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ASSESSMENT OF ANTIOXIDANT ACTIVITY IN THE SEED EXTRACT OF *DECALEPIS HAMILTONII*

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ABSTRACT

This research paper investigates the antioxidant potential of *Decalepis hamiltonii* seed extract, aiming to contribute valuable insights into the therapeutic applications of this plant species. Antioxidants are crucial in combating oxidative stress, a known contributor to various health disorders. *Decalepis hamiltonii*, commonly known as Hamilton's Tamarind, has garnered attention for its medicinal properties. The study involves the extraction of bioactive compounds from *D. hamiltonii* seeds and the subsequent assessment of their antioxidant activity using established methodologies. The research employs assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization, and FRAP (ferric reducing antioxidant power) to quantify the antioxidant capacity. Additionally, the study explores the identification and characterization of specific antioxidants within the extract using advanced analytical techniques. The findings of this research aim to provide a scientific basis for the potential utilization of *D. hamiltonii* seed extract in nutraceuticals, functional foods, or pharmaceutical formulations with antioxidant properties.

Keywords: *Decalepis hamiltonii*, antioxidant activity, bioactive compounds, DPPH, ABTS, FRAP, medicinal plants

INTRODUCTION

In the relentless pursuit of innovative and sustainable approaches to healthcare, the exploration of natural sources with therapeutic potential has gained considerable momentum **[1]**. Among the myriad of botanical treasures, *Decalepis hamiltonii*, a plant indigenous to the Indian subcontinent, has emerged as a subject of scientific interest for its diverse pharmacological properties. Notably, the seeds of *D. hamiltonii*, commonly referred to as Hamilton's Tamarind, have been deeply ingrained in traditional medicinal practices. These seeds, with a rich history of indigenous use, have been recognized for their purported health benefits, prompting a contemporary scientific investigation into their bioactive constituents **[2-4]**.

The surge in lifestyle-related diseases and the escalating demand for natural remedies have underscored the importance of exploring plant-derived compounds with therapeutic potential. The focus of this study is on elucidating the antioxidant activity inherent in the seed extract of *Decalepis hamiltonii* **[5]**. Antioxidants, substances that counteract the damaging effects of oxidative stress, have garnered immense attention for their potential in preventing and managing various health disorders, including cardiovascular diseases, neurodegenerative conditions, and cancer **[6-7]**.

Decalepis hamiltonii, belonging to the family Apocynaceae, thrives in arid regions and is renowned for its ability to withstand challenging environmental conditions. Beyond its ecological resilience, the plant has been traditionally utilized for its purported medicinal attributes, making it a compelling candidate for contemporary scientific scrutiny. The seeds, in particular, are believed to harbor a spectrum of bioactive compounds that contribute to the plant's pharmacological efficacy **[8-9]**.

As the pharmaceutical and nutraceutical industries increasingly seek sustainable and nature-inspired solutions, the investigation of plant-derived antioxidants becomes pivotal. Therefore, this study endeavors to systematically assess the antioxidant potential of the seed extract of *Decalepis hamiltonii*, employing a range of established assays to comprehensively evaluate its radical scavenging and reducing capabilities **[10]**. The outcomes of this research may unveil new dimensions in the therapeutic applications of *D. hamiltonii* seed extract, potentially informing the development of antioxidant-rich products for human consumption and pharmaceutical formulations **[11]**.

In summary, this investigation aims to bridge the traditional wisdom surrounding *Decalepis hamiltonii* with contemporary scientific rigor, offering a nuanced

understanding of its antioxidant attributes. Through this exploration, we endeavor to contribute to the evolving landscape of natural remedies and enhance our knowledge of plant-derived compounds that hold promise for promoting human health and well-being.

MATERIALS AND METHODS

Chemicals

Ascorbic acid; aluminum chloride, 2, 2′-azino-bis-(3- ethylbenzothiazoline-6-sulphonic acid) (ABTS); ferric chloride ($FeCl_3$); Folin-Ciocalteu; bovine serum albumin (BSA); potassium persulphate; 2,2′-diphenyl-1-picrylhydrazyl (DPPH); nitro blue tetrazolium (NBT); phenazine methosulphate (PMS); reduced glutathione (GSH); 1,2-dithio-bis nitro benzoic acid (DTNB); sulphosalicylic acid; thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Co. (St. Louis, MO, USA). Sulphuric acid; 2-deoxyribose; riboflavin; sodium carbonate (Na_2CO_3); sodium hydroxide (NaOH); sodium nitrite ($NaNO_2$); disodium hydrogen phosphate (Na_2HPO_4) and hydrogen peroxide (H_2O_2) were obtained from Wako Co. (Osaka, Japan). Potassium ferricyanide [$K_3Fe(CN)_6$]; triflouroacetic acid; sodium dihydrogen phosphate (NaH_2PO_4) and all solvents *n*-hexane (99.8%); chloroform (99.8%); ethyl acetate (99.8%) and *n*-butanol (99.8%) used were of analytical grade and purchased from Merck Co. (Darmstadt, Germany). Distilled deionized water (dd. H_2O) was prepared by Ultrapure TM water purification system (Lotun Co., Ltd., Taipei, Taiwan).

