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In Vitro anticholinesterase and antioxidant activity of Thunbergia coccinea leaves extract

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***Abstract*---**Herbal medicines gained an interest in the development of new drugs as a therapeutic agent for various manifestation. Relevant research reveals that *Thunbergia coccinea* is a plant which possess antipyretic, anti-inflammatory activity and it has been used as an ethnomedicinal plant. The present study explores the cholinesterase inhibitory potential relevant to neurodegenerative disorder and antioxidant activities of hydroethanolic extract of *Thunbergia coccinea* leaves. Cholinesterase inhibition was determined by Ellman method at different concentrations. The antioxidant activity was assessed by DPPH, FRAP, ABTS, TRAP, NO radical inhibition assay, Phosphomolybdate assay for total antioxidant capacity, total phenolic content, SOD scavenging activity. The hydroethanolic extract of *Thunbergia coccinea* leaves express significant amount of antioxidant and anticholinesterase activity against neurodegenerative disorders.

***Keywords*---**Thunbergia coccinea, cholinesterase inhibition, antioxidant activity, ethnomedicinal plant.

Introduction

Herbal medicines and their active ingredients have been used since ancient times. Phytotherapeutic products with plant parts in crude form or bioactive compounds are earning interest in the treatment of diseases.[1] Plant are enriched with bioactive compounds and almost all the parts of a plant can be considered as a medicine in one way or another. However, the most used parts are flowers, fruits, seeds, roots, leaves, barks, etc[2]. Number of medicinal plants have been identified and used as an alternative for treating various pathological conditions.

Alzheimer's disease (AD) the neurodegenerative disease is associated with progressive and irreversible loss of cognitive abilities, memory loss, cognitive impairment, emotional dysfunction, and ultimately death.[3] Alzheimer's disease characterized by the formation of senile plaques is composed mainly of amyloid β (Aβ), and neurofibrillary tangles (NFTs), tau protein, in the hippocampus and cerebral cortex of afflicted humans.[4,5,6]

Thunbergia coccinea, (Acanthaceae family) commonly called as scarlet clock vine or red clock vine is widely used by tribal society of Assam for the treatment of various pathological conditions. Root extract of *Thunbergia coccinea* was used to treat dysentery, stomach pain and possess anti-inflammatory property. Scarlet coloured flower of this plant is rich in antioxidants. Leaves are used as an antipyretic, analgesic, anti-inflammatory agents thus help to heal wounds. An attempt is made in the present study to explore the cholinesterase inhibitory potential and antioxidative activities of hydroethanolic extract of *Thunbergia coccinea* leaves.

Materials and Methods

Collection and identification of plant material

Thunbergia coccinea leaves were collected from Kothagiri, Nilgiri Dt, Tamilnadu. The authentication of plant the plant was confirmed by Botanical survey of India, Southern regional centre, Coimbatore by referring the deposited the specimen. The voucher number of the specimen is BSI/SRC/5/23/2017/Tech. Collected leaves were shade dried and coarsely powdered using an electrical blender.

Preparation of Extract

Bio active components in 25 g of *Thunbergia coccinea* leaves were extracted using 100 ml of hydroethanol by Soxhlet extraction. The solution was filtered using Whatman filter paper and the filtrate was made into a concentrated semi-solid residue and stored at 4^0 C for analysis[7].

Preliminary phytochemical analysis

The plant extract was screened for active secondary metabolites using the Trease and Evans method to investigate phytoconstituents in hydroethanolic extract[8].

In-vitro acetylcholinesterase inhibitor activity

Acetylcholinesterase activity was carried out by Ellman method[9] having Acetylthiocholine iodide (ATCl) as a substrate[10]. Galantamine was used as a reference standard. Percentage inhibition for test solution was then calculated using the following equation.

$$\%\ inhibition = \frac{(change\ in\ OD\ per\ min\ of\ control\ -\ change\ in\ OD\ per\ min\ of\ extract)}{change\ in\ OD\ per\ min\ of\ control}$$

Estimation of Total Phenolic Content

Phenolic compounds concentration in the Ethanolic extracts was estimated by a colorimetric assay and the estimation is based on the method described by Singleton and Rossi with some modification[11]. Gallic acid was used to calculate the standard curve. The results were measured in mean values ± standard error of mean and expressed as mg of gallic acid equivalents/g of extract.

