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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *EUPHORBIA PULCHERRIMA*

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Abstract
The primary goal of the research is to investigate the phytochemicals and antioxidant activities of *Euphorbia pulcherrima* extract in various solvents. *E. pulcherrima* leaves were extracted using a variety of solvents, including water, ethanol, and petroleum ether.
The results of the phytochemical analysis of *E. pulcherrima* indicate that the petroleum ether and ethanol extracts contain steroid, alkaloid, terpene, glycoside, phenol, tannin, saponin, flavonoid, and amino acid compounds.
The DPPH activity varies from 56.21±0.10 to 86.10±0.15. When compared to aqueous extract, the FRAP in petroleum ether and ethanol extract is significant and is 0.80 ± 0.15 mg/g and 0.77 ± 0.10 mg/g, respectively. The anti-oxidant activity of the ethanol extract was (79.85 ± 0.002 mg/g), while the petroleum ether showed a high total anti-oxidant capacity (90.75 ± 0.10 mg/g). Petroleum ether extract had a total phenolic concentration of 3.85 ± 0.15 mg/g, while ethanol extract had a total phenolic value of 2.65± 0.10 mg/g.
The results imply that all the extracts possess antioxidant qualities. The petroleum ether extract has greater antioxidant potential than the ethanol and aqueous extracts, which may be helpful for the future development of pharmaceutical, food supplement, and therapeutic goods.
Keywords: *Euphorbia pulcherrima*, Phytochemicals, DPPH, FRAP, TAC, TPC.

1. Introduction
The development of oxidative stress in the body is caused by an imbalance between the biological system's capacity for detoxification and the generation of reactive oxygen species. In the cells of all living things, a decreasing environment is maintained. By continuously supplying metabolic energy, enzymes can maintain this decreasing state.
Any redox potential disruption can harm live cells by causing the production of free radicals and peroxides, which can damage the cells' lipids, proteins, and DNA(Saleem et al. 2014) and numerous chronic diseases, including diabetes, atherosclerosis, ageing, cancer, and other degenerative disorders in humans are finally brought on by the oxidative destruction of biomolecules (Sultan A and Askin Celik 2013).
Released free radicals cause inflammatory reactions by harming crucial macromolecules and cellular membranes. Antioxidants can counteract these free radicals, protecting individuals from such illnesses. Natural anti-oxidants are being sought after because it has been discovered that the synthetic anti-oxidants that are commercially available have negative effects.

Natural antioxidants are also superior to synthetic antioxidants because they offer a variety of therapeutic benefits and do not include chemical contaminants. Natural antioxidants are therefore approved for use in medication, dietary supplements, nutraceuticals, and cosmetics with the goal of enhancing immune system function, increasing consumer health, and reducing the consequences of major diseases (Salunke et al. 2021). Artificial antioxidants like butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), which have been demonstrated in animal experiments to be toxic and carcinogenic, must be used in place of natural antioxidants (Salunke et al. 2021).

An efficient way for the body to scavenge free radicals is through exogenous antioxidant intake.

Nowadays, there is a discernible interest in antioxidants, particularly those that can stop the alleged harmful effects of free radicals on the human body and to stop the breakdown of lipids and other food components (Basma et al. 2011).

As a result, supplementing with antioxidants to reduce the oxidative damage caused by free radicals has emerged as an appealing treatment approach for lowering the risk of various diseases. It has been discovered recently that a variety of plant species serve as sources of antioxidants and have medicinal value (Thambiraj et al. 2012).

Natural phenolic compounds known as antioxidants work in three different ways to neutralise free radical electrons: It either conducts both above mentioned procedures simultaneously, or it transfers an antioxidant's electron to inhibit or diminish the effect of metals and carbonyl, which deactivates free radicals by hydrogen donation (Farhan et al. 2013).

In the tropical regions of Pakistan, India, and China, the genus Euphorbia is one of the most common genera of medicinal plants. Numerous varieties of Euphorbia have historically used as a folk remedy for a variety of conditions, including the treatment of warts, dermatitis, and the eradication of intestinal parasites, due to their significant medicinal potential (Chohan et al. 2020).

