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In vitro Antioxidant Activity, Total Phenolic and Total Flavonoid Contents of *Dracaena terniflora Roxb.* Root Extracts.

Kavitha Vasudevan*[1], Dr. R .Sanil Kumar [2], Dr. M.C Sabu [1]

[1] *Mookambika College of Pharmaceutical Sciences and Research, Mannathoor P.O, 686667 Muvattupuzha, Ernakulam, Kerala, India.*

[2] *Department of Pharmacy, Annamalai University, Annamalai Nagar-608002, Chidambaram, India.*

Abstract:

The present study was carried out to estimate the phytochemical constituents mainly total phenolic and flavonoid contents and to measure the *in vitro* antioxidant activity of various extracts of the roots of *Dracaena terniflora* Roxb.(Liliaceae) in order to validate the medicinal potential of this plant. In this study, hexane, ethyl acetate, ethanol and water were used as solvents for the successive extraction of roots of *Dracaena terniflora* Roxb (DTR).The extracts showed the presence of phenols, flavonoids, tannins, resins, terpenoids, alkaloids, steroid, anthraquinones, sugars and saponins except glycosides and anthocyanins. The total polyphenols and flavonoids were determined. Among the various root extracts, aqueous extract (DTR-A) exhibited the highest phenolic content (90 mg gallic acid equivalent/g of roots) and ethanolic extract (DTR-E) showed the highest flavonoid content (72 mg quercetin equivalent/g of roots). The antioxidant activity of ethanolic and aqueous root extract were evaluated using total antioxidant capacity, 2, 2-diphenyl-l-picrylhydrazyl (DPPH) scavenging, nitric oxide scavenging and superoxide radical scavenging assays. Both the extracts showed significant antioxidant activity. The ethanolic extract of roots exhibited higher antioxidant activity than the aqueous extract by total antioxidant assay (IC_{50} = 383.565µg/ml), DPPH assay (IC_{50} = 58.002µg/ml), nitric oxide assay (IC_{50} = 248.895µg/ml) and super oxide assay (IC_{50} = 437.856µg/ml). These results indicate that the plant can be used to reduce oxidative stress. The presence of flavonoid compounds in the root extract could be considered responsible for conferring antioxidant ability. The ethanol is a recommended solvent for extracting antioxidants from this plant.

Key words: Free radical scavenging, antioxidant activity, *Dracaena terniflora* Roxb. Folin-Ciocalteu method, Aluminium chloride method, Colorimetric method.

INTRODUCTION:

Herbal medicine plays a vital role in maintaining the health and wealth of mankind. The healing properties of the medicinal plants are due to the phytochemicals present in them. These phytochemicals work with nutrients and fibres to form an integrated part of defence system against various diseases and stress conditions (1). Plant based antioxidant rich foods traditionally formed a major part of the human diet, and are performing an important role in maintaining human health(2).Thousands of herbal and traditional compounds are being screened worldwide to validate their use as antioxidants. There exists a well maintained balance between antioxidant mechanism and free radical generation in a normal healthy individual.(3) However in diseased states, the balance will shift towards the production of excessive free radicals or reactive oxygen species (ROS) like O_2, H_2O_2, OH^- , ROO^- and leads to a condition called oxidative stress. This oxidative stress is implicated in hundreds of diseases or disorders such as diabetes, cardiovascular diseases, neurological disorders such as Alzheimer's disease and Parkinson's disease, cancer and others (4). Poly Phenolic compounds in plants have proved as prominent natural antioxidants and they seem to display important anti-inflammatory, anti-diabetic, anti hepato toxic, anti-allergic and anti-cancer activities. *Dracaena terniflora* Roxb. *(Pleomele terniflora* Roxb.) is known as Manjakkanda or Manjakantha (in Malayalam). The synonyms of this plant include Elathaani, dwarf dracaena or wild dracaena of the family Liliaceae. (formerly in Dracaenaceae or Agavaceae). It has wide spread uses in ethno medicine. It is a stout perennial subshrub. It is less than 1m tall. Stem is covered with annual leaf scar, Stem is sprawling, simple or few branched, internodes are longer. Leaves are spaced along distal part of stem. The plant prefers moist marshy land for its growth. This is found in evergreen and semi evergreen forests in India and South-East Asia (5)

