



ISSN 2320-3862

JMPS 2016; 4(3): 23-25

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Received: 04-03-2016

Accepted: 01-04-2016

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## ***In-Vitro* Antioxidant Activities of Methanolic Extract of Whole Plant of *Euphorbia Hirta* L. (Euphorbiaceae)**

**Sangeeta Devi, Davinder Kumar, Muneesh Kumar**

### **Abstract**

Plants are good source of phytochemicals such as vitamin E, vitamin C, carotenoid, flavonoids, glutathione, ascorbic acid etc. which having antioxidant properties. *Euphorbia hirta* belongs to the family *Euphorbiaceae* is a small annual herb. *E. hirta* possesses various pharmacological actions including anti-inflammatory, antifungal, antibacterial, antidiarrheal, sedative, anxiolytic, analgesic, antipyretic, antioxidant, antiasthmatic, antitumor, antimalarial, larvicidal, diuretic etc.

**Keywords:** *Euphorbia hirta*, *Euphorbiaceae*, Antioxidant, Total Phenolic Content, Total Flavonoid Content, Gallic acid

### **Introduction**

Natural antioxidants have great interest among scientist because of their anticarcinogenic and health promoting properties. Plants are good source of phytochemicals such as vitamin E, vitamin C, carotenoid, flavonoids, glutathione, ascorbic acid etc. which having antioxidant properties. <sup>[1]</sup> Antioxidants are the chemical compounds which cause delay or inhibition of oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction. <sup>[2]</sup> Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are generated as by-products of biological reaction or other factor. <sup>[3]</sup>

*Euphorbia hirta* belongs to the family Euphorbiaceae is a small annual herb. It can grow to a height of 40 cm. The stem of the plant is slender, reddish in color, covered with yellowish bristly hairs. The leaves are about 5 cm long, lanceolate, oppositively arranged and are usually greenish or reddish in color. The stem and leaves produce white or milky latex on cutting. <sup>[4]</sup>

The flowers are unisexual, small, crowded together in dense cyme and green in color. The male flowers are sessile, linear bracteoles, fringed, lack perianth, and possesses one stamen, whereas the female flowers have short pedicel, the perianth is rimmed, with superior ovary. Fruits are yellow in colour which contains three brown, four-sided, angular, wrinkled seeds. *Euphorbia hirta* L. is used in the treatment of many disease including bronchitis, skin diseases, cough, hay asthma, bowel disease, worm infestation, kidney stones, bronchial disease, to decrease lactation; as sedative, anxiolytic, analgesic, antipyretic, and as anti-inflammatory agent. <sup>[5]</sup>

*E. hirta* also possesses various pharmacological actions including anti-inflammatory, antifungal, antibacterial, antidiarrhoeal, sedative, anxiolytic, analgesic, antipyretic, antioxidant, antiasthmatic, antitumor, antimalarial, larvicidal, diuretic etc. <sup>[6]</sup>

### **Materials and Methods**

#### **Collection of Plant Material**

The Indigenous plant *Euphorbia hirta* L. were collected from different locations of Bhopal (M.P.) region. The plants were acknowledged by a senior Botanist Dr. Tayaaf Safi Principal Gandhi P.R. College Bhopal.

#### **Preparation of Extract**

Plant material was washed with water and then allowed to dry in shade for about 3 to 4 weeks. Dried plant materials were grinded by using the electronic grinder. The powder of the whole plants of *Euphorbia hirta* L. was extracted according to (Harborne and Baxter., 1995). <sup>[7]</sup> The

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dried plants sample was powered and filed into the soxhlet using petroleum ether and methanol respectively. Almost all the chlorophyll and lipid was deposited on the side of the flask and removed carefully. The extracts were stored in refrigerator till any further use.

