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Phytochemical screening of *Euphorbia hirta* L and antioxidant properties

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Abstract
Euphorbia hirta. L play an important role in traditional medicine. The current study was carried on research of phytochemical screening and assessment of antioxidant properties. The presence of alkaloids, terpenoids, tannins and flavonoids was discovered during phytochemical screening of *Euphorbia hirta*.L leaf extract. The Antioxidant Activity of were tested DPPH, H_2O_2, O^{2-}Free Radical Scavenging Assay method.

Keywords: *Euphorbia hirta* l; phytochemical screening; antioxidant; scavenging method

Introduction
The Plants have been used as the basis of advanced medicine system for thousands of countries including India and China. [1] In conformity with the world health organization many peoples currently using phytomedicine for improving health care. Peoples are using many different plant parts used to make medicine; these are acting as a curable medicine for modern civilization. [2-4] *Euphorbia hirta* L has been widely used in the treatment of many diseases like diarrhea, cold, cancer, bronchitis, etc. [5,6] and cure some skin diseases also such as acne, dark spots, etc. In the purpose, we reported here in the phytochemical investigation of *Euphorbia hirta* L. along with its Many researchers have investigated pharmacological properties. The primary constituents of the plant were isolated, described and identified as flavonoids, steroids, terpenoids, coumarins, tannins, and polyphenols. [7, 8-12]. Phytochemical approaches played a vital part in the pharmaceutical industry's hunt for raw materials and resources. Preliminary phytochemical studies aid in the identification of chemical compounds that are sources of pharmaceutically active susbstances. [13] the plants were said to be high in nutrients like crude protein.[14] Saponins, tannins, and volatile oils were discovered in the three plants after phytochemical examination. Oxidation stress is caused by the reactive species/free radicals and is stabilized by various beneficial compounds known as antioxidants. They serve as first line of defense and are critical for maintaining health have reported that Euphorbia species show high antioxidant activity.

Plant Description

Fig 1: *Euphorbia hirta.* L

Fig 2: *Euphorbia hirta*. L

Plants have long been known to contain antioxidants such as phenols and flavonoids. As a result, it could play a role in decreasing or preventing oxidation. [15] *Euphorbia hirta* L extract has been shown to contain quercitrin, luteolin, (-)-epicatechin gallate and (-)-epigallocatechin gallate in other medicinal research. [16]

In present days, antioxidants are gaining popularity, particularly those that can prevent the alleged harmful effects of free radicals in the human body, as well as the deterioration of lipids and other constitutents of foodstuffs and food materials. In both circumstances, antioxidants derived from natural sources are preferred over those derived from synthetic ones [17]. The majority of antioxidants are currently produced synthetically. The biggest downside of synthetic antioxidants is there *in vivo* side effects [18]. Previous research has shown that butylated hydroxy anisole builds up in the body, causing liver damage and carcinogenesism [19]. Therefore, strict governmental rules regarding the safety of the food have necessitated the search for safer alternatives as food preservatives [20].

Materials and Methods

Collection of plant materials

The fresh plant leaves were collected in August 2021 from Tindivanam in Villupuram, Tamil Nadu, India. Identified these fresh plant leaves was washed from clean water, air dried and then fine powder stored in airtight bottle.

Preparation of Plant powder

The plant leaves was air dried under shade for 5-10 days. Then the dried material was crushed to fine powder using an electrical grinder and stored in air tight bottles. The powder was used for further analysis.

Preparation of the Ethanolic extract

Ethanolic extract was prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1996). The leaves powder material was subjected to Batch extraction separately and successively with 140ml ethanol and 60ml distilled water. These extracts was filtered by what man filter paper. Then the extract was put in an air tight container stored.

Crude Extraction

Aqueous extraction: Five grams of dried plant materials was dissolved in 20ml of water and after dissolving the crude extract was filtered through no.1 filter paper. The extract was heating in water bath and the concentrated extract was subsequently dried aseptically at room temperature. The concentrated extract of the selected plant were subjected to different chemical test for the detection of different chemical phytoconstituents using standard methods.

Solvent extraction

Solvent extraction: Five grams of dried plants materials was dissolved in 20ml of Ethanol and after dissolving the crude extract was filtered through no.1 filter paper. The extract was heating in water bath and the concentrated extract was subsequently dried aseptically at room temperature. The concentrated extract of the selected plant were subjected to different chemical test for the detection of different chemical phytoconstituents using standard methods.