Many different chemicals that can scavenge free radicals, including phenolic compounds, are among the most frequently discovered active components in plants (medicinal herbs, fruits, vegetables etc.) like phenolic substances (e.g., quinines, phenolic acids, coumarins, flavonoids, lignans, tannins, stilbenes), nitrogen compounds (betalains, alkaloids, amines,), vitamins (vitamin C and E), terpenoids (including diterpenes, carotenoids, sesquiterpenes, triterpenes) and different endogenous metabolites (polysaccharides, curcuminoids), which some have high antioxidant action (Sultan A and Askin Celik 2013).

Due to the presence of numerous components in the plants with various modes of action, the Euphorbia genus may exhibit a wide variety and range of biological activities. Secondary metabolites are the active ingredients in many medications that are found in plants. However, research on the bioactive secondary metabolites produced by Euphorbia pulcherrima, which may be the cause of the plant's therapeutic effects, is scarce (Sharif et al. 2015).

These plants are a huge, largely unexplored source of bioactive chemicals and potent novel pharmaceuticals. Consequently, when looking for complementary therapies and natural cures for cancer, the species Euphorbia looks to be one of the most promising targets (Ben Jannet et al. 2017).

Due to their use in traditional medicine to treat skin conditions, gonorrhoea, migraines, intestinal parasites, and warts, as well as for the diverse pharmacological effects of their secondary metabolites, including antiproliferative, antimicrobial, antioxidant, antiviral, and anti-inflammatory activities, Euphorbia species have received a lot of attention in recent years. They have a wide variety of chemical ingredients, including coumarins, phenols, sesquiterpenes, diterpenes, triterpenes, and flavonoids, which contribute to their wide spectrum of pharmacological characteristics (Badaoui et al. 2020).

Since *Euphorbia pulcherrima* is rich in phenolic compounds such flavonoids, phenolic acids, and tannins, which are recognised as the primary contributor to the anti-oxidant capacity, there have only been a few reports on the anti-oxidant activity of this plant yet. The phenolic content of plants has been linked to their anti-oxidant capabilities. As part of our ongoing research to identify bioactives, we started looking at the antioxidant capacities of plant extracts. The study concentrated on species that are among the most abundant in general and that may, in theory, develop into a natural hotspot for the extraction of metabolites with antioxidant qualities for application in the pharmaceutical and food business. Additionally, it is important to locate new, inexpensive, natural sources of antioxidants.

2. Materials and Method

2.1. Collection and Processing of Plant Samples

E. pulcherrima is gathered from maharashtra, India. The *E. pulcherrima* leaves were cleaned with distilled water and dried at room temperature. Using a sterile mortar and pestle, dried E. pulcherrima leaves were crushed and ground into powder.

2.2. Extraction of Plant Samples

In a Soxhlet extractor, the plant powder was progressively extracted with water, ethanol, and petroleum ether. For the extraction, which yielded the crude extract, 100 g of leaves were employed. All extracts were dried to their respective degrees in a rotary vacuum evaporator set to 40^0C, then they were kept in the dark until analysis (Zengin et al. 2017).

The yield percentage for the E. pulcherrima aqueous extract was 4.46% and it had a dark solid appearance. The yield percentage for the ethanol extract was 5.14% and it had a gummy, dark green appearance. The yield percentage for the petroleum ether extract was 3.45% and it had a dark green, gummy appearance.

2.3 Preliminary Phytochemical analysis of *E. Pulcherrima*

Standard procedures were utilised to determine the preliminary phytochemical analysis of alkaloids, phenolic compounds, steroids, flavonoids, tannins, saponins, and steroidal glycosides in water, ethanol, and petroleum ether extract of *E. pulcherrima* (Thiruchelvi et al. 2018).

2.4 Anti-oxidant Activity of *E. Pulcherrima*

DPPH Radical Scavenging Activity:

Using the DPPH assay, the free radical scavenging capacity of *E. pulcherrima* was evaluated. The procedures outlined by Torey et al. were used to determine the quantitative assessment of radical scavenging activity. The plant extract solutions, with concentrations ranging from 0.031 to 2 mg/mL, were mixed with 5 millilitres of a 0.004% DPPH radical solution. After being vortex-mixed, the mixtures were left at room temperature in the dark for 30 minutes. At 517 nm, the optical density (OD) was determined using spectrophotometers (Shimadzu UV-Mini1240, UV/ Vis). Methanol served as a blank (Basma et al. 2011).

