RESEARCH ARTICLE

Comparative Assessment of the Antioxidant Potential of Different Parts of *Delonix regia*

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ABSTRACT

The flamboyant tree, or *Delonix regia*, has attracted a lot of interest due to the widespread belief that it can be used as a powerful antioxidant. The search for natural antioxidants has taken on paramount relevance as the world faces increasing health problems attributed to oxidative stress. *D. regia*, a native of Madagascar, can be found all across the tropics and subtropics and is highly regarded for its medicinal value and beautiful red flowers. The complex phytochemical composition of *D. regia* includes a number of bioactive compounds that might prevent oxidative damage to biomolecules. Flavonoids, tannins, phenolic acids, carotenes, and tocopherols are all examples of these substances. The antioxidant action of different parts of *D. regia* is comprehensively examined and clarified in this work, from its broad scope in direct radical scavenging to its fine-tuning of endogenous antioxidant enzymes. The antioxidant capabilities of *D. regia* have been studied for their potential to address a broad spectrum of illnesses and health conditions, such as cancer, diabetes, cardiovascular disease, neurological disorders, and a number of skin issues. The importance of utilizing nature's bounty in the hunt for novel remedies to oxidative stress-related illnesses is underscored by this study's addition to the growing body of knowledge concerning the antioxidant effects of *D. regia*. Further research is recommended to explore the pharmacological properties of *D. regia* to fully harness its medicinal potential for the advancement of human health and well-being.

Keywords: *Delonix regia*, Antioxidants.

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INTRODUCTION

The demand for natural remedies that might reduce the health hazards caused by excessive oxidative stress is greater than ever as urbanization, industrialization, and the usage of synthetic drugs continue to rise. Oxidative stress is linked to the development of numerous serious illnesses.[1] This includes everything from cancer to cardiovascular disease to neurological disorders. The underlying reason for this stress stems from an equilibrium in producing reactive oxygen species (ROS) and the body's antioxidant defense mechanisms.[2] This has led to a renewed focus in mining the plant kingdom for potent antioxidants; this area has long been underappreciated despite its reputation as a veritable gold mine for bioactive chemicals. The flamboyant tree, or *Delonix regia*, has caught the attention of scientists and herbalists for its unique combination of ecological significance and traditional medicinal benefits. The beautiful red blossoms of *D. regia*, a plant native to Madagascar that has since spread throughout the tropics and subtropics, have made the species famous.[3] This tree's long history of use in alternative medicine practices offers promising evidence that it may have medical benefits beyond its pleasing appearance. Science has just lately uncovered the pharmacological potential of *D. regia*, particularly as an antioxidant agent. *D. regia* is a gold mine of phytochemicals; many of them have been demonstrated to have potent antioxidant effects.[4] Phenolic substances include but are not limited to, flavonoids, tannins, phenolic acids, carotenoids, and tocopherols. To prevent oxidative damage to lipids, proteins, and DNA, these compounds can neutralize reactive oxygen species (ROS).[5,6] Moreover, apart from directly counteracting free radicals, *D. regia* enhances the body's inherent defence mechanisms against oxidative stress by augmenting the synthesis of internal antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).[7,8] *D. regia* has significant medicinal promise, but this can only be realized if we can better understand the processes underlying its antioxidant properties. *D. regia* contains phytochemicals that have been found to chelate transition

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metals implicated in ROS formation, prevent the onset of oxidative processes, and disrupt chain reactions. In addition, they have anti-inflammatory effects that are linked to oxidative stress. *D. regia* chemicals lead to a lower oxidative burden on cells and tissues via regulating inflammatory pathways.[9,10] The antioxidant properties of *D. regia* are diverse and show promise for various medical uses besides its traditional therapeutic applications.[11,12] Additionally, there is evidence that bioactive chemicals and extracts from *D. regia* inhibit cancer cell growth and boost the efficiency of standard cancer treatments.[13] This study explores the lush understory of *D. regia* and delves into the fascinating field of phytochemistry to decipher the plant's antioxidant benefits along with a comparative assessment of its different parts. It will shed light on the mechanisms of action and potential applications of the bioactive chemicals discovered in *D. regia* for treating diseases caused by oxidative stress. We hope that our investigation will lead to new discoveries about the medicinal potential of *D. regia* and encourage other studies that strive to use nature's gifts to improve human health.

