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COMPARATIVE EVALUATION OF PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT POTENTIAL OF SOME MEDICINALLY-IMPORTANT EUPHORBIACEOUS PLANTS

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ABSTRACT

The members of the family Euphorbiaceae planted as ornamental plants are rich sources of medicinally important phytochemical antioxidants. The study aimed the comparative evaluation of phytochemical composition and antioxidant potential of different parts of seven different plants of family Euphorbiaceae including *Euphorbia hirta*, *Euphorbia prostrata*, *Chrozophora tinctoria, Euphorbia milli, Euphorbia cotinifolia, Euphorbia tirucalli*, and *Ricinus communis*. The selected parts of the plants were extracted in 70% methanol and the extracts were subjected to phytochemical screening and phytochemical and antioxidant analysis. The data were statistically analyzed by one-way analysis of variance. The root, stem, and leaves of each of the selected plants consisted of tannins, saponins, flavonoids, and glycosides with few exceptions. The terpenoids and anthocyanins were mostly found in the studied parts of *E. tirucalli*, and *R. communis*. The total extract yield, total phemolics, total flavonoids, total tannins, and ascorbic acid content of the extracts ranged from 2.42±0.53 to 10.20±2.02, 0.07±0.01 to 0.34±0.1, 0.07±0.02 to 0.81±0.04, 0.12±0.02 to 0.29±0.05, and 0.17±0.03 to 0.51±0.04 g/100 g dry weight respectively. The content were found to be statistically different ($p<0.05$) in the studied parts of the selected plants The antioxidant potential in terms of total antioxidant activity, anti-radical capacity, and reducing power was also found to be statistically different ($p<0.05$) in different parts of the studied plants except for ascorbic acid content of stem. *E. tirucalli*, *R. communis,* and *E. milli* were found to be comparatively good in phytochemical composition while *R. communis* showed the highest antioxidant potential among the selected plants.

Keywords: Antioxidant potential, Euphorbiaceous plants, Phytochemical composition, Free radical scavenging capacity

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INTRODUCTION

Euphorbiaceae (spurge family) is the group of a fifth-most abundant family of flowering plants with about 300 genera and more than 7500 species including herbs, shrubs, and trees diversified throughout the world (Wilson *et al*., 1976). Due to the presence of diverse phytochemical composition, the members of the family Euphorbiaceae possess high medicinal value. The latex obtained from these has been found to possess anti-microbial, cytotoxic, and anti-cancer activities. The cyclic peptides isolated from the latex of these plants are effective against carcinoma cells (Wele *et al*., 2007). The latex has been used as a disinfectant and a therapeutic remedy for the treatment of toothache, heartburns, and ulcers, and fastens the wound healing process (Islam *et al*., 2019). The oil obtained from Euphorbiaceous plants is used for skin diseases, hair growth stimulants, muscle relaxation, and paralysis in various parts of the world (Salehi *et al*., 2019). The leaves are reported as an antiparasitic, pain reliever in arthritis, anti-inflammatory, treatment of solid tumor, heartburn, cardiovascular diseases and protect culture cells against human immunodeficiency virus (Thomas *et al*., 2008). Seeds have been reported to be used in the treatment of cancer, stomach ache, gout, paralysis, antidote against poison, and fungal infection (Liu and Leibowitz, 2010). Roots extract has been reported to be used as an antidote for snake venom, mouth wash for bleeding gums, and therapeutic remedy against fungal infections. It has been reported that the flowers of most plants of the family Euphorbiaceae are rich in antioxidants like diterpenoids which is a contributor to the treatment of cancer (Zhu *et al*., 2015). Flowers have also been found to stop bleeding wounds. The peptide commonly known as orbitides obtained from the family Euphorbiaceae has been reported to inhibit the human T- cell proliferation in autoimmune disorders (Ramalho *et al*., 2018).

Plants of this family contain different types of phytotoxins, including alkaloids, ricin, etc. (Charles *et al*., 2007). Some genera of the family Euphorbiaceae have been reported to contain di-terpenes, cyclic

peptides, alkaloids flavonoids, phenols, tannins, saponins, terpenoids, anthocyanins, and cardiac glycosides (Qaisar *et al.*, 2012). The phytochemical analysis of leaves confirmed the presence of glycosides, flavonoids, terpenoids, starch, steroids, reducing sugar, terpenoids, tannins, xanthoprotein, and saponins (Ruchi and Renu, 2010). The inhibition in the superoxide scavenging model was recorded as 58.7±0.62% in the ethanolic extract of the plant. Some plants also showed high phenolic acid contents (42.60 mg tannic acid equivalent (TAE)/100 g fresh weight) (Jain *et al.*, 2013).

In previous studies, work has been done on the phytochemical composition and antioxidant potential of many species of the family Euphorbiaceae, but no comparative evaluation has been done between its various species. Therefore, the present study was focused on the comparative evaluation of phytochemical composition and antioxidant potential of various parts of seven different plants of this family. The study would provide useful guidelines for the researchers and manufacturers, seeking for the potent sources of antioxidants, to select the Euphorbious plants as valuable candidates for the pharmaceutical and medicinal applications.

