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Assessment of Antioxidant and Total Polyphenolic Content of Some Plants of Euphorbiaceae Family

KEYWORDS: Phytochemicals, DPPH, Antioxidant, Total Polyphenolic Content

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ABSTRACT *Plant and herbs are being used as traditional medicines since a very long time to cure various diseases as well as in the fields of agriculture, veterinary etc. Various phytochemical and pharmacological studies have clearly shown multidisciplinary usages of these plant derived phyto-medicines. In the present work four plants of family Euphorbiaceae (Jatropha curcas, Euphorbia hirta, Euphorbia neriifolia and Ricinus communis) have been analyzed for their polyphenolic and antioxidant activity. Euphorbia hirta was found to be strong antioxidant because it showed highest percentage DPPH scavenging activity (67.56 %, IC50 value 61.34 ± 0.0004) microgram/ ml. E. neriifolia and R. communis also showed antioxidant activity lower than Euphorbia hirta (62.83%, IC50 value 58.00 ± 0.0003 microgram/ ml and 51.35 % IC50 value 98.00 ± 0.0006 respectively). While J. curcas is a moderate antioxidant as it showed the lowest DPPH scavenging activity i.e. 40.87µg/ml. R. communis showed maximum polyphenolic content(25.36±0.0003µg/ml) whereas least amount of polyphenols was presented in E. neriifolia plant(5.36±0.0005µg/ml). Quantity of polyphenols present in J. curcas and E. hirta were less than R. communis and higher than E. neriifolia (21.34±0.001µg/ml and 14.97±0.0085µg/ml) respectively.*

Introduction

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube NS et al., 2008). Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice for examining the current search for therapeutically effective new drugs such as anticancer drugs (Dewick, P.M. et al., 1996), antimicrobial drugs (Phillipson, J.D. et al., 1996), antihepatotoxic compounds. Individuals from developing as well as developed countries use traditional plant medicines. Phytochemicals can be derived from barks, leaves, flowers, roots, fruits, seeds (Criagg, G.M. et al., 2001). Studies have shown that phytochemicals isolated from plant sources have been used for the prevention and treatment of cancer, heart disease, diabetes mellitus, and high blood pressure (Waltner-Law, M.E. et al., 2002). These phytochemicals provide definite physiological action on the human body. These bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga, H.O.et al., 2005, Mann, J. et. al. et al. 1978). Phenolic compounds and flavonoids, widely distributed in plants, have been reported to exert multiple biological effects, including antioxidants, anti inflammatory, anti carcinogenic etc. (Miller, A.L. et al. 1996). Antioxidants have been reported to prevent oxidative damage caused by free radicals, it can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers (Shahidi, F. et al. 1992, Buyukokuroglu, M.E. et al. 2001). The potentially reactive derivatives of oxygen or Reactive Oxygen Species (ROS) are continuously generated in human body that is detoxified by antioxidants present inside the body (Farber, J.L. et al. 1994). If these free radicals are produced in vivo or in cell in vitro in sufficient amounts, it can chemically modify or damage to proteins, lipids, carbohydrates and nucleotides. (Travor F. Slater et al. 1984). An increase in ROS is an impairment of antioxidant defence system or an insufficient capacity to repair oxidative damage (Atli T at al. 2004). Several studies have been indicated that the antioxidant activities of some fruits and vegetables were highly correlated with their total phenolic contents (Velioglu YS et al. 1998, Emmons CL et al. 1994). The antioxidant activity of these phenolic contents is due to their redox properties which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa T et al. 1994). Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Devasagayam TPA et al. 2004). Many plant species such as compositae, Myrtacae, Umbelliferae, Asteracae and Euphorbiaceae have been reported for their medicinal properties.

Euphorbiaceae is the largest family among the Allophyta,

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with 300 genera and 5000 species, sub cosmopolitan but with strong representation in humid tropic and subtropics of both hemispheres. Different species of Euphorbia are used for the treatment of various ailments such as skin diseases, gonorrhoea, migraine, intestinal parasites (M Uzair et al. 2009). Euphorbiaceae plants have been intensively investigated and contain alkaloids, saponins, flavonoids, tannins, resins, and carbohydrate amongst others (Patricia A. Onocha et al. 2011).