DPPH scavenging (%) = (Acontrol - Asample) / Acontrol × 100

Where "A sample" denotes the absorbance of the sample after the peak has been reached (30 minutes), and "A control" denotes the absorbance of DPPH.

Ferric reducing antioxidant power (FRAP)

The methodology suggested by Celik et al. with a few small alterations was used to determine the ferric reducing antioxidant power. A volumetric flask with a capacity of 1000 mL was used to combine the samples (500 µL) with 120 µL of phosphate buffer (pH 7), which was made by combining dibasic potassium phosphate (61.5 mL, 1 M), monobasic potassium phosphate (38.1 mL, 1 M), and water. The mixture was then homogenised, and it was incubated at 50^0C for 20 min with 220 µL of 1% potassium ferrocyanide (C6FeK4N6). Then, 45 µL of distilled water, 12 µL of 10% trichloroacetic acid, and 10 µL of 0.1% ferric chloride were added. The absorbance was measured at a 734 nm wavelength. A calibration curve was created using the same standard, and the results were then reported as µg equivalent gallic acid per millilitre (Bautista-Hernández et al. 2021).

Total phenolic content assay

For this test, 0.2 ml of the sample and 0.2 ml of the 10% Folin-Ciocalteu solution were combined, and the mixture was then allowed to sit for 4 minutes in a darkened area. After the solution had been stirred for 4 minutes, 1 ml of a 15% sodium carbonate solution was added, and it was allowed to stand at room temperature and away from light for 30 minutes. The solution was then put into a cuvette, and the absorbance was measured at 760 nm with a UV/Vis spectrophotometer. The readings being performed three times. In this work, the gallic acid equivalent (GAE), a measure of the amount of total phenolic content of *E. pulcherrima*, was estimated using an equation derived from a reference curve that used Gallic acid at various doses(Mohamad and Ismail 2022).

Total Anti-oxidant Capacity

The extract (100 mg/ml) was combined with 3 ml of reagents that contained 0.6 M H2SO4, 28 mM sodium phosphate, and 4 mM ammonium molybdate, and the mixture was then incubated at 95 °C for 90 min in a water bath at 695 nm, the absorbance was measured. Ascorbic acid (0.1 mg/ml in distilled water) was employed as a reference, and values were presented as mg/g (Salunke et al. 2021).

3. Results and Discussion

3.1 Preliminary Phytochemical analysis of *E. Pulcherrima*

E. pulcherrima leaf extracts in water, ethanol, and petroleum ether underwent preliminary phytochemical investigation. Table 1 lists the secondary metabolites that were found in various extracts.

Strongly favourable results for the presence of phytochemicals were obtained from the crude ethanolic and petroleum ether extract of *E. pulcherrima* leaves. While only glycosides, saponins, and amino acids exhibited strong good benefits from the aqueous extract.

Table. 1: Preliminary phytochemical analysis of *E. pulcherrima*

Phytochemicals	Aqueous extract	Ethanol extract	Petroleum ether extract
Steroids	-	+	+
Alkaloids	-	+	+
Terpenoids	-	+	+
Glycosides	+	+	+
Phenol and tannins	-	+	+
Saponin	+	+	+
Flavonoids	-	+	+
Amino acids	+	+	+

('+' presence & '- 'absence)

3.2 Anti-oxidant Activity *E. Pulcherrima*

As shown in Table 2, the total antioxidant capacity (TAC), ferric reducing antioxidant property (FRAP), total antioxidant capacity (TAC), and total phenolic content of the aqueous, ethanolic, and petroleum ether extract of *E. pulcherrima* were all measured using a separate method.

TABLE 2: Anti-Oxidant Properties of *E. Pulcherrima* in different Extract

Sr. No	Extract	DPPH (% inhibition)	FRAP (mg/g)	Total anti-oxidant capacity (mg AA/g)	Total Phenolic content (mg GA/g)
1.	Aqueous extract of *E. pulcherrima* (g)	56.21± 0.10	0.50± 0.015	52.25 ± 0.15	1.70 ± 0.15
2.	Ethanol extract of *E. pulcherrima* (g)	74. 20± 0.10	0.77± 0.10	79.85 ± 0.002	2.65 ± 0.10
3.	Petroleum ether extract of *E. pulcherrima* (g)	86.10± 0.15	0.80 ± 0.15	90.75 ± 0.10	3.85 ± 0.15

Values are the average of three observations, with standard deviation (SD) ±

The DPPH assay is used to assess the anti-oxidant chemicals in the *E. pulcherrima* extract's ability to behave as hydrogen donors or radical proton scavengers. The anti-oxidant properties of various *E. pulcherrima* extract extracts were examined in this work. When compared to ethanol and aqueous extract, petroleum ether extract had the highest DPPH activity. The DPPH activity ranges between 56.21± 0.10 and 86.10± 0.15. All three extracts have anti-oxidant activity that is greater than 50%. As shown in Fig. 1, the results revealed that every extract tested in this study has antioxidant characteristics.