MATERIALS AND METHODS

Sampling: *Euphorbia hirta*, *Euphorbia prostrata*, and *Chrozophora tinctoria* were collected from the agricultural lands of Bahauddin Zakariya University Multan, Pakistan. At the same time, *Euphorbia milli, Euphorbia cotinifolia, Euphorbia tirucalli*, and *Ricinus communis* were purchased from local nurseries. The plants were identified by a botanist from Department of Botany, Bahauddin Zakariya University, Multan, Pakaistan (Voucher No. *Euphorbia hirta*: BZBOT0001543, *Euphorbia prostrata*: BZBOT0001549, *Chrozophora tinctoria*: BZBOT0001565, *Euphorbia milli*: BZBOT0001547*, Euphorbia cotinifolia*: BZBOT0001541*, Euphorbia tirucalli*: BZBOT0001552, and *Ricinus communis*: BZBOT0001569). The root, stem, leaves, and flowers were separated manually, washed with distilled water, and dried under shade until constant weight. The entirely dried samples were ground in an electric grinder to obtain fine powder, sieved through a fine cloth to get fine particle size, and stored in airtight containers at standard laboratory conditions.

Phytochemical screening: The presence of phytochemicals including tannins, flavonoids, terpenoids, saponins, anthocyanins, and cardiac glycosides in different parts of the selected plants was performed using procedures described earlier (Harborne, 1973; Trease and Evans, 1989; Shad *et al.*, 2013). The greenish-brown coloration of the aqueous extract (2 ml) of the plant parts on the addition of a 1% $FeCl_3$ solution (2 mL) confirmed the presence of tannins. The formation of the emulsion by vigorous shaking of olive oil with diluted aqueous extract confirmed the presence of saponins. The appearance of yellow coloration on mixing 70% ethanolic extract (5 ml) with 1% aluminium chloride solution (2-3 drops) confirmed the presence of flavonoids. The addition of concentrated sulfuric acid (3 ml) along the walls of the test tube to the mixture of 70% ethanolic extract (5 ml) and chloroform (2 ml) confirmed the presence of terpenoids in the samples by forming the brownish-red colored ring at the junction of two separate layers. The addition of 2 ml of concentrated sulfuric acid to 5 ml of 70% ethanolic extract resulted in the formation of bright-red precipitates which turned green in color on the addition of alkali (4% NaOH) indicating the presence of anthocyanins. The formation of a brown ring at the interface by mixing 5ml of the ethanolic extract with 2 ml of glacial acetic acid and a drop of 1% $FeCl_3$ solution followed by the addition of concentrated H_2SO4 (1 ml) indicated the presence of cardiac glycoside (Harborne, 1973).

Preparation of extracts and total extract yield: The dried powdered sample (5 g) of each part of the selected plants was used to extract in 70% methanol (100 ml) for 24 h, filtered through Whatman filter paper No. 42, and dried under shade. The dried extract was weighed on an electrical balance **(**YP-3002**),** and the total extract yield (TEY) was calculated as:

$$T \quad (\%) = W_e \quad / W_{sc} \quad \times 100$$

All the extracts were kept for storage in airtight containers in the dark for further antioxidant analysis. The extract solutions (10 mg/100 ml) were prepared in 70% methanol before the study of phytochemical composition and antioxidant potential (Nawaz *et al.*, 2019).

Phytochemical Analysis

Total phenolic content: The previously reported method was used to extracts the total phenolic content (TPC) of the selected parts of plants (Taga *et al.*, 1984). The absorbance of the reaction mixture containing methanolic extract (1 ml), 10% Folin-Ciocalteu reagent (1 ml), and saturated solution of sodium carbonate (2 ml) was noted at 750 nm after 30 min using a UV/Visible spectrophotometer (Jenway-6405, Japan). The regression curve (R^2=0.9897) genrated the following regression equation to calculate Gallic acid equivalent TPC (g/100g dw).

$$TP \quad (g/100\, g\, d \quad) = A \quad . \, o \quad sc \quad /10.552$$

Total flavonoids content: A previously reported method was used to evaluate the total flavonoid content (TFC) (Zhishen *et al.*, 1999). 1 ml of methanolic extract was allowed to mix with 0.3 ml of 5 percent sodium nitrate solution and 4 ml of 30 percent aqueous C_2H_5OH solution. Then the contents of the reaction were allowed

to stay for six minutes at 25ºC temperature along with the addition of 0.3 ml of 10 percent aluminium chloride solution, 0.4 ml of 30 percent C_2H_5OH, and 4 ml of 4 percent sodium hydroxide solution. After 12 min, the absorbance of the reaction mixture was taken at 510 nm wavelength. Catechin equivalent TFC (g/100 g dw) was calculated from the following regression equation (Catechin anhydrate: R^2=0.9852).