Plant extract of *Jatropha curcas* is used to treat allergies, burns, cuts, wound, inflammation, leprosy, leucoderma, and smallpox. Water extract of branches is used to treat HIV and tumor (Shivani Sharma et al. 2012). *E. hirta* shows antidiarrheal, antispasmodic, anti inflammatory, antifungal, anticancer, antimalarial, antiamoebic, antibacterial, and antihelmentic effects (A.N.M. Mamun-Or-Rashid et al. 2013). *E. neriifolia* shows diuretic and antiseptic properties, and also used in the treatment of bleeding piles, ear ache and chronic respiratory troubles (Shaikh Arshad Ahmed et al. 2011). *Ricinus communis* is used as purgative and laxative (Jitendra Jena et al. 2012).

In the present study we have chosen four above plants of family Euphorbiaceae for evaluating their antioxidant and total polyphenolic content.

Material and Methods

Chemicals

Methanol (HPLC grade), Water (HPLC grade), Tris HCl, Folin & Ciocalteu's Phenol Reagent (PCP), DMSO HPLC grade, Sodium carbonate AR grade and Ascorbic acid were obtained from SRL, India, while 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) were purchased from Alfa acer, Britain. All chemicals were analytical grade.

Sample collection of Plant Materials

The plant leaves of *Jatropha curcas*, *Euphorbia Hirta*, *Euphorbia neriifolia*, and *Ricinus communis* were collected in March 2013 from the campus of M.G.C.G.V. Chitrakoot Satna (M.P.) and identified. All plant leaves were collected, washed with fresh water and dried under shade at room temperature separately. The leaves were grinded coarsely and then powdered, filtered through sieve (30No.) stored in sterile and air tight container for further use.

Preparation of Plant extracts

100 mg powdered sample of plant leaves were extracted with 10 ml HPLC grade methanol through open air reflux process at 40°C for 6 hours till dried than make the volume again 10 ml with methanol and reflux, this process was repeated several times. The extracts were filtered through filter paper (Watman no.1) to remove free un-extractable substances. The filtrates of plant extract were evaporated at room temperature at dryness, finally dissolve with 10 ml with DMSO and preserved at 4-5°C for further process. The crude samples were subjected to antioxidant and total polyphenolic content.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The assay for free radical DPPH was done by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. In brief, a 96-well microplate, 25 µl of various dilutions (10-100 µg/ml) of methanolic extract 125 µl of tris–HCl buffer (0.1M, pH 7.4) and 125 µl of DPPH solution (0.004% w/v in methanol) were added. The reaction mixture was shaken well. The DPPH decolourization was recorded at 518 nm on a BioTek Synergy H4 hybrid multimode micro plate reader (BioTek instruments, Inc Winoosci, VT, USA.), after 30 min incubation in dark. The percentage of DPPH scavenging by plant extracts obtained in terms of ascorbic acid equivalent concentration. Quantification was performed with respect to the standard curve of Ascorbic acid (y= 0.731x+14.60; R^2 =0.947). Results were expressed as milligram of Ascorbic acid equivalent per ml of extract. Experiment was done in triplicates. DPPH radical's concentration was calculated using the following equation:

DPPH scavenging effect (%) = Ao – A1 / Ao X 100

Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample.

Determination of Total polyphenolic content

Total polyphenolic content of plant leaves extracts was measured by using Folin-Ciocalteu reagent. The 25 µl of plant extract diluted with 125 µl water followed by addition of 150 µl of Folin-Ciocalteu reagent (1N) & 25 µl of Na_2CO_3 (20%w/v) and incubated at 45°C for 60 min then absorbance was measured spectrophotometrically at 765nm (BioTek Synergy H4 multimode micro plate reader, BioTek instrument, Inc Winoosci, VT, USA). Absorbance was recorded triplicates. Quantification was performed with respect to the standard curve of Catechol (y= 0.003x+0.024; R^2 =0.965). Result was expressed as milligram of Catechol equivalent per ml of extract.

