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SCREENING OF *EUPHORBIA HIRTA EXTRACTS* FOR ANTIOXIDANT ACTIVITY

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ABSTRACT

Objective: To investigate the total phenol content, the total flavonoid content and antioxidant properties of Ethanolic and Aqueous extracts of the whole plant of Euphorbia hirta. **Methodology:** We determined the total phenolic and flavonoid content by using Folin-Coicalteu assays and Aluminium chloride colorimetric method. In addition, the antioxidant activity of two extracts was tested by DPPH (1, 1'-diphenyl-1-picrylhydrazyl) free radical scavenging assay, hydroxyl radical scavenging assay and superoxide radical scavenging assay. **Results:** The total phenol contents in aqueous and ethanol extracts were calculated as 277.64±2.46 and 293.74±2.48, mg/g, respectively. Analysis of the phenolic contents in these extracts of *Euphorbia hirta* have revealed that the ethanolic extract contained the maximum phenolic content in terms of gallic acid equivalents, than aqueous extract. The total flavonoid content in aqueous and ethanol extracts were calculated as 27.37±0.39 QE mg/g and 41.32±1.69 QE mg/g. The flavonoid contents of ethanol extract had the higher amount of flavonoid contents than aqueous extract. The antioxidant activity of two extracts was tested by DPPH (1,1'-diphenyl-1-picrylhydrazyl) free radical scavenging assay, hydroxyl radical scavenging assay and superoxide radical scavenging assay. From this result it states that ethanolic extract has a strong percentage of inhibition with lower IC_{50} value than aqueous extract. These results indicate that there was a direct correlation between total phenol and antioxidant activity. **Conclusions:** The result concludes that the whole plant of Euphorbia hirta posses antioxidant activity.

KEYWORDS: Antioxidant activity, Total phenol content, Total flavonoid content, *Euphorbia hirta,* Aqueous extract, Ethanolic extract.

INTRODUCTION

Natural products including plants have been the basis of treatment of human diseases for thousands of years. Almost 60% of all new chemical entities introduced world wide as a drug in last two decades may be traced to or inspired by natural product.[1] Many plants contain substantial amount of antioxidants like Vit C, Vit. E, carotenoids, flavonoids and tannins. These phytoconstituents can be utilized to scavenge the excess free radicals from the human body.[2] Approximately 1-3% of the consumed oxygen in living cells is converted to several harmful reactive oxygen species (ROS) and free radicals under physiological conditions. The major site of ROS production in the cell is a mitochondrial respiratory chain. Therefore, mitochondria are suggested as prime targets for oxidative damage.[3] Reactive oxygen species (ROS) are greatly reactive molecules, which includes superoxide radicals (O_2-), hydrogen peroxide (H_2O_2), peroxyl radicals (Roo-), reactive hydroxyl radicals (OH-) as most common reactive oxygen species and nitric oxide, peroxy nitrite as the nitrogen derived free radicals. An antioxidant can be defined as "Any substance that is capable of delaying or inhibiting the oxidation of the oxidizable substrate when present in a low concentration compared to that of an oxidizable substrate.[4-5] To inhibit the oxidative chain reaction, adequate antioxidants are supplied as natural or synthetic food additives to humans and animals as natural or as septic preventive pharmaceuticals. However, synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl O-toluene (BHT), propyl gallate [PG], metal chelating agents (EDTA), tertiary butyl hydroquinone (TBHQ), and Nordihydroguaiaretic acid (NDGA) have many side effects. Hence, plant based natural antioxidants are supplied in the form of leafy vegetables, fruits, seeds, cereals and algae, acts as a good source to produce a wide range of natural antioxidants. Plants contain a wide variety of natural antioxidants termed 'Phytochemicals'. These include flavonoids, classes of phenolic compounds, carotenoids, terpenoids, vitamin C & E and polyphenols. Among the phytochemicals of plants, phenolics are the most abundant natural antioxidant acts as reducing agents, hydrogen donators, free radical scavengers and singlet oxygen quenchers and as cell saviors. The relationship between level of a phenolic compound and antioxidant

potential of plants has been reported previously. The phenolic compound with antioxidant activity is predominantly due to their redox properties.[6] In India and other parts of the world, several ethno botanical surveys were performed and result have shown that *Euphorbia hirta* Linn. belonging to Euphorbiaceae family is a ruderal plant, known and widely used in the treatment of various ailments such as ear aches, boils and promotes wood healing AKE-ASSI (2011). Also this species is known to have analgesic, antipyretic, expectorant, anti-syphilis and antiviral (AKEASSI, 2011). This species has been the subject of many scientific studies highlighting various activities. The majority of these works concern antimicrobial, anti malarial, antioxidants, anti-diabetic, anti-hypertensive, anthelmintics, anti-allergic, anti-tumour, anti-anxiety, sedative and immunomodulatory, anti-inflammatory, anti- tetanus, galactogenic, diuretic, cardiovascular and vasodepressor activities (LANHERS et al., 2005). LANHERS et al. Recently, modern pharmacological investigations showed that *E. hirta* and its active components possessed wide pharmacological actions, such as anti-inflammatory, antifungal, antibacterial, anti diarrheal, sedative, anxiolytic, analgesic, antipyretic, antioxidant, anti-asthmatic, antitumor, antimalarial, larvicidal, diuretic, and increases electrolytes, among others (Lanhers et al, 1990; Lanhers et al, 1991; Johnson et al, 1999. The present work has been design to evaluate total phenol content total flavonoid content, and Antioxidant activity of ethanolic and aqueous extracts of whole plant of *Euphorbia hirta.*

