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In Vitro Antioxidant Activities of Methanolic Extract of *Triumfetta rotundifolia* (Linn.) Family (Tiliaceae)

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ABSTRACT

In the present study, in vitro antioxidant of the methanolic extracts of Triumetta rotundifolia(Linn.) leaves were investigated. Methanolic extracts were prepared from fresh dried leaves of Triumfetta rotundifolia by hot continuous percolation method in Soxhlet apparatus.The antioxidant activity of methanolic extracts of Triumfetta rotundifolia(Linn) estimated by total reduction capability, superoxide anion scavenging activity, free radical scavenging activity, hydrogen peroxide scavenging activity was determined in treated with different concentrations of vitamin C and butylated hydroxyl anisole (BHA) as standard antioxidant compound.

Keywords: Triumfetta rotundifolia, Antioxidant activities,superoxide anion scavenging activity

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INTRODUCTION

Plants, owing to its medicinal value have continued to play a dominant role in the maintenance of human health since ancient times. The world health organization estimates that the plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [1]. Turkish people have a tradition of using a number of plant species for the treatment of infectious diseases and various ailments [2]. Traditional and folkloric medicines play important role in health services around the world. About three quarter of the world's population rely on plant and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. The subcontinent is rich in medicinal plants and is one of the richest countries in terms of genetic diversity of medicinal plants. It exhibits a wide range in topography and climate, which have a bearing on its vegetation and floristic composition. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties [3]. Several plants have been used in folklore medicine [4]. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. Triumfetta rotunifolia(Linn.) belongs to the family Tiliaceae is widely used in Indian traditional medicines and the leaf paste is used to treat rheumatic pain, cough, fever and severe cold [5].

Leaf paste is taken along with pepper to treat dyspepsia [6] Bark paste, mixed with hot milk is used internally for treating urinary infections [7]. From the above information the present investigation was undertaken which deals with the studies of the different extracts of Triumfetta rotundifolia against various gram positive and gram negative bacteria and fungal organism, the result of which are being reported in the present communication.The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [8]. Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals. Traditionally, leaves are used as an application to boils and abscesses. The plant is useful in cold and eye disease. The plant is being used very specifically in the indigenous systems of medicine such as Ayurveda, Siddha and Unani. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [9]. In the present paper, we have examined the chemical composition of the methanol extract of Triumfetta rotundifolia (Linn.) In addition, the antioxidant activities of the methanol extract of Triumfetta rotundifolia (Linn.) were also investigated.

MATERIALS AND METHODS

Plant materials

Whole fresh plant leaves of Triumfetta rotundifolia (Linn.) were collected from kalakatu, Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Goverment of India, Palayamkottai. The whole plant leaves were

dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials (1kg) were successively extracted with petroleum ether (40-60°C), ethyl acetate (40-60°C) and methanol (70-80°C) for 48 hrs by continuous hot percolation method in soxhlet apparatus (Harrbone JB, 1984).The methanolic extract was collected and evaporated to dryness by using a vaccum distillation unit. The yield of the crude methanolic extract was found to be 16.08%.

In vitro antioxidant assay

Total Reduction Capacity

The reduction capacity of a compound indicates its antioxidant potential. The transformation of Fe3+ - Fe2+ was investigated for measuring the reductive ability. Total reduction capability of various extract of Triumfetta rotundifolia (Linn.) was estimated using the method described by Oyaizu [11]. Various concentrations of Triumfetta rotundifolia (Linn.) (25-75 µg.ml-1) were prepared in 2.5 ml of 0.2M phosphate buffer pH 6.6. To 2.5 ml of 1% potassium ferricyanide [K3Fe-(CN) 6] and 2.5 ml of 10% trichloroacetic acid was added. The mixture is incubated at 50^{O}C for 20 min and centrifuged at 2000 rpm for 30 minutes. Supernatant solution (2.5 ml) of 10% trichloro acetic acid was added. The mixture was incubated at 50^{O}C for 20 min and centrifuged at 2000 rpm for 30 minutes. Supernatant solution (2.5ml) was mixed with equal volume of distilled water and 0.5ml of 0.1% FeCl3. The absorbance of the solution was measured at 700 nm using spectrophotometer (Shimadzu 160 UVPC). Higher absorbance was the indication of grater reduction power and it was compared with the absorbance capacity of standard antioxidants, ascorbic acid and butylated hydroxyl anisole (BHA).