According to tribal literature available, the herbal preparation containing *Dracaena terniflora* Roxb. is used for treating spermaturia.(6) The fresh juice of this plant is used in the treatment of diabetes by the Kurunarippullu tribes of Wayanad.(7)(8) Roots boiled with rice are taken internally for jaundice and root of this plant is used for the treatment of various liver disorders especially jaundice by the various tribal communities of Kerala.(9)Traditionally the Root extract is used for piles, Fruits boiled in coconut oil are used against head ache.(10) Despite the widespread folklore uses of *Dracaena terniflora* Roxb. In the management of various diseases there is no documented report regarding the antioxidant activity of this plant.

Thus the aim of this study was to evaluate the *in vitro* antioxidant activity of the roots of *Dracaena terniflora* Roxb.

MATERIALS AND METHODS:

Plant material:

The whole plant of *Dracaena terniflora* Roxb. were collected in March 2017 from the forests of Wayanad and identified by Dr. Sr. Tessy Joseph, H.O.D department of botany, Nirmala College, Muvattupuzha, kerala, India where a herbarium specimen was deposited (Voucher number NCH/2017/538).

Preparation of extracts:

Fresh plant materials were washed thoroughly in running tap water to remove adhering impurities, shade dried to constant weight. The roots and aerial parts were separated.

Roots were coarsely powdered separately and passed through a 40-mesh sieve. It was stored in a tightly closed container. Fifty grams of the dried root powder was successively extracted by Soxhlet apparatus using different organic solvents with analytical reagent (AR) quality. These solvents (low polarity to high polarity) were hexane (68^0C), ethyl acetate (76-78^0 C), ethanol (78.37^0 C), and finally water (100^0C) used successively for the extraction. To ensure the complete extraction process, exhaustive extraction was applied with each solvent for 10 hours. Extracts of different organic solvents were collected separately into dry clean beakers, after that they were recovered from the solvents by evaporation in a rotary evaporator at 60^0C, final drying were done by keeping the extract in desiccators for 1 hour and finally the extracts were weighted and the percentage of each extract was determined and showed in table.1 Dried extracts were kept at 20^0c until further tests were carried out. (11)

Extract % = Weight of extract in grams / Weight of sample in grams* 100

Preliminary Qualitative phytochemical analysis (12) (13)

The preliminary phytochemical analysis of all the extracts was carried out using standard procedures to identify the various constituents and the inferences were tabulated in table.2.

Determination of Total phenolic content (TPC) (14) (16)

Folin-Ciocalteau method was used for the determination of the total phenolic content of the plant extracts using gallic acid as an internal standard with slight modification as previously reported. 1 ml of the extract (1 mg/ml) was mixed with 2.5 ml of Folin-Ciocalteau phenol reagent. After 5 min, 4 ml of 7.5% Na_2CO_3 solution was added to the mixture and made up to the mark with distilled water. The mixture was incubated in the dark for 90 minutes at room temperature. A set of standard solutions of gallic acid (100, 200, 400, 800 and 1000 µg/ml) were prepared in the same manner as described for the extracts. The absorbances of the extracts and standard solutions were read against the reagent blank at 750nm with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The total phenolic content was determined from the calibration curve and expressed as milligram of gallic acid equivalent (GAE) per gram of the extracts. The determination of the total phenolic in the extract was carried out in triplicates.

Determination of total flavonoid content (TFC) (15) (16)

The total flavonoid content was determined according to the aluminium chloride colorimetric method. Each plant extracts (2 ml, 0.3 mg/ml) in methanol were mixed with 0.1 ml of 10% aluminium chloride hexa hydrate, 0.1 ml of 1 M potassium acetate and 2.8 ml of deionized water. After the 40 minutes incubation at the room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. A set of standard solutions of quercetin (100, 80, 60, 40 and 20 µg/ml) were prepared in the same manner as described for the extracts. The absorbances of the extracts and standard solutions were measured against the reagent blank at 415 nm with a UV/Visible spectrophotometer. The total flavonoid content was determined from the calibration curve and expressed as milligram of quercetin equivalent (QE) per gram of extracts. The determinations of total flavonoid in the extracts and standards were carried out in triplicates.