2. MATERIALS AND METHODS

Plant material

Euphorbia hirta the whole plant was collected, from in and around Palakkad, Kerala, India and identified and authenticated by Dr. P Jayraman, Director of plant Anatomy Research Centre, and Chennai. The plant authenticated specimen is deposited in the Department of Pharmacognosy, Sanjo College of pharmaceutical studies, Palakkad. Authentication specimen number is SCPS/P.COG/004/2017 the fresh leaves and stems were kept for shade drying. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Preparation of Extract

The powdered whole plant of *E.hirta* was subjected to batch extraction in Soxhlet apparatus. The solvent used as ethanol and water. The powdered crude drug was evenly packed in Soxhlet extractor for extraction with solvent. The temperature was maintained on an. electric heating mantle with thermostat control. Appearance of brown solvent in the siphon tube was taken as the termination of extraction. The filtrate was concentrated using a rotary evaporator at low temperature (40-45^{0}C) and pressure and percentage yield was calculated.[7]

Chemicals

Carbonate solution, Gallic acid, Aluminium chloride, Potassium acetate, Quercetin, DPPH(2,2 diphenyl-1-picrylhydrazyl), Ascorbic acid, Methanol, BHT (butylated hydroxyl toluene), Potassium dihydrogen phosphate, Potassium hydroxide, Deoxy ribose, Ferric chloride, Hydrogen peroxide, hydrochloric acid, EDTA (ethylene diamine tetra acetic acid), TCA (trichloro acetic acid), TBA (thio barbituric acid). All the chemicals used were AR grade and were obtained from SD fine chemicals, New Delhi.

Determination of total phenol content

The total phenolic contents of the ethanol and aqueous extracts of *Euphorbia hirta* were estimated using the Folin-Ciocalteau reagent method as described by Mc.Donald S et al & Miliauskas.G et al.[8-9] Each extract of 0.5 mL [1.0 mg/L] in methanol and 0.1 mL of Folin – Ciocalteu reagent [10%v/v] was mixed, added into the test tubes. The mixture was allowed to stand for 15min. To the mixture, 2.5 ml of sodium carbonate solution was added and mixed well. The mixture was further incubated for 30 min at room temperature. The total phenolic content was determined at 760 nm using UV-VIS spectrophotometer. A calibration curve was made by preparing 1 mL aliquots of 100, 200, 300, 400 and 500 µg/mL solutions of Gallic acid the results were expressed as gallic acid equivalents in milligram per gram [mg GAE/g] of the sample. Absorbance = mx + a. Gallic acid acts as a common reference compound. The estimation was carried out in triplicate. The amount of phenol in plant extracts was calculated by the following formula: **C = C1 x V/m**

Where,
C = Total Phenolic content mg g-1 of extracts in GAE [Gallic acid equivalent];
C1 = The Concentration of Gallic acid established from the calibration curve mg/ml
V = The Volume of extract solution [ml]
m = The Weight of the plant extract [g].

Determination of total flavonoid content

The total flavonoid content of the ethanolic and aqueous extracts of *E.hirta* were determined by the aluminium chloride colorimetric method.[10] Extracts of *Euphorbia hirta* (0.5 mL of 1:10 g/mL) in methanol; 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water constitutes the reaction mixture. The mixture was allowed to stand for 30 min at room temperature without any disturbance. The absorbance of all the mixtures was measured using UV-VIS spectrophotometer at 415 nm against blank containing water instead of the sample. Quercetin acts as a standard compound for the quantification of total flavonoid. A standard curve was prepared with quercetin of known concentrations. The total flavonoid content was determined as mg/g of quercetin equivalents [mg QE/g] of plant material in

triplicate. The total content of flavonoid in plant extracts was calculated by the following formula:

C = C1 x V/m

Where,
TC= Total flavonoid content mg g-1 of extracts in quercetin equivalent;
C1 = The Concentration of quercetin solution established from the calibration curve gm/L;
V = The Volume of extract solution [mL]
m = The Weight of the pure plant extract [g].

Antioxidant activity: *In-vitro*

DPPH radical scavenging assay: (1,1-Diphenyl -2-Picryl hydrazyl)

DPPH radical scavenging activity of ethanolic and aqueous extracts of *E.hirta* was determined by the following method described by Blois DS.[11] DPPH; the purple colour stable free radical readily shows a maximum absorption at 517 nm and undergoes reduction by an antioxidant. DPPH [1,1-diphenyl-2-picrylhydrazyl] purple colour is converted to 1-1-diphenyl-2-picrylhydrazine, a yellow colour by reacting with antioxidants. This test provides information on the free radical scavenging potential of the *E. hirta* extracts. Different volumes [2, 4, 6, 8, 10 µL] of alcoholic and aqueous extracts were mixed with 1.0 mL of 0.1 mM DPPH radical in methanolic solution. The reaction mixture was shaken vigorously and incubated in room temperature in dark place for 30 min. After 30 mins, of reaction period the decrease in absorbance at 517nm was monitored by placing in a UV-VIS spectrophotometer. Ascorbic acid, a stable antioxidant was used as a standard reference positive control. A decrease in DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. The inhibition percentage (1%) of DPPH radical scavenging activity was calculated according to the equation.