Preliminary Phytochemical Analysis

The preliminary phytochemical investigation of the plant leaf of *Euphorbia hirta*. L were carried out with the standard protocol. The extracts are subjected to preliminary phytochemical analysis (Kokate *et al*., 1995).

1. S**aponins test**

The plant extract by adding 7ml of H_2O in a tube and shake well, till the forming of foam occurs the saponin present.

2. Flavonoids test

The plant extract by adding 15ml distilled water and 10ml of dilute ammonia solution was added from aqueous filtrate 2ml conc. H_2SO_4 added. The appearance of the yellow colour in presence of flavonoids.

3. Steroids test

The plant extract it dissolve in 0.8ml dichloromethane and dilute solution then 0.8ml of anhydrides was added with few drops of concentrated oil of vitriol. When a blue green colour presence of steroids.

4. Tannins test

The plant extract by adding a small quantity of H_2O and heated with water both mixed and additionally add a drop of ferric chloride to the presence of tannins.

5. Alkaloids test

5ml of 4% HCl added to the extract at form solid and heat gently. Wagner and Mayer reagent were added to the mixture to blend. The cloudiness of the resultant precipitate indicate the alkaloids.

6. Carbohydrate test

1ml of Fehling's solution-A is mixed with 1ml of Fehling's solution-B. The mixture is added to a little of the substance dissolved in water, shake well and heated in a boiling water bath. When red brown precipitate is formed in the presence of carbohydrates.

7. Phenol test

To a little of the substance in water of alcohol a few drop very dilute solution of neutral ferric chloride is added. When violet colour is produced in the presence of phenol.

8. Amino acid and Proteins test

Taken 2ml of extract with few drops of aqueous ninhydrin solution and keep in a boiling water both for 5 minutes and indicate the purple colour.

9. Terpenoids test

Take 1ml of plant extract and treat it with 0.8ml of conc. HCl and indicate for the formation of yellow precipitate or colouration.

10. Quinones test

Take 5ml of plant extract and add 10ml distilled water and indicate form of cloudiness.

11. Coumarins test

Take 2ml of plant extract and add 2.5ml of 15% NaOH then indicate form of yellow color the presence of coumarins.

Result and Discussion

Phytochemical Screening

Basic phytochemical screening is using simple chemical tests to find the presence of classes of molecules recognized to have medicinal potential in a plant extract, such as alkaloids, acetogenins, polyketides, isoprenoids, and carbohydrate. The general composition of an unknown plant product can be established using qualitative chemical's constituents. Preliminary qualitative phytochemical screening was performed using standard techniques for steroids, terpenoids, alkaloid, flavonoids, Tannins, Saponins, Coumarins, Carbohydrates, proteins and amino acids.

