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Phytochemical and Antioxidant Activities of Methanolic Extract of the Plant *Euphorbia thymifolia* Linn

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Abstract

The present study was designed for phytochemical and antioxidant activity of methanolic extract of the whole plant *Euphorbia thymifolia* linn. Belonging to the family Euphorbiaceae. It cures many diseases. The phytochemicals present in this methanolic extract were studied by standard Protocols and the *invitro* antioxidant activities were studied using different methods i.e., H_2O_2 scavenging activity, Reducing power method and Phosphomolybdinum method. These three methods show significant activity with standard drugs.

Keywords: *Euphorbia thymifolia* linn.; Phytochemical screening; Antioxidant activities

Introduction

Euphorbia thymifolia linn. belongs to the family Euphorbiaceae. The plant is used as laxative, diuretic, antihelmintics, constipation, skin diseases, bitter and antiviral. Phytochemical screenings, antibacterial activity and Physico chemical constants of ethanolic extract of *Euphorbia thymifolia* linn. [1]. Review of literature did not reveal any information on studies of methanolic extract of this plant. Hence, in the basic present work in phytochemical screenings and antioxidant activities of the methanolic extract of whole plant of *Euphorbia thymifolia* Linn. was studied.

Materials and Methods

Plant material

The Whole parts of *Euphorbia thymifolia* linn. was collected from local areas of Anantapur and was identified and authenticated by Dr. Reddy Raju Venkatapathi Raju, Botanist, SK University, Anantapur, Andhra Pradesh, India. A voucher specimen has been preserved in our laboratory for future reference. The Whole plant was shade dried under reduced pressure, powdered by a mechanical grinder and were passed through 40-mesh sieve and stored in airtight container for further use.

Preparation of extract

About 1 kg of the powdered plant material was exhaustively extracted using Methanol solvent in a Soxhlet extractor. The extract was concentrated and dried by using Rotavapour (Heidolph) under vacuum. The yield of the concentrated Methanolic extract was 11.8%.

Preliminary phytochemical screening

The crude drug was dissolved in distilled water and subjected to preliminary phytochemical screening. The study was carried out by using standard procedure described by Kokate [2] and Harborne [3].

Scavenging of hydrogen peroxide

A solution of H_2O_2 (20 mm) was prepared in phosphate buffer saline (PBS, pH 7.4). Various concentration (10 µg-100 µg) of standard and extracts was prepared, 1 ml of the extract and standard was dissolved in methanol in a separate volumetric flask and to this solution 2 ml of H_2O_2 solution in PBS was added, the absorbance was measured at 230 nm, after 10 min against blank solution. The % inhibition of OD was calculated by the formula.

The percentage inhibition was calculated by using the formula.

$$\%\text{inhibition} = A_{control}\text{-}A_{sample}/A_{control} \times 100\%$$

Determination of reducing power

Method based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in anti-oxidant activity [4]. Different concentration of extracts (20 µg-100 µg) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 m; pH 6.6) and 2.5 ml of potassium ferricyanide $[K_3Fe(CN)_6]$ (1%), the resulting mixture was incubated at 50°C for half an hour. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) were added. The absorbance was measured at 700 nm in UV-Vis spectrophotometer against blank. Increasing of the reaction mixture indicates increasing reducing power [5]. The % inhibition of OD was calculated by the formula.

The percentage inhibition was calculated by using the formula.

$$\%\text{inhibition} = A_{control}\text{-}A_{sample}/A_{control} \times 100\%$$

Estimation of phosphomolybdenum

In this method quantitative determination of anti-oxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. An aliquot of 0.3 ml of sample solution containing a reducing species in DMSO was combined in a test tube with 3 ml of reagent solution (0.6 m H_2SO_4, 28 mm sodium phosphate and 4 mm ammonium molybdate) then the tubes were covered with aluminium foil and kept in a water bath at 95°C for 90 min. Then the samples were cooled to room temperature, absorbance of each solution was measured at 695 nm against blank. The total anti-oxidant was expressed as mm equivalent to DMSO [6]. The % inhibition of OD was calculated by the formula. The results are tabulated in Tables 2-4 and (Figures 1-3) respectively.

The percentage inhibition was calculated by using the formula.

$\%inhibition = A_{control}\text{-}A_{sample}/A_{control} \times 100\%$

Results and Discussion

Phytochemical screening of Methanol extract of *Euphorbia thymifolia* linn. reveals the presence of Alkaloids, carbohydrates, glycosides, saponins and flavonoids as majorly compounds tabulated in Table 1. Antioxidant studies prove to show potent antioxidant activity for Methanol extract of Peristrophe paniculata Brummitt. Presence of the flavonoids in past reported to possess antioxidant properties [7]. Hydrogen peroxide scavenging Methanol, extract showed high activity, the reducing anti-oxidant activity shows the reducing property of the plant extracts on potassium ferricyanide. The absorbance is directly proportional to the reduction of ferric ions to ferrous ions, thus an increase in the absorbance denotes the reducing property of the plant extracts, Methanol extract show lesser anti-oxidant activity. In Phosphomolybdenum method the extract shows potent anti-oxidant activity. The extract shows significant activity in all three methods. The results are shown in Tables 2-4 and Figures 1-3.