DPPH radical scavenging activity [I%] = [Abs control –Abs sample] / [Abs control] × 100

Where Abs control and Abs sample are the absorbance values of the blank sample and of the tested samples (Aqueous, Ethanol extracts of *Euphorbia hirta*).

The antiradical activity was expressed in terms of the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50% [IC50].

The IC50 value for each extract or reference compounds was determined graphically by plotting the percentage of DPPH scavenging as a function of extract concentration.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of *Euphorbia hirta* extract was assayed by following the 2-deoxyribose oxidation method. The 2- deoxyribose gets oxidized and degraded to melandialdehyde in the presence of hydroxyl radical generated by the Fenton reaction.[12] Therefore, the hydroxyl scavenging effect of *Euphorbia hirta* extracts on hydroxyl radicals was estimated by malondialdehyde chromogen formation due to 2-deoxy-2-ribose degradation 0.2 mL potassium dihydrogen phosphate-potassium hydroxide [100 mµ], 0.2 mL deoxyribose (1.5 mM) 0.2 mL $FeCl_3$ (500 mM), 0.1 mL EDTA (1 mM), 0.1 mL ascorbic acid [1 mM] and 0.1 mL H_20_2 [10 mM] constitutes the reaction mixture. $FeCl_3$ and EDTA are mixed before adding to the reaction mixture. 0.1mL of aqueous, ethanolic extracts were mixed with the reaction mixture. The mixture was incubated for 1 hr at temperature 37ºC. To the reaction mixture, 1.0 mL of TBA (1% W/V), and 1.0 mL of TCA (2.8% W/V) was added after incubation. Then the mixture was heated on a water bath at 80ºC for 20 minutes to develop the colour. A pink colour was developed. After cooling, the absorbance of the solution was measured at 532 nm spectrophotometrically against blank. Quercetin acts as. as a standard. The hydroxyl radical scavenging activity of extracts, standard [I%] was determined and IC50 value was also calculated.

Scavenging effect (%) = [(Control absorbance – Sample absorbance) / (Control absorbance)] ×100

Superoxide Scavenging Activity

Superoxide anion scavenging activity of ethanolic and aqueous extracts of *E.hirta* was measured by the method determined by Aruoma.[13] Phenazine Metho Sulfate – Nicotinamide Adenine Dinucleotide (PMS-NADH), a non –enzymatic system generates superoxide anion radicals through the reaction of PMS, NADH and oxygen. The generated superoxide anion O_2 reduces NBT to form a blue formazan. The blue formazan formed was determined spectrophotometrically at 560nm.

0.3 ml of *Euphorbia hirta* extract at different concentration [20, 40, 60, 80, 100 µg/mL] was mixed with the reaction mixture. The reaction mixture [3.0 ml of Tris HCl buffer 100 mm, pH 7.4] constitute 0.75 mL of nitro blue tetrazolium [NBT] 300 µm, 0.75 ml of nicotinamide adenine dinucleotide [NADH] 936 µM solution and 0.75 mL phenazine methosulfate 120 µM, respectively. The absorbance of the reaction mixture was recorded at 560nm after 5 minute against control samples. BHT acts as a standard positive control.

The degree of scavenging activity was calculated as a scavenging percentage.

Inhibition (%) = [Absorbance Control –Absorbance Sample / Absorbance Control] ×100

Where Abc and Abs were the absorbance values for control and test sample, respectively. IC50 value was calculated.

RESULTS

Total Phenol Content and Total Flavonoid Content

Table 1: Total phenol content and total flavonoid content of Ethanolic and Aqueous extracts of *E. hirta.*

EXTRACTS	TOTAL PHENOL CONTENT(mg of GAE/g)	TOTAL FLAVONOID CONTENT(mg of QE/g)
Ethanol	293.74±2.48	41.32±1.69
Aqueous	277.64±2.46	27.37±0.39

Values represent mean ± SD. (n=6)
Total phenols are expressed as Gallic acid equivalent
Total flavonoid are expressed as mg of total flavonoid content/g of sample based on Quercetin as standard
GAE = Gallic acid equivalent
QE = Quercetin equivalent

The total Phenol content of *Euphorbia hirta* was measured by Folin – Ciocalteau reagent and are presented in (Fig. 1). Results of the total phenolic content of aqueous and ethanoilc extracts of *Euphorbia hirta*, expressed as milligrams of gallic acid equivalents [GAE]. (Table -1) The absorbance of various dilutions of gallic acid was found as standard curve equation with y= 0.003x + 0.020, R2 = 0.993. The total phenol contents in aqueous extract, and ethanol extract were calculated as 277.64±2.46, 293.74±2.48, respectively. Analysis of the phenolic contents in the extracts of *Euphorbia hirta* revealed that the ethanolic extract contained the maximum phenolic content in terms of gallic acid equivalents, than aqueous extract.