Superoxide Anion Activity

Superoxide anion is an oxygen-centred radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non-enzymatic electron transfers that univalently reduce molecular oxygen [12]. Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleodide (PMS-NADH) and oxygen. It was assayed by the reduction of nitro blue tetrazolium (NBT). In these experiments the superoxide anion is generated in 3 ml of Tris-Hcl buffer (100mM, pH 7.4) containing 0.75 ml of NBT (300µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 ml of various concentration of extracts of Triumfetta rotundifolia (Linn.) such as 25, 50, 75, 100, 250 and 500 µg.ml-1. The reaction begins with the addition of 0.75 ml of PMS (120 µM) to the mixture. After 5 minute of incubation at room temperature, the absorbance at 560nm was measured using a spectrophotometer (Shimadzu 160 UVPC). The percentage of super oxide anion scavenging activity was calculated using the following equation. The percentage

scavenging of superoxide anion radical = [(A0-A1)/A0×100], where A0 is the absorbance of the control reaction (blank, without extract) and A1 is the absorbance of the standard sample.

Free Radical Scavenging Activity

DPPH is used as a free radical to evaluate antioxidant activity of natural compounds. The degree of its discoloration is attributed to the hydrogen donating ability to test compound [13]. The free radical scavenging activity of Triumfetta rotundifolia (Linn.) was estimated by using 1, 1- diphenyl – picryl – hydrazil (DPPH) as per the method described by shimada co-workers. In this experiment 0.1mM solution of DPPH in methanol was prepared. 1ml of the DPPH solution was added to 3 ml of various concentrations of Triumfetta rotundifolia (Linn.) such as 25, 50, 75, 100,250 and 500 µg.ml-1. The mixture was shaken vigorously and kept at room temperature for 30 min. The absorbance was measured at 517 nm using UV-Visible spectrophotometer (Shimadzu 160 UVPC). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of DPPH scavenging effect was calculated.

Hydrogen Peroxide Activity

The ability of the extracts of Triiumfetta rotundifolia (Linn.) to scavenge hydrogen peroxide is determined as per the method described by Ruch and co-investigators [14]. A solution of hydrogen peroxide (40mM) is prepared in phosphate buffer (pH 7.4). Various concentrations of Triumfetta rotundifolia(Linn.) 25, 50, 75, 100, 250, 500 µg.ml-1 in distilled water was added to hydrogen peroxide solution (0.6ml, 40mM). After 19 minute the absorbance of hydrogen peroxide was measured at 230 nm against a blank solution consisting of phosphate buffer without hydrogen peroxide. The concentration was determined spectrophotometrically at 230nm (Shimadzu 160 UVPC). The percentage of scavenging activity of hydrogen peroxide was calculated.

RESULTS AND DISCUSSION

Effect on Total Reduction Capacity

The effect of methanolic extract of Triumfetta rotundifolia(Linn.) with the concentrations of 25, 50, and 75µg.ml-1 were tested for total reduction capability. From the study observed that DVET showed significant ($p<0.01$) reduction potential and the results are comparable to that of standard antioxidants such as l-ascorbic acid and BHA. The order of total reduction capability of DVET and the standard antioxidants are BHA> DVET> l-ascorbic acid. The results were shown in table-1.The reduction property of DVET increased proportionately to its concentrations. DVET showed higher antioxidant effect than l-ascorbic acid and lower antioxidant effect than BHA at all concentrations. For the measurements of the reductive ability, the Fe3+ -Fe2+ transformation in the presence of DVET was investigated and found to have significant reducing ability. The reducing capacity of a compound may serve as significant indicator of its potential antioxidant activity. The antioxidant activity of a compound has been attributed to various mechanisms among such as prevention of chain initiation, binding of

transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [15].