**Antioxidant Activities:**

**Total Phenolic Content (TPC) Estimation:** <sup>[8, 9]</sup>

The amount of total phenolic content was determined by the method as reported by Chun *et. al.* (2003) <sup>[8]</sup> using Folin Ciocalteu Reagent. Gallic acid was used as a standard and the total phenolic content was expressed as mg/g gallic acid equivalent (GAE). Different concentrations i.e. 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were also prepared in methanol. Concentrations of 0.1-1mg/ml of plant leaf extract were also prepared in methanol. 0.5 ml of each extract sample was taken, mixed with 2.5ml of (a 10 fold) dilute folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate solution. The tubes were covered with paraffin and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm spectrophotometrically using methanol as blank. All determinations were performed in triplicate.

The folin-Ciocalteu reagent was found to be sensitive to reducing compounds including polyphenols. It produced a blue color upon reaction. This blue color was measured spectrophotometrically.

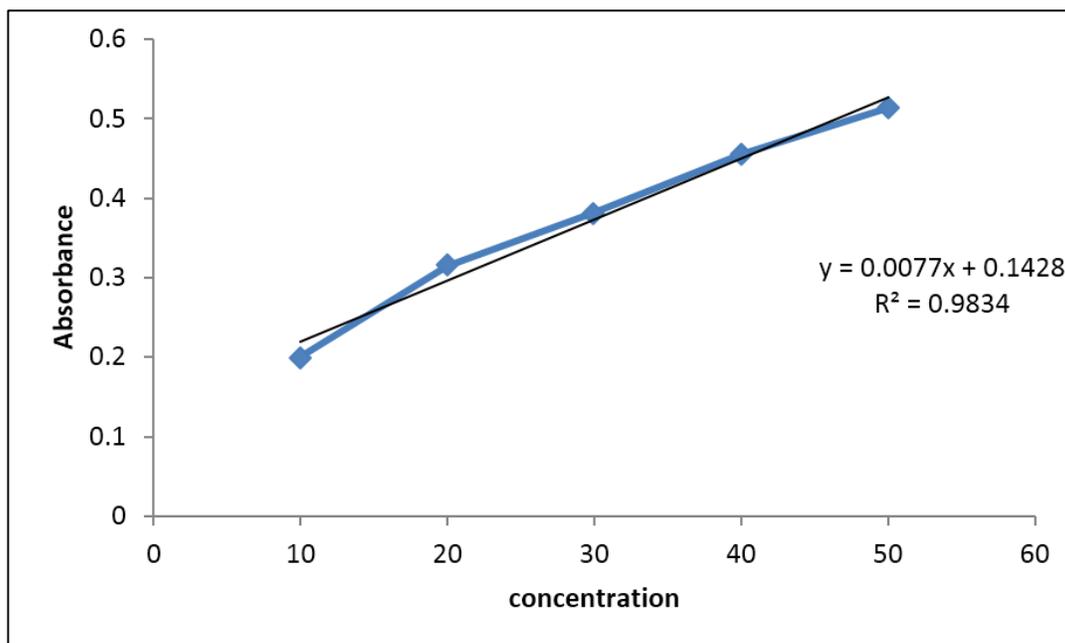
**Total Flavonoid Content (TFC) Estimation:** <sup>[10, 11]</sup>

Total flavonoids were measured by a colorimetric assay method as reported by Zhishen *et. al.* (1999). An aliquot of leaf extract sample and standard solution of rutin (1-100µg/lit) was added to a 75µl of NaNO<sub>2</sub> solution and mixed for 6 min, before adding 0.15ml AlCl<sub>3</sub> (100g/L). After 5 min, 0.5ml of 0.1M NaOH solution was added. The final volume was adjusted to 2.5ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510nm against the same mixture, without the leaf extract, as a blank. Total flavonoid content was determined as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of rutin. All samples were analysed in three replications.