In-vitro Antioxidant activity

DPPH Radical Scavenging Activity

The free radical scavenging ability of the extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical method[12]. The radical scavenging capacity was calculated using the following equation

$$\%DPPH\ Radical\ Scavenging\ Activity = \{(A_0 - A_1) / A_0\} \times 100$$

In the above equation, A_0 is the absorbance of the control, and A_1 is the absorbance of the standard. The % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

ABTS Radical Scavenging Activity

ABTS radical scavenging activity was determined according to Re et al using ascorbic acid as standard[13]. ABTS scavenging ability was expressed as IC_{50} (µg/ml) and the inhibition percentage calculated using the following formula

$$\%\ Inhibition = \{(A_0 - A_1) / A_0\} \times 100$$

In the above equation, A_0 is the absorbance of the control, and A_1 is the absorbance of the standard. The % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

Ferric Reducing Antioxidant Power

The ability to reduce ferric ions was measured using the method by Benzie and Strain. Ascorbic acid was used as a standard[14]. It is based on the reduction of a ferric 2, 4, 6-tripyridyl-s-triazine complex to the ferrous form. FRAP value was expressed as mmol/100 g on dry weight basis using the calibration curve of Fe^{2+}.

Total Reducing antioxidant potential

The total reducing antioxidant potential is estimated using the method by Benzie and Strain, where this method determines the ability to inhibit the reaction between peroxide radical and molecule. The absorbance was measured against the corresponding blank at 490nm. Ascorbic acid was used as a standard[15].

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition was estimated by Griess Ilosvoy reaction. This method is based on the estimation of nitric ion which is produced by the interaction of nitric oxide with oxygen. Nitric oxide scavengers reduce the formation of nitric ion by inhibiting oxygen. Ascorbic acid was taken as a standard[16]. The percent inhibition as calculated using the formula:

$$\%\ Inhibition = \{(A_0 - A_1) / A_0\} \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the standard. Then % inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

Phosphomolybdate Assay for Total Antioxidant Capacity

The total antioxidant capacity can be useful to determine the antioxidant enriched compounds on prevention of alzheimer's disease. The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard[17]. The antioxidant capacity of plant extract was measured using following formula:

$$\%\ Inhibition = \{(A_0 - A_1) / A_0\} \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then % inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

Superoxide anion scavenging assay

The scavenging activity of *extract* towards superoxide anion radicals was measured in spectrophotometer. The estimation of scavenging ability of the plant extract against radical was determined using a method by Ni-Shimiki et al (1972)[18]. The superoxide anion scavenging activity was calculated by the following equation.

$$\%\ Inhibition = \{(A_0 - A_1) / A_0\} \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