MATERIALS AND METHODS

Assay of Reducing Power

Concentrations of sample (20–320 g/mL), buffer (0.1 M sodium phosphate, pH 6.6), and a reducing agent, i.e., potassium ferricyanide solution 1% w/v ($K_3Fe(CN)_6$ were all added to make a mixture of 2.5 mL and subjected to incubation at 50°C for 20 minutes to assess the reducing power. Centrifuged at 5000 rpm for 10 minutes, the blend underwent subsequent treatment with 2.5 mL of trichloroacetic acid (10% w/v). The absorbance was measured at 700 nm after mixing the upper layer (4 mL) with 0.4 mL of sterile 0.1% w/v of ferric chloride ($FeCl_{3)}$. The IC_{50} value was determined relative to ascorbic acid.[14]

Assay of H_2O_2 Scavenging Activity

Extract concentrations between 20 and 320 g/mL were added to 0.1 M phosphate buffer (adjusted to a pH of 7.4) & 0.6 mL of 40 mM H_2O_2 solution in a 25 mL tube, agitated vigorously, and subjected to incubation for 10 minutes. The absorbance of the reaction mixture was determined at 230 nm. The extracts represented a standard of excellence. The method below was used to calculate the scavenging efficiency against H_2O_2.

$$\text{Scavenging outcome \%} = [1- (A_1-A_2)/A_0] *100$$

Absorbance is denoted by the letters A0, A1, and A2, where A0 refers to the absorbance of water and A1 and A2 refers to the absorbance of sample with H_2O_2 and sample alone (phosphate buffer instead of H_2O_2 solution). The concentration of compounds that inhibited H_2O_2 generation by 50% was determined to be the IC_{50} value.[15]

Activity of Scavenging Nitric Oxide Radicals

Scavenging activity against nitric oxide (NO) was evaluated using the Griess reagent. To a volume of 3.0 mL, various extract concentrations (20–320 g/mL) were added, and the resulting mixture was incubated at 25°C for 150 minutes. Step one involved making a solution of sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS). The samples underwent processing using 5 mL of Griess reagent, composed of 1% sulphanilamide, 2% H_3PO_4, and 0.1% naphthalene-diamine dihydrochloride. The absorbance was measured at 546 nm and compared to that of ascorbic acid standard solutions that had been treated with Griess reagent similarly.

The calculation of inhibition percentage was established utilizing the subsequent formula:

$$\text{\% inhibition} = [(A_0-A_T)/A_0 \text{ x}100]$$

where A_T denotes absorbance after adding the extract and A0 denotes absorbance of the blank (extract-free) control. The experiment was conducted in triplicate, and the average values were graphed.[16,17]

Antioxidant Capacity (TAOC) Assay

The TAC was analysed with a tweaked version of a technique called the phosphomolybdate method. The first step was to combine a 0.3 mL sample with 3 mL of phosphomolybdate and heat the mixture at 95°C for 10 minutes. After that, the absorbance was measured at 695 nm. Afterward, the ascorbic acid calibration curve (10–320 g/mL) was used to establish the TAC in terms of mg AAE/g raw sample weight.[17]

Determination of DPPH Radical-Scavenging Capacity

The extract solutions in water (20–320 g/mL) were prepared, and a 0.1 mM DPPH solution was prepared in ethanol before being added to 1.0 mL of the extract solution in water. The absorbance measurements at 517 nm were calibrated after 30 minutes using ascorbic acid. The lower the absorbance value, the greater the capacity to scavenge free radicals. The formula below was used to calculate the free radical scavenging activity of a sample based on the percentage of free radicals it blocked

$$\text{\% inhibition}= [(A_0-A_t)/A_0 \text{ x } 100]$$

where A0 refer to blank absorbance (without extract) and At stands for the absorbance when the extract is present. The trials were conducted three times, and the graph was generated using the mean outcomes.[18]

Estimation of Total Phenolic Compounds

The Folin-Ciocalteu reagent was used in this method. The procedure called for a volumetric flask containing 1-mg/mL of extract solution. The final reaction mixture was obtained by combining 0.5 mL of plant extracts solution, 2.5 mL of Folin-Ciocalteu reagent diluted in 10% water, and 2.5 mL of $NaHCO_3$ aqueous solution at 7.5% concentration. The samples were kept at 45°C for 45 minutes. At 760 nm, we found that the blue color was most absorbent. Total phenol content was reported per mg of dry extract and calculated as gallic acid equivalents (mg/mg) (GAE). Triangulated results were used for all calculations.[19]

RESULTS AND DISCUSSION

Assay of Reducing Power

Extracts of flowers, leaves and stem were tested for their ability to inhibit free radical formation at doses ranging from 20 to 320 g/mL. Concentration was observed to boost the reducing power of DRFE, DRLE, and DRSE extracts. Extracts showed the greatest reducing power at 320 g/mL. Percent inhibition at 320 g/mL was higher for DRFE (1.93%) followed by DRLE (1.83%) and DRSE (1.74%) while at the same concentration percent inhibition for ascorbic acid was 2.03%. IC_{50} was found to be 32.61 µg/mL with DRFE, 29.17 with DRLE and 27.0 µg/mL with DRSE extract, it was 18.3 µg/mL for ascorbic acid (standard) (Figure 1).

