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Research Article

Antioxidant Activity of Isolated Bioactive Compound from *Mollugo nudicaulis*

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ABSTRACT

Mollugo nudicaulis (*M. nudicaulis*) is a medicinal plant with a long history of use in traditional medicine. In the present study focused on exploring the *in-vitro* antioxidant potential of 12-(10-carboxydecanoyloxy)-12-oxo-dodecanoic acid (compound-1) isolated and characterized as a specific natural compound from the aerial parts of *M. nudicaulis*. The results of these *in-vitro* antioxidant assays were promising. Compound-1 demonstrated significant antioxidant activity when compared to the standard drug across all tests, effectively scavenging free radicals. These findings suggest that compound-1 from *M. nudicaulis* has considerable potential as a natural antioxidant. Its effectiveness indicates that it could be valuable in various applications within the pharmaceutical and food industries. The natural antioxidant properties of compound-1 could contribute to the development of new therapies and preservation methods, providing a natural alternative to synthetic antioxidants. However, to fully understand the potential of compound-1, further studies are necessary. These future investigations should aim to elucidate the precise mechanisms by which compound-1 exerts its antioxidant effects and explore its broader therapeutic applications. Overall, this study highlights the promising pharmacological potential of *M. nudicaulis*, particularly in the realm of natural antioxidants.

Introduction

Oxidative stress arises when the body's Reactive oxygen species (ROS) production is out of balance with its antioxidant defense mechanisms. ROS reactively responds to external triggers such as UV radiation, pollution, and poisons.[1] While ROS plays crucial roles in cellular signaling and pathogen defense, excessive levels could lead to oxidative damage in biomolecules, resulting in cellular malfunction and the development of numerous illnesses.[2] The role of antioxidants is crucial in neutralizing or impeding the formation of ROS, which is vital for maintaining cellular homeostasis. A variety of mechanisms are involved.[3] These compounds, crucial for cellular health, can be classified into endogenous, synthesized within the body, and exogenous, sourced from dietary intake or supplementation. Both enzymatic and non-enzymatic antioxidants work synergistically to uphold redox balance and shield cells from oxidative stress.[4] This intricate antioxidant network acts as a frontline defense, safeguarding cellular components from damage induced by ROS, thereby preserving overall physiological function and contributing to longevity and well-being.[5] Natural antioxidants have various advantages over synthetic counterparts, including increased bioavailability, reduced toxicity, and potential synergistic effects with other bioactive chemicals found in whole foods.[6] Plant-based antioxidants have been intensively researched for their health benefits and potential chronic diseases.[7]

Flavonoids, phenolic acids, carotenoids, and tocopherols are among the major classes of phytochemicals known for their antioxidant activity. These compounds scavenge free radicals, inhibit lipid peroxidation, and modulate

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cellular signaling pathways involved in oxidative stress responses. Furthermore, several fatty acids and derivatives have been shown to have antioxidant characteristics, however, their mechanisms of action may differ from typical antioxidants.[8]

Mollugo nudicaulis is an herbaceous plant that has an extensive historical background of therapeutic usage in traditional systems of medicine. It is a part of the Molluginaceae family. It's found widely in tropical and subtropical regions, where it often grows as a weed in agricultural fields, wastelands, and disturbed habitats. Various parts of *M. nudicaulis*, including the leaves, stems, and roots, have been employed in traditional folk remedies for the treatment of diverse ailments.[9]

Phytochemical studies on *M. nudicaulis* have revealed the presence of numerous bioactive constituents with pharmacological potential. These include alkaloids, flavonoids, terpenoids, saponins, and phenolic compounds, many of which exhibit antioxidant, anti-inflammatory, antimicrobial, and antiproliferative activities. The bioactive compound of 12-(10-carboxydecanoyloxy)-12-oxododecanoic acid (Fig. 1) was isolated and identified from ethanolic extract of *M. nudicaulis.* [10] The chemical diversity of *M. nudicaulis* underscores its importance as a potential source of novel therapeutic agents for drug discovery and development.[11]

However, to date, there have been no reports on the antioxidant activity of compound-1. Thus, the main aim of this research is to assess the antioxidant potential compound-1 derived from *M. nudicaulis*.

