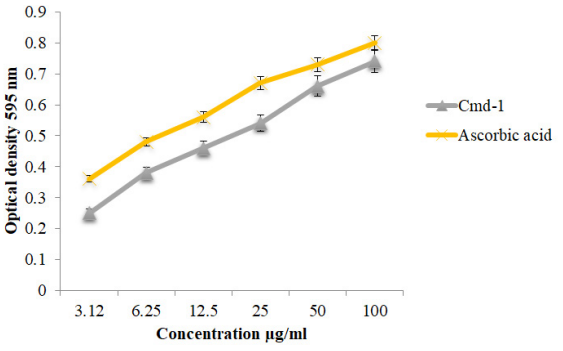

Figure 5: FRAB assay

Finwally, the FRAP assay affirmed the antioxidant potential of Cmd^{-1} by demonstrating its capability to reduce ferric ions to ferrous ions, as indicated by the increased absorbance at 595 nm. Cmd^{-1} displayed the maximum absorbance of 0.81 ± 0.01, whereas ascorbic acid demonstrated 0.85 ± 0.03 at a concentration of 100 µg/mL, as illustrated in Fig. 5. These results are consistent with the findings of other assays, collectively reaffirming the potent antioxidant activity of Cmd^{-1}. This underscores the efficacy of Cmd^{-1} as a natural antioxidant compound and supports its possible applications in battling oxidative stress-related disorders. The alignment of results across different assays underscores the trustworthiness of the results and further highlights the promising therapeutic potential of Cmd^{-1} in oxidative stress management.[20]

The findings of this study underscore the substantial antioxidant potential of linolenyl alcohol extracted from *C. trifolia*. Cmd^{-1} demonstrated robust free radical scavenging activity, inhibition of nitric oxide production, and effective reduction of ferric ions, indicative of its promising therapeutic applications in combating oxidative stress-related diseases. Further elucidation of the underlying mechanisms of Cmd^{-1}'s antioxidant action and exploration of its potential pharmacological and nutraceutical applications are warranted, paving the way for the development of novel antioxidant-based therapies.

Conclusion

In conclusion, this study highlights the remarkable antioxidant potential of linolenyl alcohol (Cmd-1) from *C. trifolia*. Through a range of assays, Cmd-1 exhibited strong scavenging abilities against various free radicals and effectively inhibited nitric oxide production. Its potent reducing power further underscores its antioxidant efficacy. These findings suggest Cmd-1 is a promising natural antioxidant for mitigating oxidative stress-related damage and preventing associated diseases. Its potential applications in pharmacology and nutraceutical industries are evident, though additional investigation is warranted to elucidate its mechanisms and confirm safety. This study adds to the increasing body of evidence supporting the therapeutic utility of natural antioxidants, with Cmd-1 showing promise for preventive healthcare and therapeutic interventions against oxidative stress-related disorders.

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