Results and Discussion

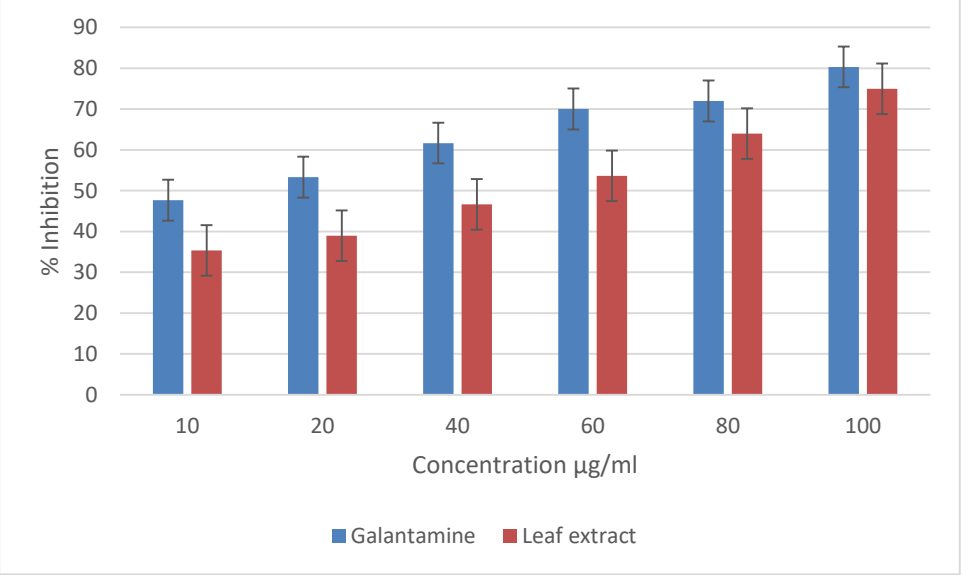
The analysis of ethnomedicinal plant is widely done to study various disorders since many years. The biochemical substances present in plants are considered as an major source of drug development since it exhibits less adverse effect comparatively to synthetic drugs, therefore plant derived drugs are widely used as an complementary medicine for treating various ailments. The medicinal

properties of plants are mostly attribute to secondary metabolites[19]. The results of the present study are discussed in following headings.

In-vitro Acetylcholine inhibitor assay

A concentration dependant assay was carried out with hydroethanolic extract which is shown in figure 1. In this assay it has been found that hydroethanolic extract of *Thunbergia coccinea* leaves exhibit potential on inhibition of acetylcholinesterase enzyme. This enzyme plays a key role in the cholinergic system in the brain. Therapies designed to reverse the cholinergic deficit in alzheimer's disease is mostly based on inhibitors of acetylcholinesterase, which enhance cholinergic transmission with modest and transient therapeutic effects[20]. Most of the acetylcholinesterase inhibitor which are used for the treatment of neurogenerative disorders have side effects such as toxicity, loss of efficiency, insomnia which on further uses lead to adverse effects. However, acetylcholinesterase induces formation of amyloid plaques which eventually increases cytotoxicity and cell functions are disrupted. Leaves extract of *Thunbergia coccinea* is used to determine the inhibition of acetylcholinesterase activity. The result obtained from the leaves extract against acetylcholinesterase enzyme inhibition activity and the percentage inhibition was evaluated and it is represented in figure 1. The percentage inhibition of the hydroethanolic extract of Thunbergia coccinea leaves were found to be 25.51 µg/ml and percentage inhibition of standard galantamine was found to be 43.69 µg/ml. Hydroethanolic leaves extract shows low potent inhibition when compared to galantamine

Figure 1: *In vitro* acetylcholine inhibitory assay of hydroethanolic extract of *Thunbergia coccinea* leaves

