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PHYTOCHEMICAL INVESTIGATIONS AND IN-VITRO ANTIOXIDANT ANALYSIS OF EUPHORBIA MILII DES MOUL

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Abstract
The Crown of Thorns, or Euphorbia milii Des Moul, is a stunning plant that has long been appreciated for its latent capacity as a source of bioactive chemicals with potential medical uses. The present study aims to investigate the presence of the phytochemical and in-vitro antioxidant study of hydroalcoholic (HA) and ethyl acetate (EA) preparations of E. milii leaves. The hydroalcoholic (HA) and ethyl acetate (EA) extracts shown strong antioxidant activity when examined in a laboratory setting using DPPH, H2O2, NO, reducing power, total phenol, and TOC assays. Also compounds like saponin, alkaloids, flavonoids, and phenolics were all identified through phytochemical testing. In addition to expanding our understanding of the pharmacological characteristics of Euphorbia milii, the results of this study have the potential to change the way medicinal botanicals are viewed forever. Nature's treasure trove, concealed within the leaves of Euphorbia milii, offers a pharmacological narrative that transcends traditional boundaries, suggesting new dimensions for antioxidant-based therapeutic strategies. The insights gained from this study may pave the way for the development of novel botanical interventions in preventive healthcare and disease management.

Keywords: Antioxidants, Medicinal plants, Euphorbia milii

1. INTRODUCTION

The investigation of botanical sources is becoming an ever more pressing necessity in the search for novel therapeutic agents that can be utilized in a variety of different pharmacological contexts. An mysterious succulent known as Euphorbia milii Des Moul, which has a long and illustrious history steeped in the tradition of being used as an ornamental plant, has just recently fascinated the scientific world with its latent potential for using it in medicinal applications [1-3]. This research article embarks on a journey of phytochemical investigations and in-vitro antioxidant analysis, seeking to unravel the molecular intricacies that underlie its pharmacological prowess [4-6]. The Crown of Thorns, or Euphorbia milii Des Moul, is a plant that shows how nature may produce a complex variety of bioactive chemicals. The plant's tolerance for harsh environments suggests it likely includes many phytochemicals, one of which may hold the key to unlocking its medicinal properties[7, 8]. Among the many kinds of secondary metabolites discovered in Euphorbia milii are phenolic chemicals, alkaloids, diterpenoids, and flavonoids. The metabolic actions of these many secondary metabolites are distinct from one another. To find out what these chemicals are and how much of them there is in Euphorbia milii, we wish to use state-of-the-art analytical techniques to carefully examine the plant's phytochemical composition [9-13]. This will lay the groundwork for comprehending the potential medicinal uses of the plant. Central to the allure of Euphorbia milii is the prospect of capitalising on the plant's antioxidant capability. This is a trait that is essential for warding off the oxidative stress that has been linked to a variety of pathological disorders. The byproducts of cellular metabolism known as reactive oxygen species (ROS) are a persistent danger to the integrity of cells and constitute a hazard to cellular health [1, 5]. The in-vitro antioxidant study of Euphorbia milii is of the utmost importance in determining whether or not it is able to deactivate these harmful ROS, hence reducing the amount of oxidative damage caused [10, 14-16]. Our research intends to measure and qualify the antioxidant potential of Euphorbia milii by conducting a number of rigorous studies, such as radical scavenging assays, determination of total antioxidant capacity, and assessment of enzymatic antioxidant activities [16-18]. Our goal is to shed light on the therapeutic implications of this botanical entity in the prevention and management of illnesses that are associated to oxidative stress by clarifying the various mechanisms via which it combats oxidative stress [19-21]. As we get started on this scientific journey, it becomes clear that combining phytochemical research with antioxidant testing is a potentially fruitful path to go in the direction of deciphering the medicinal mysteries that are concealed inside Euphorbia milii Des Moul [22-24]. The results of this research have the

potential to not only further our understanding of the pharmacological properties of the plant, but also to open the way for the creation of revolutionary therapeutic approaches based on antioxidants. Within the leaves of Euphorbia milii, we anticipate discovering a pharmacological narrative that surpasses traditional boundaries, providing new dimensions to the landscape of therapeutic botanicals.

