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PHYTOCHEMICAL ESTIMATION AND ANTIOXIDATIVE POTENTIAL OF *THUNBERGIA MYSORENSIS* (WIGHT) T. ANDERS. EX BEDD IN UTTARAKHAND, INDIA

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ABSTRACT

Free radicals and reactive oxygen species are well known inducers of molecular, cellular and tissue pathogenesis posing several threats to the human society. Antioxidants are compounds capable either to delay or inhibit to the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are involved in the defence mechanism of the organism against the pathologies associated with the attacks of free radicals. Many plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and per-oxy nitrite which results in oxidative stress leading to cellular damage. *Thunbergia mysorensis*(Wight) T. Anders.exBedd (Acanthaceae family), is an extensive, glabrous climber with pendant branches and it is used to treat various diseases such as cough, jaundice, live disease, fever etc. Therefore, the present study comprises of collection of the plant material from Dehradun, Uttarakhand followed by ethanol extraction for qualitative and quantitative estimation of its phytoconstituents and antioxidant potential. The percentage yield of crude extract was 4.7% and dark green in colour. Phytochemical studies showed the presence of protein, alkaloid, amino acid, carbohydrate, flavonoids, tannin, phenolics. The phenol and flavonoid concentration of plant extract was 0.264µg/ml,0.259µg/ml respectively, and its reducing power activity is 0.311µg/ml. The IC50 value of the plant sample was 125µg/ml against the IC50 value of the standard (Ascorbic acid) was 237.5µg/ml. These finding suggested for the first time that *Thunbergiamysorensis*(Wight) T. Anders. ExBedd cultivation should be promoted as a medicinal plant as it is rich in phenolic and flavonoids compounds which are good sources of antioxidants. Thus in future, the plant extract could be used to control various diseases such as cough, jaundice, liver diseases, fever, arthritis etc. and the presence of phenols and flavonoids makes this plant as a source of good antioxidants.

KEY WORDS: ***Thunbergia mysorensis*(Wight) T. Anders. exBedd, Antioxidant activity, phenol, flavonoids**

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INTRODUCTION

Free radicals and reactive oxygen species are well known inducers of molecular, cellular and tissue pathogenesis leading to several threats to the human society such as atherosclerosis, arthritis, cardiovascular diseases, central nervous system injury, gastritis, cancer, aging and Acquired Immune Deficiency SyndromeAIDS[1-4].Along with lack of effective therapies, oxidative damage plays a decisive etiological factor in many chronic conditions, the expediency of antioxidants in protection against these diseases is defensible.[5-6]Antioxidants are compounds capable either to delay or to inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are involved in the defence mechanism of the organism against the pathologies associated to the attacks of free radicals. Enzymes, like superoxide dismutase, catalase, glutathione peroxidase or non enzymatic compounds, such as uric acid, bilirubin, albumin etc have endogenous antioxidative potential. These endogenous antioxidative compounds lose its potential for controlling and providing the complete protection of the organisms against the reactive oxygen species, the need for exogenous antioxidants like natural antioxidants arises as a nutritional supplements or pharmaceutical products for their role in preventing human diseases[7-8]. The fruit juices, beverages and hot drinks obtained from the natural sources were found to reduce the morbidity and mortality caused by degenerative disorders as they are rich in antioxidants, like polyphenols, vitamin C, vitamin E, β-carotene and lycopene [9].There is an increasing interest in the measurement and use of plant antioxidants for scientific research as well as industrial (dietary, pharmaceutical and cosmetic) purposes in present time. This is basically due to strong biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis[10]. Therefore, the need exits for safe, economic, powerful, and natural antioxidants to replace these synthetic ones [11]. Plants are the rich source of antioxidant compounds which have an ability to protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals which results in oxidative stress leading to cellular damage. Epidemiological studies have indicated the relationship between the plant antioxidants and reduction of chronic diseases. [6,12-13] Therefore, there is a demand at present to evaluate the antioxidative potential of plants parts and to determine of free radical radial quenching ability.*Thunbergia* is a genus of flowering plant which includes about 100 species of annuals, perennials, shrubs, and twining climbers. It belongs to Acanthaceae family native to tropical regions of Africa, Madagascar, Australia and South Asia. Different species of *Thunbergia* used to treat various diseases such as cough, jaundice, liver disease, fever etc. The extracts of plants possess several biological activities like antioxidant, anti-diabetic, antifungal, anthelmintic, anti-inflammatory, antidote and detoxification, cytotoxicity, hepatoprotective, antinoceptive, antibacterial, antifungal etc[14-18]. *Thunbergia mysorensis* (Wight) T. Anders.exBedd commonly known as Clock wine is an extensive, glabrous climber with pendant branches. The leaves are strongly three-nerved, opposite, elliptic lanceolate, acuminate, dentate and glabrous. The inflorescence is an elongated pendant raceme. The flowers are bright yellow, bracteates, the bracts being small and deciduous [19]. In the view of the above literature, most of the studies on *Thunbergiamysorensis* (Wight) T. Anders.exBeddreferred to the cultivation of the plant as ornamental which prompted us to evaluate the qualitative and quantitative determination of its phyto-constituents along with their antioxidant potential.