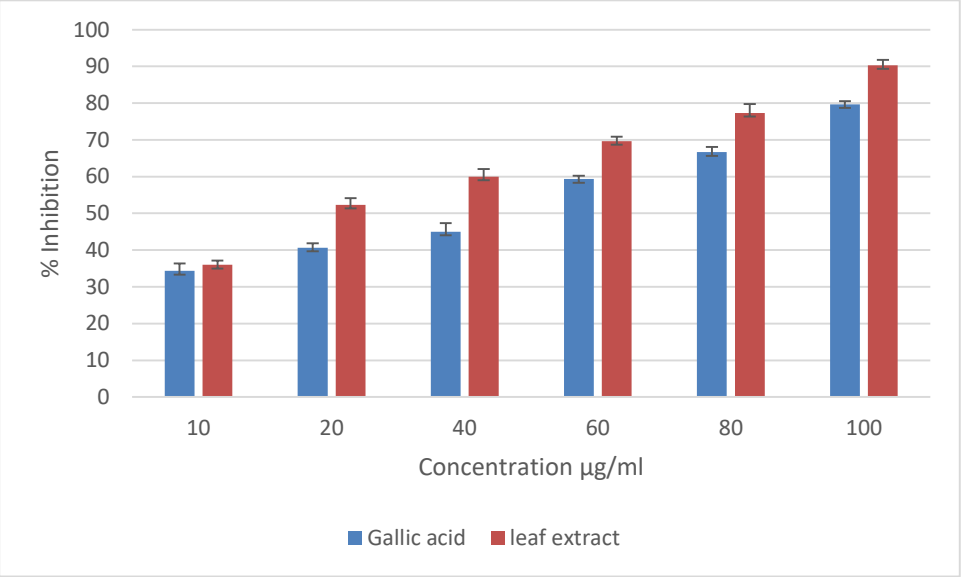


Estimation of Total Phenolic Content

The antioxidant activity of phenolic compounds is mainly due to their redox potential, which can play a key role in adsorbing and neutralising free radicals,

quenching oxygen or decomposing peroxides[21]. Naturally occurring phenolic contents from the plant possess a vital defensive response against oxidative stress, inflammatory responses, prevention against ageing etc[22]. It is widely known as phenols are constituents of plant which are found abundantly among them and hence, they are in a great interest in scientific research for the health benefits as an antioxidant[23]. The concentration of the sample to scavenge the radical is determined using IC_{50} inhibition of plant extract and standard gallic acid, where it is found to be 25.18 µg/ml and 42.96 µg/ml respectively. The ability to scavenge hydroxyl radicals by phenols present in this plant are represented in the figure 2. The outcome of the present investigation revealed that the hydroethanolic extract of *Thunbergia coccinea* leaves extract.

Figure 2: Total phenolic content of hydroethanolic extract of *Thunbergia coccinea* leaves

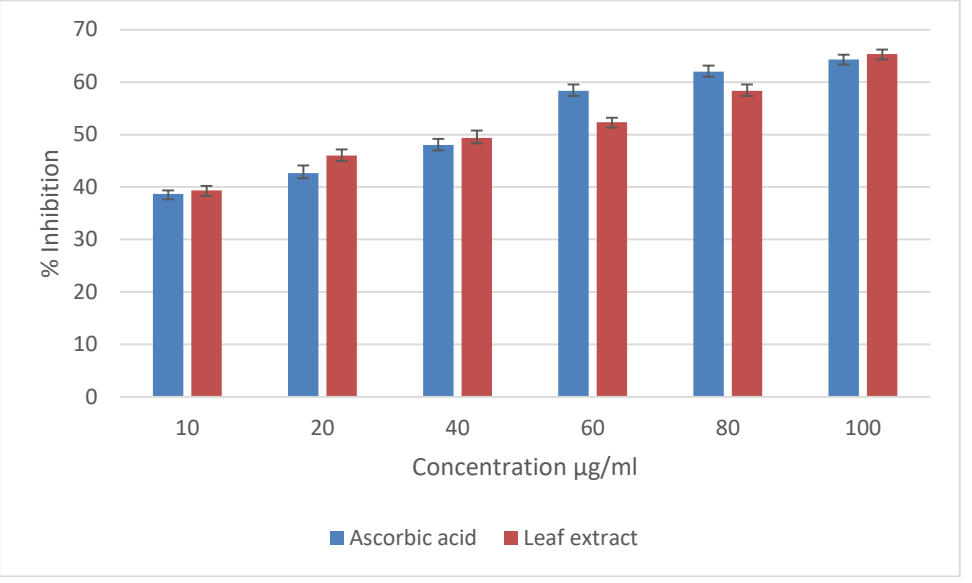


In-vitro antioxidant activity

DPPH radical scavenging activity

Different methods were used to study the scavenging and reducing capacity of a leaves extract of *Thunbergia coccinea*. The assay is based on the ability of antioxidant to quench DPPH, a stable free radical, to decolorize in the presence of antioxidants[24].The odd electrons in the DPPH radicals are responsible for absorbance. Changes in the absorbance are measured quantitatively based upon the acceptance of electron by DPPH from antioxidant compound of leaves extract. *In vitro* antioxidant assay of *Thunbergia coccinea* leaves extract reveals the presence of antioxidant potential. In this assay the scavenging ability of hydroethanolic *Thunbergia coccinea* leaves extract and standard ascorbic acid. The IC_{50} value of DPPH are found to be 44.8 µg/ml and 43.91 µg/ml for hydroethanolic leaves extract and ascorbic acid respectively which is represented in figure 3. This shows leaves of *Thunbergia coccinea* have potency to scavenge the DPPH radicals.