Materials and Methods

Compound Isolation

In our previous studies, the compound-1 was extracted from *M. nudicaulis* using established extraction techniques. This purified compound was utilized for subsequent *in-vitro* analysis.[12]

DPPH radical assay

Compound-1 DPPH radical activity was evaluated through Blois' technique (1958). Compound-1 concentrations (6.25–200 µg/mL) were combined with a methanolic DPPH radical solution. Following a 30-minute incubation period in a light-protected environment, the mixture was subjected to spectrophotometric analysis in a specific absorption of 517 nm utilizing a UV spectrophotometer. As a standard antioxidant reference, ascorbic acid was employed. The scavenging efficacy was quantified as the percentage suppression of DPPH radical, reflecting the ability of a tested compound to neutralize free radicals and thereby evaluate its potential antioxidant activity.[13]

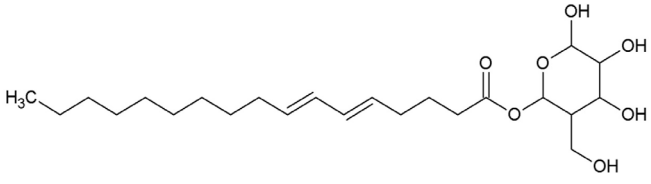

Fig. 1: The molecular structure of compound-1

Nitric oxide scavenging assay

Compound-1 was evaluated for its ability to scavenge nitric oxide following the protocol described by Garratt (1964). Sodium nitroprusside dissolved in phosphate-buffered saline (PBS) was employed to generate nitric oxide, which reacts with Griess reagent to form chromophores. Compound-1 concentrations (6.25–200 µg/mL) were tested with sodium nitroprusside solution, and chromophore absorbance was determined at 546 nm. A %suppression of nitric oxide generation was used to calculate scavenging activity.[14]

Hydroxyl scavenging assay

The assessment of compound-1 hydroxyl scavenging activity followed the methodology outlined by Elizabeth and Rao (1990). This method relies on the ability of the test substance to compete with deoxyribose for hydroxyl radicals generated via the Fenton reaction. A reaction mixture comprising compound-1 (ranging from 6.25–200 µg/mL), $FeSO_4$, hydrogen peroxide, deoxyribose, and ascorbic acid underwent 37°C of incubation for 1-hour. The degree of deoxyribose degradation was quantified spectrophotometrically at 532 nm. By measuring the suppression of hydroxyl radicals, the scavenging activity was quantified and it provided insights into the compound's potential as an antioxidant.[15]

Reducing power assay

The determination of compound-1 reducing power adhered to the protocol elucidated by Oyaizu (1986). This method hinges on the compound's capability to convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}), quantifiable via spectrophotometry. After combining phosphate buffer with potassium ferricyanide, the solution with diverse concentrations of compound-1 (ranging from 6.25–200 µg/mL) was raised at 50°C for 20 minutes. The subsequent introduction of trichloroacetic acid and ferric chloride facilitated the reaction, with absorbance measured at 700 nm. Enhanced absorbance readings correlate with heightened reducing power, providing insights into the compound's potential to counteract oxidative stress through electron donation mechanisms.[16]

FRAP reducing assay

The assessment of compound-1 FRAP adhered to the methodology proposed by Chakraborty *et al*. (2010). The FRAP assay gauges compound-1 ability to transform the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex into its ferrous state under acidic conditions. Various concentrations of compound-1 (ranging from 6.25–200 µg/mL) were blended with the FRAP reagent and subjected to

a 30 minutes gestation in 37°C. Following incubation, a measurement of absorbance was made at 593 nm. FRAP values were deduced utilizing a $FeSO_4$ standard curve and expressed as µM Fe(II) equivalents, delineating the compound's capacity to mitigate oxidative stress through ferric ion reduction.[17]

Together, these assays illuminate the antioxidant potential of compound-1 derived from *M. nudicaulis*, offering valuable insights into its possible uses in the pharmaceutical and nutraceutical sectors.

Statistical Analysis

Three independent replicates were used to calculate the mean and standard deviation. To discern disparities among multiple groups, ANOVA was executed. Statistical significance was attributed to *p-values* below 0.05, ensuring robust analysis and interpretation of the experimental findings. This stringent analytical approach enabled a comprehensive assessment of the experimental data, facilitating the identification of significant variations among treatment groups and robust conclusions regarding the efficacy of compound-1 as an antioxidant.[18]

Results and Discussion

By examining its performance across various antioxidant mechanisms, researchers gained valuable insights into the potential therapeutic applications of compound-1. These *in-vitro* antioxidant assays serve as essential tools for elucidating the compound's antioxidant potential and pave the way for further exploration in pharmaceutical and biomedical research for combating oxidative damage-related conditions.

DPPH Scavenging Assay

The compound-1 DPPH scavenging activity was determined by measuring its ability to neutralize the stable DPPH radical, which is a commonly used method for assessing the free radical scavenging activity of the sample.[19] A concentration-dependent increase in DPPH scavenging activity was observed, with higher concentrations demonstrating greater activity. The IC_{50} value of compound-1 was 29.45 ± 0.15 µg/mL, which is illustrated in Fig. 2.