**Results and Discussions**

**Table 1: Total phenolic content of methanolic extract *Euphorbia hirta* L.**

S. No.	Conc. of gallic acid (µg/ml)	Absorbance of gallic acid	Conc. of test sample (µg/ml)	Absorbance of test sample
1.	10	0.2	100	0.196
2.	20	0.315		
3.	30	0.382		
4.	40	0.455		
5.	50	0.514		
Total phenolic content- 7.714 µg/100 µg gallic acid equivalent				



**Fig 1: Graph represent regression curve of gallic acid**

**Table 2: Total flavonoid content of methanolic extract *Euphorbia hirta* Linn.**

S. No.	Conc. of Rutine (µg/ml)	Absorbance of Rutin	Conc. of test sample (µg/ml)	Absorbance of test sample
1.	10	0.025	100	0.028
2.	20	0.032		
3.	30	0.044		
4.	40	0.058		
5.	50	0.066		
Total Flavonoid content- 16µg/100 µg rutin equivalent				

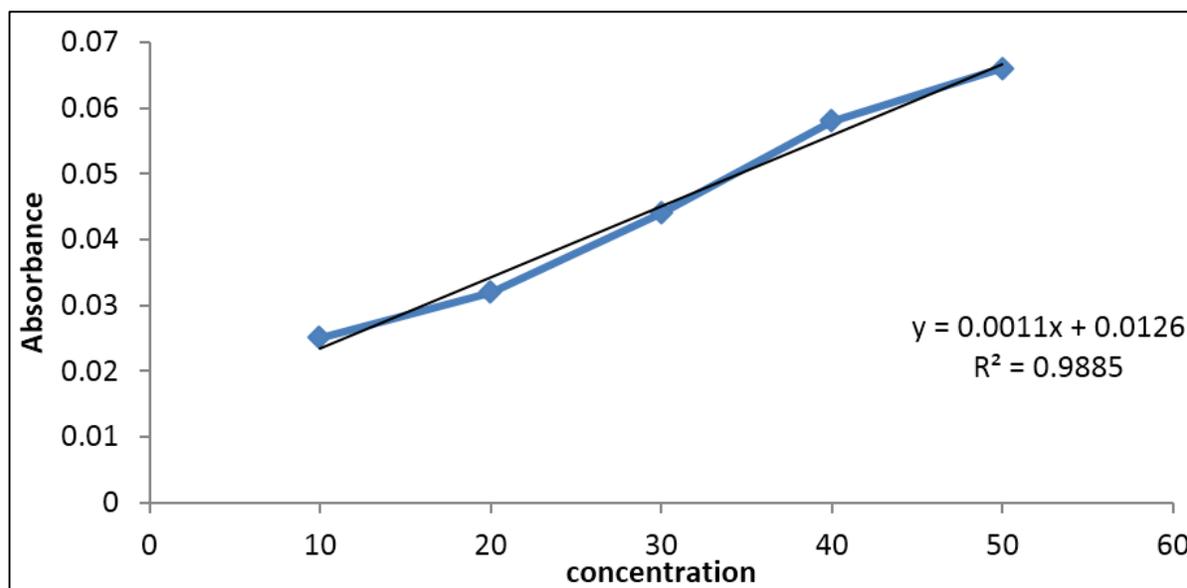


Fig 2: Graph represent regression curve of rutin

Phenolic compounds are bioactive compounds with antioxidant properties. The phenolic compounds contain hydroxyl functional groups which are responsible for its antioxidant property however other factors may also cause increase or decrease in antioxidant activity such as: presence of electron withdrawing or releasing group in the aromatic ring.<sup>[12]</sup>

On the basis of our results it is concluded that *Euphorbia hirta* exhibit potent antioxidant property. The antioxidants act as defence mechanism that protects against oxidative damage, and include compounds to remove or repair damaged against diseases. The present study thus scientifically validates and strengthens the candidature of *Euphorbia hirta* in the preparation of medicinal aids for the diseases arising due to oxidative stress.

### Conclusion

This investigation supports the view that *Euphorbia hirta* whole plant extracts are promising source of natural antioxidants. The high antioxidant activities of *Euphorbia hirta* whole plant extract appeared to be attributed to its high phenolic content and flavonoids content. Therefore, isolation and identification on individual active compounds in wax gourd seed and their *In vivo* antioxidant activities need to be investigated further.

### Acknowledgement

Authors are beholden to Principal Govt. College for Women Parade Jammu, University of Jammu, India for providing laboratory facilities for this research work.

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