Statistical Analysis of Data

Data reproduced during the experimental work, were analysed using Origin Pro8.5 software. All parameters were triplicatly recorded for accurate result. Tables show mean value and standard deviation (±) of reproduced data. Graphs were also plotted using OriginPro 8.5 software.

Result and Discussion

In the present situation, there is a strong need for an effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases like cancer, cardiovascular diseases, age related muscular degeneration, atherosclerosis, etc (O.L. Aruoma et al. 1998). Continuous efforts have been carried out to determine the presence of bioactive compounds in various plant materials, in particular, the agro-industrial by-products since they are renewable and abundantly available (Balasundram N. et al. 2006).

The extraction method was designed in such a way that maximum extraction of polyphenolic content present in plant leaves with the help of methanol in an open reflection apparatus at 40 °C.

Antioxidants avert the oxidation of cellular organalles and minimize the hazardous effects of free radicals, and thus helpful in the effective prevention of a varity of lifestyle related diseases and aging.

The results shown in **Graph-1** indicates the % 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *E. hirta* , *E. neriifolia*, and *R. communis* methanolic extracts of leaves at different concentrations. The inhibition of DPPH radical scavenging activity increased in a dose-dependent manner. **Table-1** shows the % of free radical scavenging activity of methanolic leaf extracts of four plants and **Table-2** shows the absorbance of DPPH at different concentration of treated extracts. While **Graph-2** was plotted between the absorbance and different conc. of plant extracts. **Table-3** shows the IC_{50} values of plant extracts. Ascorbic acid was used for reference standard to quantify the IC_{50} value.

DPPH scavenging activity of selected plant leaves are tabulated in Table 2, the lowest IC_{50} shows the most potent antioxidant activity i.e. *E. neriifolia* 66.70 µg /ml than 72.06 µg/ml of *E. hirta* and 96.08 µg /ml of *R. communis*. While *J. curcas* shows moderate antioxidant activity. The DPPH scavenging of methanolic extract of *Jatropha curcas* leaves which does not show significant DPPH activity. Maximum % of free radical scavenging activity shows by this plant leaves is 40.87 %.

Plant of *Jatropha curcas* used traditionally to cure diseases like cancer, piles, snakes bites, paralysis, and dropsy (Oku-

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jagu TF et al. 2006). However, limited information is available on the pharmacological properties of *Jatropha* species which showed that many species possess antimicrobial activity (Aiyelaagbe OO et al. 2007, Aiyelaagbe OO et al. 2001, Thomas OO, 1989). G. E. Diwani *et al* (2009) studied the antioxidant activity of Egyptian *Jatropha curcas* and found that extract of root shows IC_{50} value 0.521 mg/ ml. Sanjana Safi *et al* (2012) determined in-vitro antioxidant activity of Indian Sub continental *Jatropha curcas*, methanolic and chloroform extract of root indicated the IC_{50} value 35.62µg/ml and 43.81 µg /ml respectively.

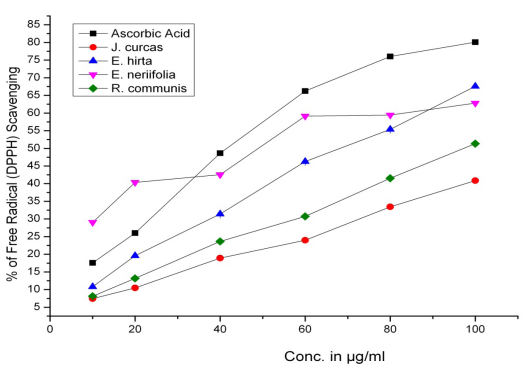
P Jagdeesan *et al* (2011) studied the antioxidant activity of *Euphorbia hirta* and it showed 78.33 µg /ml IC_{50} value for DPPH scavenging activity. Basma *et al* (2011) investigated the antioxidant activity of whole plant of *Euphorbia hirta* and leaf extract showed the lowest IC_{50}value 0.803 mg /ml.

