ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

In-vitro Antioxidant Capacity of Leaves of *Tephrosia villosa* Linn. (Leguminosae) in Various Solvent Extract

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Abstract

Introduction: Leaves of *Tephrosia villosa* belonging to the family fabaceae being used for the treatment of dropsy and diabetes. The present research work was under taken to investigate the *in-vitro* antioxidant activity of petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and water extracts. **Methods:** *In vitro* antioxidant activities were carried out for six solvent extracts such as *T. villosa* leaf petroleum ether extract, *T. villosa* leaf chloroform extract, *T. villosa* leaf ethylacetate extract, *T. villosa* leaf ethanol extract, *T. villosa* leaf methanol extract, and *T. villosa* leaf water extract by standard methods. Five various antioxidant methods such as DPPH radical scavenging ability, superoxide radical scavenging potential, ABTS scavenging capacity, metal chelating ability, and reducing power assay (FRAP) were estimated. **Results:** Further investigation was carried out for *in-vitro* antioxidant activity and radical scavenging assay by calculating its % inhibition by means of IC50 values, all the extracts concentration has been adjusted to come under the linearity range and here reference standards ascorbic acid has been taken for the method suitability. **Conclusion:** The results revealed that leaves of this plant have antioxidant potential. Among these results, methenolic and water extract has more potent than the other extract. *T. villosa* Linn. (Fabaceae) leaves possess the antioxidant substance which may be potential responsible for the treatment of oxidative stress related diseases.

Keywords: *Tephrosia villosa. Antioxidant, DPPH, ABTS, invitro*

Asian Pac. J. Health Sci., (2022); DOI: 10.21276/apjhs.2022.9.4.51

Introduction

Compounds of antioxidant in food play an important role as a health-protecting factor. Primary sources of naturally occurring antioxidants are fruits, whole grains, and vegetables. The most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Plant sourced food antioxidants such as carotenes, phenolic acids, Vitamin C, Vitamin E, phytate, and phytoestrogens have been recognized as having the potential to lower disease risk. Some compounds, such as gallates, have strong antioxidant activity, while the weak antioxidants are mono-phenols. Ability to trap free radicals is the main characteristic of an antioxidant. From a wide variety of sources, highly reactive free radicals and oxygen species are present in biological systems. These free radicals may oxidize nucleic acids, proteins, lipids, or DNA and can begin degenerative disease. Antioxidant compounds include polyphenols, flavonoids, and phenolic acids, scavenge free radicals such as hydroperoxide or lipid peroxyl and peroxide, and thus slow down the oxidative mechanisms that lead to degenerative diseases. By inhibiting the initiation or propagation of oxidizing chain reactions, antioxidants compounds inhibit or setback the oxidation of other molecules. Cell death or tissue damage occurs when exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing bio molecules. Oxidative damage plays an appreciable pathological role in human disease. Free radicals lead to cellular necrosis, which is implicated in some membrane pathophysiological conditions including rheumatoid arthritis, atherosclerosis, as well as toxicity of many ischemia xenobiotics, and reperfusion injury of many tissues, central nervous system injury, ageing, gastritis, respiratory diseases, inflammatory response syndrome, cancer, liver diseases, and AIDS.[1-3] Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as superoxide, peroxyl radicals, singlet, oxygen and peroxynitrite, and hydroxyl radicals.[4,5] The antioxidants can counterbalance the sick effects of free radicals by chain breaking or scavenging (like Vitamin A, C, β-carotene, etc.) or some other mechanism of action, these antioxidants must be continuously replenished since they are "used up" in the process of neutralizing free radicals.[6]

Human beings are frequently revealed to free radicals which are generated from normal metabolic processes in the body. Free radicals are not constantly harmful; however, chromosomal instability and mutation occur when the high level of free radicals may disturb the normal cellular mechanism by inducing oxidative damage to protein, lipids, and DNA.[7,8] Furthermore, over production of free radicals may decrease the potency of the biological defense system to detoxify these radicals which result in oxidative stress which might play a role in the risk of development of several metabolic diseases such as cardiovascular diseases, obesity, cancer, neurodegenerative diabetes, and aging-related disorders.[9,10] Antioxidant mechanism prevents free radicals formation and thus suppresses the life-threatening diseases. By maintaining normal cell cycle regulation can prevent cancer sequence, inducing apoptosis and inhibiting cell proliferation.[11,12] Several plants and

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How to cite this article: Nisha KM, Kiruthika ML, Sheela DS, Priya ES, Kalimuthu K. *In-vitro* Antioxidant Capacity of Leaves of *Tephrosia villosa* Linn. (Leguminosae) in Various Solvent Extract. Asian Pac. J. Health Sci., 2022;9(4):265-271.