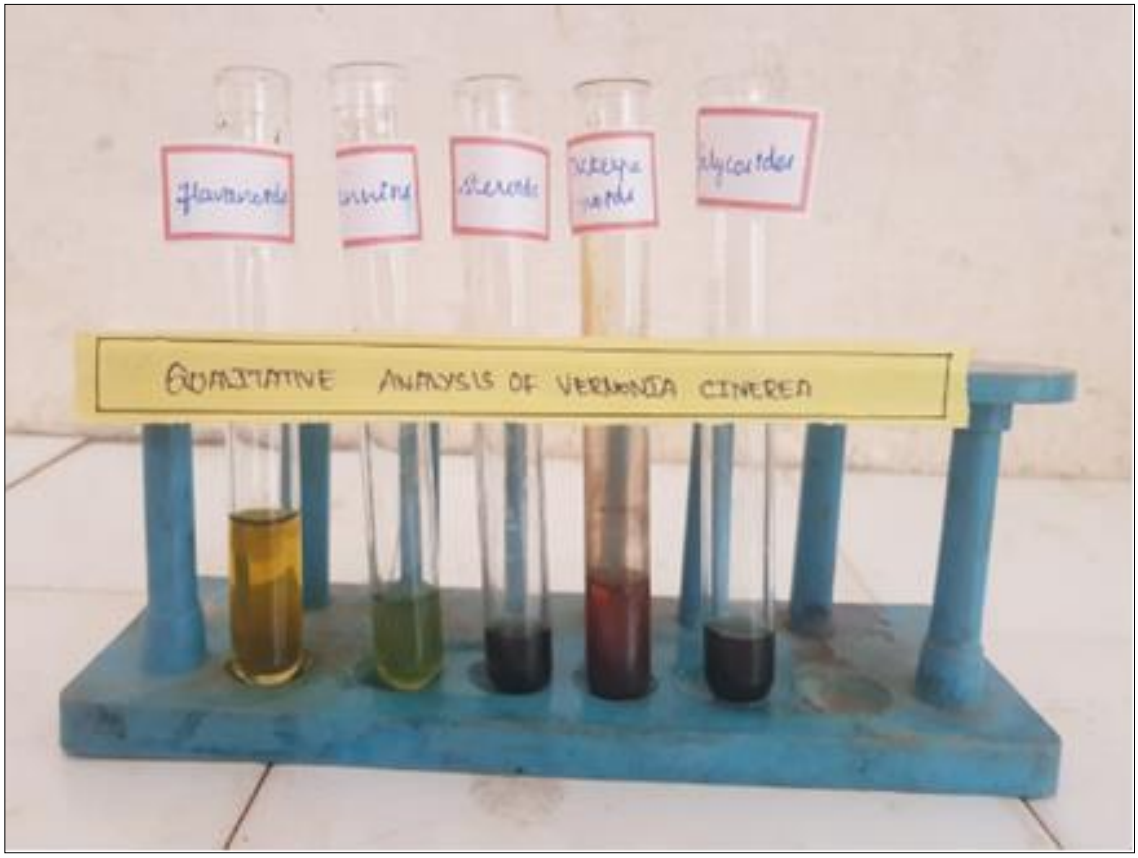

Fig 3: Qualitative analysis of *Euphorbia hirta*. L

Table 1: Preliminary Phytochemical Analysis of Flowers and Leaves of *Euphorbia hirta*. L

S.NO	Phytochemical Constituents	Aqueous Extract	Ethanol Extract
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Flavonoids	_	+
4.	Phenol	+	+
5.	Amino acids/ Proteins	+	_
6.	Saponins	+	+
7.	Tannins	+	_
8.	Terpenoids	_	+
9.	Quinones	_	_
10.	Coumarins	_	+

+= indicate presence of phytochemical, - = indicates absence of phytochemicals Currently most research work has been focused on the investigation of phytochemicals of *Euphorbia hirta* plant. They found many phytochemicals in *Euphorbia hirta* leaf extract and the extraction was carried out by waterbath method. Thus, the study suggests that the plant extract exhibits phytochemical.

In Vitro Antioxidant Activity

In vitro antioxidant activity of the extract *Euphorbia hirta* was evaluated DPPH, H_2O_2, O^{2-} free radical scavenging assays. The IC50 values were calculated and compared to the conventional standard antioxidant's ascorbic acid (Table 2). The ABTS assay is a newer one that uses a more powerful, chemically generated radical for screening complex antioxidant mixtures including plant extracts, drinks, and biological fluids. The solubility in both the organic and aqueous mediums, as well as the stability over a wide pH range, piqued researcher' interest in using an ABTS•+ to estimate antioxidant activity [17]. when the DPPH-free radical react with hydrogen donors, it is reduced to a corresponding hydrazine. The DPPH radical is purple in color and turns yellow when it react with hydrogen donors. It's a discoloration test that involves adding the antioxidant to a DPPH solution in ethanol or methanol and measuring the decrease in absorbance at 490 nm. Most human diseases, including cardiovascular disease and cancer, appear to be characterized by the presence of free radicals, particularly their enhanced generation.

The addition of sodium hydroxide to air-saturated dimethyl sulfoxide (DMSO) produces superoxide radicals in the alkaline DMSO method. At normal temperature, the produced superoxide remains stable in solution, reducing nitro blue tetrazolium to formazan dye, which can be detected at 560 nm. Several oxidizing enzymes create superoxide peroxide *in vivo*, which prevents the synthesis of a red dye formazan [19]. There is mounting

evidence that hydrogen peroxide causes serious harm to biological systems, either directly or indirectly through its reduction product, the hydroxyl radical (OH). The decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm when a scavenger is incubated with hydrogen peroxide in tis method. [20]