$$T \quad (g/100\,g\,d \quad) = A \quad .o \quad sc \quad /3.875$$

Total tannins content: The total tannins content (TTC) was determined using the method described earlier (Fagbemi *et al.*, 2005). Methanolic extract (1 ml) was allowed to mix with 1 ml of each of the saturated solution of sodium carbonate and Folin-Ciocalteu's reagent. The reaction mixture was diluted with distilled water (8 ml). After centrifugation at 3000 × g after 30 min at 25±5ºC, the absorbance was recorded at 725 nm. The Tannic acid equivalent TTC (g/100 g dw) was calculated from the following regression equation (Tannic acid standard curve: R^2=0.9867).

$$T \quad (g/100\,g\,d \quad) = A \quad .o \quad sc \quad /6.795$$

Ascorbic acid content: The ascorbic acid was extracted from the powdered sample (1 g) in a mixture of meta-phosphoric acid and glacial acetic acid (50 mL) for 1 h and filtered through Whatman filter paper No. 1. The 10 ml of extract was titrated against a 5% standardized solution of 2, 6-dichlorophenolindophenol dye till pale pink color that persisted for 30 sec. The AAC was calculated as g/100 g dw by standardization of dye with 0.2% ascorbic acid solution (Rankin *et al.*, 1976).

Antioxidant Analysis: The antioxidant potential of the extracts was determined in terms of Trolox equivalent total antioxidant activity (TAOA), Free radical scavenging capacity (RSC), and metal-reducing power (RP).

Total antioxidant activity: Trolox equivalent TAOA was determined by two methods including phosphomolybdenum assay and 2, 2-diphenyl picrylhydrazyl (DPPH) assay as described earlier (Nawaz *et al.*, 2016). In phosphomolybdenum assay, the methanolic extract (1 ml) was added to a freshly prepared mixture (1 ml) of 0.6M H_2SO_4, 28mM Na_2PO_4, and 4 mM ammonium molybdate solutions. The contents were heated to 95ºC for 90 min, cooled to room temperature, and the absorbance was recorded at 695 nm. Trolox equivalent TAOA (g/100 g dw) was calculated using the regression equation (Absorbance=4.095×Conc. of Trolox) (Trolox standard curve: R^2 = 0.9879). In the DPPH assay, the 40µM methanolic DPPH solution (3 ml) was mixed in methanolic extract (1 ml). After 30 min the absorbance was recorded at 517 nm, and the Trolox equivalent TAOA (g/100 g dw) was calculated practicing the regression equation (Absorbance=6.497×Conc. of Trolox+0.267) obtained by the standard curve of Trolox (R^2 = 0.9798).

Free radical scavenging capacity: The extract (1 ml) was treated with 40µM DPPH solution (3 ml) and the change in the optical density of DPPH radical solution was noted at 517 nm after 30 min. Methanol was used as blank while the DPPH solution was used as control. The DPPH radical scavenging capacity (%) was calculated as:

$$D \quad r \quad s \quad c \quad (\%) = A_c - A_s/A_s \times 100$$

where A_c is the absorbance of control and A_s is the absorbance of the sample (Nawaz *et al.*, 2016).

For determination of hydroxyl radical scavenging capacity, the extract (1 ml) was mixed with equivolume of 9mM salicylic acid, 8.8mM hydrogen peroxide, and 9mM ferrous sulfate solutions. The reaction mixture was kept for incubation at 37ºC temperature for 30 min and absorbance was determined at 510 nm. The mixture excluding the sample was used as a control and that without salicylic acid was treated as blank. The hydroxyl radical scavenging capacity calculated as:

$$H \quad r \quad sc \quad c \quad (\%) = [1 - (A_{sc} \quad - A_b \quad)/A_c \quad] \times 100$$

Metal reducing power: The reducing power was calculated by the method described by Nawaz *et al* (Nawaz *et al.*, 2016). An aliquot of extract (2.5 ml) was mixed with 2.5 ml of each of 0.2M phosphate buffer (pH 6.6) and 1 percent solution of potassium ferricyanide. For 20 minutes, the mixture was allowed to heat at 50ºC along with the addition of 10% trichloroacetic acid solution (2.5 mL). The centrifugation of contents was carried out at 3000 x g for 10 min, and 5 ml of supernatant was diluted with 5 ml deionized water, mixed with 1 mL of 0.1% $FeCl_3$ solution, and the absorbance was recorded at wavelength 700 nm. A higher value of absorbance was taken as higher reducing power.

Statistical Analysis: The experiments were performed in triplicate and the results were shown as mean ± standard deviation. The data collected were subjected to a one-way analysis of variance (ANOVA) and difference among the means was determined by Tukey′s multiple range tests at ($p \leq 0.05$).