Source of support: Nil

Conflicts of interest: None.

Received: 04/02/2022 **Revised:** 16/03/2022 **Accepted:** 05/04/2022

their active compounds are reported to possess the antioxidant activity which controls many disease conditions; therefore, natural antioxidant compounds have been investigated for various types of the treatment modalities including cancer.[13,14] Many of the natural antioxidants, especially flavonoids exhibit a wide range of biological effects, including antiviral, antibacterial, and anti-inflammatory, antithrombotic, vasodilatory, and anti-allergic actions. Antioxidant activity is necessary for many biological functions such as anticarcinogenicity, antiaging, and antimutagenicity, originate from this property.

Free radicals and peroxidative damage have been caught up in several human and animal pathological disorders, including microbial infectious diseases and inflammatory ailments.[15] The toxic effects of the free radicals can be diminished by natural antioxidants available in plants. Among the various natural compounds extracted from plants that have confirmed biological activities, many are receiving particular attention as radical scavengers.[16] Natural products present in higher plants are an important source of therapeutic agents and many research groups are currently screening different biological activities of plants.[17] Numerous studies have proven the antioxidant or radical scavenging properties of medicinal plant extract.[18]

For the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes, oxidative stress is the major driving factor responsible.[19] The condition is brought by unnecessary generation of free oxygen and nitrogen species or their inefficient quenching in the cell. Free oxygen and nitrogen species are unbalanced molecules that are present in the environment (exogenous) and are also generated in the body (endogenous) during the normal aerobic metabolic processes in the body.[20] Exogenous sources of free radicals include contact to ozone and ionizing radiation such as X-rays and cigarette smoke while endogenous sources of free radicals include the xanthine oxidase pathway and electron transfer chain reactions in the mitochondria, during disease states such as ischemia, inflammation, and reperfusion injury. Polyphenols and Vitamins (A, C, and E) are the major groups of phytochemicals that contribute to antioxidant capacity of plants. Phenolic compounds of plants are hydroxylated derivatives of benzoic acid and cinnamic acids, which have antioxidant and anticarcinogenic effects.[21] They include phenols, flavonoids, tannins, anthocyanidins, and coumarins. These phytoactive complexes are important in plant defense mechanisms against biotic and abiotic stresses.[22] When plants or plant products rich in these phytoactives are consumed and give the same beneficial effects to humans.[23] For occurrence, flavonoids have for long been recognized to possess anti-inflammatory, antiviral, antiallergic, antiaging, antiproliferative, and immunomodulatory properties.[24] Important source of antioxidants is found in medicinal plants. The secondary metabolites such as phenolics and flavonoids from plants have been reported to be effective free radical scavengers. They are found in almost all parts of plants such as leaves, fruits, seeds, roots, and bark.[25]

Materials and Methods

Plant Collection and Authentication

The plant parts of *Tephrosia v*illosa were collected from Nambiyur, Tamil Nadu. The authenticity of the selected plant materials was duly identified and confirmed (vide no: BSI/SRC/5/23/2016Tech/207) by comparison with reference specimen preserved at Botanical Survey of India, Southern Circle, Coimbatore.

Extract Preparation

100 g of *T. villosa* dried leaves were subjected to Soxhlet extraction. Six polar solvents such as petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and water were used for this extraction. After extraction, the solvent was evaporated by vacuum solvent evaporator. Then, the extracts were stored at 4° C for investigation of further studies.

