IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
**Research Article**
September 2015 Vol.:1, Issue:3


# Estimation of Total Phenolic, Flavonoid Content and Antioxidant Potential of Various Parts of *Euphorbia thymifolia*

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

**Shelar Uttam B.*, Thorve Sandip S.**

*P.G. Department of Chemistry, Shri Shiv Chhatrapati College Junnar, Pune, Maharashtra, India – 410 502*

**Submission:** 29 August 2015
**Accepted:** 3 September 2015
**Published:** 15 September 2015



**Keywords:** Phenolic, flavonoid, antioxidant, root.

**ABSTRACT**

The total phenolic, flavonoid content and antioxidant potential of various part of *Euphorbia thymifolia* was reported. The antioxidant potential of different extracts (ethyl acetate, ethanol and aqueous) of leaves, aerial parts and roots were evaluated by scavenging assays such as DPPH. For total phenolic content, Folin-Ciocalteau assay was performed and determination of flavonoids was performed according to the colorimetric assay. Among all parts studied, the root extract of *Euphorbia thymifolia* reported the highest antioxidant capacity for scavenging activity and also exhibited highest total phenolic and flavonoid content as compared to leaves and fruit. All the extracts showed concentration dependent scavenging activity. The aqueous roots extract of *E. thymifolia* possesses significant anti-oxidant activity and higher content of phenolic and flavonoids. Thus it can be concluded that the aqueous roots extract of this plant might play a vital role as therapeutic agents to prevent oxidative stress and related disorders.

## INTRODUCTION

*Euphorbia thymifolia* is commonly known as laghududhika or choti-dudhi. It is a small branched, his idly pubescent, prostate annual herb, commonly known as laghududhika or choti-dudhi.[1] The leaves, seeds and fresh juice of whole plant are used in worm infections as stimulant, astringent. It is also used in bowel complaints and in many more diseases therapeutically. *E. thymifolia* is traditionally used as a blood purifier, sedative, haemostatic, aromatic, stimulant, astringent in diarrhea and dysentery, anthelminthic, demulcent, laxative and also in cases of flatulence, constipation; in chronic cough; as an antiviral in bronchial asthma and paronychia[1-2]. Antioxidants are important factor to maintain optimal cellular and human body health. Antioxidant compounds are gaining importance due to their dual role in food and pharmaceutical industries as lipid stabilizers[3]. The importance of the reactive oxygen species (ROS) has attracted increasing attention over the last decade. ROS includes free radicals such as superoxide anion radicals ($O_2^-$), hydroxyl radicals ($OH^-$), hydrogen peroxide ($H_2O_2$) and singlet oxygen ($O_2$) along with various forms of activated oxygen[4-5]. They are involved in various physico-chemical processes and diseases such as aging[6], cancer[7], atherosclerosis[8] etc. Many plants contain substantial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids, polyphenols, tannins and thus can be utilized to scavenge the excess of free radicals from the human body.

In present study, different parts of *Euphorbia thymifolia* were studied for the presence of total phenols and flavonoids contents and antioxidant potential of various parts.

## MATERIALS AND METHODS

### Plant materials

Plant materials were collected from local medicinal plant vendor. Care was taken to select healthy plant materials (Leaves, aerial parts and roots). The powder was produced using grinder mill.

**Extraction**

The 50 g of each powdered material was extracted with ethyl acetate, ethanol and water using Soxhlet apparatus. The extract was stored in a glass bottle in refrigerated condition throughout the period of experiment.

**Phytochemical analysis (qualitative)**

Phytochemical analysis was carried out by using ethyl acetate, ethanol and aqueous extract to identify the constituents using standard procedures as described by Sofowara, Trease and Harborne[9-11].

**Estimation of Total phenol content**

Total phenolic content was estimated using the Folin-Ciocalteu method of Yu et al.[12]. Extract (100µL) was mixed thoroughly with 2 ml of 2% $Na_2CO_3$. After 2 minutes 100µl of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 mins and the absorbance was measured at 743 nm against the blank. A calibration curve was established using varying concentration of gallic acid. The values were expressed in mg/g of sample.

**Estimation of Total Flavonoid content**

The determination of flavonoids was performed according to the colorimetric assay of Chang et al.[13]**.** To 1ml of extract, 3 ml of methanol, 0.2 ml of 1 M potassium acetate, 0.2 ml of 10% aluminium chloride and 5.6 ml of distilled water was added and left at room temperature for 30 minutes. Absorbance of the mixture was read at 415 nm using UV spectrophotometer. Calibration curve was prepared using quercetin as standard.

**Determination of Total Antioxidant Capacity**

The assay is based on the reduction of molybdenum (VI) to molybdenum (V) by the extract and the subsequent formation of a green phosphate Mo (V) complex at acid pH Preito et al.[14]. An aliquot of sample solution (100 µg/ml was combined with reagent solution (0.6 M Sulfuric acid, 28mM Sodium Phosphate and 4mM Ammonium molybdate). The tubes were capped and incubated in a boiling water bath at $95^0$C for 60-90 mins. The samples were then cooled at room

temperature and the absorbance was measured at 695 nm against the blank in spectrophotometer. The values were expressed as equivalents of BHT.