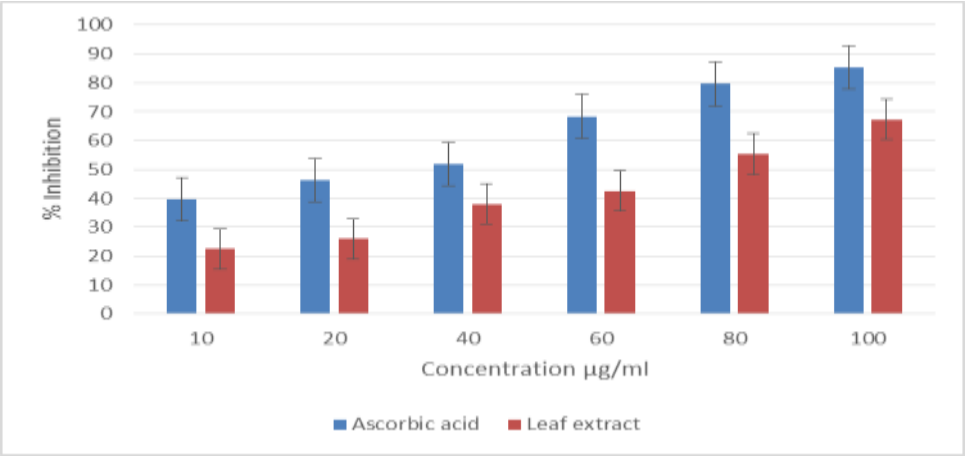
Figure 3: DPPH radical scavenging activity of hydroethanolic extract of *Thunbergia coccinea* leaves.



ABTS Radical scavenging activity

The Proton radical scavenging is an important characteristic of antioxidants. ABTS is a protonated radical which has a characteristic absorbance maximum at 734 nm that decreases with the scavenging of the proton radicals[25]. The hydroethanolic extract of *Thunbergia coccinea* leaves extract exhibit the potent ABTS radical scavenging activity in concentration dependant manner. Radical scavenging ability of hydroethanolic extract of *Thunbergia coccinea* leaves extract and standard ascorbic acid is represented in table 3. The IC_{50} value obtained in the ABTS radical scavenging ability was found to be high in *Thunbergia coccinea* leaves 68.15 µg/ml followed by standard 29.30 µg/ml which is represented in figure 4. This indicate *Thunbergia coccinea* leaves good radical scavenging property.

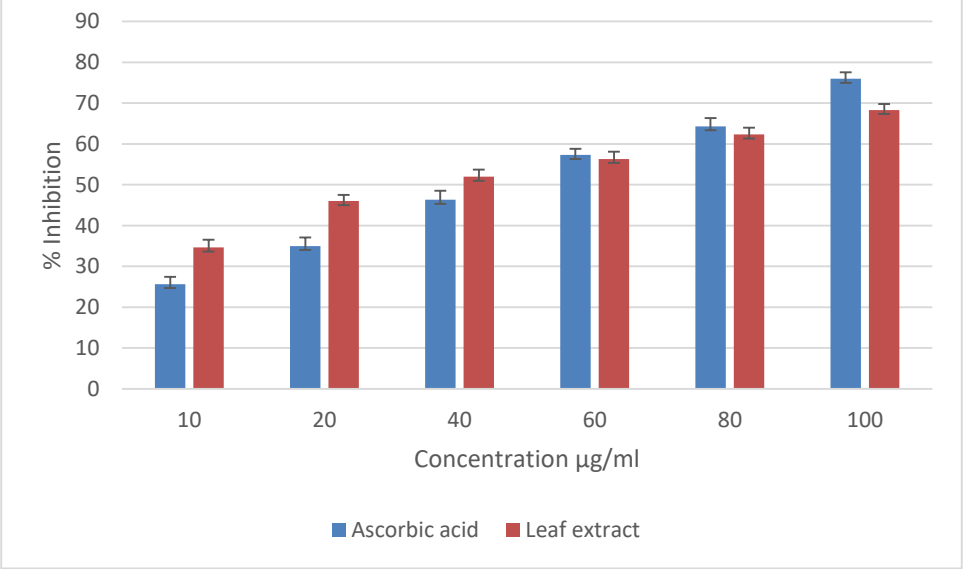
Figure 4: ABTS Radical scavenging ability of hydroethanolic extract of *Thunbergia coccinea* leaves