Determination of *in vitro* Antioxidant Activity

The *in vitro* antioxidant activities were carried out for six solvent extracts such as *T. villosa* leaf petroleum ether (TVLPE) extract, *T. villosa* leaf chloroform (TVLC) extract, *T. villosa* leaf ethylacetate (TVLEA) extract, *T. villosa* leaf ethanol (TVLE) extract, *T. villosa* leaf methanol (TVLM) extract, and *T. villosa* leaf water (TVLW) extract by standard methods. Five various antioxidant methods such as DPPH radical scavenging ability, superoxide radical scavenging potential, ABTS scavenging capacity, metal chelating ability, and reducing power assay (FRAP) were estimated using standard protocols with ascorbic acid used as standard. Various concentrations of all the six extracts were studied for radical scavenging potentials. Finally, the percentages of inhibition were calculation using the formula.

$$\text{Percentage of inhibition } (\%) = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

DPPH Radical Scavenging Activity

The hydrogen donating capacity was assessed using the stable DPPH• method (Blois, 1958.[26] Briefly, a solution of 0.1 mM DPPH• was prepared using methanol. The samples (50–250 μg/mL) were mixed with 5.0 mL of DPPH• solution. Reaction mixture was mixed well and incubated at 27° C for 20 min; the absorbance was measured at 517 nm. Moreover, the results were compared with the rutin, quercetin, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) activity. DPPH• percent discoloration of the samples was calculated using the formula:

DPPH radical scavenging activity (%) = [(Control OD-Sample OD)/Control OD] × 100.

Antioxidant activities of the extracts were expressed as IC_{50} values which were calculated from the linear deterioration of the percentage antioxidant activity against concentration of the extracts and greater antioxidant activity is indicated by lower IC_{50} value.

Superoxide Radical Scavenging Activity

Beauchamp and Fridovich, 1971[27] generated the modified method of Superoxide radicals. The assay was based on the capacity of the sample to slow down formation by scavenging superoxide radicals generated by riboflavin-light-NBT in the system. Each 3 ml reaction mixture contained various concentrations (200–1000 μg) of sample extract, 50 mM sodium phosphate buffer (pH 7.6), 12 mM EDTA, 0.1 mg NBT, and 20 mg riboflavin. Reaction was started by revealing the reaction mixture with sample extract for 90 s. Immediately, after elucidation, the absorbance was measured at 590 nm. The entire reaction assembly was kept in a box creased

with aluminum foil and the identical tubes with reaction mixture kept in dark served as empty. Superoxide anion generation inhibition percentage was calculated as:

Superoxide radical scavenging activity (%) = (control OD-sample OD/control OD) × 100.

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

Reducing Power Assay

The Fe3+ reducing power of the extract was determined according to the method suggested by Oyaizu, 1986.[28] 5.0 mL of 0.2 M phosphate buffer of pH 6.6 and 5.0 mL of 1% K3 Fe $(CN)_6$ were mixed with the plant extracts (100500 µg/mL) and the mixtures were incubated at 50° C for 20 min. The reaction was completed by adding 5.0 mL of 10% TCA (w/v) and the mixture was centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5.0 mL) was mixed with 1.0 mL of 0.1% (w/v) FeCl3 and 5.0 mL of distilled water and the absorbance was read at 700 nm., BHA and BHT, rutin, and quercetin served as the reference material. Increased reductive capability is indicated by increased absorbance.

ABTS Radical Scavenging Activity

Antioxidant activity was performed using an improved ABTS▪+ method proposed by. The ABTS radical cation (ABTS▪+) was generated by a reaction of 7 mM ABTS▪+ and 2.45 mM potassium persulfate and the mixture was incubated in dark for 1216 h at room temperature. Before assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to obtain an absorbance of 0.700 ± 0.02 at 734 nm. 10 µL/mL of sample was added to 1.0 mL of diluted ABTS▪+ solution. Absorbance was read at 734 nm, after 30 min of incubation. Ascorbic acid was used for control (Siddhuraju and Manian, 2007).[29]

Metal chelating Ability for Ferrous Ions

The ferrous chelating potential of the six solvent extracts was assessed according to the method suggested by Yamaguchi *et al*., 2000.[30] The reaction was initiated with the adding of 250 µg of sample extract, 0.25 mL of 1 mM FeSO4 solution, 1.0 mL of 0.2 M Tris-HCl buffer (pH 7.4), 1.0 mL of 2, 2′ bipyridyl solution, 0.4 mL of 10% hydroxylamine hydrochloride, and 2.0 mL of ethanol in sequence, the final volume was made up to 5.0 mL with deionized water, the absorbance was determined at 522 nm. Moreover, to benchmark, the chelating abilities EDTA was used. Higher ferrous ion chelating ability was indicated by lower absorbance of the reaction mixture.