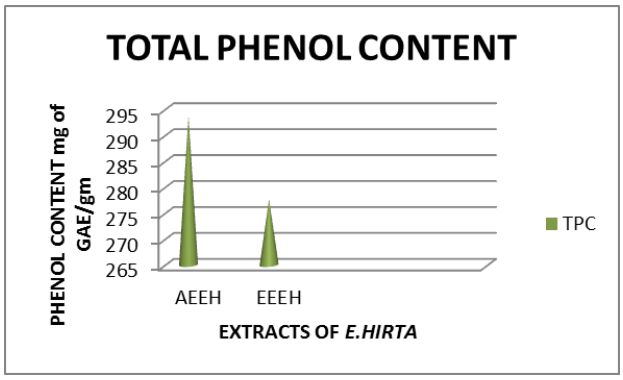

Fig. 1: Total phenol content of Ethanolic and Aqueous extracts of *E. hirta.*
EEEH- Ethanolic extract of *E.hirta,*
AEEH- Aqueous extract of *E.hirta*

The total flavonoid content of the extracts of *Euphorbia hirta* measured by Aluminium chloride colorimetric method was calculated as quercetin equivalent/g extract. (Table -1) The total flavonoid content of extracts with reference to standard curve was reported to be [Y = 0.012x + 0.008 and R2 = 0.993] 27.37±0.39 QE mg/g for aqueous, and 41.32±1.69QE mg g-1 for ethanol extracts respectively According to the results of this study, total flavonoid contents of ethanol extract had the highest amount of flavonoid contents than aqueous extract.(Fig - 2).

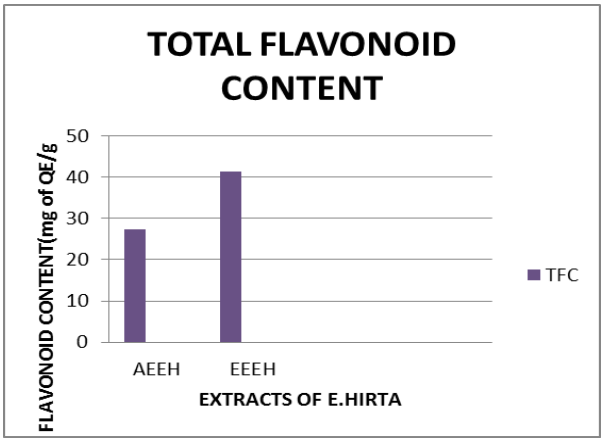

Fig. 2: Total flavonoid content of Ethanolic and Aqueous extracts of *E. hirta.*
EEEH- Ethanolic extract of *E.hirta,*
AEEH- Aqueous extract of *E.hirta*

Antioxidant Activity-(Invitro)

1) DPPH Scavenging Activity

Table 2: DPPH radical scavenging activity of extracts of *E.hirta.*

Concentration µg/ml	% INHIBITION		
	Ascorbic acid	Ethanol	Aqueous
2	48.17±0.03	44.17±0.11	24.47±0.29
4	60.85±0.07	56.78±0.06	33.53±0.18
6	70.58±0.07	66.52±0.05	44.59±0.40
8	81.84±0.09	77.77±0.06	54.69±0.17
10	90.76±0.21	86.70±0.06	64.18±0.14

Values are expressed as mean ± S.D (n=6).

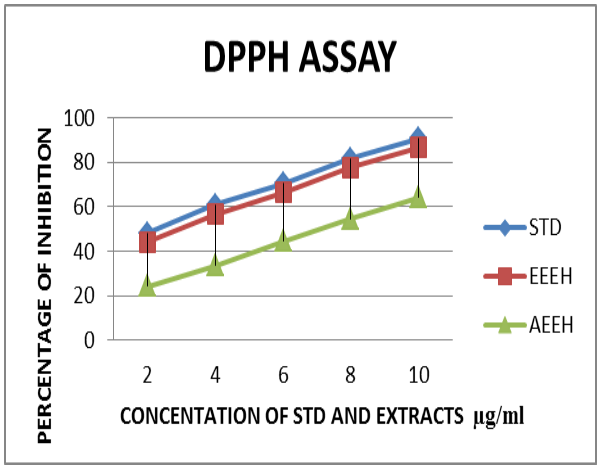

Fig. 3: DPPH radical scavenging activity of extracts of *E.hirta.*

STD- Ascorbic acid,
EEEH- Ethanolic extract of *E.hirta*
AEEH-Aqueous extract of *E.hirta*