2. MATERIAL AND METHOD

2.1. Plant material and Preparation of Herbal extract

The plant material of *E.milii* was collected and authenticated. Extraction of the leaves begin with rinsing them with clean water to eliminate any dirt or other debris that had stuck to them. Then, they were dried in an oven set to 35 to 40 degrees Celsius. The local collection of E.milii leaves was followed by shade drying and grinding into a coarse powder. Using the Soxhlet apparatus, a mixture of hydroalcohol in a 60:40 ratio and 100% ethyl acetate, was used to extract 250g of dried plant leaves. When the extraction was complete, the extracts were filtered using Whatman filter paper No. 1, dried in a water bath until they were semisolid, and their yields were recorded. They were then stored at 4 °C until needed [25-27].

2.2. Method:

2.2.1. Phytochemical analysis

Each consecutive extract was made using a 1 percent (W/V) stock concentration of ethyl acetate and hydro-alcohol using the matching solvents. Using conventional procedures, the presence of active phytochemicals such as saponin, alkaloid, terpenoid, steroid, glycoside, flavonoid, tannin, protein, carbohydrate, amino acid, and fixed oil was qualitatively assessed in these extracts and those used as controls [28, 29].

2.2.2. In-vitro antioxidant activity:

2.2.2.1. Assay of reducing power

The reducing power was determined by combining 2.5 mL of varying sample concentrations (ranging from 20-320 µg/mL), 2.5 mL of 0.1 M sodium phosphate buffer (pH 6.6), and 2.5 mL of K3Fe (CN)6 (1 percent, w/v). The mixture was then incubated at 50° C for 20 minutes. Mix in 2.5 ml of 10% w/v trichloroacetic acid, and spin at 5000 rpm for 10 minutes. The absorbance at 700 nm was then measured after mixing 4 ml of the upper layer with 0.4 ml of fresh FeCl3 (0.1 percent, w/v). Using ascorbic acid as a reference, the inhibitory concentration (IC50) value was determined [30].

2.3. Assay of H2O2 scavenging activity

This experiment involved combining extracts with varying concentrations (ranging from 20-320 µg/mL) with 2.5 mL of 0.1 M phosphate buffer (pH 7.4) and 600 µl of a solution containing 41 mM of H2O2. The mixture was then violently agitated and left to incubate at room temperature for 10 minutes. After that, we measured the absorbance of the reaction mixture at 230 nm. The positive control was VC. To determine the H2O2 scavenging activity, the following was done:

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

In this case, A0 represents the control absorbance (water instead of sample), A1 represents the sample absorbance, and A2 represents the sample absorbance alone (phosphate buffer instead of H2O2 solution). A compound's IC50 value indicates the concentration at which it inhibited H2O2 by half [31].

2.4. Nitric oxide radical scavenging activity

The Griess reagent was used to determine the nitric oxide scavenging activity. First, 5 millimolar sodium nitroprusside was made in phosphate buffer saline (PBS). Then, 3.0 millilitres of extract with varying concentrations ranging from 20 to 320 micrograms per millilitre was added. The combination was then incubated at 25°C for 150 minutes. After that, 5 millilitres of Griess reagent containing 1% sulphanilamide, 2% H3PO4, and 0.1% naphthalene-diamine dihydrochloride was added to the samples. As a positive control, we evaluated the absorbance at 546 nm of ascorbic acid standard solutions treated in the same manner with Griess reagent.

The following formula was used to measure the percentage of inhibition:

$$\%\ inhibition = [(A_0-A_T)/A_0\ x100]$$

in which A0 denotes the absorbance of the control (empty, extract-free) and At denotes the absorbance

when the extract is present. We performed each test three times and then graphed the means [32, 33].

2.5. TAOC

The first step was to heat a 0.3 mL plant sample with 1 mg/mL of phosphomolybdate to 95 °C for 10 minutes. Following this, the absorbance at 695 nm was measured. The TAC was then determined as milligrams of AAE per gramme of raw sample weight by referring to the ascorbic acid calibration curve (10-320 g/mL) [34].