Plant Material

The *D. hamiltonii* plant seed were procured from IndiaMart, following which they were thoroughly washed and excess water was drained off. Subsequently, the seed were subjected to shade drying, and the dried plant material was finely powdered using an electric mixer grinder, thus preparing it for further analysis and experimentation.

Phytochemical Screening

Standard techniques were employed to conduct preliminary phytochemical tests on the methanolic extracts of *D. hamiltonii* seed. These tests were conducted to identify the potential presence of various compounds, including alkaloids, steroids, saponins, glycosides, anthraquinones, tannins, terpenoids, coumarins, carbohydrates, and flavonoids.

Determination of antioxidant Activity [12-14]

The assessment of the antioxidant activity was performed using the free radical scavenging method, specifically through the use of DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays, along with the ferric reducing antioxidant power (FRAP) assay.

DPPH scavenging activity (DPPH)

The antiradical capacity of the substances was assessed by monitoring the reduction in the absorption of DPPH (1,1-Diphenyl-2-picrylhydrazyl). In this process, 950 µl of a methanol solution of DPPH (0.1 mM) was combined with 50 µl of the plant seed extract. After a 30-minute incubation period, the absorbance of the resulting mixture was measured at 517 nm. The ability to scavenge the DPPH radical was determined using the following formula:

$$\%\ \boldsymbol{Inhibition\ of\ DPPH} = \frac{\boldsymbol{A_c - A_s}}{\boldsymbol{A_c}} \boldsymbol{X\ 100}$$

A_c = Absorbance of control

A_s = Absorbance of sample

ABTS scavenging activity (ABTS)

The method utilized the scavenging of the $ABTS^{\bullet+}$ [(3-ethyl benzothiazoline 6-sulfonic acid) diammonium salt] radical cation, generated through the mixing of ABTS (7 mmol/L) and potassium persulfate (2.45 mmol/L) solutions. Subsequently, the mixture was incubated in darkness at room temperature for 16 hours. The resulting product was appropriately diluted to achieve an optimal absorbance of 0.7 at 734 nm. The decrease in absorption at 734 nm over a 30-minute period was used to monitor the decolorization of the $ABTS^{\bullet+}$ solution by 100 µg/mL of the test sample or a reference compound (Trolox). The antioxidant activity was expressed as µM trolox equivalent antioxidant capacity (TEAC)/mg dry weight (DW).

Ferric reducing antioxidant power (FRAP)

The method employed is founded on the principle of the reduction of ferric tripyridyltriazine (Fe3 – TPTZ) to ferrous complex tripyridyltriazine (Fe2 – TPTZ) by an antioxidant in an acidic pH environment. The resulting ferrous Fe (II) complex -TPTZ generates a blue color with maximum absorbance at 593 nm. The methodology outlined by Benzie and Strain20 was adopted for this purpose.

The FRAP (ferric reducing antioxidant power) mixture was composed of 10 parts of acetate buffer solution (300 mM) at pH 3.6, 1 volume of 10 mmol/l 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/l HCl, and 1 volume of a solution of FeCl3.6H2O (20 mM). Subsequently, 2 ml of the FRAP mixture was combined with 10 µl of the plant extract. Following an incubation period of 15 minutes at room temperature, the absorbance was measured at 593 nm. The calibration range was prepared using Trolox. The results were expressed as µmol Trolox equivalent antioxidant capacities (TEAC) per milligram of dry weight (DW).

RESULT AND DISCUSSION

Phytochemical test

The phytochemical analysis of the *D. hamiltonii* aqueous seed extract revealed the presence of alkaloids, tannins, flavonoids,

sterols, terpenoids, and saponins. These chemical constituents have been well-documented to exhibit noteworthy biological activities (as shown in **Table 1**).