RESULTS AND DISCUSSION

Phytochemical Screening: Phytochemical screening confirmed the presence of tannins, saponins, and flavonoids in each of the studied parts of all of the selected plants. The leaves of *E. cotinifolia*, stem and leaves of *C. tinctoria,* and each part of *R. communis* also contained terpenoids. The root of *E. milli*, *C. tinctoria,* and *R. communis*, stem, and leaves of *C. tinctoria* and leaves of *R. communis* also contained anthocyanins. The

cardiac glycosides were confirmed in the root of *E. milli*, *E. cotinifolia*, *C. tinctoria,* and *R. communis*, the stem of *E. prostrata* and *C. tinctoria,* and *R. communis* and leaves of *E. milli*, *E. cotinifolia*, and *R. communis*. The results are presented in Table 1.

The phytochemicals are the medicinally important bioactive secondary metabolites of the plants which possess various biological activities. The flavonoids possess anti-inflammatory, anti-carcinogenic, and anti-mutagenic properties (Kumar and Pandey, 2013). Tannins bind to proteins, show adherence to mucosal cells, and prevent the growth of microorganisms (Haslam, 1996). Terpenoids possess anti-inflammatory, anti-viral, anti-tumor, and anti-bacterial properties (Sun and Liu, 2011) while anthocyanins show anti-diabetic activity, improve sight, cognitive function, and immunity (Miguel, 2011). Cardiac glycosides are the steroids that play an essential role in the intervention of congestive heart failur. The presence of these phytochemicals in the Euphorbiaceous plants highlights their medicinal and pharmaceutical importance. Our results correlate with those reported earlier (Qaisar *et al*., 2012; Suurbaar *et al*., 2017; Tadesse *et al*., 2016).

Table1. Phytochemical screening of different parts of the selected Euphorbiaceous plants.

Plant studied	Parts used	Tannins	Saponins	Flavonoids	Terpenoids	Anthocyanins	Cardiac Glycosides
E. hirta	Stem	+	+	+	-	-	-
E. prostrata	Stem	+	+	+	-	-	+
E. milli	Root	+	+	+	-	+	+
	Stem	+	+	+	-	-	-
	Leave	+	+	+	-	-	+
E. cotinifolia	Root	+	+	+	-	-	+
	Stem	+	+	+	-	-	-
	Leave	+	+	+	+	-	+
E. tirucalli	Root	+	+	+	-	-	
	Stem	+	+	+	-	-	-
C. tinctoria	Root	+	+	+	-	+	-
	Stem	+	+	+	+	+	+
	Leave	+	+	+	+	+	-
R. communis	Root	+	+	+	+	+	+
	Stem	+	+	+	+	-	+
	Leave	+	+	+	+	+	+

Phytochemical Composition: The total extract yield (TEY) of methanolic extracts obtained from root, stem, and leaves of the selected Euphorbiaceous plants ranged from 3.75±0.83 to 7.68±1.20, 2.42±0.53 to 10.20±2.02, and 4.11±0.02 to 8.85±0.93 g/100 g dw respectively (Table 2). The TPC of the root, stem, and leaves of the selected plants ranged from 0.07±0.01 to 0.33±0.1, 0.14±0.01 to 0.34±0.1, and 0.15±0.01 to 0.31±0.07 g/100g dw respectively. The TFC of root, stem, and leaves of the selected plants ranged from 0.08±0.03 to 0.54±0.04, 0.08±0.01to 0.70±0.02, and 0.07±0.02 to 0.81±0.04 g/100g dw respectively. The TTC of roots, stem, and leaves of the selected plants ranged from 0.16±0.01 to 0.27±0.03, 0.12±0.02 to 0.27±0.03, and 0.15±0.02 to 0.29±0.05 g/100g dw respectively. The AAC of roots, leaves, and stem of the selected plants ranged from 0.22±0.02 to 0.32±0.01, 0.23±0.05 to 0.30±0.04, and 0.17±0.03 to 0.51±0.04 g/100g dw respectively. The experimental results of the phytochemical composition of the selected plants are presented in (Table 3).

Table2. Total extract yield (g/100 g dw) of different parts of the selected Euphorbiaceous plants.

Plant name	Root	Stem	Leave
E. hirta	ND	10.20±2.02^{a}	ND
E. prostrate	ND	8.35±1.10^{ab}	ND
E. milii	5.21±1.01^{b}	4.28±0.38	8.85±0.93^{a}
E. cotinifolia	7.68±1.20^{a}	6.33±1.01^{b}	8.32±0.78^{a}
Euphorbia tirucalli	5.34±0.94^{b}	5.23±1.15^{bc}	ND
C. tinctoria	4.55±1.41^{bc}	4.06±0.72^{c}	5.34±0.23^{b}
R. communis	3.75±0.83^{c}	2.42±0.53^{d}	4.11±0.12^{c}
p-value	0.002	0.00	0.00

ND: Not determined; Values expressed as mean ±SD of three replicates; Means followed by different letters in a column are significantly different at 95% confidence level ($p \leq 0.05$) using Tukey's multiple range test.