2.5.1. Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity [35]

The first step was to make an ethanol solution of 0.1 mM DPPH. Then, 1.0 ml of this solution was mixed with 3.0 ml of extract solution in water at various concentrations (20-320 µg/ml). Following the 30-minute period, the absorbance was assessed at 517 nm using ascorbic acid as the standard chemical. Free radical scavenging activity is proportional to the absorbance value. Use this formula to determine the sample's free radical scavenging activity, which is defined as the percentage by which it inhibits free radicals:

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

where A0 is the absorbance when the extract is not present and At is the absorbance when the extract is present. Every test was carried out three times, and the average results were used to create the graph.

2.6. Estimation of total phenolic compounds

As a standard phenolic compound, gallic acid was used to do the estimation with the Folin-Ciocalteau reagent. The procedure called for a volumetric flask to be filled with an extract solution with a concentration of 1 mg/mL. Mixed with 2.5 mL of a 10% Folin Ciocalteu reagent in water and 2.5 mL of a 7.5% NaHCO3 aqueous solution, the final reaction mixture consisted of 0.5 mL of plant extract solution. The samples were left at 45°C for 45 minutes. The wavelength at which the blue colour was detected was 760 nanometers. In milligrammes per gramme of dried extract, the total phenol concentration was reported. The results were verified three times. Milligrams of gallic acid equivalents were used to estimate the overall amount of phenolic compounds in the plant extract (GAE) [36].

3. RESULTS

3.1 Phytochemical analysis

The study of plants and their bioactive components is known as phytochemical analysis, and it is an important branch of science. Phytochemicals include a wide variety of secondary metabolites from plants, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, among many more. To put traditional applications of medicinal plants into context, establish a scientific basis for them, and pave the path for the development of novel pharmaceuticals or alternative treatments, phytochemical study is essential (Table1).

Table 1 Phytochemical analysis

S. No	Phytoconstituent	*E.milii*	
		EA (Ethyl acetate)	**HA (Hydroalcohol)**
1	Saponin	+	+
2	Alkaloid	-	+
3	Terpenoid	-	-
4	Steroid	-	-
5	Glycosides	-	+
6	Flavonoids	+	+
7	Tannins	+	+
8	Proteins	-	-
9	Carbohydrates	+	+
10	Amino acids	+	-
11	Fixed oils	-	-

3.2 In-vitro antioxidants:

DPPH free radical scavenging activity

The IC50 value were found to be EA (197.34 µg/ml), HA (172.85 µgml) while that of ascorbic acid was found to be 119.85 µg/ml. Both plant extracts appear to have DPPH scavenging ability

that is on par with that of the gold standard, ascorbic acid, according to the results (Figure 1).

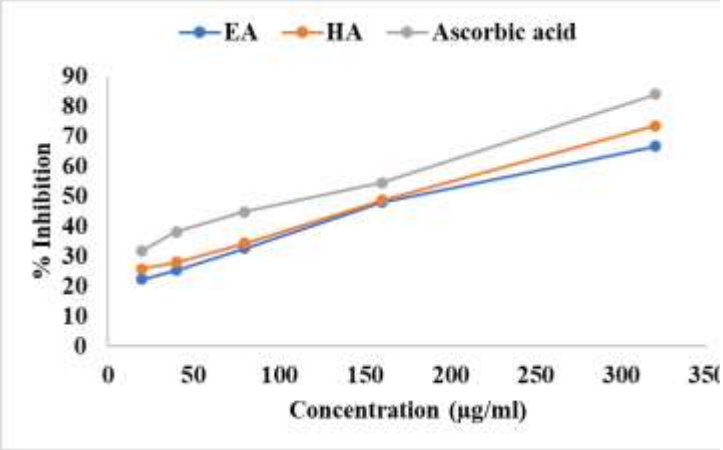

Figure 1: DPPH free radical scavenging activity

3.3 Reducing power activity

The reducing power of EA and HA extracts were assessed at different concentrations (10µg/ml to 320µg/ml). The reducing power of EA and HA extracts of was found to be increased with concentration. The highest reducing power of extracts was obtained at 320 µg/ml. EC50 was found to be 27.27 µg/ml with EA, 28.77 µg/ml with HA. It was 19.32 µg/ml for ascorbic acid (standard) (Figure 2).