Acute toxicity test

No mortality was observed in mice following the oral administration of the aqueous extract, even at high dosages of up to 5,000 mg/kg. These findings suggest that the oral LD50 (Lethal Dose 50, the dose at which 50% of the test subjects would be expected to die) was determined to be greater than 5,000 mg/kg, as outlined in **Table 2**.

Determination of Antioxidant Activity

The findings in **Table 3** illustrate the antioxidant activity assessed via three distinct methodologies. The DPPH radical scavenging assay revealed an IC_{50} of 120.2 ± 0.17 µg/ml for the plant seed. Moreover, for the ABTS test, the seed demonstrated values of 11430.04 ± 10.31 µM TEAC/mg DW. Notably, no significant differences were observed between the male and female trees in either the DPPH or ABTS tests. Concerning FRAP, the seed' MEOAs exhibited significantly high values of 60.40 ± 3.51 µM TEAC/mg DW.

Literature suggests that the antioxidant potential of plant extracts is contingent on both the test employed and the specific phenolic compounds present. For instance, quercetin displayed relatively low antioxidant activity in the FRAP test, whereas it exhibited markedly high activity in the DPPH and ABTS assays. Polyphenols, which encompass more than one hydroxyl group attached to one or more benzene rings, are typically encountered as esters or glycosides rather than free compounds. Consequently, the antioxidant activity of polyphenols is influenced by the arrangement and number of hydroxyl groups in the phenolic rings, as well as their connections with saccharides. Polyphenols may function as reducing initiators, chelating agents, or through the prevention of oxidative reactions induced by active singlet oxygen.

In our study, the observed antioxidant activity can be attributed to the presence of various compounds such as cardenolide, triterpenoids, pregnanes, carbohydrates, phenolic acids, flavonoids, and amino acids. Previous chemical analyses have indicated that *D. hamiltonii* i is abundant in diverse compounds, including flavonoids, triterpenes, pregnanes, cardenolides, cardiac glycosides, and carbohydrates. Notably, the presence of a single hydroxyl in the B ring has been highlighted. These data collectively suggest that the antioxidant activity observed in our study results from the synergistic interplay of various phenolic compounds, even in the presence of other non-phenolic antioxidants.

Table 1: Preliminary Phytochemical Screening of *D. hamiltonii*

S. No.	Chemical Constituents	Ethanolic	Aqueous	Pet. Ether	Chloroform
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	+	+	+	+
4	Steroids	+	+	+	+
5	Flavonoids	+	+	+	+
6	Saponins	+	+	+	+
7	Fixed oils and fats	-	-	-	-
8	Tannins	+	+	+	+
9	Proteins and amino acids	-	-	+	-
10	Terpenoids	-	-	-	-

Abbrevations: (+) is positive; (-) is Negative

Table 2: LD50 and ED50 of *D. hamiltonii*

Plant Name	LD 50	ED 50
D. hamiltonii	5000 mg/kg	200mg/kg

Table 3: Antioxidant activity of *D. hamiltonii* seed extract assessed by DPPH, ABTS and FRAP methods

Activity	DPPH (%)	IC_{50} (µg/ml)	ABTS (µM TEAC/ mg DW)	FRAP (µM TEAC/mg DW)
D. hamiltonii	81.53 ± 0.62	120.2 ± 0.17	1430.04 ± 10.31	60.40 ± 3.51

Values are expressed in mean ± SEM. Means in each column followed by different letters are significantly different (P<0.05)

CONCLUSION

Based on the results obtained, it can be inferred that the selected medicinal plant extracts, along with most of the sub-fractions, demonstrated varying degrees of antioxidant activities, ranging from moderate to high. Interestingly, the pure isolated compounds from these fractions exhibited relatively weak antioxidant properties, with the exception of 5-O-caffeoyl quinic acid, isolated from the active fraction of *D. hamiltonii* seed, which displayed potent antioxidant activity against the DPPH free radical. The antioxidant capacity of these plants appears to stem from the presence of bioactive compounds, making them a promising source of natural antioxidants that can be harnessed for various industrial and domestic applications. Our investigation identified the presence of cardenolide, triterpenoids, pregnanes, carbohydrates, phenolic acids, flavonoids, and amino acids within the *D. hamiltonii* plant. Previous chemical analyses have indicated that *D. hamiltonii* is a plant rich in various compounds, including flavonoids, triterpenes, pregnanes, cardenolides, cardiac glycosides, and carbohydrates. Similar trends were observed for the antioxidant activity, with the exception of the FRAP test, which demonstrated heightened activity in the flowers.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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