Results

DPPH Radical Scavenging Activity

The percentage of inhibition of each extracts were noticed at higher concentration. The maximum percentage of inhibition 51.22, 45.01, 37.14, 51.44, 62.32, and 56.03 were reported in TVLPE, TVLC, TVLEA, TVLE, TVLM, and TVLW extract, respectively. Among the six extracts TVLM showed highest DPPH scavenging inhibition with 62.32% at 250 µg/ml concentration. The second best highest inhibition was observed in TVLW extract with 56.03% at the same concentration. Minimum percentage of inhibition was observer in TVLC extract with 10.22% at 50 µg/ml concentration. TVLM extract has potential DPPH scavenging activity and less IC_{50} value at 100.12 µg/ml followed by TVLW extract IC_{50} value at 50.01 µg/ml concentration [Table 1]. The percentage of inhibition of TVLM was compared with standard of ascorbic acid (67.51%) and IC_{50} value was <50 [Table 2 and Figure 1].

Superoxide Radical Scavenging Activity

The scavenging ability of superoxide anion radicals was investigated by five different concentrations of six solvent extracts. Superoxide radical scavenging activity of extracts was measured by reduction of nitroblue tetrazolium (NBT). Based on this view, more NBT reduction was observed in TVLM extract than other extracts. The maximum percentage of superoxide scavenging ability of TVLM extract noticed was 61.38 at 1000 µg/ml. The potential IC_{50} value of TVLM extract was 760.12µg/ml which compared to positive control ascorbic acid (580 µg/ml). The second best highest inhibition percentage observed in TVLW extract with 56.06 at 1000 ug/mL concentration and IC_{50} value was 980.01 ug/mL [Table 1]. The scavenging percentage of other extracts such as TVLPE, TVLC, TVLEA, and TVLE was 50.27, 45.28, 51.36, and 55.25, respectively [Table 3 and Figure 2].

Reducing Power Assay

Table 4 exhibits the FRAP scavenging activity of six solvent extracts of *T. Villosa*. Iron reduction capacity of the extracts was in concentration depended manner. Among the six extracts, HPLM extract was shown good reducing absorbance (2.12) than other extracts [Figure 3]. All the extracts absorbance were compared with standard (ascorbic acid) absorbance value, the standard absorbance value was 2.16. The other treatments of TVLPE, TVLC, TVLEA, TVLE, and TVLW extracts showed the reducing power absorbance of 1.12, 1.61, 1.34, 1.95, and 1.82, respectively [Table 4].

ABTS Radical Scavenging Activity

ABTS radical scavenging ability of TVLPE, TVLC, TVLEA, TVLE, TVLM, and TVLW extracts was evaluated by percentage inhibition of free radicals. The maximum ABTS radical scavenging percentage was recorded from TVLM extract with 65.75% at 250 ug/mL

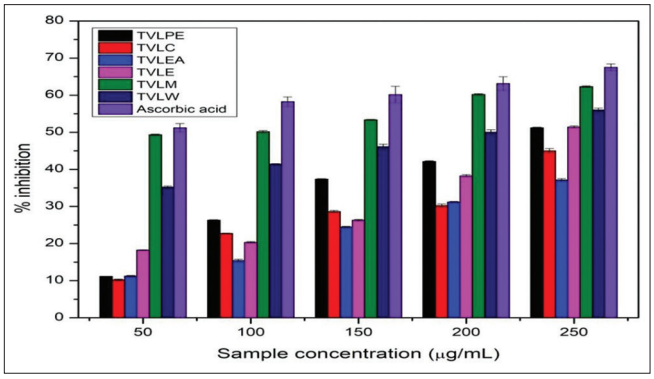

Figure 1: DPPH radical scavenging activity of six solvent leaf extract of *Tephrosia villosa*

concentration. Lowest IC_{50} value of TVLM extract was observed at 200.34 µg/ml which result more or less equal to ascorbic acid 98.2 µg/ml [Table 1]. The second highest percentage inhibition (51.32%) was observed in TVLW extract at 250 µg/ml concentration and IC_{50} value was 80.01 µg/ml. Other solvent extracts such as TVLPE, TVLC, TVLEA, and TVLE extract inhibition percentage were 52.39, 31.35, 48.21, and 50.28, respectively, at 250 ug/mL concentration and IC_{50} values found was 280.12, >250, >250, 250.12 ug/mL, respectively [Table 5 and Figure 4].