Ferric Reducing Antioxidant Power (FRAP)

A FRAP method is based on the ability of an antioxidant to reduce (electron transfer) Fe^{3+} to Fe^{2+} ions in the presence of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm[26]. Compounds that are active in Fe^{3+} reduction also stimulate the formation of OH[27]. In FRAP method, reducing ability of an antioxidant present in *Thunbergia coccinea* leaves extract was found to be IC_{50}=41.84 µg/ml against standard ascorbic acid IC_{50}= 50.20 µg/ml, represented in figure 5. Therefore, leaves extract of *Thunbergia coccinea* was found to have considerable antioxidant property.

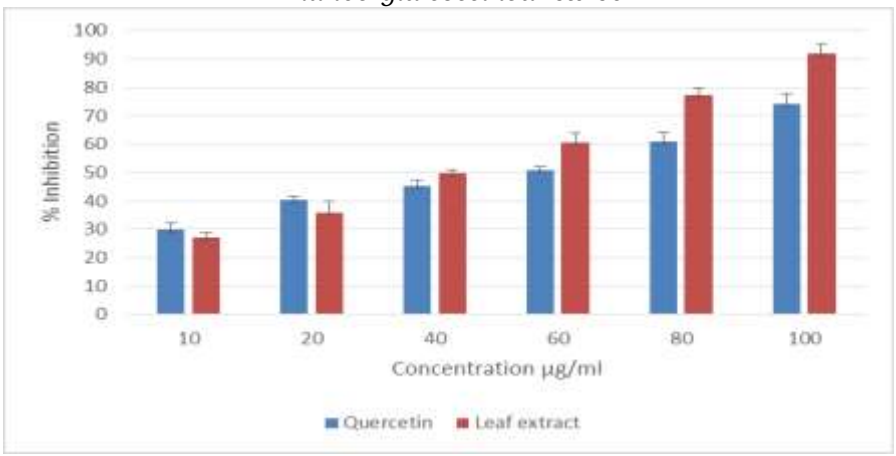
Figure 5: Ferric reducing antioxidant power of hydroethanolic extract of *Thunbergia coccinea* leaves



Total Reducing Antioxidant Potential (TRAP)

The TRAP test is based on the antioxidants capacity to inhibit the reaction between peroxyl radicals and a target molecule[28]. The total reducing antioxidant potential of *Thunbergia coccinea* leaves extract possess good antioxidant potential in a concentration manner. The result from this analysis has revealed that total reducing potential of *Thunbergia coccinea* leaves extract with reliable inhibition with IC_{50} value of 41.6 µg/ml against IC_{50} value of standard quercetin 50.91 µg/ml. The total reducing power of the hydroethanolic leaves extract of *Thunbergia coccinea* and standard quercetin is represented in figure 6. It was found that the leaves extract has considerable amount of scavenging activity.