***In-vitro* antioxidant activity**

Total antioxidant Capacity (17)

The total antioxidant capacity of the methanol extract was evaluated by the phosphomolybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid.

Procedure:

Different concentrations of the sample such as125µg/ml-2000µg/ml from a stock concentration of 10mg/ml are combined with 3 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing reaction solutions were incubated at 95^0C for 90 minutes. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid using standard plot.

DPPH radical scavenging assay (18)

The radical scavenging activity of different extracts was determined by using DPPH radical scavenging assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured by the spectrophotometric method at 517 nm.

0.1mM DPPH was prepared by dissolving 4mg of DPPH in 100ml of methanol. Different volumes of extracts, 1.25µl-20 µl (12.5 - 200 µg/ml) from a stock solution of 10mg/ml were made up to a final volume of 20µlwith DMSO and 1.5 ml of DPPH (0.1mM) solution was added. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of absorbance was measured at 517 nm by using a spectrophotometer. The solution without any extract and with 3ml DPPH was taken as control. The experiment was replicated in three independent assays. Ascorbic acid (10mg/ml in DMSO) was used as reference.

Inhibition of DPPH free radical in percentage was calculated by the formula:

(%) inhibition = $(A_{control}-A_{test})/A_{control}\times100$

Where, $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of samples.The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the graph after plotting inhibition percentage against extract concentration.

Nitric oxide scavenging activity (18)

This procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete

with oxygen leading to reduced production of nitric ions. Large amount of NO may lead to tissue damage. Nitric oxide scavenging was measured spectrophotometrically. Gallic acid (10mg/ml) was used as standard.

Procedure:

Sodium nitroprusside (5mmol/l) in phosphate buffer saline (PBS) was mixed with different concentrations of the sample such as 125µg/ml -2000µg/ml) from a stock solution of 10mg/ml and incubated at 25 °C for 30 min. A control without the test compound, but an equivalent amount of methanol was taken. After 30 minutes, 1.5mL of the incubated solution was removed and diluted with 1.5mL of Griess reagent (1% sulphanilamide, 2% $H_3PO_4$and 0.1% napthylethylenediaminedihydrochloride). The absorbance of the chromospheres formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive control. All the tests were performed in triplicates and the graph was plotted with the mean values. The percentage of inhibition was measured by the following formula:

Radical scavenging activity (%) = $(A_{control}-A_{test})/A_{control}\times100$

Where $A_{control}$ is the absorbance of the control (without extract) and A_{test} is the absorbance in the presence of the extract/standard.

Superoxide radical scavenging assay (18)

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan product. Ascorbic acid (10mg/ml) was used as the standard.

Procedure:

Different concentrations of extracts such as 125-2000µg/ml from a stock solution of 10mg/ml, 0.05ml of Riboflavin solution (0.12mM), 0.2 ml of EDTA solution [0.1M], and 0.1 ml NBT (Nitrobluetetrazolium) solution [1.5mM] were mixed in test tube and reaction mixture was diluted up to 2.64ml with phosphate buffer [0.067M]. A control without the test compound, but an equivalent amount of distilled water was taken. The absorbance of solution was measured at 560nm using DMSO as blank after illumination for 5 minutes in fluorescent light and also measured after illumination for 30 minutes at 560nm on UV visible spectrophotometer. Difference in OD was determined.

The percentage inhibition of superoxide anion generation was calculated as:

%inhibition = (control OD – sample OD / control OD) ×100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

Statistical analysis:

All the experiments were carried out in triplicate. Values are expressed as mean ± SEM.

RESULTS AND DISCUSSION

Extraction yield of successive extracts of *Dracaena terniflora* Roxb. Root.

The extraction yield of different fractions of extracts of *Dracaena terniflora Roxb.* Root followed in the order of hexane < ethyl acetate< water < ethanol. (Table: 1).The extraction with ethanol resulted in the highest amount of total extractable compounds whereas the extraction yield with hexane was only small in comparison with that of the other solvents.