Metal Chelating Ability for Ferrous Ions

The metal-chelating ability of the six extracts of *T. Villosa* is evaluated by the decrease in the formation of the ferrous-ferrozine complex. Among the six extracts, the TVLM gave the highest activity 62.82% followed by TVLW extract (58.12%) at 250 ug/mL concentration. The lowest IC_{50} value (180.42 and 175.03 ug/mL) is also observed at TVLM and TVLW extracts, respectively [Table 6]. The percentage inhibition of standard ascorbic acid was 68.29% with IC_{50}value 185.3 ug/mL. The other extracts of TVLPE, TVLC, TVLEA, and TVLE extracts IC_{50} value were >250, 250.32, 248.35, and 243.27 ug/mL concentration, respectively [Table 6 and Figure 5].

Discussion

Decreased capacity of antioxidant defenses and increased production of reactive oxygen/nitrogen species in the body leads to oxidative stress.[31,32] Generation of reactive oxygen/nitrogen species (ROS/RNS) is expected in healthy cells and for aerobic organisms, and it occurs at a controlled speed.[33] Successive variation of membrane lipids, proteins, and nucleic acids occurs under conditions of oxidative stress, when ROS/RNS production is

Table 1: Inhibition Concentration (IC_{50} value) value of different antioxidant activity of various solvent extracts of *Tephrosia villosa*

Sample	*Antioxidant method (IC_{50} value µg/ml)*			
	DPPH	*Superoxide*	*ABTS*	*Ferros ions*
TVLPE	249.12±1.17	1000.12±0.04	280.12±0.21	<250
TVLC	>250	>1000	>250	250.32±0.09
TVLEA	>250	998.62±0.11	>250	248.35±0.41
TVLE	248.32±0.11	900.23±0.16	250.12±0.04	243.27±0.02
TVLM	100.12±0.05	760.12±0.09	200.34±0.07	180.42±0.08
TVLW	50.01±0.66	980.01±0.21	80.01±0.03	175.03±0.05
Ascorbic acid	<50	580±0.01	98.2±0.03	185.3±0.12

Table 2: DPPH radical scavenging activity of various solvent extract of *Tephrosia villosa*

Con.mg/ml	*TVLPE*	*TVLC*	*TVLEA*	*TVLE*	*TVLM*	*TVLW*	*Ascorbic acid*
50	11.14±0.01	10.22±0.24	11.22±0.21	18.21±0.12	49.31±0.21	35.23±0.31	51.19±1.14
100	26.31±0.14	22.71±0.12	15.43±0.35	20.33±0.19	50.16±0.31	41.42±0.11	58.22±1.30
150	37.35±0.12	28.62±0.31	24.45±0.21	26.31±0.21	53.33±0.13	46.12±0.67	60.18±2.19
200	42.13±0.21	30.23±0.46	31.22±0.14	38.24±0.32	60.21±0.22	50.01±0.66	63.12±1.84
250	51.22±0.15	45.01±0.59	37.14±0.37	51.44±0.28	62.32±0.19	56.03±0.45	67.51±0.90

Table 3: Superoxide scavenging activity of different solvent extracts of *Tephrosia villosa*

	Superoxide scavenging activity of different solvent extracts						
Con. mg/ml	*TVLPE*	*TVLC*	*TVLEA*	*TVLE*	*TVLM*	*TVLW*	*Ascorbic acid*
200	17.82±1.19	12.18±1.30**0**	10.21±1.08	21.29±1.17	31.12±2.10	28.21±0.23	34.09±0.31
400	21.23±1.27	18.21±1.30**0**	18.35±1.10	32.8±0.48**0**	39.31±1.23**1**	34.52±0.61	46.59±1.82
600	32.48±2.14	29.22±1.15	25.74±1.34	39.57±1.32	45.12±1.71	34.52±0.61	51.96±2.15
800	38.31±2.23	34.35±1.11	39.25±1.86	41.86±1.47**1**	52.71±0.21	47.43±0.72	60.96±1.41
1000	50.27±0.41	45.28±1.13	51.36±1.05	55.25±1.42	61.38±0.01	56.06±0.13	65.01±0.61