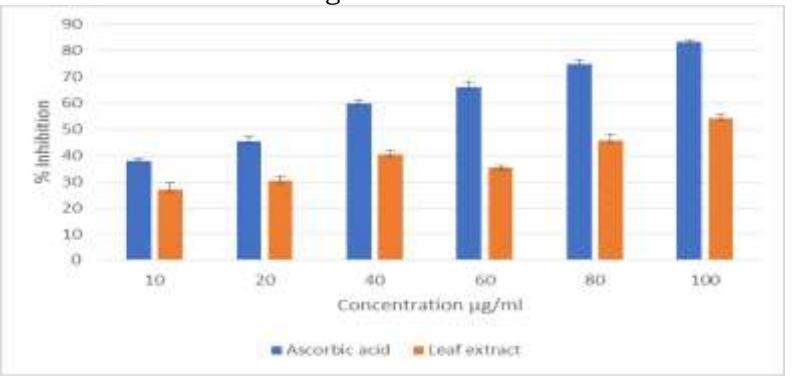
Figure 6: Total reducing antioxidant potential of hydroethanolic extract of *Thunbergia coccinea* leaves



Nitric Oxide Radical Inhibition Assay

Nitric oxide radical scavengers compete with reactive oxygen species to reduce the production of nitrite ion. Overabundance in production of nitric oxide is associated with several diseases[29]. The development of new therapeutics for the prevention of overproduction of nitric oxide is being a target for treating neuroinflammatory disorders[30, 31]. From the analysis it is observed that hydroethanolic extract of *Thunbergia coccinea* leaves shows highest inhibitory effect with the IC_{50} value of 92.63 µg/ml. In contrast the inhibitory effect of standard ascorbic acid was found to be IC_{50}=28.67 µg/ml. It was found that the leaves extract is likely to have nitric oxide scavenging ability. The plant products have a property to counteract the effect of nitric acid formation. The leaves extract of *Thunbergia coccinea* shows ability to reduce production of nitrite ions[32]. Nitric oxide radical scavenging ability of the leaves extract at different concentration is shown in figure 7.

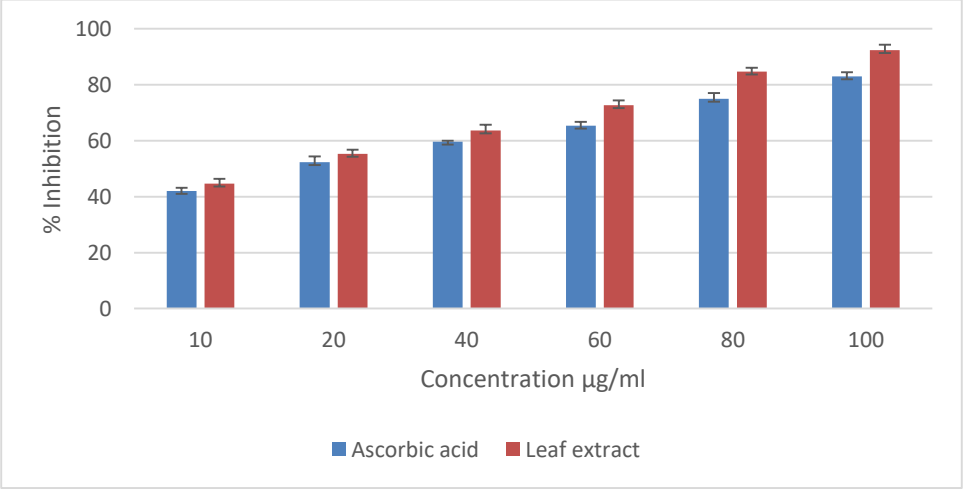
Figure 7: Nitric oxide radical inhibition assay of hydroethanolic extract of Thunbergia coccinea leaves



Phosphomolybdate assay for total antioxidant capacity

Antioxidant capacity of hydroethanolic extract of *Thunbergia coccinea* leaves was determined as per phosphomolybdate assay. The phosphomolybdate method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and formation of green Mo(V) complex with a maximal absorption at 695 nm[33]. The yield of hydroethanolic extract of *Thunbergia coccinea* and its total antioxidant capacity are given in figure 8. The study reveals that total antioxidant capacity of the extract changes depending on their concentration and it is observed that hydroethanolic leaves extract of *Thunbergia coccinea* shows significant inhibitory activity with the IC_{50} value of 14.73 µg/ml against standard ascorbic acid with the IC_{50} value of 21.25 µg/ml. The *in vitro* antioxidant activity of the aqueous and alcoholic extract in the same plant revealed the presence of significant amount of radical scavenging ability where alcoholic extract shown the highest inhibitory effect in alcoholic extract of *Thunbergia coccinea*[34].