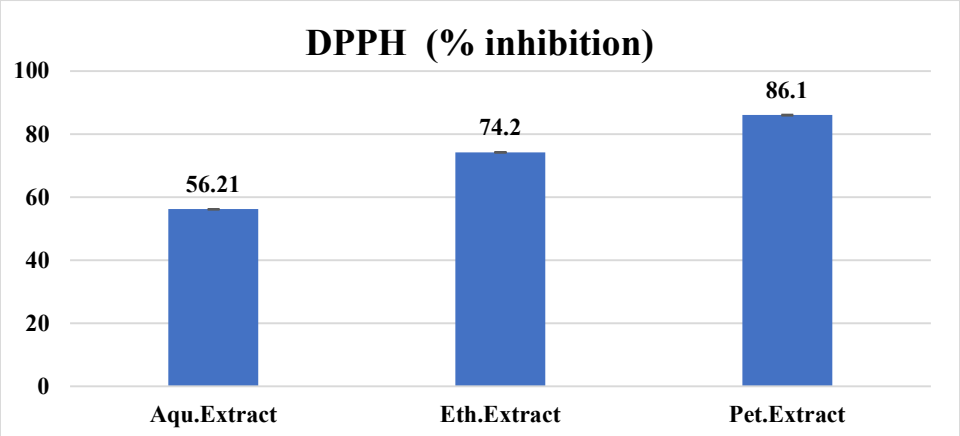

Fig. 1: DPPH Radical Scavenging Activity (% Inhibition) of *E. pulcherrima*

When compared to aqueous extract, petroleum ether and ethanol extract had large amounts of ferric reducing antioxidant property (FRAP), 0.80 ± 0.15 mg/g and 0.77±0.10 mg/g, respectively. The outcomes of the FRAP experiment are shown in Fig 2.

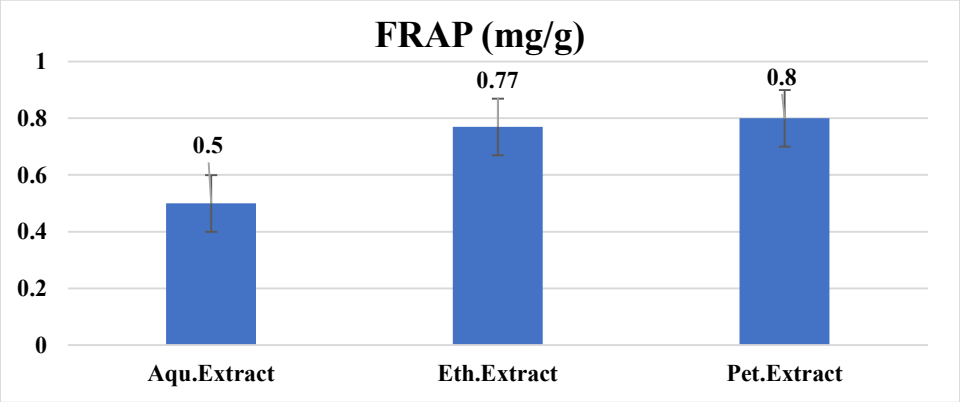

Fig. 2: Ferric Reducing Antioxidant Property (FRAP) of *E. pulcherrima*

The anti-oxidant activity of the petroleum ether was high (90.75± 0.10 mg/g), whereas that of the ethanol extract was low (79.85 ± 0.002 mg/g), and that of the aqueous extract was moderate (52.25 ± 0.15 mg/g). The results of the total anti-oxidant activity in various extracts are shown in Fig. 3.