Hydrogen Peroxide-Scavenging Activity

Maximum concentrations of DRFE, DRLE and DRSE extracts were shown to inhibit hydroxyl radicals by 64.09, and 62.98% and DRSE 62.37%, respectively. In contrast, ascorbic acid showed 77.71% inhibition. Increasing the concentration of the extracts enhanced their ability to scavenge these radicals. The IC_{50} value of DRFE, DRLE and DRSE extracts was found to be 209.49, 213.16, and 220.47 µg/mL. IC_{50} value of ascorbic acid was determined to be 150.02 µg/mL (Figure 2).

Activity of Scavenging Nitric Oxide Radicals

Griess reagent was used to test for nitric oxide scavenging activity. After preparing 5 mM of sodium nitroprusside in phosphate buffer saline (PBS), we mixed it with 3.0 mL of extract at concentrations ranging from 20 to 320 g/mL, and then the solution was subjected to incubation at 25°C for 150 minutes. At maximum concentration, percent inhibition was recorded as DRFE (65.59%), DRLE (64.21%), and DRSE (63.32%), whereas ascorbic acid (75.56%). IC_{50} value was found to be DRFE 212.97 µg/mL, DRLE 217.36 µg/mL, and for DRSE 219.93 µg/mL while that of ascorbic acid 156.84 µg/mL (Figure 3).

Total Antioxidant Capacity (TAOC) Assay

DRFE, DRLE, and DRSE all showed potential antioxidant efficacy compared to ascorbic acid, which is 148.23, 143.47 and 136.33 mg/AAE (Ascorbic acid equivalent), respectively (Figure 4).

DPPH free Radical-scavenging Capacity

DPPH assay showed that all the extracts were effective in neutralizing free radicals. The results showed that ascorbic acid had a strong inhibitory impact at concentrations ranging from 10 to 320 g/mL. Maximum inhibition effects were observed highest in *D. regia* flower extract (DRFE) as 73.50% followed by *D. regia* leaf extract (DRLE) as 70.52% and *D. regia* stem extract (DRSE) as 66.66%. The IC_{50} values of DRFE, DRLE and DRSE extracts were found to be 173.87, 184.47 and 197.34 µg/mL, respectively, while for ascorbic acid, it was found to be 123.12 µg/mL. The plant extracts had strong DPPH scavenging activity, with the former being on par with the gold standard ascorbic acid (Figure 5).

Total Phenolic Content

The total phenol content of DRFE, DRLE and DRSE extracts was found to be 530.5, 516.50 and 506.5 mg/Gallic acid equivalent (GAE), respectively (Figure 6).