DPPH method is based on the free radical scavenging activity of leaf extracts of *E.hirta* on DPPH radical. The reaction capability was determined by a decrease in absorbance at 517 nm. At 2 to 10 µg concentrations, the percentage inhibition of the extracts (Aqueous and Ethanol) and standard(Ascorbic acid) on DPPH radical increases with concentration. Hence, the extract exhibited a concentration- dependent radical scavenging activity that is, higher the concentration, higher the radical scavenging activity. The DPPH radical scavenging activity of *Euphorbia hirta* extracts is shown in Table 2) and Fig. 3. Results revealed that ethanolic extract 86.70±0.60% showed potent inhibition of DPPH radical compared to aqueous extract 64.18±0.14% at 10µ/ml concentration. The percentage inhibition of ascorbic acid was found to be 90.76±0.21%. The scavenging effects of samples and standard on the DPPH radical were in the following order Ascorbic acid > Ethanol > Aqueous. The concentration of extract required to scavenge 50% of the DPPH· radicals the IC_{50} values are presented in (Table 5 and Fig. 6). In general, the lower the IC50 value, the stronger the scavenging activity. In the present investigation, the IC50 value of DPPH radical scavenging activity for the aqueous extract was 7.07±0.06 µg/mL, and ethanol extract was 2.87±0.04 µg/mL whereas that of the standard, ascorbic acid exhibited 2.13±0.02 µg/mL. These observations revealed that methanol and aqueous extract have a weaker antioxidant activity than ethanol. The present result could be attributed to the presence of phenolic compounds.

2) Hydroxyl Scavenging Activity

Table 3: Hydroxyl scavenging activity of extracts of *E.hirta.*

Concentration µg/ml	% INHIBITION		
	Ascorbic acid	Ethanol	Aqueous
2	20.16±0.12	26.02±0.08	18.06±0.05
4	34.20.±0.12	39.82±0.08	32.16±0.05
6	49.33±0.17	54.06±0.11	44.65±0.11
8	56.17±0.11	63.54±0.07	59.15±0.12
10	73.77±0.22	80.05±0.11	70.05±0.11

Values are expressed as mean ± S.D (n-6).

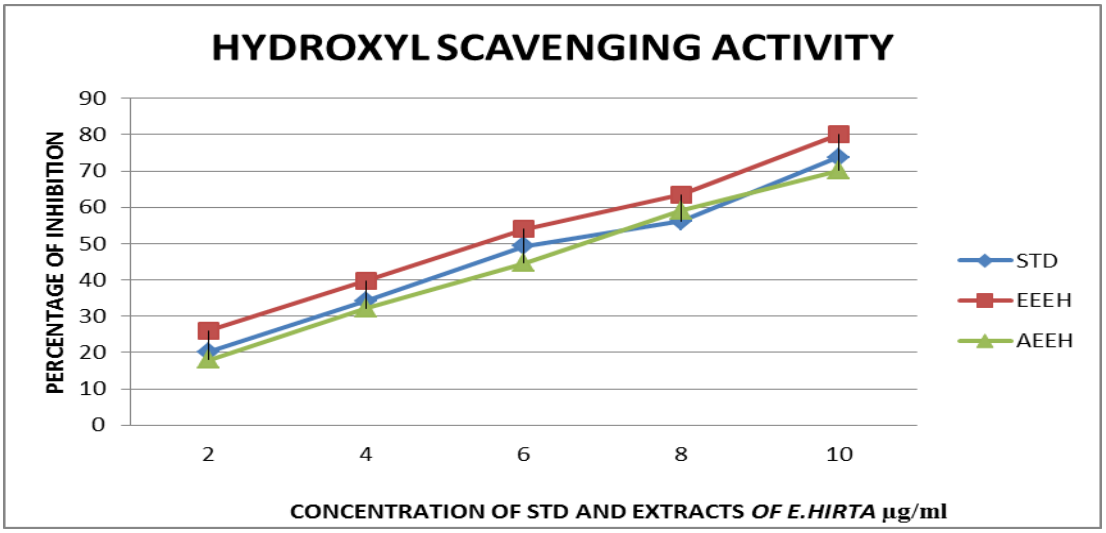

Fig. 4: Hydroxyl scavenging activity of Extracts of *E.hirta.*

STD- Ascorbic acid,
EEEH- Ethanolic extract of *E.hirta*
AEEH-Aqueous extract of *E.hirta*

Hydroxyl radical scavenging activity measures the degree of inhibition of the 2- deoxyribose degradation by the Fonton reaction. The hydroxyl radical scavenging activity of *Euphorbia hirta* extract was shown in Table 3 and Fig. 4 and the results were ranked as Ethanol extract [80.05 ± 0.11%] > Ascorbic acid [73.77±0.22%] > Aqueous extract [70.05±0.112%]. The ethanolic extract of *Euphorbia hirta* to quench the hydroxyl radical mediated deoxyribose damage seems to be directly related to the propagation prevention in the process of lipid per oxidants. The activity was assessed at a concentration of 2,4,6,8,10 µg/mL and the sample exhibits minimum activity at 2 µg/mL and maximum activity at 10 µg/mL The ethanol extract seems to be a good scavenger of reactive oxygen species. In the present investigation, the IC_{50} value of hydroxyl radical scavenging activity for ethanol, was 5.55±0.03µg/ml, and aqueous was 6.75±0.04 µg/mL (Table 5 and Fig. 6). These antioxidant activities are comparable to that of IC50 value of L ascorbic acid acid 15.72±0.04 µg/mL, which serves as a positive control. The ethanolic plant extract showed antioxidant activities with an increased absorbance, thus proving their capacity to scavenge hydroxyl radicals.

3) Superoxide radical scavenging activity

Table 4: Superoxide radical scavenging activity of extracts of *E.hirta*.