Table 4: Reducing power activity of different solvent extracts of *Tephrosia villosa*

	Reducing power activity of different solvent extracts						
Con. mg/ml	*TVLPE*	*TVLC*	*TVLEA*	*TVLE*	*TVLM*	*TVLW*	*Ascorbic acid*
50	0.12±0.05	0.29±0.04	0.31±0.03	0.38±0.05	0.40±0.07	0.38±0.21	0.62±1.32
100	0.21±0.04	0.91±0.02	0.43±0.05	0.82±0.09	0.86±0.21	0.91±0.31	1.22±1.90
150	0.65±0.02	1.12±0.11	0.85±0.01	1.11±0.41	1.33±0.23	1.01±0.11	1.52±2.18
200	0 0.88±0.11	1.53±0.16	1.02±0.04	1.37±0.02	1.81±0.12	1.52±0.91	1.92±1.22
250	1.12±0.05	1.61±0.09	1.34±0.07	1.81±0.08	2.12±0.07	1.82±0.41	2.16±0.20

Table 5: ABTS activity of different solvent extracts of *Tephrosia villosa*

	ABTS activity of different solvent extracts						
Con. mg/ml	*TVLPE*	*TVLC*	*TVLEA*	*TVLE*	*TVLM*	*TVLW*	*Ascorbic acid*
250	49.39±0.05	31.35±0.21	48.21±0.32	50.28±0.01	65.75±0.03	50.32±0.01	68.21±0.08
200	49.17±0.09	28.28±0.32	35.78±0.18	42.38±0.04	50.21±0.32	45.41±0.15	70.16±0.02
150	42.28±0.11	20.12±0.43	30.21±0.13	38.71±0.06	38.71±0.11	38.11±0.12	60.72±0.01
100	35.78±0.15	15.72±0.09	21.92±0.15	28.38±0.08	35.28±0.21	30.34±0.51	52.31±0.03
50	21.92±0.22	10.18±0.05	17.62±0.18	20.71±0.03	31.32±0.24	22.31±0.21	42.72±0.03

Table 6: Chelating ability for ferrous ions activity of different solvent extracts of *Tephrosia villosa*

	Chelating Ability for Ferrous Ions activity of different solvent extracts						
Con. mg/ml	*TVLPE*	*TVLC*	*TVLEA*	*TVLE*	*TVLM*	*TVLW*	*Ascorbic acid*
50	15.28±0.09	11.23±0.12	28.41±0.03	11.21±0.23	21.32±0.03	30.12±0.42	25.92±0.14
100	17.37±0.24	22.35±0.23	32.52±0.06	22.11±0.24	36.78±0.07	35.21±0.23	38.71±0.16
150	20.78±0.12	31.46±0.31	34.72±0.09	29.35±0.21	48.37±0.12	42.34±0.56	45.82±0.11
200	22.32±0.11	42.64±0.04	42.82±0.06	39.64±0.12	51.48±0.11	50.12±0.31	54.71±0.05
250	38.68±0.12	50.72±0.32	51.71±0.12	52.92±0.13	62.82±0.13	58.12±0.11	68.29±0.06

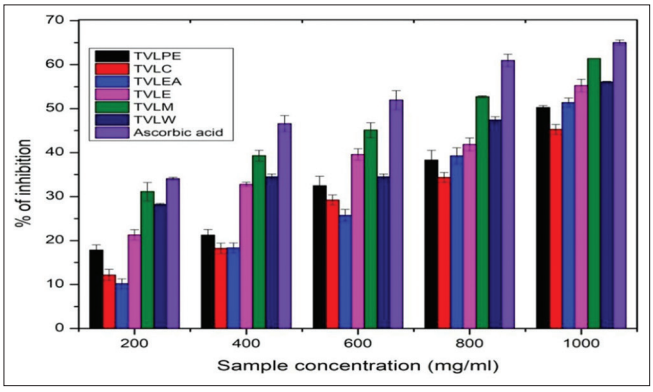

Figure 2: Superoxide radical scavenging activity of six solvent leaf extract of *Tephrosia villosa*