A statistically significant variation ($p<0.05$) was observed in TEY, TPC, TFC, TTC, and AAC of various part of the selected plants. The stem of *E. hirta* was found to be comparatively high in TEY while the lowest extract yield was obtained from the stem of *R. communis*. The stem of *E. prostrate* and leaves of *E. milli* and root and

leaves of *E. cotinifolia* also showed statistically similar extract yield to that of *E. hirta* stem. The relatively higher extract yields make *E. hirta* and *E. prostrate* stem, *E. milli* leaves and *E. cotinifolia* root and leave the valuable candidates for pharmaceutical application. The TPC was found to be highest in *E. prostrata* stem and lowest in *C. tinctoria* root. Although, the comparatively lower but statistically similar value of TPC to that of *E. prostrata* stem was found in stem of *E. hirta,* stem and leaves of *R. communis,* and each part of *E. milli* and *E. cotinifolia*. Our results correlate with previous studies (Prasad, 2014). The TFC was found to be highest in *E. milli* leaves and lowest in *C. tinctoria* leaves. However, comparatively lower but statistically similar values of TFC to that of *E. milli* leaves were observed in *E. hirta* stem and *E. cotinifolia* leaves. All parts of *C. tinctoria* possessed comparatively lower values of TPC and TFC among the selected plants. TPC and TFC of the extracts were positively correlated with the total yield of the respective extract. The plant parts showing comparatively higher extract yield were found to possess a higher value of TPC and TFC. The results were in correlation with those reported in previous studies (Cimanga *et al*., 2018).

The TTC were found to be highest in *E. milli* leaves and lowest in *C. tinctoria* stem. Statistically similar value of TTC to that of leaves of *E. milli* was found in *E. cotinifolia* leaves, *R. communis* leaves, *E. prostrate* stem, *E. hirta* stem, and *E. milli* root. However, all parts of *C. tinctoria* possessed comparatively lower values of TTC among the selected plants. The plant parts showing relatively higher extract yield were found to maintain a higher amount of TTC. The highest value AAC was observed in *E. milli* leaves and while *C. tinctoria* leaves were found to be lowest in AAC. Our results correlate with previous studies (Basma *et al.*, 2011; Sharifi-Rad *et al.*, 2015). Although, comparatively lower but the statistically similar value of ascorbic acid content to that of leaves of *E. milli* was found in leaves of *R. communis*. The plant parts showing comparatively higher extract yield were found to possess a higher value of ascorbic acid content.

Antioxidant Potential: The antioxidant potential of the selected plants was determined in terms of total antioxidant activity (TAOA) by phosphomolybdenum and DPPH methods, free radical scavenging activity against DPPH and hydroxyl radicals, and metal reducing power.

Total antioxidant activity by phospho-molybdenum assay: In the case of the phospho-molybdenum method, TAOA of roots, stem, and leaves ranged from 0.025±0.007 to 0.08±0.009, 0.025±0.008 to 0.085±0.009, and 0.034±0.008 to 0.15±0.006 mg Gallic acid Eqv./100 g dry weight respectively.

Table 3. Phytochemical composition (g/100g dw) of different parts of the selected Euphorbiaceous plants

Plant	Part used		
	Root	Stem	Leave
Total phenolic content			
E. hirta	ND[*]	0.31±0.01	ND
E. prostrata	ND	0.34±0.04	ND
E. milli	0.33±0.06[**]	0.26±0.05	0.27±0.05
E. cotinifolia	0.30±0.08	0.25±0.06	0.31±0.07
E. tirucalli	0.16±0.02	0.17±0.02	ND
C. tinctoria	0.07±0.01	0.14±0.01	0.15±0.01
R. communis	0.17±0.01	0.26±0.03	0.30±0.04
p-value	0.004	0.007	0.002
Total flavonoids content			
E. hirta	ND	0.70±0.08	ND
E. prostrata	ND	0.53±0.01	ND
E. milli	0.47±0.2	0.39±0.02	0.81±0.04
E. cotinifolia	0.54±0.04	0.38±0.02	0.75±0.05
E. tirucalli	0.16±0.01	0.10±0.01	ND
C. tinctoria	0.08±0.03	0.08±0.01	0.07±0.02
R. communis	0.12±0.01	0.19±0.03	0.65±0.06
p-value	0.003	0.00	0.00
Ascorbic acid content			
E. hirta	ND[*]	0.28±0.01	ND
E. prostrata	ND	0.30±0.04	ND
E. milli	0.26±0.03	0.30±0.04	0.51±0.04
E. cotinifolia	0.22±0.05	0.24±0.01	0.21±0.01
E. tirucalli	0.22±0.02	0.25±0.03	ND
C. tinctoria	0.23±0.01	0.23±0.05	0.17±0.03
R. communis	0.32±0.01	0.28±0.04	0.43±0.02
p-value	0.00	0.461	0.00
Total tannins content			
E. hirta	ND	0.25±0.03	ND
E. prostrata	ND	0.27±0.03	ND
E. milli	0.27±0.03	0.19±0.02	0.29±0.05
E. cotinifolia	0.24±0.04	0.21±0.02	0.28±0.03
E. tirucalli	0.19±0.04	0.18±0.01	ND
C. tinctoria	0.16±0.01	0.12±0.02	0.15±0.02
R. communis	0.22±0.02	0.22±0.04	0.27±0.03
p-value	0.006	0.00	0.00

[*]ND: Not determined,;[**]Values expressed as mean ±SD of three replicates

[***] Mean±SD followed by different letters in a column are significantly different at 95% confidence level (p ≤ 0.05) using Tukey's multiple range test.