TABLE: 1

Sl.no	Solvent	colour	% Yield(w/w)	Form
1	Hexane	Golden Yellow	0.14	Sticky Mass
2	Ethyl Acetate	Orange	2.8	Sticky Mass
3	Ethanol	Reddish Brown	6.31	Sticky Mass
4	Water	Brownish black	5.87	Sticky Mass

Preliminary qualitative phytochemical analysis

The present study revealed that the successive extracts of roots of *Dracaena terniflora* contained alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids, carbohydrates, proteins, coumarins and Triterpenoids as in (Table:2). However N- hexane and ethyl acetate extracts showed the fewer amounts of primary and secondary metabolites. Compared to all other solvent extracts, aqueous and ethanolic root extracts had higher concentration of primary and secondary metabolites with high degree of precipitation (+++). Triterpenoids and coumarins were determined to be present with lesser amount (+), in all extracts. Anthocyanins and glycosides were absent in all the extracts.

TABLE: 2

Sl. No	Phytoconstituents	Dracaena terniflora root extract (DTR)			
		Hexane	Ethyl Acetate	Ethanol	water
1	Phenols	+	+	++	+++
2	Flavanoids	+	+	+++	++
3	Tannins	+	+	++	+
4	Alkaloids	+	+	++	+
5	Glycosides	-	-	-	-
6	Carbohydrates	-	-	+++	++
7	Proteins	-	-	++	+
8	Anthocyanins	-	-	-	-
9	terpenoids	+	+	+++	+
10	Saponins	+	+	++	+
11	Steroids	+	+	++	++
12	Coumarins	+	+	+	+
13	Triterpenoids	+	+	+	+

+++: highly present, ++: moderately present, +: Low, -: absent, DTR: root extract.

Total phenolic (TPC) and flavonoid (TFC) content:
Total phenolic and flavonoid contents of various root extracts of *D.terniflora Roxb* is tabulated in table: 3.Among the various solvent extracts, aqueous root extract of *Dracaena terniflora Roxb.* exhibited the highest phenolic content (90 mg gallic acid equivalent/g of roots of the extract) followed by ethanol (31 mg GAE/g), ethyl acetate (19mgGAE/g) and hexane extract (11mgGAE/g). Ethanolic extract showed the highest flavonoid content (72 mg quercetin equivalent/g of extract) followed by hexane (58mgQE/g), water (48mgQE/g) and ethyl acetate (36mgQE/g) extracts.

TABLE: 3

Solvents	Total phenolic content (mgGAE/g)	Total flavonoid content (mg QE/g)
hexane	11.2 ± 0.1	58.17±0.06
Ethyl acetate	19.22 ±0.08	36.23±0.15
Ethanol	31.27 ±0.15	72.07±0.15
Water	90.17 ±0.12	48.73 ±0.15

Values are means of three analyses of the extract ± standard deviation (n=3) GAE: Gallic acid equivalent, QE: Quercetin equivalent.

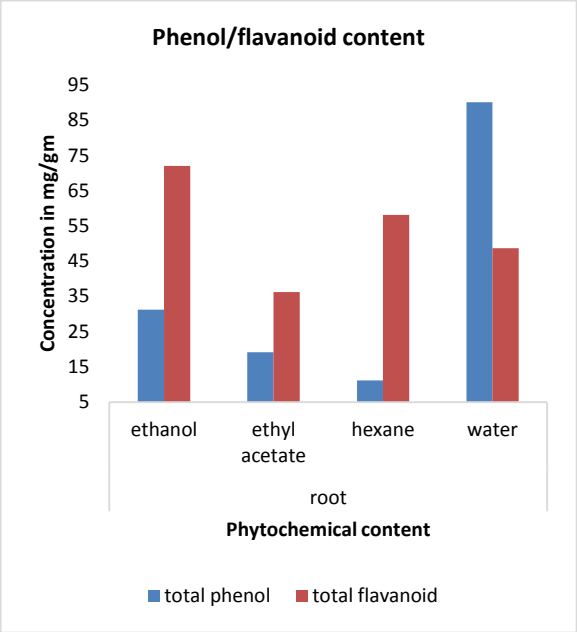

FIGURE: 1 Total Phenolic and Flavonoid content of different extracts.