The presence of phytochemicals such as alkaloids, carbohydrates, flavonoids, gums and mucilages, phenolic compounds, saponins, tannins, and terpenoids in the *Euphorbia hirta*.L extract results in enhanced antioxidant activity. The *Euphorbia hirta* extract by chemical method shows the least antioxidant activity. Based on the above antioxidant results, all the *Euphorbia hirta* show significant antioxidant activities compared to standard antioxidants ascorbic acid. The order of the antioxidant activity remains same for all the tested methods. The *Euphorbia hirta* shows excellent antioxidant activity compare to standard ascorbic acid , because it contains a large number of phytochemicals like alkaloids, flavonoids, phenolic compounds and terpenoids etc.,

The in vitro approaches rely on samples being added to a free radical generating system, the inhibition of free radical action being evaluated, and the inhibition being connected to the samples antioxidant activity. The generated radical, the reproducibility of the generating procedure, and the endpoint employed for the determination vary substantially amongst approaches. Despite the fact that in vitro methods provide a valuable indication of antioxidant activity, data derived from in vitro methods are challenging to apply to biological system and do not always indicate a similar *in vivo* antioxidant activity. It's important to remember that all of the methodologies established have advantages and disadvantages, and that a single measurement of antioxidant capacity is rarely enough. To properly analyses in vitro antioxidant activity of a specific molecule or antioxidant capacity of a biological fluid, a variety of approaches may be required. Standard methods were used to investigate the antioxidant potential of *Euphorbia hirta*. The *Euphorbia hirta* and standard solutions concentrations employed were 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 µg mL^{-1}, respectively. To extract agglomeration, sonicate a dilute solution of *Euphorbia hirta* in a sonicator bath at room temperature for 30 minutes. The absorbance was compared to the equivalent blank solution using spectrophotometry. The percentage inhibition was calculated by using the following formula:

$$\text{Radical scavenging activity \%} = \frac{OD\ control - OD\ sample}{OD\ control} \times 100$$

In vitro Antioxidant Activity

DPPH Assay

IN a 96-well micro titer plate, the assay was performed. In each well of the micro titer plate, 10 µL of each of the samples for standard solution was added individually to 200 µL of 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) solution. After 30 minutes of incubation at 37 degree Celsius, the absorbance of each solution was measured at 490 nm.

The antioxidant activity of extract *Euphorbia hirta* was reinvestigated by DPPH radical scavenging assay method. Table 2 shows the anti-oxidant activity of *Euphorbia hirta* equal concentrations of 100 nM measured at 230 nm

Hydroxyl Radical Scavenging Assay

To a reaction mixture containing ferric chloride (0.5 mL,0.1 mM); EDTA (0.5 ml, 0.1mM); ascorbic acid (0.5 mL,0.1 mM); hydrogen peroxide (0.5 mL, 2 mM); and p-nitrosodimethyl aniline (p-NDA; 0.5 mL, 0.01 mM) in phosphate buffer (pH 7.4, 20 mM), various concentrations of samples or standard (0.5 mL) were added to make a final volume of 3 mL. Sample blank was prepared by adding 0.5-mL sample and 2.5 mL of phosphate buffer. Absorbance of these solutions was measured at 440 nm.

The antioxidant activity of extract *Euphorbia hirta* was reinvestigated by H_2O_2 radical scavenging assay method. Table 2 shows the anti-oxidant activity of *Euphorbia hirta* equal concentrations of 100 nM measured at 230 nm.

Superoxide Radical Scavenging Assay (Alkaline DMSO Method)

To the reaction mixture containing 1 mL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL water) and0.3 mL of the sample in freshly distilled DMSO at various concentrations, 0.1 mL of nitro blue tetrazolium (NBT; 1 mg mL^{-1}) was added to make a final volume of 1.4 mL. The absorbance was measured at 560 nm.

The antioxidant activity of the *Euphorbia hirta* was again evaluated by superoxide scavenging assay method. In the present study, superoxide scavenging activity *Euphorbia hirta* at equal concentration of 100 nM was determined at 560 nm. Table 2.