Ramesh Kumar Singh *et al* (2010) evaluate the In-vitro antioxidant activity of *Ricinus communis* stems, DPPH scavenging activity with different solvent systems, benzene and 50% methanol successive extracts showed the maximum antioxidant activity with IC_{50} values of 36.19 ± 2.332 µg /ml and 34.40 ± 5.98 µg /ml respectively. But the methanol and chloroform extract also showed antioxidant activity with IC_{50} values of 64.18 ± 3.20 and 66.17 ± 6.30 µg/ml respectively and the distilled water crude extracts showed IC_{50} values of 106.14 ± 4.33 µg/ml. Adel Kadri *et al* (2011) found that essential oil isolated from aerial part of *Ricinus communis* exhibits a moderate DPPH radical scavenging ability, extracted oil shows maximum scavenging activity of 46.30 ± 2.43% at 300 mg /ml.

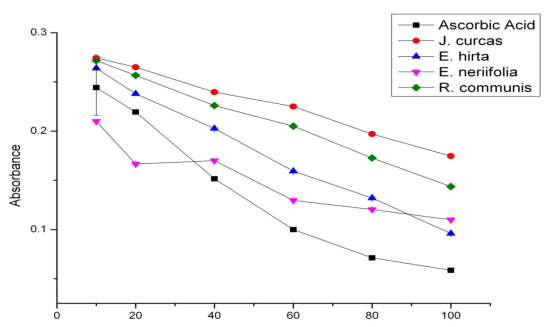
Pracheta *et al* (2011) determined first time antioxidant scavenging activity, reported that ethanolic extract of *Euphorbia neriifolia* leaves shows strong antioxidant. Datta Samaresh *et al* (2012) determined *In Vitro* Free Radical Scavenging of Methanolic Extract of *Euphorbia neriifolia* Linn. stem. The IC_{50} value for DPPH scavenging of *E. neriifolia* extract and standard ascorbic acid were found to be 85.11 ± 0.53 µg /ml and 8.54 ± 0.85 µg /ml.

Table.1: % DPPH scavenging activity of plant leaves methanolic extracts and ascorbic acid

S. N.	Conc.	(% of free radical Scavenging) Ascorbic Acid	Error ± SD	J. curcas	Error ± SD	E. hirta	Error ± SD	E. neriifolia	Error ± SD	R. communis	Error ± SD
1	10	17.56	0.03	7.43	0.0006	10.81	0	29.05	00	8.1	0.0006
2	20	26.01	0.0006	10.47	0	19.59	0	40.34	0.0006	13.17	0.002
3	40	48.64	0.002	18.91	0.002	31.41	0.0006	42.56	0	23.64	0
4	60	66.21	0	23.98	0	46.28	0.0006	59.12	0.0006	30.74	0
5	80	76.01	0.0006	33.44	0	55.4	0.001	59.45	0.0006	41.55	0.0012
6	100	80.06	0.0006	40.87	.0006	67.56	0	62.83	0	51.35	0.0006



Graph- 1 representing graph between conc. v/s % inhibitions of DPPH



Graph –2 : Graph between conc. v/s absorbance

Table.2: Absorbance of DPPH of plant leaves methanolic extracts and ascorbic acid

S.N.	Conc.	(Absorbance of DPPH)w Ascorbic Acid	Error ± SD	J. curcas	Error ± SD	E. hirta	Error ± SD	E. neriifolia	Error ± SD	R. communis	Error ± SD
1	10	0.244	0.03	0.275	0.0006	0.264	0	0.210	0	0.272	0.0006
2	20	0.219	0.0006	0.265	0	0.238	0	0.167	0.0006	0.257	0.002
3	40	0.152	0.002	0.240	0.002	0.203	0.0006	0.170	0	0.226	0
4	60	0.100	0	0.225	0	0.159	0.0006	0.130	0.0006	0.205	0
5	80	0.071	0.0006	0.197	0	0.132	0.001	0.120	0.0006	0.173	0.0012
6	100	0.059	0.0006	0.175	.0006	0.096	0	0.110	0	0.144	0.0006

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Table. 3 : The IC_{50} value of Selected plant extracts and ascorbic acid

S. No.	Plants Name (Botanical name)	IC_{50} Values µg/ml
1	Moti doodhi (E. Hirta)	61.34±0.0004
2	Sehud (E. Neriifolia)	58.00±0.0003
3	Arand (R. communis)	98.00±0.0006
4	Ascorbic Acid	48.67±0.01
5	Jatropha	Nil