IN VITRO ANTIOXIDANT ACTIVITY
Antioxidant capacity of DTR was examined by using five different assays such as total antioxidant, DPPH, nitric oxide scavenging and super oxide scavenging models respectively. Of all the four successive solvent extracts of *Dracaena terniflora Roxb*. aqueous extract showed highest phenol content and ethanolic extract exhibited high flavonoids. Hence both the extracts were selected for performing the antioxidant studies to compare their effect against oxidative stress. While performing various free radical scavenging activity studies, it was found that there was a significant linear correlation between the free radical scavenging activity determined by using the total antioxidant, DPPH, NO, super oxide methods, and total polyphenolic content (phenols and flavonoids) of the extracts.

Total antioxidant activity (Phosphomolybdenum assay)
Results in Table: 4 indicated that the DTR-E extract of the roots of *D.terniflora Roxb* showed the highest total antioxidant activity with IC_{50} value of 383.565µg/ml. Also, there are no significant differences between the IC50 values of DTR-A extract and ascorbic acid (730.196µg/ml and 737.394µg/ml respectively).

TABLE: 4 Total antioxidant activity of Roots of **D. terniflora Roxb.**

Sl. No	Concentrations(µg/ml)	DTR-E	DTR-A	Ascorbic acid
1	125	18.47 ±0.03	10.30 ±0.15	14.50 ±0.18
2	250	42.66 ± 0.015	34.78 ±0.10	29.28 ±0.22
3	500	59.66 ± 0.015	42.68 ±0.28	42.63 ±0.24
4	1000	68.65 ± 0.021	60.32 ±0.14	57.55 ±0.32
5	2000	74.61 ± 0.032	71.49 ±0.20	88.38 ±0.23

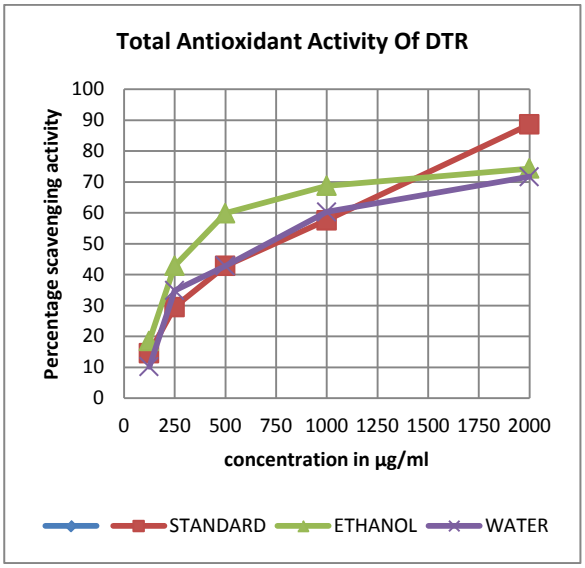

Figure: 2 Total antioxidant activity of Roots of *D. terniflora Roxb.*

DPPH scavenging:
The aqueous (DTR-A) and ethanolic(DTR-E) extracts of the roots of *Dracaena terniflora Roxb* showed promising free radical scavenging effect against DPPH in a concentration dependent manner upto a concentration of 200 µg/ml and tabulated in table: 5. DTR-E showed more scavenging activity than DTR-A. IC_{50}values of DTR-E, DTR-A and ascorbic acid were 58.002µg/ml, 80.7313µg/ml and 34.30µg/ml respectively. The ethanolic root extract of *D. terniflora Roxb* showed very high free radical scavenging property with less concentration of the extract as compared with DTR-A as evidenced from the IC_{50} values obtained.

TABLE: 5 DPPH Radical scavenging activity of roots extracts of *D.terniflora Roxb*.