The TAOA of different parts of the selected plants, as determined by phosphomolybdenum assay, was found to be statistically different (p<0.05) with the highest value in leaves of *Ricinus communis* and *E. milli* and lowest in the root of *E. tirucalli* and stem of *C. tinctoria.* The lowest antioxidant activity was observed in all parts of *E. tirucalli* and *C. tinctoria*. The plant parts showing higher total antioxidant activity were found to possess a higher value of flavonoid content, tannin content, and ascorbic acid content. The results of TAOA

are presented in *Figure* 1A, B. Molybdenum (VI) is a metal that is involved in redox reactions, and its high concentration in the body causes many disorders like hypothyroidism, sterility, anemia, joint deformities, and liver abnormalities, etc. (Shields, 2013). The phospho-molybdenum method is based on the principle that the plants in the presence of antioxidants reduce the molybdenum (VI) to molybdenum (V) and thus leading to the formation of green phosphor-molybdate (V) complex. Plants that contain antioxidants like phenols and flavonoids etc. can cause this reduction and thus prevent us from hazardous effects of the metal (Mashwani *et al.*, 2013).

Total antioxidant activity by DPPH assay: DPPH is a reactive nitrogen species that are produced in our body as a result of redox reaction and if not controlled properly can lead to oxidative stress (Lin *et al.*, 1995). In our study, by DPPH method, TAOA of roots, stem, and leaves ranged from 0.68±0.0002 to 1.2±0.0003, 0.5±0.0003 to 1.1±0.0004, and 0.668±0.0002 to 4.0±0.0003 g/100 g dw respectively. The total antioxidant content of different parts of the selected plants, as determined in terms of DPPH assay, was found to be statistically different ($p<0.05$) with the highest value in leaves of the *Ricinus communis* and lowest in the stem of *E. hirta* (*Figure* 1 A, B). The plant parts showing comparatively higher TAOA were found to possess a higher value of tannin and ascorbic acid content.