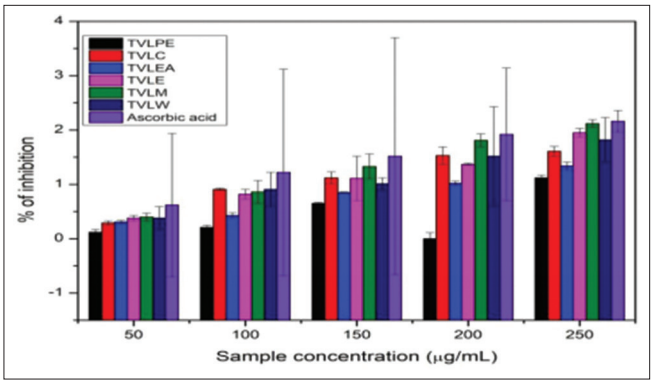

Figure 3: Reducing power assay of six solvent leaf extract of *Tephrosia villosa*

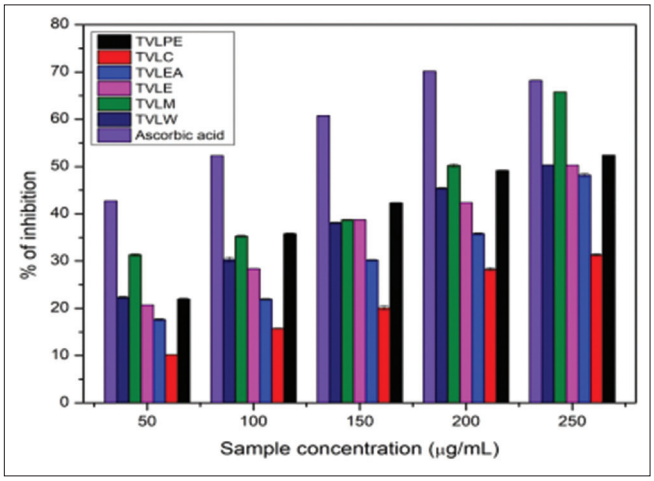

Figure 4: ABTS radical scavenging activity of six solvent leaf extract of *Tephrosia villosa*

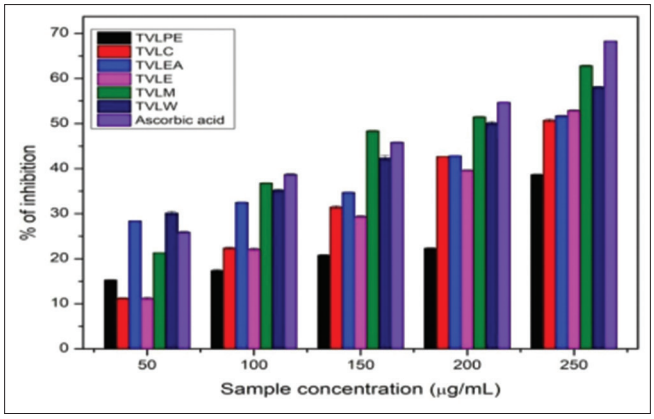

Figure 5: Metal chelating ability for ferrous ions of six solvent leaf extract of *Tephrosia villosa*

dramatically increased,[34] oxidative damage of these biomolecules is related with aging and a variety of pathological actions, including ischemia reperfusion injury, carcinogenesis, and neurodegenerative disorders.[35] Humans have evolved complex antioxidant systems, which work to avert deleterious effects of oxidative stress to protect the body against ROS and RNS.[36] Antioxidant defense systems of the body are of exogenous and endogenous origin.[37] Endogenous sources of antioxidant defenses include glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase enzymes, which catalyze free radical quench reactions, while β-carotene, L-ascorbic acid (Vitamin C), α-tocopherol, and tocotrienols (Vitamin E), which are derived from dietary foods, we consume are exogenous sources of antioxidants.[38-40]

Artificial antioxidants such as BHA, BHT, and propyl gallate are presently used against oxidative stress and have been related with adverse health effects including malignancies and hepatic damages, they have limited influence in humans.[41] At present, there is an increase of interest to replace artificial antioxidants with naturally occurring antioxidants from plants since they are very much safer, easily available, and affordable.[42,43] Several *in vitro* examination techniques are taken thought for assessing antioxidant activities.[44]

The antioxidant activity of six solvent leaf extracts of *T. villosa* reported the potential activity of free radical scavenging. Among the six solvent extracts, TVLM and TVLW extracts were reported maximum percentage of inhibition in DPPH, superoxide, FRAP, ABTS radical scavenging activity, and metal chelating ability for ferrous ions assays.