**Evaluation of Anti-oxidant activity by radical scavenging assay**

**DPPH radical scavenging activity**

Free radical scavenging capacity of various parts of *Euphorbia thymifolia* was determined using DPPH[15]. DPPH radical scavenging activity was done by serial dilution by taking diluted methanol (1:20) as standard. 10 ml of various diluted extracts of various concentrations (100, 200 and 400 µg/ml) were added to 1 ml DPPH solution (0.004%) and incubated for 10 mins at room temperature. Absorbance of test and reference standard, ascorbic acid was measured at 517 nm. The amount of DPPH scavenging was calculated by using the formula:

$$\% \text{ DPPH radical scavenging} = [(\text{Absorbance of control} - \text{Absorbance of test sample})/(\text{Absorbance of control})] \times 100$$

**STATISTICAL ANALYSIS**

The data from the experiments were presented as mean ± S.E.M (n=3). Student's *t*-test was used for statistical analysis. Values were considered statistically significant when $P < 0.5$.

**RESULTS AND DISCUSSION**

Reactive oxygen species [ROS] have been implicated in several diseases like cancer, diabetes, atherosclerosis and cariovascular disease. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and buytlated hydroxyanisole (BHA) which are commonly used in processed foods, possessed a number of side effects which makes limited their use as antioxidant agent. In the present study the antioxidant potential of various parts of *Euphorbia thymifolia* was performed in search of potential antioxidant agent from natural source[16-18].

**Phytochemical analysis (qualitative)**

Phytochemical analysis of *Euphorbia thymifolia* shows the presence of flavonoids, Saponins, terpenoids, phenolic compounds and alkaloids.

**Total Phenol Content**

The Phenolic contents present in extracts of various parts of *Euphorbia thymifolia* were presented in Table 1. Root shows promising results wherein the ethyl acetate shows 32.34±0.67mg/g, ethanol has 95.40±0.96 mg/g and aqueous extract shows 82.48±0.94 mg/g of phenolic content in roots. The ethanolic extracts of leaves, fruit and root have noticed higher percentage of total phenolic compounds at all tested concentrations.

**Total Flavonoid Content**

The Flavonoids contents present in extracts of various parts of *Euphorbia thymifolia* were presented in Table 1. Similarly as like phenol content, root also shows higher concentration of flavonoid content. Ethyl acetate extract of roots shows 70.76±1.28 mg/g, Ethanol extract shows 76.81±0.67 mg/g and aqueous extract shows 78.65±0.47 mg/g.

**Table 1: Total phenol and Flavonoid content of *Euphorbia thymifolia***

| S. No. | Parameter | Concentration mg/g of extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | LEAVES | | | AERIAL PARTS | | | ROOT | | |
| | | Ethyl acetate | Ethanol | Aqueous | Ethyl acetate | Ethanol | Aqueous | Ethyl acetate | Ethanol | Aqueous |
| 1 | Total Phenol (gallic acid/gm of extract) | 20.66±1.79 | 80.68±0.94 | 91.78±0.91 | 22.78±1.66 | 80.14±0.24 | 91.75±0.74 | 28.34±0.67 | 80.48±0.94 | 95.40±0.96 |
| 2 | Total Flavonoid (Quercetin/gm of extract) | 13.27±1.38 | 68.34±0.92 | 70.67±0.88 | 14.34±1.28 | 64.65±0.34 | 68.37±0.28 | 17.76±1.28 | 68.65±0.47 | 76.81±0.67 |

**The Total Antioxidant Capacity**

The total antioxidant capacity was presented in Table 2. The total antioxidant capacity was measured as equivalent of BHT (mg/g of extract). The Ethyl acetate extract of root has 124±1.68, Ethanol extract 126.16±1.27 mg/g and aqueous extract 138.78±1.63 mg/g.

**Table 2: Total Antioxidant level of *Euphorbia thymifolia***

| S. No. | Parameter | Concentration mg/g of extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | LEAVES | | | AERIAL PARTS | | | ROOT | | |
| | | Ethyl acetate | Ethanol | Aqueous | Ethyl acetate | Ethanol | Aqueous | Ethyl acetate | Ethanol | Aqueous |
| 1 | Total Antioxidant (BHT/gm of extract) | 121±1.28 | 116.16±1.27 | 132.33±1.62 | 122±1.78 | 112.78±0.47 | 128.28±1.28 | 124±1.68 | 126.16±1.27 | 138.78±1.63 |

**DPPH Radical Scavenging Activity**

Among the extracts tested the highest antioxidant activity was exhibited by fruit and root extracts. In the present investigation, the ethanolic extracts of fruit and root extract were notably, exhibited scavenging activity compared to other extracts. The values were obtained with leaves and root are 0.40±0.11 and 0.45±0.15 respectively which were significant to $p<0.5$ and are noticed at 400 µg/ml concentration of extract (Table. 3).