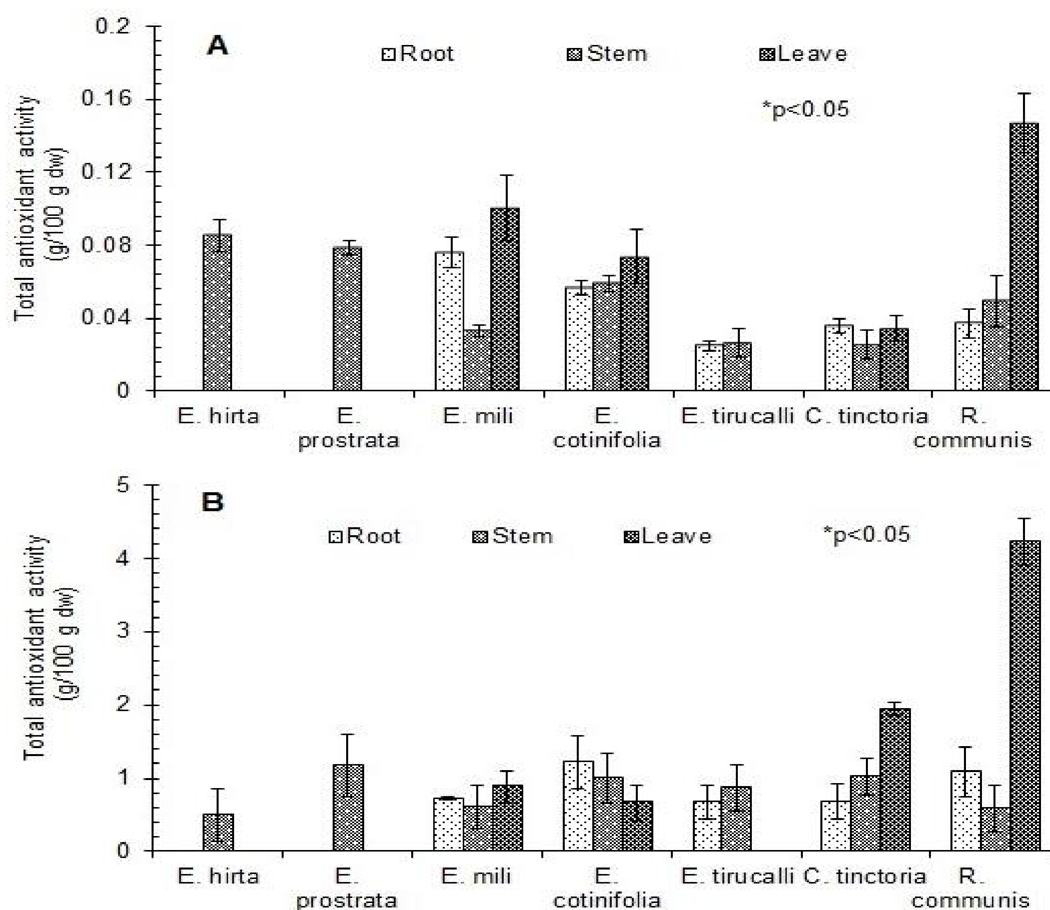

Figure 1: Total antioxidant activity of different parts of the selected Euphorbiaceous plants

A) Phosphomolybdenum assay, B) DPPH assay

*The values are statistically different at 95% confidence level.

DPPH radical scavenging capacity: The ability of antioxidants to capture the reactive nitrogen specie was estimated by DPPH radical scavenging capacity (%). In our results, DPPHRSC of roots, stem, and leaves ranged from 70.28 ± 2.2 to 75.20 ± 2.07, 64.27±2.3 to 75.14±0.01 and 18.1±2.64 to 77.5±0.4 % respectively. The DPPHRSC of different parts of the selected plants was found to be statistically different ($p<0.05$) with the highest value in leaves of *E. cotinifolia* which is comparable with the findings reported earlier (Mariani *et al.*, 2013) and lowest value in leaves of *C. tinctoria*. Our values of DPPHRSC for roots of *R. communis* and leaves

of *C. tinctoria* are found to different than those reported earlier (Sharifi-Rad *et al.*, 2015). All the results of the free radical scavenging capacity of all the selected seven specie have been shown in *Figure* 2 A.

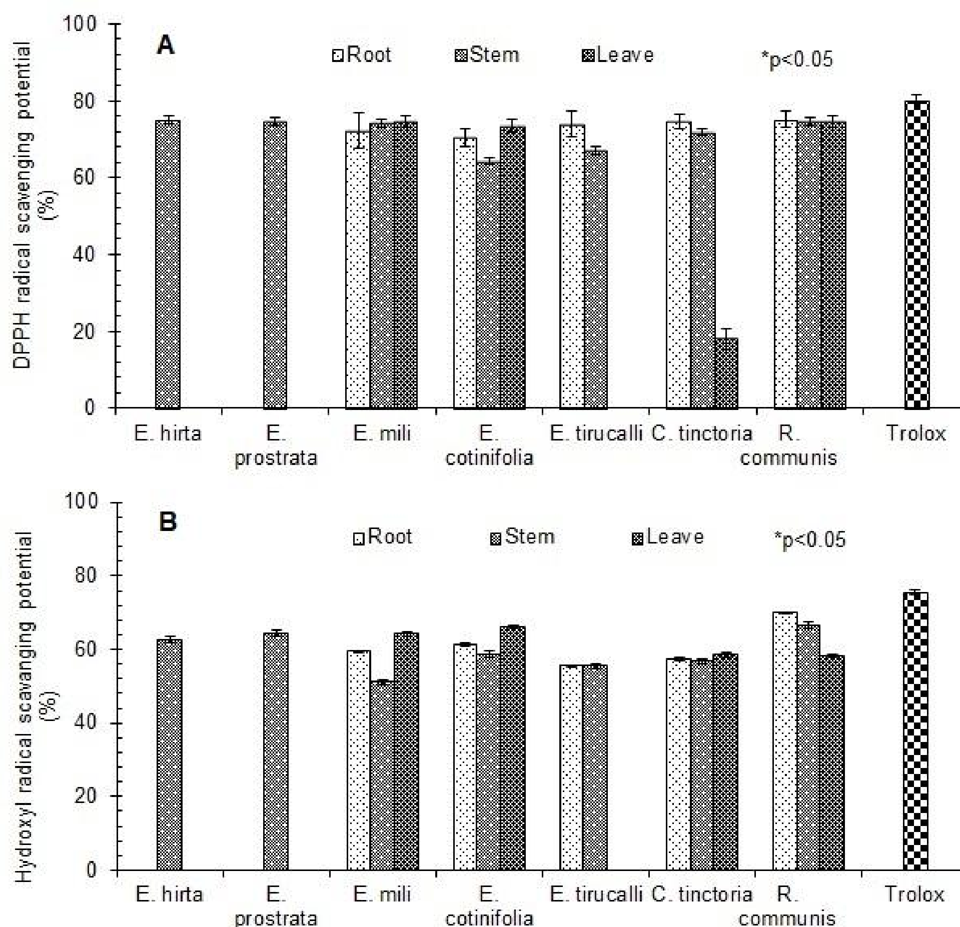

Figure 2 Free radical scavenging potential of different parts of the selected Euphorbiaceous plants
A) DPPH radical scavenging capacity, B) Hydroxyl radical scavenging capacity
*The values are statistically different at 95% confidence level.