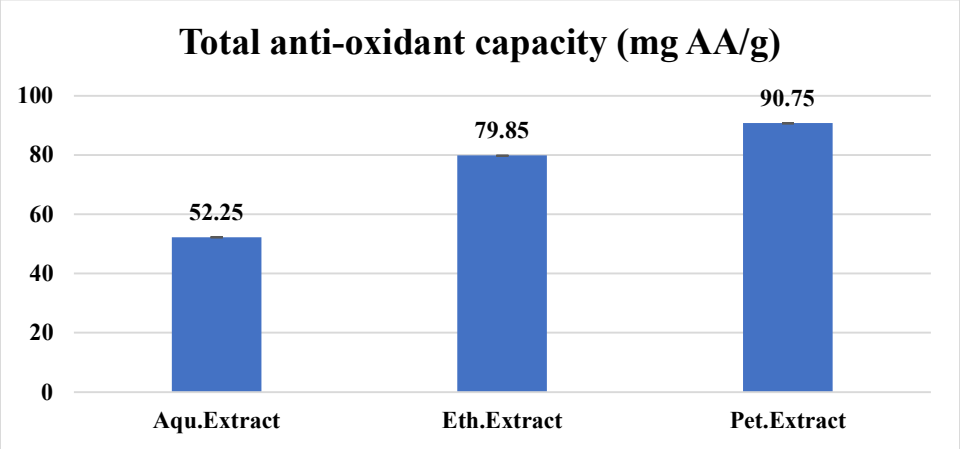

Fig. 3: Total Anti-Oxidant Capacity of *E. pulcherrima*

In this experiment, the petroleum ether extract had a higher total phenolic content than the ethanol and aqueous extracts. The petroleum ether extract had a total phenol concentration of 3.85±0.15 mg/g, while the ethanol extract had a phenol level of 2.65±0.10 mg/g. Compared to ethanol and petroleum ether extract, aqueous extract has reduced phenolic content. The total phenolic content of several extracts from *E. pulcherrima* is shown in Fig. 4.

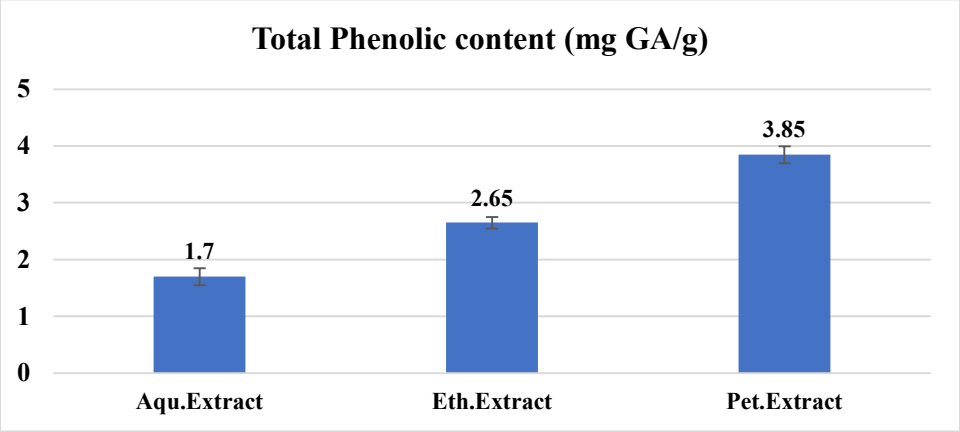

Fig. 4: Total Phenolic Content of *E. pulcherrima*

4. Conclusion

Many human diseases are regarded to have a significant role for free radicals and other reactive species. Due to the harmful impact that free radicals play in biological systems, radical scavenging activities are crucial. The preliminary phytochemical analysis of the petroleum ether and ethanol extract reveals the presence of steroids, alkaloids, terpenoids, glycosides, tannins, phenol, saponin, flavonoids, and amino acids that act as sources of antioxidants and as free radical scavengers. When compared to the aqueous extract, the petroleum ether and ethanol extracts contained significant amounts of DPPH, FRAP, and TAC.

According to the results, all the extracts contain antioxidant capabilities that may be useful for the future development of medicinal applications, dietary supplements, and therapeutic goods. These

natural economic resources must be exploited, which will call for additional research in the future. *E. pulcherrima* can be used as a source of natural antioxidant chemicals, according to the study's findings. However, additional investigation is needed to determine the precise method by which *E. pulcherrima's* antioxidant qualities work.

Conflict of Interests

The authors affirm that there are no competing interests.

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