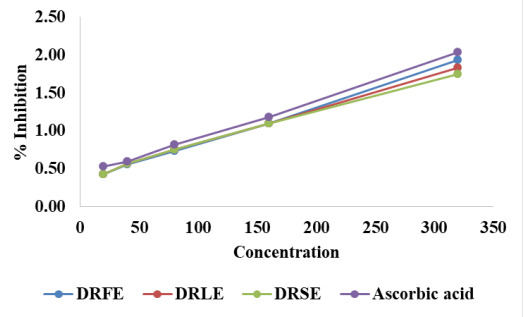

Figure 1: Reducing power activity

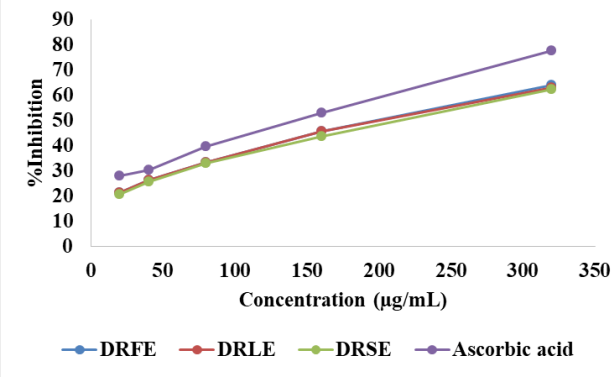

Figure 2: Hydrogen peroxide-scavenging activity

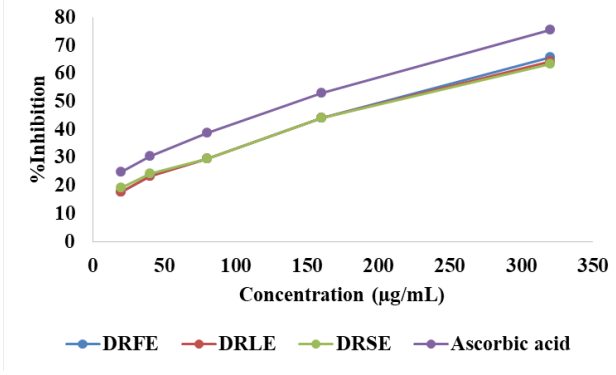

Figure 3: Nitric oxide radical scavenging activity

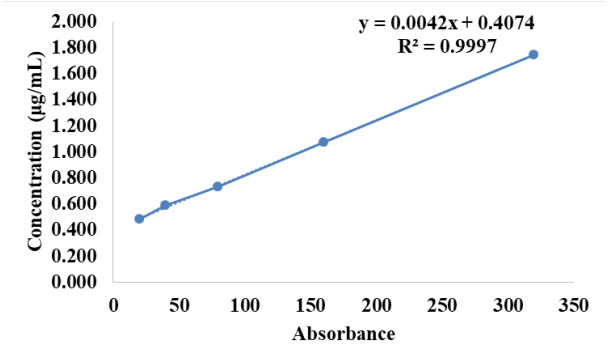

Figure 4: Total antioxidant capacity

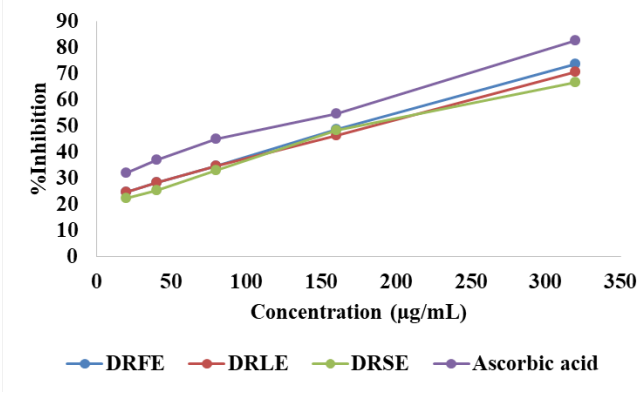

Figure 5: DPPH free radical-scavenging capacity

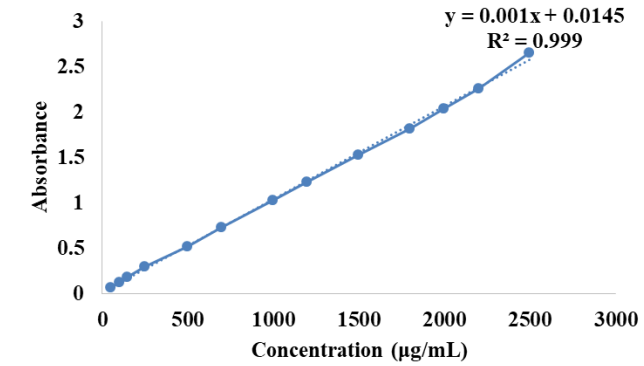

Figure 6: Total phenolic content

CONCLUSION

In conclusion, the flamboyant tree (*D. regia*) emerges as a potentially useful natural source of antioxidants with the potential to battle health concerns associated to oxidative stress. The red blossoms and lengthy history of medicinal use of this stunning plant are only the beginning; it has been discovered to have a plethora of bioactive compounds that, when combined, give it potent antioxidant qualities. To further its anti-inflammatory and antioxidant properties, *D. regia* does more than only neutralize free radicals; it also promotes the body's own synthesis of antioxidant enzymes. *D. regia's* versatility makes it a promising candidate for a multitude of medical applications. Animal studies on *D. regia* have shown encouraging results for a variety of medical uses, including the prevention of diabetes and CV diseases, the treatment of neurodegenerative illness, and the improvement of skin health. Because of its potential medical benefits beyond its conventional usage, further investigation of *D. regia's* pharmacological characteristics is necessary. At a time when there is a growing interest in natural medicines and sustainable healthcare solutions, *D. regia* shines as a light of hope, urging us to find the unfulfilled potential in our natural surroundings. More research into this remarkable plant is required to unlock new treatments for diseases caused by oxidative stress. Finally, the antioxidant activities of *D. regia* are a remarkable example of the intricate relationship between natural settings and human health. This research not only strengthens our belief in *D. regia's* therapeutic potential but also stresses the need to protect and responsibly utilize the planet's many natural resources for the benefit of future generations.

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