Nitric Oxide Scavenging Assay

Compound-1 nitric oxide scavenging ability was assessed using the Griess reagent method, which assesses the suppression of nitric oxide generation by sodium nitroprusside. There are many free radicals in nature, including nitric oxide, involved in various physiological and pathological processes, and its overproduction can lead to oxidative stress and inflammation.[20] The results demonstrated a dose-dependent inhibition of nitric oxide production by the compound-1, with higher concentrations showing greater inhibition. Compound-1 has a strong nitric oxide scavenging effect (Fig. 3) compared to the conventional antioxidant, quercetin (IC_{50} = 31.8 ± 0.1 µg/mL).

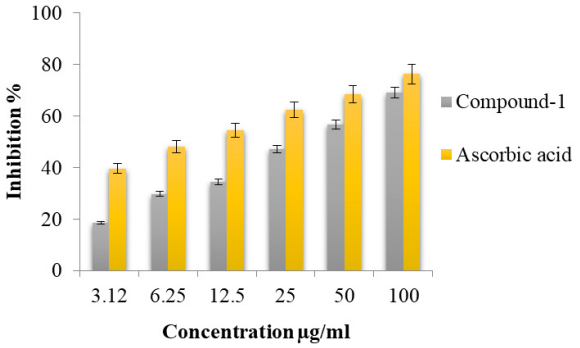

Fig. 2: DPPH scavenging potential of compound-1 compared with standard drug (ascorbic acid)

Hydroxyl Scavenging Assay

Compound-1 hydroxyl scavenging activity was assessed by assessing its capacity to block deoxyribose breakdown by hydroxyl radicals produced during the Fenton reaction. Hydroxyl radicals are extremely reactive and can cause oxidative damage to biomolecules like DNA, proteins, and lipids.[6] The results revealed a dose-dependent inhibition of deoxyribose degradation by the compound-1, indicating its potent hydroxyl scavenging activity. The IC_{50} value of compound-1 was determined to be 22.3 ± 0.05 µg/mL, suggesting its efficacy in scavenging hydroxyl radicals and protecting against oxidative damage (Fig. 4).

Reducing Power Assay

In order to determine the electron-donating capacity of compound-1, ferric ions (Fe^{3+}) were reduced to ferrous ions (Fe^{2+}), indicating its reducing power.[4] The results revealed a concentration-dependent increase in reducing power, with higher concentrations of the compound-1 demonstrating a stronger reducing power of 0.86 ± 0.12 (the absorbance values) at 700 nm were significantly higher when compared to the control, indicating its potent reducing capacity (Fig. 5).

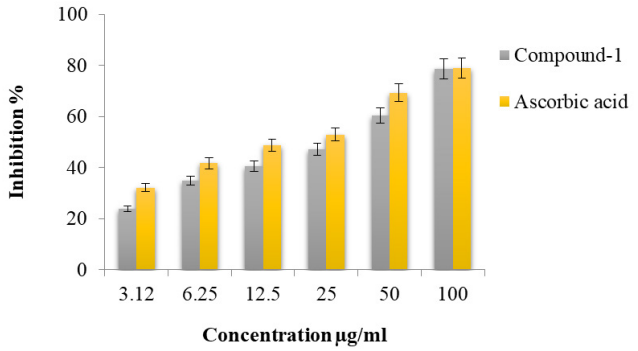

Fig. 3: Nitric oxide scavenging activity of compound-1 compared with standard drug (ascorbic acid)

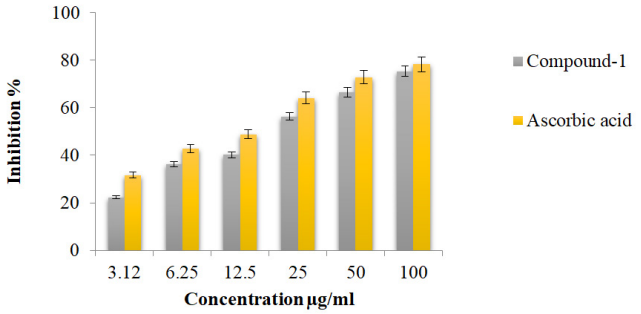

Fig. 4: Hydroxyl radical scavenging activity of compound-1 compared with standard drug (ascorbic acid)

FRAP Reducing Assay

FRAP assays measure antioxidant effectiveness by developing a blue ferrous complex with maximum absorbance at 593 nm after antioxidants convert ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous. The results demonstrated a concentration-dependent increase in FRAP values, indicating the reducing power of compound-1 (0.79 ± 0.2). The FRAP values were significantly higher for compound-1 compared to the control, suggesting its potent antioxidant activity (Fig. 6).

