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Determination of Total Phenolic Content and DPPH Radical Scavenging Activity of *Euphorbia hirta*

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ABSTRACT

The present study was aimed to evaluate the total phenolic content present in entire herb of *Euphorbia hirta* by using Folin ciocalteau procedure and to determine its antioxidant property by using DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging method. The content of phenolic compounds indicated that these compounds contribute to the antioxidant activity. The *Euphorbia hirta* can be regarded as promising entrant for natural plant sources of antioxidants with high value.

Keywords: Total phenol, DPPH, Antioxidants, Folin ciocalteau, Antioxidant activity

1. INTRODUCTION

Phenol flavonoids are well known for their antioxidant activity. The term antioxidant refers to the activity of various vitamins, minerals and other phytochemicals to defend against the damage caused by reactive oxygen species(ROS). By their capability to respond with and damage many structures in the body. ROS are involved in various physiological processes and disease such as cancer. They are natural disease preventing and antiageing substances.[1] Phenolic compounds are secondary metabolites that synthesize in plants. According to WHO more than 21000 plants are used for medical treatment in all over the world.[2] Phenolic compounds are antioxidants with redox properties that allow them to act as singlet oxygen quenchers, reducing agents and hydrogen donators.[3] Present study deals with the plant *Euphorbia hirta* (family Euphorbiaceae) synonym of *E pilulifera* which is commonly known as asthma weed. The plant is used for various actions likewise, sedative antidiabetic analgesic, antipyretic, antimicrobial, anxiolytic, wound healing etc and antioxidant property[4,5,6,7]. Hence the objective of this investigation was to evaluate total phenolics content in ethanolic extract of *Euphorbia hirta* and to examine the DPPH radical scavenging activity.

2. MATERIALS AND METHODS

2.1 Plant material

The whole plant of *Euphorbia hirta* was collected from various localities of Bhopal (M.P) during the month of March. The plant was identified and authenticated by Dr. H.B Singh, raw material, Herbanium and Museum NISCAIR, New Delhi with Ref No. NISCAIR / RHMD /Consult /2013/2214/220.

2.2 Chemicals

Ethanol, DPPH, folin ciocalteu's phenol reagent, Ascorbic acid, distilled water and sodium carbonate. All the solvents and chemicals were of analytical grade.

2.3 Preparation of the Plant Extracts

Whole plant of *Euphorbia hirta* was dried in shade. The shade dried plant material was crushed to get a coarse powder. About 250g of dried powder was extracted with ethanol by soxhlet apparatus. Extract was concentrated till extract become only 15 to 20ml. Resulting crude extracts was collected and kept in vacuum oven to remove final traces of solvent. The ethanolic extract on dry basis was found to be 80g.

2.4 Determination of total phenolic content

The total phenolic content was determined by using Folin ciocalteu reagent method as described by Singleton in 1965 [8] with little modification. For determination of phenolic content from ethanolic extract 1ml of diluted extract 1:10 mg/ml was mixed with 5ml of Folin ciocalteu reagent which is diluted with distilled water 1:10 and 4ml of Na_2CO_3 (7.5%) w/v. Left it for 15minutes.After 15min the absorbance of sample was measured at 765 nm via a double beam UV–visible spectrophotometer. Sample was analyzed in triplicates. For blank measurements 1ml water with 5 ml folin ciocalteu and 4 ml Na_2CO_3. For standard measurement and comparison gallic acid was taken as standard. Various dilutions were made of both standard and sample. Different concentrations of the standard gallic acid and extract solution viz. Weight of standard = 3.8mg in 10ml (100µl) solution of folin and sodium carbonate. i.e. concentration is 380 µg.

Weight of sample (extract) is .0286mg in 10ml (100µl) solution of folin and sodium carbonate i.e. concentration is 2860µg (Table 1 and Fig 1.).

Concentration of sample in µg = Conc. of Gallic acid x
Absorbance of sample / Absorbance of Gallic acid

Thus in total extract i.e. 80g total phenolic content value is 2.30%.