All the six solvent extracts were response to decrease DPPH color from dark purple to light purple. DPPH assay results of TVLM extract showed the maximum percentage of inhibition 62.32% at 250 ug/mL concentration and IC_{50} value was observed as 100.12 ug/mL concentration followed by TVLW extracts IC_{50}

value observed was 50.01 ug/mL concentration. However, these results were in comparable to the results of the previous studies using plant extracts of *Tephrosia perpuria* (T.P) in which methanolic leaf extract significantly quenched the DPPH free radicals at concentration of 186.3 ± 14.0 µg/mL with IC_{50} value concentration of 9.6 µg/mL[45] and concentration of water extract of *T.P* root IC_{50} value is 79 µg/mL.[46] Inhibition percentage of TVLPE, TVLC, TVLEA, and TVLE is 52.22%, 45.01%, 37.14%, and 51.44 %, which was compared by Crotalaria pallida[47] in which concentration of 0.1 mg/ml, the scavenging activity of ethanol extract reached above 80% while petroleum ether, ethyl acetate, chloroform, and water extract also reached 60%. The scavenging activity increases with increasing concentration of the extract and the result is comparable with the antioxidant activity of methanol extract of *Abrus pulchellus* wall.[48]

Kannat *et al.* 2007[49] reported that the superoxide radical is known to be a very harmful and its scavenging is necessary because it acts as the precursor for other major ROS such as hydrogen peroxide, hydroxyl, and singlet oxygen. Several biological reactions produce superoxide radical which is a highly toxic species. Although they cannot directly initiate lipidoxidation, superoxide radical anions are potent precursors of highly reactive species such as hydroxyl radical and thus the study of scavenging of this radical is important. Since six solvent extracts obtained by the Soxhlet extraction of leaf of *T. villosa* showed appreciable percentage of scavenging activity against superoxide radical, it can be used against adverse effects caused by superoxide radical in the body. The maximum percentage of inhibition 61.38% was observed at 1000 µg/ml concentration and its IC_{50} value observed was 760.12 µg/mL concentration in TVLM extract followed by TVLW extracts with 56.06% and IC_{50} value 980.01 µg/mL concentration.

Reducing power activity is often used to evaluate the ability of natural antioxidant to donate electron.[50,51] Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts.[50,52] Among the six extracts, TVLM extract showed good reducing absorbance (2.12) than other extracts whereas when compared with the reducing power of *Tephrosia purpurea* leaf extracts concentration was 65.7 ± 4.2 µg QE/mg[45] The reducing power activity increased consistently with the increase in the volume of extract concentration. It is known further that the reducing power activity of TVLM extract was more or less similar to the standard ascorbic acid.

ABTS is decolored by measuring the reduction of the radical cation as a percentage inhibition of absorbance at 734 nm.[53] ABTS was generated by incubating ABTS chromophore through the reaction.[54] The presence of specific chemical compounds in the extracts of *T. villosa* may inhibit the potassium persulfate activity and hence reduced production of ABTS. This study reports that the TVLM extract has the highest antioxidant activity (65.75%) at 250 ug/mL concentration compared to other extracts. The lowest IC_{50} value was observed in TVLM extract at 200.34 ug/mL which was followed by TVLW extract inhibition percentage 51.32% at the concentration of 250 µg/ml and IC_{50} value was 80.01 µg/ml.

In ferrous chelating assay, iron is essential for life because it is required for oxygen transport, respiration, and activity of many enzymes. However, it is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins, and other cellular components.[55] The metal chelating ability of the six solvent extracts was measured by the formation of ferrous ion ferrozine complex. Ferrozine combines with ferrous ions forming a red colored complex which absorbance at 562 nm. Iron reduction capacity was noticed extracts concentration dependent manner. Iron binding capacity in terms of percent inhibition TVLM extract 62.82% at 250 ug/mL concentration was higher than the other extract.

Conclusion

T. villosa Linn. (Leguminosae) leaves possess the antioxidant substance which may be potential responsible for the treatment of diabetes. Hence, there are many scopes in leaves portion and more number of studies can be undertaken such as oxidative stress hepatoprotective and anticancer activities.

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