Sl. No	Concentrations (µg/ml)	DTR-E	DTR-A	Ascorbic acid
1	12.5	27.57±0.5	19.37±0.04	21.64±0.26
2	25	45.32±0.17	26.60±0.03	40.28±0.33
3	50	49.36±0.1	43.61±0.04	60.34±0.40
4	100	62.26±0.09	55.37±0.24	83.50±0.32
5	200	71.07±0.12	65.25±0.08	96.19±0.16

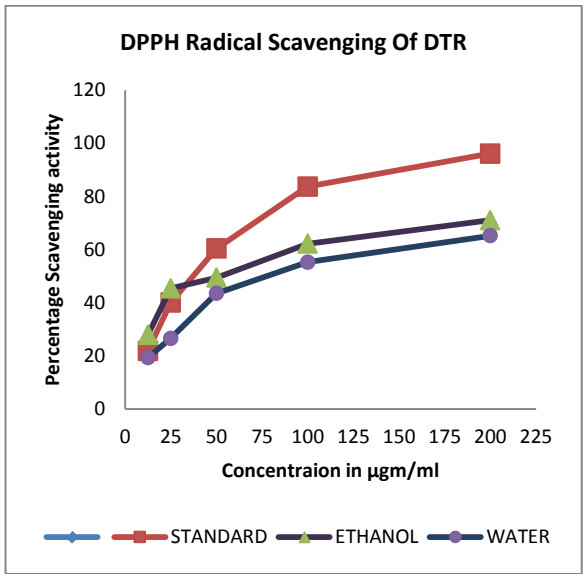

Figure: 3 DPPH Radical scavenging activity of roots extracts of *D. terniflora Roxb*

Nitric oxide scavenging:

Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with super oxides. These compounds are responsible for altering the structural and functional behaviour of many cellular components. Table 6: illustrate the percentage inhibition of nitric oxide generation by ethanolic, aqueous *D. terniflora* root extracts and gallic acid respectively. The IC_{50} value for NO inhibition of ethanolic extract (DTR-E) was found to be lowest among the two extracts (248.895µg/ml) and that of standard gallic acid was 102.39µg/ml. The result indicates that the ethanolic extract of *D. terniflora Roxb.* root (DTR-E) has comparable NO scavenging activity with standard gallic acid.

TABLE: 6 Nitric oxide scavenging activity of roots of *D. terniflora Roxb.*

Sl. No	Concentrations(µg/ml)	DTR-E	DTR-A	Gallic acid
1	125	25.46 ±0.15	13.60±0.19	57.27 ± 0.20
2	250	50.22 ±0.25	19.20 ±0.19	64.47 ±0.16
3	500	54.64 ±0.37	34.36 ±0.28	68.15 ±0.05
4	1000	62.95 ±0.95	42.57 ±0.33	71.72 ±0.23
5	2000	71.89 ±0.48	56.11 ±0.12	92.58 ±0.32

All values in this table represent the mean ±SD (n=3)

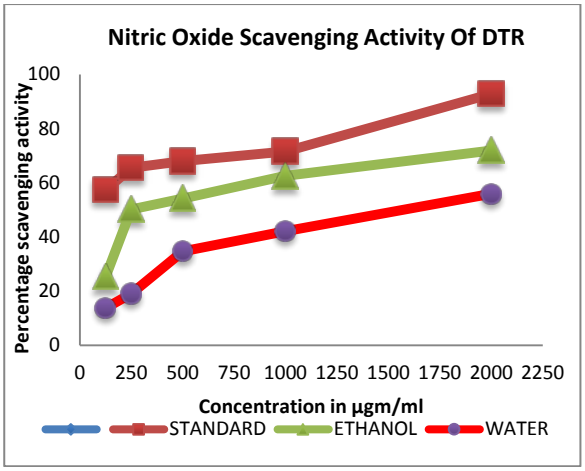

Figure: 4 Nitric oxide scavenging activity of roots of *D. terniflora Roxb*.