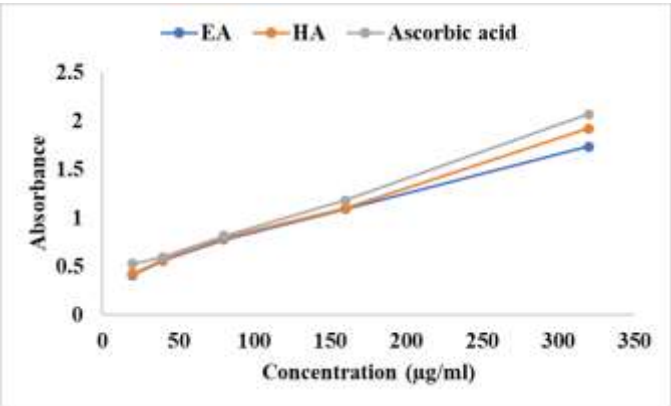

Figure 2: Reducing power activity

3.4 Hydrogen peroxide-scavenging activity

The highest level of hydroxyl radical inhibition observed in the EA, HA extract was determined to be 62.37%, 68.531% respectively, at the maximum concentration of 320 µg/ml. In contrast, ascorbic acid showed 79.41% inhibition. The ability of the extracts to scavenge these radicals was shown to be concentration dependent. Extracts of EA and HA were determined to have an IC50 value of 220.44 µg/ml, 193.11 µg/ml. The IC50 value of ascorbic acid was found to be 145.92 µg/ml (Figure 3).

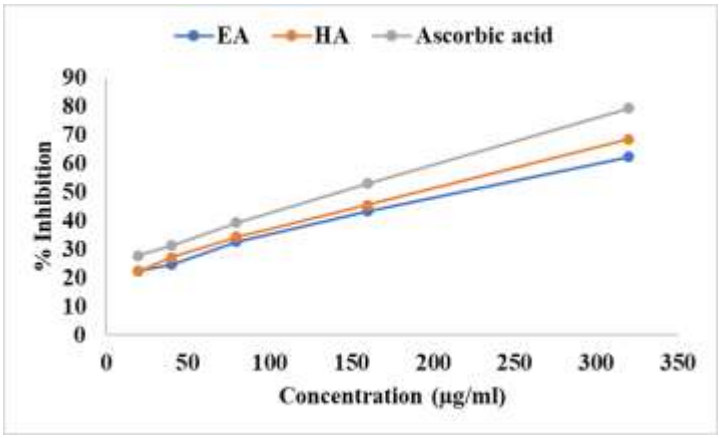

Figure 3: Hydrogen peroxide-scavenging activity

3.5 Total antioxidant capacity (TAC)

When measured against an ascorbic acid reference standard, EA, HA displayed potential antioxidant ability, that is 80.73, 139.26 mg/AAE (Ascorbic acid equivalent), respectively (Ogah et al. 2020) (Figure 4).

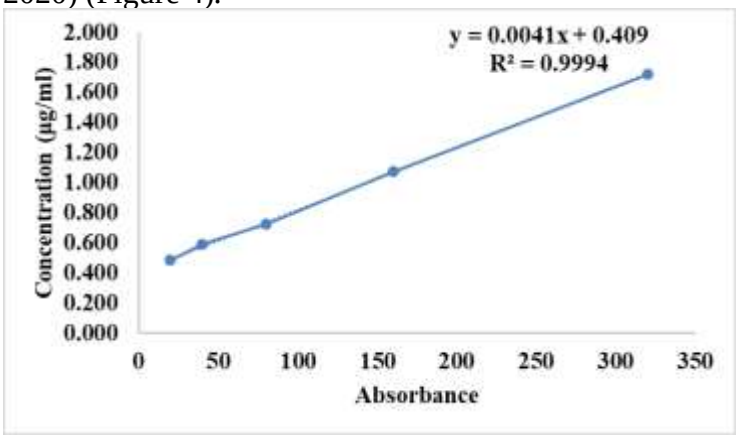

Figure 4: Total antioxidant capacity (TAC)

3.6 Nitric oxide radical scavenging activity

The Griess reagent was used to determine the nitric oxide scavenging activity. Before incubating the mixture at 25°C for 150 minutes, 5mM of Sodium nitroprusside was first produced in phosphate buffer saline (PBS). Then, 3.0 ml of extract with varying dilutions, ranging from 20-320 µg/ml, was added (Marcocci et al., 1994; Shukla et al., 2009). In NO scavenging activity, IC50 value of

EA 216.17, HA 196.45 µg/ml while that of ascorbic acid was found to be 154.44 µg/ml (Figure 5).