Table- 1: Effect of methanol extract of *Triumfetta rotundifolia* (Linn.) in total reduction capability

Concentration ($\mu g.ml^{-1}$)	Absorbance@ 700nm		
	l-Ascorbic acid	BHA	DVET
25	0.931±0.014**	1.63±0.051**	1.132±0.020**
50	1.431±0.002**	2.07±0.034**	1.741±0.002**
75	2.040±0.002**	2.84±0.042**	2.243±0.009**

The effect was compared with standard antioxidants such as l-ascorbic acid and BHA. The values are expressed as mean ± S.E.M. n=3. The statistical analysis was carried out using one way ANOVA, where ***p*<0.01.

Effect on Superoxide Anion Radical Scavenging Activity

Table-2: Effect of methanol extract of *Triumfetta rotundifolia* (Linn.) in superoxide anion radical scavenging activity

Concentration ($\mu g.ml^{-1}$)	% inhibition of superoxide generation		
	l-Ascorbic acid	BHA	DVET
25	10.231±1.214**	15.632±1.251**	0.932±0.302
50	15.831±0.682**	19.873±0.630**	3.774±0.052
75	18.240±0.002**	26.843±1.242**	17.543±0.854**
100	28.478±0.249**	52.584±1.870**	27.642±0.315**
250	70.158±2.297**	82.232±0.271**	61.458±0.431**
500	78.835±0.289**	88.693±1.412**	76.799±0.456**

The effect was compared with standard antioxidants such as l-ascorbic acid and BHA. The values are expressed as mean ± S.E.M. n=3. The statistical analysis was carried out using one way ANOVA, where **p<0.01.

The effect of methanolic extract of Triumfetta rotundifolia (Linn.) with the concentrations of 25, 50, 75, 100, 250 and 500 µg.ml-1 were tested for their superoxide anion radical scavenging activity. The percentage of inhibition of superoxide radical generation of DVET at the concentrations of 25, 50, 75, 100, 250 and 500 µg.ml-1 was determined and the results were compared with the same concentrations of standard antioxidants such as l-ascorbic acid and BHA. From the study it has been observed that the methanol extract has showed a significant (p<0.01) activity when compared to the standard antioxidants such as l-ascorbic acid and BHA. The percentage of inhibition of superoxide generation by DVET at 500µg.ml-1 concentration was found to be 72.03%, whereas, l-ascorbic acid and BHA were found to be 78.82% and 88.68%, respectively. The orders of superoxide radical scavenging activity of petroleum ether extract and standard antioxidants are BHA> l-ascorbic acid> DVET. The crude extract of DVET showed almost equivalent antioxidant activity of standard antioxidants. The results were shown in Table-2. Superoxide anion radicals are produced endogenously by flavoenzymes like xanthine oxidase, which convert hypoxanthine to xanthine and subsequently to uric acid in ischemia. In the phenazine methosulfate-nicotinamide adenine dinucleodide (PMS-NADH) system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT [15]. The reduction in absorbance at 560 nm with

antioxidants indicates the consumption of superoxide anion in the reactive mixture. The crude extract of DVET showed almost equivalent antioxidant activity of standard antioxidants. Superoxide anions generation found to be reduced in the presence of DVET in hypoxanthine/ xanthine oxidase reaction. In this system there are two possibilities: either the plant extracts scavenge the O2- or they inhibit the xanthine oxidase activity.

Effect on Free Radical Scavenging Activity

Table-3: Effect of Free radical scavenging activity of methanol extract of Triumfetta rotundifolia (Linn.)

Concentration ($\mu g.ml^{-1}$)	% inhibition of free radical generation		
	l-Ascorbic acid	BHA	DVET
25	51.710±0.406**	43.632±1.551**	29.142±2.291
50	55.831±0.482**	57.573±0.330**	28.174±0.402
75	57.740±1.572**	60.643±1.242**	31.343±0.934
100	59.878±1.749**	66.254±1.870**	49.752±2.735**
250	66.458±1.797**	69.732±0.271**	65.658±0.331**
500	68.235±1.289**	71.243±1.282**	67.739±1.432**

The effect was compared with standard antioxidants such as l-ascorbic acid and BHA. The values are expressed as mean ± S.E.M. n=3. The statistical analysis was carried out using one way ANOVA, where **p<0.01.