Concentration µg/ml	% INHIBITION		
	BHT	Ethanol	Aqueous
2	48.47±0.35	42.58±0.49	30.00±0.13
4	57.28.±0.18	53.78.±0.12	42.18±0.29
6	68.32±0.22	64.76±0.16	53.22±0.35
8	81.44±0.61	76.83±0.68	65.36±0.29
10	95.28±0.57	90.78±0.14	80.25±0.17

Values are expressed as mean ± S.D (n=6).

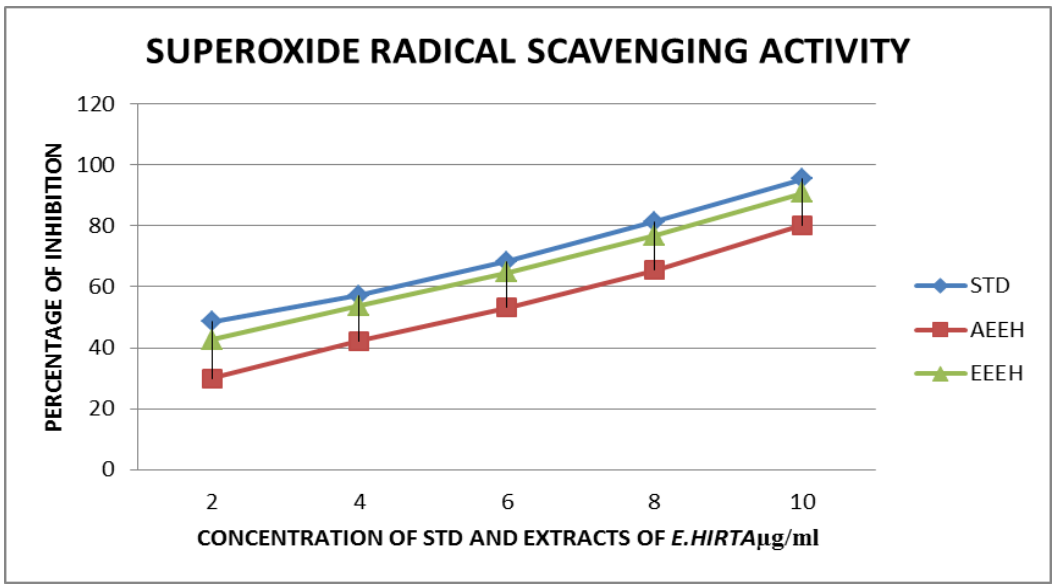

Fig. 5: Superoxide radical scavenging activity of extracts of *E.hirta*.

STD- Butylated hydroxyl toluene
EEEH- Ethanolic extract of *E.hirta*
AEEH-Aqueous extract of *E.hirta*

In this assay, superoxide ions [O_2-] are produced by phenazine methosulphate / beta – nicotinamide adenine di nucleotide which converts NBT to a reduced NBT-diformazon, a blue coloured formation, measured at 560nm. The antioxidant reduces the rate of NBT-diformazon formation, which leads to a declined absorbance. Ethanolic extract of *Euphorbia hirta* displayed a superior superoxide scavenging activity with an IC50 value 3.36±0.03 µg/mL than aqueous extract of *Euphorbia hirta*. Ethanol extract at 2, 4, 6, 8 and 10 µg/mL concentration produced 42.58±0.49, 53.78±0.12, 64.76±0.168, 76.83±0.068, 90.78±0.14 percentage of inhibition. The aqueous extract showed a inhibition of 80.25±0.17%, at the concentration of 10 µg/mL with a IC50 of 5.32±0.04 µg/mL. The superoxide scavenging activity of an extract of *Euphorbia hirta* and standard BHT is shown in Table 4 and Fig. 5. BHT was used as standard and produced 95.28±0.57% inhibition at the concentration of 10 µg/ml. IC50 value of BHT was found to be 2.58±0.05 µg/ml (Table 5 and Fig. 6).

The superoxide scavenging activities of samples and standard exist in the order of Butylated Hydroxytoluene > Ethanol > Aqueous.

Table 5: Antioxidant activities of the *E. hirta* extract on DPPH, OH- and So2.

Standard/Extracts	IC_{50} VALUE µg/ml		
	DPPH	Hydroxyl Radical Assy	Superoxide Radical Assay
STD	2.13 ±0.02	6.26±0.04	2.58±0.05
Aqueous extract	7.07.±0.06	6.75.±0.04	5.32±0.04
Ethanolic extract	2.87±0.04	5.55±0.03	3.36±0.03

Values are expressed as mean ± S.D (n=6).