**Table 3** ***Antioxidant activity of various parts of E. thymifolia***

| | **DPPH** | | |
|---|---|---|---|
| | ***100*** | ***200*** | ***400*** |
| | ***Ethyl acetate extract*** | | |
| ***Leaves*** | ***0.11***±0.14 | ***0.18***±0.16 | ***0.26***±0.21* |
| ***Aerial parts*** | ***0.20***±0.21 | ***0.25***±0.24 | ***0.30***±0.22* |
| ***Roots*** | ***0.22***±0.11 | ***0.28***±0.14 | ***0.34***±0.21* |
| | ***Ethanol extract*** | | |
| ***Leaves*** | ***0.15***±0.23 | ***0.24***±0.15 | ***0.32***±0.23* |
| ***Aerial parts*** | ***0.25***±0.11 | ***0.32***±0.05* | ***0.45***±0.15* |
| ***Roots*** | ***0.26***±0.12 | ***0.34***±0.14* | ***0.40***±0.11* |
| | ***Aqueous extract*** | | |
| ***Leaves*** | ***0.22***±0.21 | ***0.28***±0.16 | ***0.30***±0.28 |
| ***Aerial parts*** | ***0.28***±0.12 | ***0.30***±0.16 | ***0.38***±0.18 |
| ***Roots*** | ***0.30***±0.06 | ***0.32***±0.14* | ***0.38***±0.17 |

***Values are mean ± S.E.M. n=3, *significance is set at p>0.5., # Conc µg/ml***

## CONCLUSION

The present study shows that *E. thymifolia* found to contain a substantial amount of phenolic and flavonoid content. The aqueous roots extract also shows good antioxidant potential. All the extracts showed concentration dependent scavenging activity. The aqueous roots extract of *E. thymifolia* possesses significant anti-oxidant activity and higher content of phenolic and flavonoids. Thus it can be concluded that the aqueous roots extract of this plant might play a vital role as therapeutic agents to prevent oxidative stress and related disorders.

## REFERENCES


1. Nadkarni KM, Nadkarni AK. 3rd ed. I. Bombay: Popular Prakashan; 2007. Indian Materia Medica; p. 529.
2. Khare CP. Berlin: Springer Verlag; 2004. Indian Herbal Remedies: Rational Western Therapy, Ayurvedic and Other Traditional Usage; pp. 210–1.
3. Ramadan MF, Morsel JT. Screening of the antiradical action of vegetable oils. J Food Compos Anal. 2006; 19: 838-842.
4. Rangari VD. Pharmacognosy and Phytochemistry. Part-II. 1st Ed. Career Publications:Nashik. 2003.
5. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1998; 29:1199-1200.
6. Finkol T, Holbnok NJ. Oxidant, oxidative stress and the biology of aging. Nature. 2000; 239-247.
7. Senthil K, Aranganathur S, Nalio N. Evidence of oxidative stress in the circulation of ovarian cancer patients. Clin Chim Acta. 2004; 3(39): 27-32.
8. Upston JM, Kritharides L, Stocker R. The role of vitamin-E in atherosclerosis. Prog Lipid. Res. 2003; 42:405-422.
9. Sofowara A. Medicinal plants and Tradional medicine in Africa spectrum Books Ltd. 1993.
10. Trease GE, Evans WC. Pharmacognosy. 11th Ed. Brailliae Tiridal Can. Macmillan Publisher's: 1989.
11. Harborn JB. Phytochemical methods: A guide to modern techniques of plant analysis. 1973.
12. Yu L, Haley S, Perret J, Harris M, Wilson J and Qian M. Free radical scavenging properties of wheat extracts. J Agri Food Chem. 2002;50:1619-1624.
13. Chang C, Yang M, Wen HJ. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Food Drug Analysis. 2002; 10:178-182.
14. Preito P, Pinedo M and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. Anal Biochem. 1999; 269:337-341.
15. Naznin A, Hasan N. *In vitro* Antioxidant Activity of Methanolic Leaves and fowers Extracts of *Lippia Alba*. Res J Medand Medical Sci. 2009;: 4(1): 107-110.
16. Klein SM, Cohen G, Cederbaumm AI. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating systems. Biochemistry, 1981; 20(21): 6006-6012.
17. Siddiqua A, Premakumari KB, Sultana R, Vithya and Savitha. Antioxidant Activity and Estimation of Total Phenolic Content of *Muntingia calabura* by Colorimetry. Int.J. Chem Tech Res. 2010; 2(1): 205-208.
18. Chavan C, Mulik S, Chavan M, Adnaik RS. Screening of Antioxidant Activity and Phenolic Content of Whole Plant of *Barleria prionitis* Linn. Int J Res Ayur & Pharm. 2011; 4: 1313-1319.