The effect of methanolic extract of Triiumfetta rotundifolia(Linn.) with the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were tested for their free radical scavenging activity. The percentage of inhibition of superoxide radical generation of DVET at the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were determined and the results were compared with the same concentrations of to that of standard antioxidants such as l-ascorbic acid and BHA. From the study it has been observed that the DVET at 500µg.ml-1 significantly reduced the concentration of DPPH radical formation and the results were compared to the standard antioxidants such as l-ascorbic acid and BHA. The orders of free radical scavenging activity of DVET and standard antioxidants at a concentration of 500µg.ml-1 were; BHA > l-ascorbic acid > DVET and the values are 71.24%, 68.26%, 67.77% respectively. Free radical scavenging activity of DVET increased proportionately, the results were shown in Table-3.

The model of scavenging the stable DPPH radical is the widely used method to evaluate antioxidant activities in a relatively short time compared to other methods. The effect of antioxidants on DPPH radical scavenging is supposed due to their hydrogen donating ability. DPPH is a stable free radical and accepts hydrogen radical to become a stable free radical and accepts an electron on hydrogen radical to become a stable diamagnetic molecule [16]. The decrease in absorbance of DPPH radical caused by antioxidants, due to the reaction between antioxidant molecule and radical progresses, results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. These results revealed that the DVET is a free radical inhibitor.

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Effect on Hydrogen Peroxide Scavenging Activity

Table- 4.4: Effect of Hydrogen peroxide scavenging activity of methanol extract of *Triumfetta rotundifolia* (Linn.)

Concentration ($\mu g.ml^{-1}$)	% inhibition of hydrogen peroxide generation		
	l-Ascorbic acid	BHA	DVET
25	13.720±0.046**	15.232±2.251**	12.39±2.653**
50	24.431±0.682**	18.573±0.480**	26.59±0.872**
75	35.940±0.172**	37.643±0.342**	36.25±1.254**
100	49.178±0.189**	48.654±0.270**	47.29±0.525**
250	56.458±0.797**	58.742±0.771**	57.29±0.532**
500	58.835±0.389**	67.643±0.282**	61.40±0.087**

The effect was compared with standard antioxidants such as l-ascorbic acid and BHA. The values are expressed as mean ± S.E.M. n=3. The statistical analysis was carried out using one way ANOVA, where **p<0.01.

The effect of methanolic extract of Triumfetta rotundifolia (Linn.) with the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were tested for their hydrogen peroxide radical scavenging activity. The percentage of inhibition of hydrogen peroxide radical scavenging of DVET at the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were determined and the results were compared with the same concentrations of to that of standard antioxidants such as l-ascorbic acid and BHA. From the study it has been observed that the methanolic extract of Triumfetta rotundifolia (Linn.) showed a significant ($p<0.01$) promising activity at all concentrations and the result was similar to l-ascorbic acid and BHA at the same concentrations. The percentage of hydrogen peroxide scavenging activity of l-ascorbic acid and BHA and DVET at a concentration of 500µg.ml-1 was found to be 58.84%, 62.64% and 61.97% respectively. The order of hydrogen peroxide scavenging activity of DVET and standard antioxidants were; BHA> DVET> l-ascorbic acid. The results were shown in Table-4. H2O2 is highly important because of its ability to penetrate biological membranes. H2O2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. These results revealed that the DVET have hydrogen peroxide scavenging activity. The crude extract itself shows equivalent antioxidant potential like standard antioxidant (l-ascorbic acid). If the compound is purified it can be a better alternative and potential antioxidant for medicinal purposes.

CONCLUSION

To conclude the study, the extract has demonstrated significant in-vitro antioxidant activity, when compared with standard drugs. This study scientifically supports the usage of leaves antioxidants as a traditional medicine. Further study is required to find out the right molecule which is responsible for the plant's medicinal value. We hope that our study emphasizes the accuracy and efficacy of traditional remedies, and that it inspires people to realize the importance of protecting natural resources for sustainable use, not in the least for its potent pharmaceuticals. This study also illustrates the strong dependence of certain people on traditional medicine and the creativity in which plants and their secondary metabolites can

be utilized. Moreover comparative study of medicinal plants gives a vast idea about the plants nature and their medicinal value from its essence of traditional usage.

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