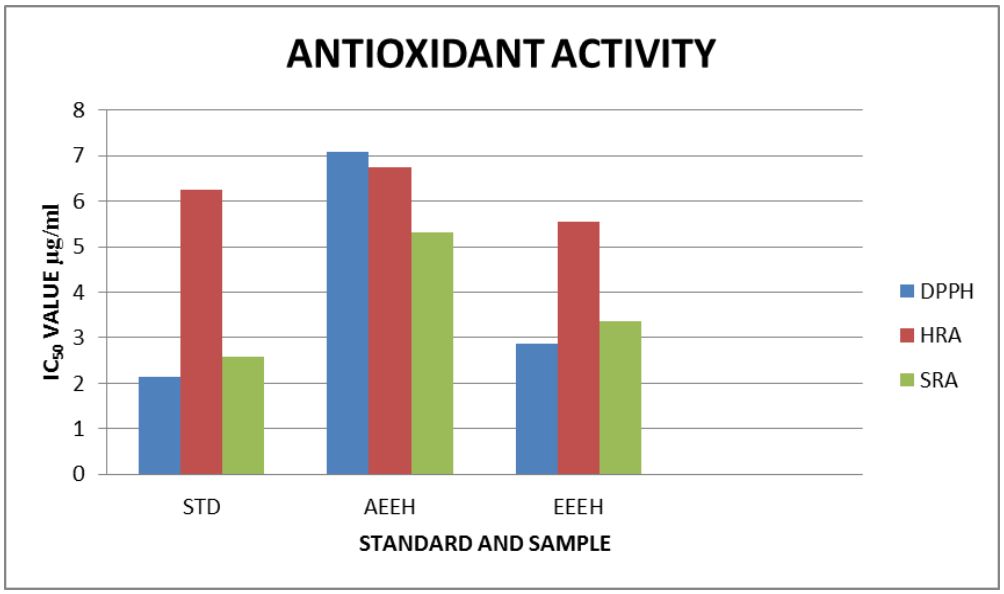

Fig. 6: Antioxidant activities of the *E. hirta* extract on DPPH, OH- and So2.

DPPH- *1,1*-Diphenyl -2- Picryl hydrazyl assay
HRA- Hydroxyl scavenging radical assay
SRA-Superoxide radical assa

DISCUSSION

DPPH, a stable synthetic free radical has been widely used to determine antioxidant activity of natural compound.[14-21] The DPPH method is an ideal method because it is fast, easy and consistent and also does not disintegrate with water, ethanol. In the present study, *Euphorbia hirta* was screened for its antioxidant and radical scavenging activity by DPPH.[22] DPPH scavenging assay is based on the reduction of methanolic DPPH solution in the presence of hydrogen-donating antioxidant, leads to the formation of a non-radical form DPPH-H (Diphenyl picrylhydrazine). There occur a change in colour from purple to yellow and this is proportional to the concentration and scavenging potential of the extract[23] in the term of hydrogen donating ability.[24] The DPPH free radical has its maximum absorbance at 517 nm and was measured by spectrophotometrically. The decrease in absorbance of DPPH at 517 nm[25] indicates a significant antiradical activity of plant extracts. Ascorbic acid used as a standard. Lower IC_{50} indicates a higher antioxidant acivity with DPPH radical scavenging activity donate hydrogen[26] to free radicals, particularly to the lipid peroxides or hydrogen peroxides radicals, major propagators of the chain autoxidation of lipids, to form a nonradical species, results in the lipid per oxidation propagation phase inhibition. Here, IC_{50} means the concentration of sample required to scavenge 50% DPPH radicals in the specified time. The IC_{50} value of *Euphorbia hirta* leaves extracts varied from 2.87±0.04 µg/mL to 7.07±0.06 µg/mL and that of the standard 2.13±0.02 µg/mL. Different parts of *Cichorium intybus* L[27] hydro alcoholic extract of chicory (*Cichorium intybus* L.) leaves,[28] ethanol extract of *Diospyros kaki* L. leaves,[29] ethanol extract of *Desmodium gangeticum* leaves,[30] also showed the similar results in leaves with higher DPPH radical inhibition and lower IC_{50} value representing a best free radical scavenging activity in accordance with our findings. However, the hydrogen donating capabilities were not the same among the *Euphorbia hirta* extracts. The difference in inhibition percentage was due to the difference in secondary metabolites. Similar results and the relationship between the secondary metabolites and antioxidant activity were also previously been reported by.[31-34] In the present study, among the tested extracts, ethanol showed higher inhibition percentage than the other extracts[35] also reported that the ethanol extract had maximum radical scavenging capacity of 13.86% at the lowest concentration. The ethanol extract of *Ulmus davidiana* stem bark exhibited the highest free radical scavenging activity compared to other extracts.[36] The free radical scavenging activities of various extracts of *Euphorbia hirta* depends on the ability of antioxidant compounds to

lose hydrogen and the structural confirmation of these compounds.[37] Since the extracts from this plant showed antioxidant activity it could be used as a medicine for the treatment of various diseases.

Hydrogen peroxide itself is not very reactive, but it gives rise to hydroxyl radical. Therefore, hydrogen peroxide removal is a very important process in a cell system.[38] Hydroxyl radical is short lived extremely reactive free radicals, formed in the biological systems, capable of damaging a wide range of biomolecule found in living cells, such as sugars, amino acids, lipids and nucleotides[39-40] by diffusion limited reaction. Since this radical causes damage to DNA strand, it leads to carcinogenesis, mutagenesis and cytotoxicity. Moreover, hydroxyl radicals are the strong reactive species, capable of initiating lipid per oxidation process by obtaining hydrogen atoms from unsaturated fatty acids. Therefore, it is very important to identify the compounds that have the excellent scavenging capacity to protect living systems from OH· The hydroxyl radical scavenging capacity of an extract seems to be directly lipid per oxidation.[41] Under certain pathological and stress conditions hydroxyl produces radicals are in turn involved in enormous biological damage. From the result, it is clear that the extract of *Euphorbia hirta* neutralizes the hydroxyl radical - induced deoxyribose cleavage in a concentration - dependent manner. In the present study, among the leaf extracts, IC_{50} value of ethanol extract [13.92±0.03 µg/mL] was efficient in quenching the hydroxyl radical formation than aqueous extract [16.94±0.045 µg/mL], and standard, Ascorbic acid [15.72±0.04 µg/mL]. To support our findings, Parameshwari et al.[42] had reported that the antioxidant activity of *Boerhavia erecta* ethanolic extract exhibited a concentration - dependent radical scavenging activity, compared with that of the standard compound against hydroxyl free radicals. Pardeep Sharma et al.[43] also identified a higher activity in an ethanolic extract of the green hull of Juglans regia showed a higher activity.