Hydroxyl radical scavenging capacity: The ability of antioxidants to capture the reactive oxygen species was estimated by hydroxyl radical scavenging capacity (%). In our results, hydroxyl radical scavenging capacity in roots, stem, and leaves ranged from 55.5±0.4 to 69.9±0.24, 51.0±0.4 to 66.5±0.4 and 58.2±0.6 to 66.2±0.4 % respectively. Hydroxyl radical scavenging capacity of different parts of the selected plants was also found to be statistically different ($p<0.05$) with the highest value in roots of *Ricinus communis* and lowest in root and stem of *E. tirucalli*. Although, the comparatively lower but statistically similar value of roots of *R. communis* was found in the stem of *E. prostrate* and *Ricinus communis* and leaves of *E. milli* and *E. cotinifolia* (*Figure* 2 B).

Metal reducing power: The results for the metal-reducing power of roots, stem, and leaves of the selected plants are presented in *Figure* 3. The reducing power of the selected extractes ranged from 0.54±0.03 to 0.95±0.25, 0.30±0.02 to 0.60±0.05, and 0.163±0.008 to 0.75 ±0.042 nm respectively. The metal-reducing power of different parts of the selected plants was found to be statistically different ($p<0.05$) with the highest value in roots of *E. tirucalli* and lowest value in leaves of *C. tinctoria*. Although, the comparatively lower but statistically similar value of reducing power to that of roots of *E. tirucalli* was found in roots and leaves of *Ricinus communis* and leaves of *Euphorbia milli*. Our results are comparable with those reported earlier (Munro *et al.*, 2015; Tewari *et al.*, 2017). There is an association between reducing power and antioxidant activity. The plants with high reducing power indicate that they contain compounds that donate electrons and can reduce the oxidized metals and reactive metabolites produced during lipid peroxidation (Chanda and Dave, 2009).

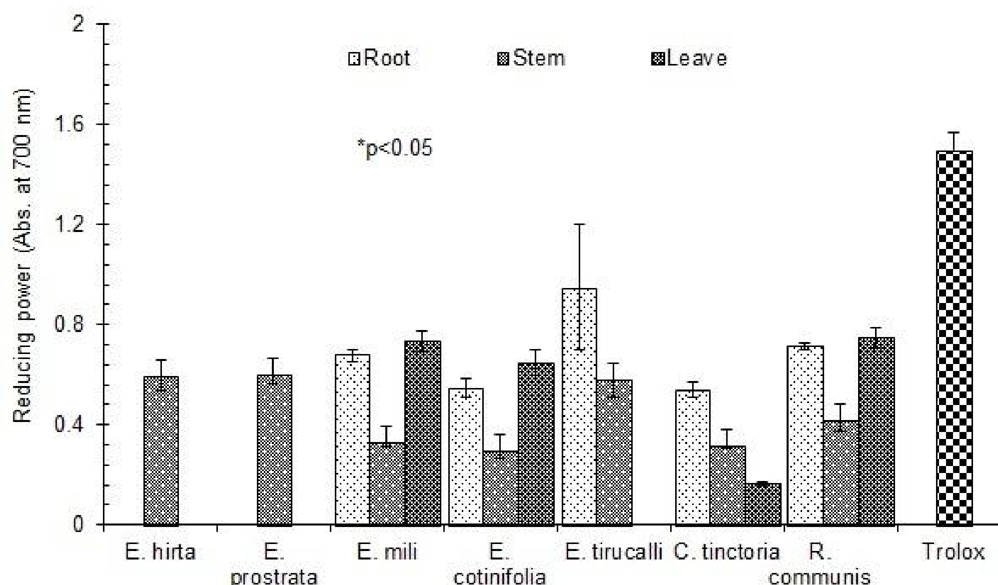

Figure 3 Reducing powers of different parts of the selected Euphorbiaceous plants
*The values are statistically different at 95% confidence level.

Conclusion: The studied parts of the selected plants of the family Euphorbiaceae contained a rich amount of phytochemicals with strong antioxidant potential. The root, stem, and leaves of each of the selected plants consisted of tannins, saponins, flavonoids, and glycosides with few exceptions. The selected plants possess good antioxidant ability in terms of the DPPH method, phosphomolybdenum method, DPPH radical scavenging capacity, hydroxyl radical scavenging capacity, and metal-reducing power. Among the seven species of euphorbiaceous plant, *E. tirucalli*, *R. communis* and *E. milli* were found to possess comparatively good phytochemical composition while *R. communis* showed relatively highest antioxidant potential among the selected plants. The significant amount of phytochemicals and antioxidant property make the euphorbiaceous plants suitable candidates for both pharmaceutical and medicinal applications.

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