2.5 Determination of Antioxidant property by using DPPH radical scavenging method

DPPH assay was performed according to method described by Nitin verma in 2008 [9] with some modification. DPPH is a stable free radical 1, 1 diphenyl -2 – picryl hydrazyl. DPPH estimated the hydrogen donating or the radical scavenging ability of the extract. Briefly 3ml of sample extract and standard of various concentrations (10, 20, 30, 40, 50 µg/ml) were added with 1ml of DPPH. 0.1mM solution of DPPH is prepared in methanol solution. Here, the standard compound used was L-ascorbic acid. Mixture was shaken vigorously and allowed to stand at room temperature for 30 minute. Then the absorbance was recorded at 517nm by an UV visible spectrophotometer and the percentage inhibition activity was calculated (Fig 2).[10]

Molecular weight of DPPH is 394.32g/mol i.e. 0.0003943 dissolved in 10ml methanol. 0.1Mm solution is formed. From this taken 1ml of DPPH with 3ml of Standard / Extract. Standard stock can be prepared by dissolving 10mg Ascorbic acid in 100ml distilled water. Concentration become 100µg. From this stock various dilution are prepared viz. 10,20,30,40,50 µg/ml.

Weight of Standard = 14.2 mg
Weight of Sample /Extract =13.2 mg (Table 2 & Fig 2)

DPPH radical scavenging activity (%) = [(Abs control – Abs sample) /Abs control] x100

Here, Ascorbic acid was used as reference compound. Hence DPPH scavenging activity shown by extract is 15.2%.

Table 1. Concentration of standard (µl) Vs Absorbance

S.No.	Conc.in µl	Conc.in µg	Absorbance
1	100	38	0.0588
2	200	76	0.2531
3	300	114	0.3884
4	400	152	0.4657
5	500	190	0.5316

Absorbance of sample is 0.2048 at 66 µg

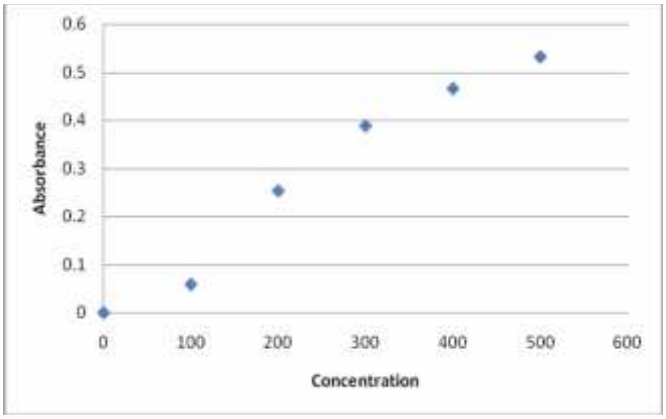

Fig 1: Concentration of standard (µl) Vs Absorbance

Table 2. Concentration of standard (µg) Vs Absorbance

S.No.	Conc.in µg	Absorbance
1	10	0.094
2	20	0.217
3	30	0.325
4	40	0.371
5	50	0.544

Absorbance of sample at 20 µg is 0.219

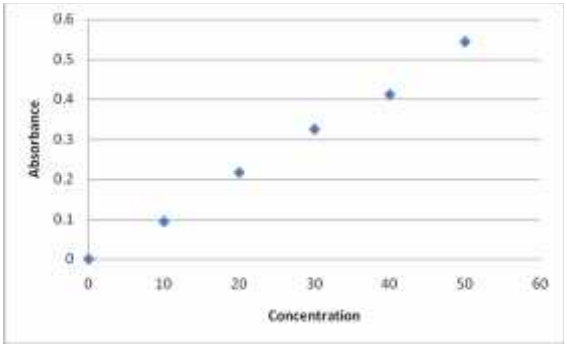

Fig 2: Concentration of standard (µg) Vs Absorbance

3. RESULT & DISCUSSION

Ethanolic extract of *Euphorbia hirta* were prepared to examine the total phenolic content and for antioxidant activity measurement the yield of extract obtained from 250g of dry plant material was found to be 80g. The total phenolic contents in the examined extract using the Folin ciocalteu reagent ranged to 2.30%. The total phenolic content in plant extracts depends on the type of extract that is the polarity of solvent which is used in extraction. High solubility of phenols in polar solvents provides concentration of these compounds in the extracts obtained using polar solvents for the extraction[10] and the free radical scavenging by DPPH assay was found to be 15.2%.

4. CONCLUSION

Based on the results of our study we conclude that the significant importance of the species *Euphorbia hirta* for its therapeutic use. Based on this information, it could be concluded that this plant is natural sources of antioxidant substances of high importance in preventing or slowing oxidative stress related degenerative diseases. However further studies of this plant species should be directed to bring out *in vivo* studies of its medicinal active components in order to prepare natural pharmaceutical products of high value.

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