Superoxide a highly toxic radical, produced in all aerobic cells by several enzymatic and non – enzymatic pathways. This radical have the potential to react with biological macromolecules including DNA and thereby promotes tissue damages.[44-46] Although superoxide anion is a weak oxidant it acts as an important precursor for generation of powerful and dangerous other ROS such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which initiate free radical chain reaction.[47] Both these contribute to oxidative stress. Superoxide anion, formed from dissolved oxygen by PMS-NADH coupling reaction reduces yellow coloured NBT2+ into a blue formazon. The decrease in absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture and was measured spectrophotometrically. As the data showed, among the aqueous and ethanolic extracts of *Euphorbia hirta* at different concentration [2 – 10 µg/mL], ethanol extract exhibited good superoxide scavenging activity 3.36±0.03 µg/mL compared to aqueous extract. The IC_{50} value of standard was found to be 2.58±0.05 µg/mL. The antioxidants are able to inhibit the reduction reaction of blue NBT formation.[48] The superoxide scavenging activity of the ethanolic extract of *Euphorbia hirta* has the potential to scavenge superoxide radical ions to form stable radicals, thus terminates the radical chain reaction, as stated earlier in previous reports like Seaweeds;[49] *Boerhavia erecta*[50]; *Rheum ribes*[51]; *Achillea santolina*[52]; *Wagatea soicata*.[53] N Phenolics or polyphenols are the most abundant secondary plant metabolites that are ubiquitously present in plants and their products. These phytochemical compounds are derived from phenylalanine and tyrosine[54] and they fall into several categories. Among them, flavonoids are the chief and potent antioxidant compound.[55] The result of the present study showed that leaves of *Euphorbia hirta* are good sources of phenolic compounds. Phenolic compounds react with FCR in Folin Ciocalteau reagent method under the basic conditions and leads to dissociation of the phenolic anion, results in a phenolate anion. The produced phenolate anion reduces molybdenum oxide to a blue coloured molybdenum oxide in FCR with a maximum absorption at 750 nm. The intensity of the blue colour formation is directly proportional to the total quantity of phenolic compounds present in the *Euphorbia hirta* extracts.[56-57] The result of ethanol extract was 293.74±2.48 mg GAE/g, and the result of aqueous extract was 277.64±2.46 mg GAE /g. Total phenolic content of ethanol extract was determined higher than another extract. The results correlated well with the previous reports about total phenolic content in *Acacia species*. Flavonoids[58-59] are the naturally occurring secondary metabolites in plants shown to be highly effective scavengers, including singlet, oxygen and various free radicals. The results of *Acacia nilotica* leaves[60] also showed a maximum phenolics and flavonoids content in ethanol extracts as like as our result than other extract. Several studies in the literature report a positive correlation between antioxidant activity and the phenolic contents.[61-69] In the present result, the antioxidant activity of *Euphorbia hirta* on DPPH scavenging activity, hydroxyl scavenging activity and superoxide radical scavenging activity are mainly due to the presence of phenolic compounds as major components. These phenolic compounds act as primary antioxidant or free radical terminators. These results are in good accordance with previous studies of[70-74] which showed that high total phenolic content increases the antioxidant activity. *Euphorbia hirta* has been found to have great medicinal importance due to the presence of phenolic compounds. In this study, different solvents with different polarities have been used to extract different classes of compounds. The results showed that the ethanol extract had highest extraction yield, total phenol and flavonoid content and highest activity in all the antioxidant assays tested. These results revealed that the extracts obtained from ethanol, a polar solvent was found to have maximum activity in comparison with the other extracts obtained. This was in accordance with[75]

who reported that some species of Lamiaceae exhibited a high antioxidant activity in ethanol extract.

CONCLUSION

In conclusions, the present study indicates that *E. hirta* possess a considerable amount of both phenol and flavonoid content. Comparatively, the data obtained clearly indicates that the ethanol extract possesses higher contents than aqueous extract. Additionally, the ethanol extract was able to scavenge 1,1-diphenyl-2- picryl-2-picrylhydrazyl, hydroxyl scavenging radicals and superoxide anion radicals effectively when compared with aqueous extract. Our findings provide a basic relationship between total phenol and free radical scavenging activity. Thus, this investigation would be useful to treat and prevent the free radical damages occurring in humans worldwide. Therefore, in future, it is noteworthy to isolate and identify the active components in the ethanol extract of *Euphorbia hirta* Linn.

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