The compound-1 exhibited significant antioxidant activity as assessed by a series of *in-vitro* assays. It showed strong DPPH scavenging activity, with a concentration-dependent rise and an IC_{50} value comparable to that of ascorbic acid, the standard antioxidant. Furthermore, compound-1 demonstrated strong nitric oxide scavenging action, reducing nitric oxide generation in a concentration-dependent manner, outperforming the conventional antioxidant, quercetin. Additionally, compound-1 exhibited substantial hydroxyl scavenging activity, effectively protecting against oxidative damage to biomolecules. Its strong reducing power was evidenced by a concentration-dependent increase, indicating its ability to donate electrons and neutralize free radicals.[21] Furthermore, compound-1 demonstrated notable ferric-reducing antioxidant power (FRAP), indicative of its potent

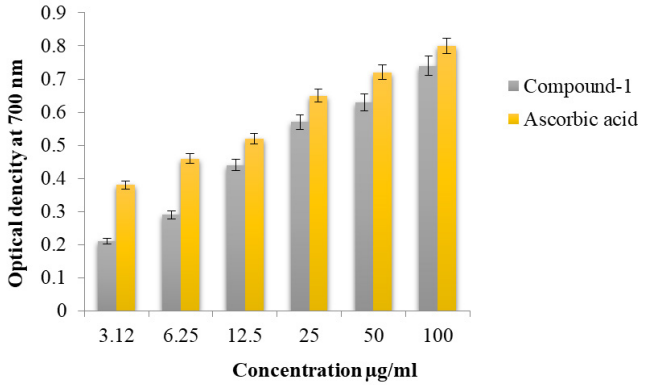

Fig. 5: Reducing power potential of compound-1 compared with standard drug (ascorbic acid)

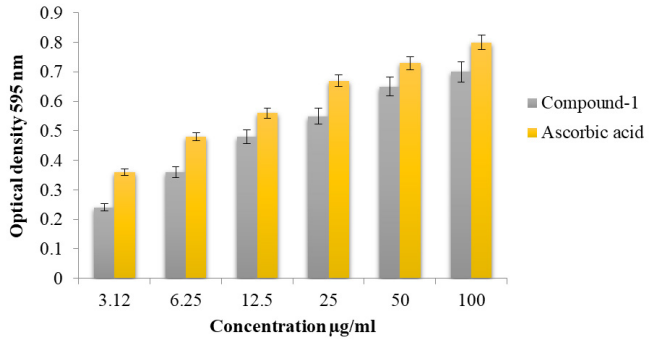

Fig. 6: FRAB potential of compound-1 compared with standard drug (ascorbic acid)

antioxidant activity. These findings highlight the potential of compound-1 as a natural antioxidant and warrant further investigation into its therapeutic applications in oxidative stress-related disorders.

The antioxidant mechanisms attributed to compound-1 likely entail intricate modulation of cellular signaling pathways associated with oxidative stress and inflammation. A significant pathway implicated in its antioxidant effects is the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling cascade.[22] By modulating the Nrf2 pathway and subsequent upregulation of antioxidant enzymes, compound-1 exerts multifaceted antioxidant effects, safeguarding cells from oxidative damage and promoting overall cellular homeostasis. This mechanistic insight underscores the potential therapeutic utility of compound-1 in combating oxidative stress-related disorders and underscores its significance in pharmacological research aimed at developing novel antioxidant interventions.

Overall, the multifaceted antioxidant mechanisms of compound-1, encompassing free radical scavenging, metal chelation, modulation of redox-sensitive transcription factors, enhancement of endogenous antioxidant defenses, anti-inflammatory activity, and hormetic responses, underscore its potential therapeutic applications in oxidative stress-related disorders.

Conclusion

In this study, compound-1 showed considerable antioxidant activity in a variety of *in-vitro* antioxidant activity analyses. It exhibited concentration-dependent DPPH scavenging activity comparable to ascorbic acid. Furthermore, compound-1 showed potent nitric oxide scavenging activity, surpassing quercetin, and effectively inhibited hydroxyl radicals, indicating its ability to protect against oxidative damage. Moreover, compound-1 displayed strong reducing power and notable FRAP, highlighting its potent antioxidant activity. These findings underscore the potential of compound-1 as a natural antioxidant and support its further exploration for therapeutic applications in oxidative stress-related disorders. Further elucidation of

its mechanisms of action and *in-vivo* efficacy is warranted to fully understand and harness its therapeutic potential.

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