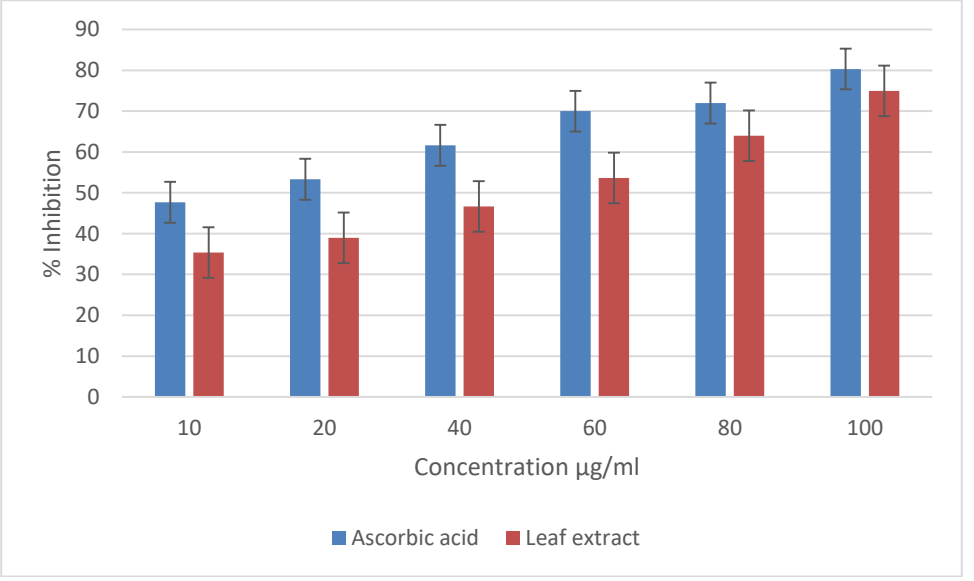
Figure 8: Total antioxidant capacity of hydroethanolic extract of Thunbergia coccinea leaves by phosphomolybdate method



Superoxide Anion Scavenging Assay

Superoxide radical is considered a major biological source of reactive oxygen species[35]. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress[36]. Superoxide anion scavenging assay is done to determine the efficiency of leaves extract to prevent against superoxide anion. These superoxide anions damage the cellular components which can be eliminated by flavonoids[37]. The radical scavenging effect of leaves extract of different concentration was compared with the similar doses of ascorbic acid. In this assay scavenging ability of leaves extract and standard ascorbic acid is evident in the figure 9. It indicates that hydroethanolic *Thunbergia coccinea* leaves extract exhibit the maximum superoxide radical scavenging activity of 46.4 µg/ml which is higher than standard ascorbic acid which have a scavenging activity of 10.85 µg/ml. Relevant study in methanolic extract of *Thunbergia coccinea* shows lower activity compared to the standard ascorbic acid[38]. In present study it is evident that *Thunbergia coccinea* leaves have a potent superoxide radical scavenging ability.

Figure 9: Superoxide radical scavenging assay of hydroethanolic extract of *Thunbergia coccinea* leaves



Conclusion

The research was carried out to evaluate the *invitro* anti-cholinesterase and antioxidant activity of the hydroethanolic extract of *Thunbergia coccinea*. The present finding showed that the leaves extract of *Thunbergia coccinea* can exhibit significant anticholinesterase property. The result of antioxidant studies such as DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing antioxidant power, total reducing antioxidant potential, nitric oxide radical inhibition assay, phosphomolybdate assay of total antioxidant capacity, total phenolic content, superoxide anion scavenging assay indicated that the

leaves extract of *Thunbergia coccinea* possess significant antioxidant properties. Further studies are conducted to find out the bioactive compounds and potential to prevent neurodegenerative disorder.

Conflict of Interest

The authors declare no conflict of interest.

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