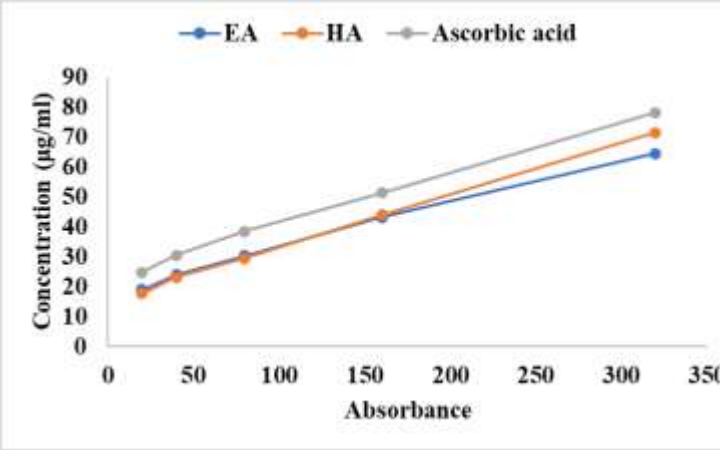

Figure 5: Nitric oxide radical scavenging activity

3.7 Total phenol content

The total phenol content of EA, HA extracts was found to be 499.1 and 507.1 mg/Gallic acid equivalent (GAE), respectively (Figure 6).

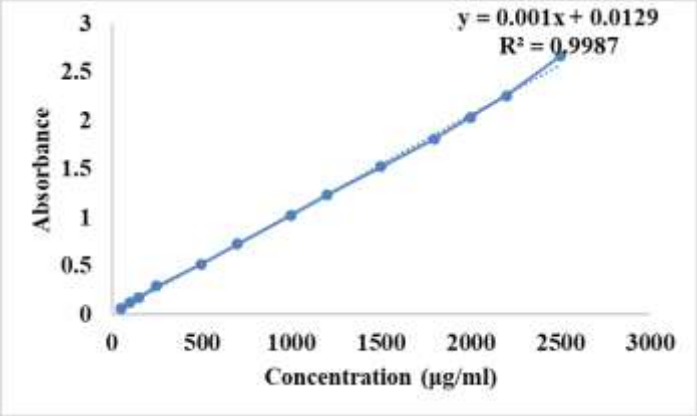

Figure 6: Total phenol content

4. CONCLUSION

In conclusion, our comprehensive exploration into the phytochemical composition and in-vitro antioxidant potential of Euphorbia milii Des Moul has illuminated the multifaceted pharmacological prowess of this botanical treasure. The richness of secondary metabolites, including flavonoids, alkaloids, and phenolic compounds, underscores the complexity and potential therapeutic value inherent in this succulent plant. The in-vitro antioxidant analyses have unequivocally demonstrated Euphorbia milii's capacity to neutralize Reactive Oxygen Species (ROS) and mitigate oxidative stress, positioning it as a promising candidate for addressing conditions associated with oxidative damage. The radical scavenging assays, assessments of total antioxidant capacity, and evaluations of enzymatic antioxidant activities collectively underscore the robust antioxidant potential of Euphorbia milii. The synergy between the identified phytochemicals and antioxidant activities opens new avenues for the development of botanical interventions in preventive healthcare and disease management. As we consider the broader implications of our findings, Euphorbia milii emerges not merely as an ornamental gem but as a potential source of natural compounds with therapeutic applications. As researchers and practitioners navigate the intricate landscapes of botanical medicine, the insights gained from this study provide a solid foundation for further investigations and potential translational applications. Harnessing the phytochemical richness of Euphorbia milii may offer innovative solutions to combat oxidative stress-related disorders and contribute to the expanding repertoire of natural therapeutic interventions. In the pursuit of sustainable healthcare solutions, the integration of Euphorbia milii into the pharmacological arsenal represents a paradigm shift, emphasizing the importance of exploring nature's reservoirs for novel bioactive compounds. As we conclude this exploration, the Crown of Thorns beckons attention not only for its aesthetic appeal but for its potential to redefine our approach to health and wellness through the harnessing of phytochemicals and antioxidants.

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