Table 2: The percentage of antioxidant scavenging against DPPH radical, Hydrogen peroxide radical and Superoxide radical in *Euphorbia hirta* compared with standard

Compound	Free radical Scavenging Activity (%)		
	DPPH	H_2O_2	O_2-
Euphorbia hirta	58.4	62.3	63.2
Ascorbic Acid	68.7	70.0	73.2

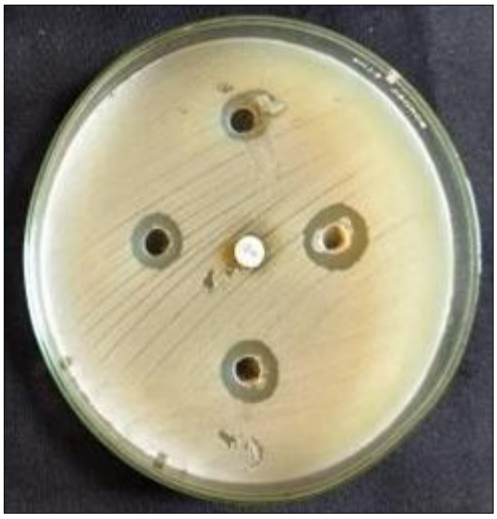

Fig 4: Antioxidant activity of Euphorbia. Hirta L by Diffusion Method

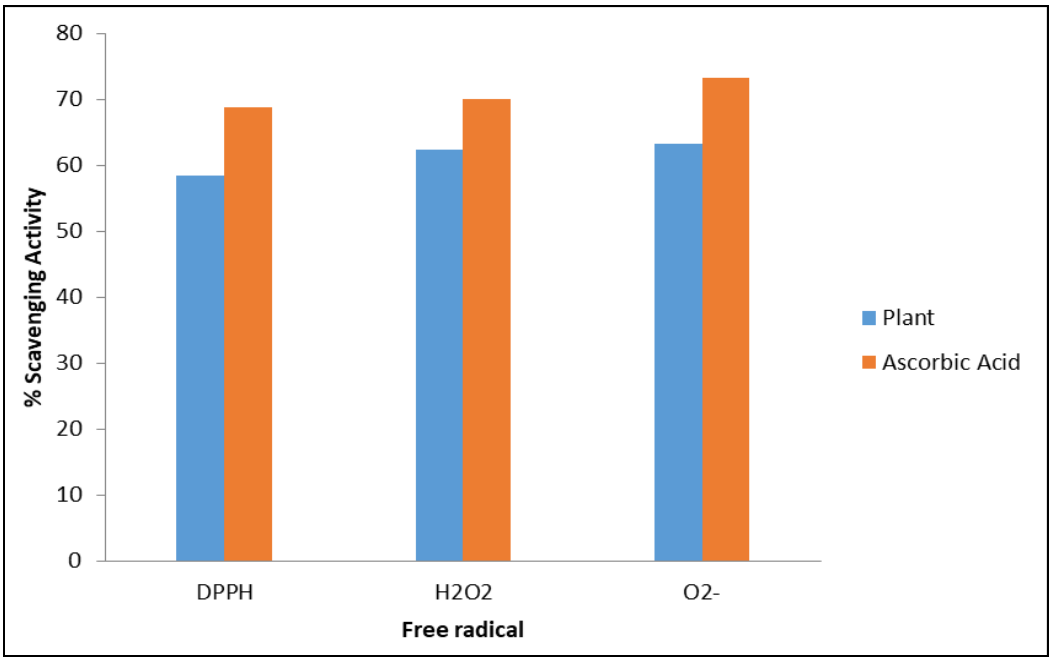

Fig 5: In vitro antioxidant activity various free radical assay method compared with Standard Ascorbic Acid.

The Antioxidant Activity of extract *Euphorbia hirta* was investigated by DPPH, H_2O_2 and superoxide radical scavenging assay method. Figure.4 shows the anti-oxidant activity of *Euphorbia hirta* compared with standard ascorbic acid. According to free radical scavenging superoxide radical shows more antioxidant activity than hydrogen peroxide radical scavenging. DPPH free radical scavenging activity shows the better antioxidant activity compare to other free radical method. It shows the 58% of activity compared to standard ascorbic acid 68%. H_2O_2 free radical scavenging activity shows the better antioxidant activity compare to other free radical method. It shows the 62% of activity compared to standard ascorbic acid 70%. Superoxide free radical scavenging activity shows the best antioxidant activity compare to other free radical method. It shows the 63% of activity compared to standard ascorbic acid 73%.

Conclusion

The extract of *Euphorbia hirta*.L showed potential antioxidant activities were tested DPPH, H_2O_2, O^{2-} free radical assay method. It shows more efficient antioxidant activity by in vitro antioxidant studies using methodologies reveal that *Euphorbia hirta*.L has potential biological action when compared to established medicines. *Euphorbia hirta* display remarkable excellent activities because it includes various phytochemicals such as alkaloids, flavonoids, phenolic compounds, and terpenoids it has been used in all biological investigations.

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