Total Polyphenolic Content

Phenolic compound occur ubiquitously in plants and a variety of biological activities have been attributed to them. These advantageous properties include antimicrobial, antiviral, anti-ulcerogenic, cytotoxic, anti-neoplastic, mutagenic, antioxidant, anti-hepatotoxic, anti-hypertensive, hypolipidemic, anti-platelet and anti-inflammatory activities. Many of these biological functions corresponded to their free radical scavenging and antioxidant activities (Muraoka S et al. 2004).

Phenolic compounds are commonly present in both edible and non-edible plants and exhibit multiple biological effects including antioxidant activity (Kahkonen *et al.*, 1999). However, presence of flavonoids in extracts could be also responsible for the potent antioxidant activity (Padmaa M Paarakh et al. 2010). The phenolic contents of the selected plant extracts were determined by FC reagent and expressed as Catechol equivalents in µg/ml of crude extract.

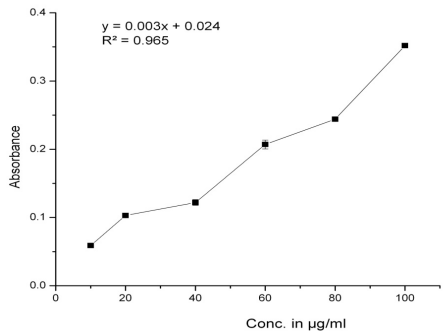
The result of **Graph-3** indicates the standard of Catechol which is used for the quantification of total polyphenolic content equivalent to Catechol µg/ml in methanolic extracts of selected plant leaves. **Table-4** presents the total polyphenolic content in Catechol equivent µg/ml.

Sanjana safi *et al* extracted the root of *Jatropha curcas* with different solvent systems and obtained highest amount of phenolic contents were found in methanolic crude extract and chloroform soluble fraction having TPC value of 36.37 and 27.01 mg of GAE/ gm of extractive respectively. G. El Diwani *et al* (2009) determined the ethanol extracts from roots, stem, leaves and nodes of *Jatropha curcas* and concluded the polyphenol content of ethanol extracts was high in root > leaf and lower in stem > nodes at concentration tested 50 and 100 ppm.

Basma A.A. *et al* (2011) studied the total phenolic content of leaves, stem, flower and root of *E. hirta* and found that leaves extract had the highest total phenolic content 206.17±1.95 mg equivalent to gallic acid/g of sample. Habila J.D. *et al* (2011) found that *E. hirta* possess polyphenolic content 7 mg/GAE.

Datta samaresh *et al* (2012) analysed the methanolic extract of *E. neriifolia* stems and concluded that the plant contains 675.6 µg of phenolic compound in 10 mg of the extract. Pracheta *et al* (2011) investigated and find out the ethanolic extract of *E. neriifolia* leaves contains 0.60±0.09 GAE/g of sample.

Durre Shahwar *et al* (2010) investigated the total phenolic contents of *Ricinus communis leaves* which possess the highest phenolic content (547.0 GAE/g of crude extract).



Graph -3: The Standard curve between of Catechol conc. v/s absorbance.

Table. 4: concentration of polyphenols present in the leaf extracts

S. No.	Plants Name (Botanical name)	Total polyphenolic content equivalent to Catechol µg/ml
1	Moti doodhi (*E. Hirta*)	14.97±0.0085
2	Sehud (E. *Neriifolia*)	5.36±0.0005
3	Arand(*R. communis*)	25.36±0.0003
4	Safed arand (*J. curcas*)	21.34±0.001

Conclusion

The overall result, obtained by the present study indicates that *E. hirta* possess strong antioxidant activity as compared to all the other three plants, while *R. communis* is the richest source of polyphenolic contents. These herbal antioxidants may play significant role in preserving the food stuff and can be used for the herbal medicines for antioxidant purpose. Further, there is still a need of a thorough investigation to identify, isolate, purify and characterize some more biologically active constituents which will be helpful to cure chronic diseases.

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