Super oxide scavenging:

Superoxide radical will reduce NBT to a blue-coloured formation that was measured at 560 nm. The superoxide scavenging activity of root extracts of *D. terniflora Roxb.* (DTR-E and DTR-A) and of standard Ascorbic acid were estimated at concentrations of 125µg/ml-2000µg/ml and were shown in table: 7. The DTR-E and DTR-A extract exhibited moderate super oxide radical scavenging activity, while comparing with ascorbic acid. IC_{50} value of DTR-E, DTR-A and standard Ascorbic acid were 437.856µg/ml, 499.073µg/ml and 254.50µg/ml respectively.IC_{50} values of DTR-E and DTR-A extracts were almost identical with each other. That means both the extracts were exhibited similar super oxide scavenging effect.

TABLE: 7 Superoxide scavenging activity of root extracts of *D. terniflora Roxb*

Sl. No	Concentration (µg/ml)	DTR-E	DTR-A	Ascorbic acid
1	125	25.38 ±0.20	21.43 ±0.07	41.32 ±0.11
2	250	40.79 ±0.18	38.36 ±0.09	47.64 ±0.14
3	500	53.23 ±0.15	50.86 ±0.04	62.47 ±0.27
4	1000	63.49 ±0.02	62.57 ±0.1	76.28 ±0.08
5	2000	71.45 ±0.39	70.36 ±0.22	90.61 ±0.48

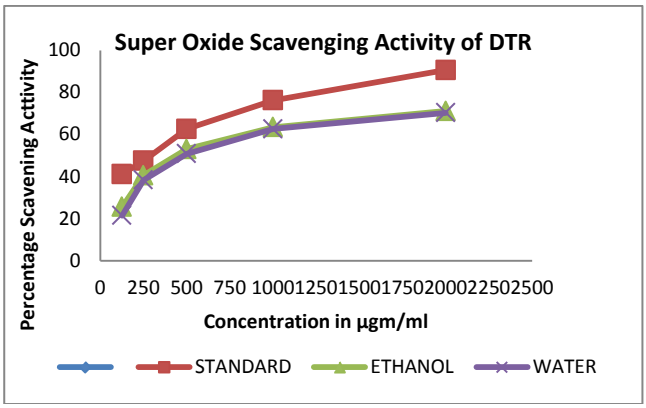

Figure: 5 Superoxide scavenging activity of root extracts of *D. terniflora Roxb*.

CONCLUSION

In the present study, dried roots of the plant *D.terniflora Roxb* were successively extracted with four solvents viz. hexane, ethyl acetate, ethanol and water according to their increasing order of polarity. It was observed that the ethanolic extract of roots of the plant *D.terniflora Roxb* extended highest extractive yield when compared with hexane, ethyl acetate and Water. Successive root extracts of *D.terniflora Roxb* were subjected to phytochemical screening and revealed the presence of phenols, flavonoids, tannins, resins, terpenoids, alkaloids, steroid, anthraquinones, sugars and saponins except glycosides. The various phytochemical compounds detected are known to have beneficial importance in medical science. Among the various root extracts, aqueous extract exhibited the highest phenolic content (90 mg gallic acid equivalent/g of roots) and ethanolic extract showed the highest flavonoid content (72 mg quercetin equivalent/g of roots). Therefore both extracts were chosen for in depth study. The results of different antioxidant assays portrayed that the DTR-E extract has maximum antioxidant activity than that of the aqueous root extracts (DTR-A). After analysing the IC_{50} values of the extracts it was confirmed that total antioxidant activity and nitric oxide scavenging activity of ethanolic root extract of *Dracaena terniflora Roxb* (DTR- E) were remarkably prominent when compared with their respective standards. The confirmed antioxidant activity of ethanolic root extract of *Dracaena terniflora Roxb* is probably due to the presence of poly phenols available in them. Total antioxidant scavenging of aqueous root extract of *Dracaena terniflora Roxb* (DTR-A) was almost similar to that of the standard ascorbic acid. All the other assays confirmed the free radical scavenging activity of the root extract of *Dracaena terniflora Roxb*. Based on these results, it is suggested that the ethanolic extract of underground parts of *Dracaena terniflora Roxb* can be utilized in food and pharmaceutical manufactures as an effective and safe source of natural antioxidants. However, further studies should be performed for isolation and identification of the antioxidant compounds of these extracts and evaluate their antioxidant potential in an *in vivo* system.

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