Type of Phyto chemical constituents	Methanolic extract of *Euphorbia thymifolia*
Alkaloid	+ve
Carbohydrates	+ve
Glycosides (Anthraquinone, cardiac)	+ve
Saponin glycosides	+ve
Proteins	-ve
Volatile oils	-ve
Fats and fixed oils	-ve
Steroids	-ve
Flavonoids	+ve
Saponins	+ve
Tannins	-ve
+ve: Indicates the presence of Phytochemical constituents.	
- ve: Indicates the Absence of Phytochemical constituents.	

Table 1: Preliminary phytochemical screening tests.

Conc.	Control	Standard	ETM
		Absorbance	Absorbance
10 µg	0.982 ± 0.01	0.680 ± 0.03 (30.75)	0.355 ± 0.02 (63.84)
25 µg	0.982 ± 0.01	0.696 ± 0.02 (29.12)	0.429 ± 0.02 (56.31)
50 µg	0.982 ± 0.01	0.728 ± 0.01 (25.86)	0.495 ± 0.03 (50.50)
75 µg	0.982 ± 0.01	0.746 ± 0.01 (24.03)	0.532 ± 0.01 (45.82)
100 µg	0.982 ± 0.01	0.792 ± 0.01 (19.34)	0.613 ± 0.01 (37.57)

Table 2: Anti-oxidant activity of solvent extract of *Euphorbia thymifolia* linn. using H_2O_2-Scavenging Profile Method.

Conc.	Control	Standard	ETM
		Absorbance	Absorbance
20 µg	0.776 ± 0.01	0.530 ± 0.05 (31.70)	0.572±0.02 (26.28)
40 µg	0.776 ± 0.01	0.455 ± 0.02 (41.36)	0.595±0.01 (23.32)
60 µg	0.776 ± 0.01	0.407 ± 0.02 (47.55)	0.603±0.01 (22.29)
80 µg	0.776 ± 0.01	0.242 ± 0.01 (68.81)	0.647±0.01 (16.62)
100 µg	0.776 ± 0.01	0.233 ± 0.01 (69.97)	0.691±0.01 (10.95)

Table 3: Anti-oxidant activity of solvent extract of *Euphorbia thymifolia* linn. using reducing power method.

Conc.	Control	Standard	ETM
		Absorbance	Absorbance
20 µg	0.882 ± 0.01	0.580 ± 0.05 (34.24)	0.531 ± 0.05 (39.79)
40 µg	0.882 ± 0.01	0.595 ± 0.02 (32.53)	0.549 ± 0.04 (37.75)
60 µg	0.882 ± 0.01	0.627 ± 0.02 (28.91)	0.639 ± 0.04 (27.55)
80 µg	0.882 ± 0.01	0.634 ± 0.02 (28.11)	0.686 ± 0.05 (22.22)
100 µg	0.882 ± 0.01	0.683 ± 0.01 (22.56)	0.692 ± 0.06 (21.54)

Table 4: Anti-oxidant activity of solvent extract of *Euphorbia thymifolia* linn. using Phosphomolybdenum Method.

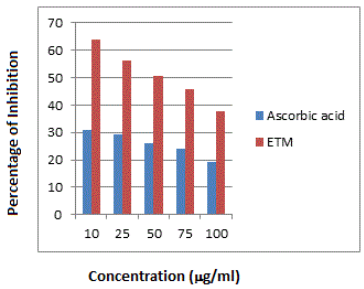

Figure 1: H_2O_2 Scavenging method.

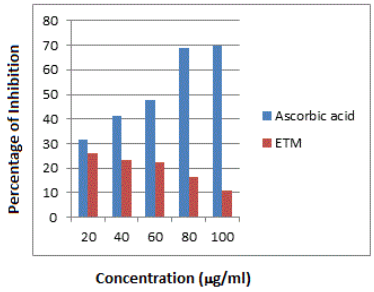

Figure 2: Reducing power method.

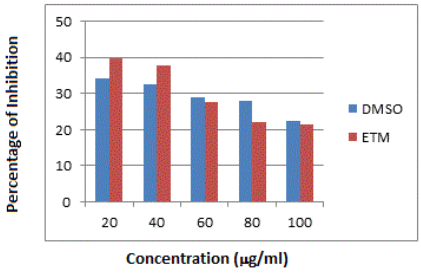

Figure 3: Phosphomolybdenum Method.

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