MATERIAL AND METHODS

Location of the experiment and climatic condition

The present study was carried out at Botany laboratory of Division of Life Sciences, Sri Guru Ram Rai Institute of Technology and Science, SGRRU, Patel Nagar, Dehradun, Uttarakhand. Located amongst Shivalik Ranges on the foothills of the Himalayas, the Doon Valley is nestled between two of India's mightiest rivers-the Ganges on the east and the Yamuna on the west. Dehradun is a picturesque city with mild climate. It is the capital of Uttarakhand, and is located between the latitude 29 ^{O}55' and 38^{O}31' N and longitude 77^{O}35' and 78^{O}20', covering an area of 2002.4sq.km with an elevation of 2000m above the sea level.

Material

The material for the present study comprised of aerial parts of plant named *Thunbergiamysorensis*(Wight) T. Anders. exBedd collected from different places of Dehradun, Uttarakhand, India.The plant material was identified from Dr.Chhaya Singh, Professor (Assistant), Deaprtment of Botany, Shri Guru Ram Rai University, Dehradun, Uttarakhand and was deposited in the departmental herbarium.

Experimental Methodology

Collection and processing of plant Thunbergiamysorensis (wight) T. Anders.exBedd

The plant samples were collected from different locations of Dehradun, Uttarakhand and dried in shade at 25^{0}C to 35^{0}C for 15-20 days in the laboratory and then crushed to coarse powder using grinder. The dried plant material was stored in paper bags.50gm of the dried plant material powder was successively extracted with 250mL of 70%ethyl alcohol solvents for 48 hours. The extract was filtered and evaporated on the water bath till it was finally reduced to dryness to get dry extracts. The extract was then transferred to previously weigh airtight container and stored in the refrigerator until it was screened for their phyto-constituents and antioxidant activity.

Phytochemical analysis

The ethanolic extract of *Thunbergia mysorensis*(Wight) T. Anders. ex Beddwas subjected to preliminary qualitative phytochemical investigation including test for alkaloids, proteins, carbohydrates, flavonoids, cardiac glycosides, saponins, steroids and triterpenoids, Tannins, phenols and oil followed by quantitative estimation of phenols and flavonoids[20].

Determination of total phenolic content

The total phenol content was determined using Folin-Ciocalteu reagent according to the method described by Singleton and Rossi (1965)[21] with some modifications. 0.1ml of sample and 20 microliter of 2N Folin-Ciocalteu reagent were added to a 5ml test tube. The solution was mixed and was allowed to stand for 3-5 min. at room temperature. 0.3ml of 20% sodium carbonate solution (w/v) was added, and the solution was mixes and kept aside (15min). Then 5ml of distilled water was added. The blue color was measured against reagent blank at two wavelengths i.e. 725nm using a spectrophotometer. The total phenol content of the plant sample was determined by comparing it with the optical density values of different concentrations of the standard phenol compounds Gallic acid. A calibration curve of Gallic acid was constructed by plotting absorbancev/s concentration. The total phenolic content was expressed as gram of Gallic acid equivalent (GAE) per 20gram. Sample measurements were done in triplicate and the mean and standard deviations were calculated in each case.

Determination of total flavonoid content

The total flavonoid content is determined with aluminium chloride ($AlCl_3$) method using Quercetin as a standard[22]. The extract (0.25ml each) was mixed with 1.25ml double distilled water which was followed by addition of 75µl of 5% $NaNO_2$. This mixture was incubated for 5 min. at room temperature and then 0.15ml of 10% $AlCl_3$ was added. The reaction mixture was treated with 0.5ml of 1mM NaOH after incubation of 6min. at room temperature. Then the reaction mixture was treated or diluted with 5ml of double distilled water followed by an incubation of 20 min. at room temperature. The absorbance was measured at 510nm. The flavonoid content was expressed in mg of Quercetinequivalents (QE) /gm of samples. Evaluations were performed in triplicate.

Determination of reducing power activity

The reducing power of the sample was determined by the Oyaizu (1986)[23]method with some modification. Reducing power activity is based on ferricyanide in stoichiometric excess relative to the amount of antioxidant[23]. Sample 50 µl with different concentration were mixed with 0.25ml of 0.2M sodium phosphate buffer (pH 6.6) and 0.25ml of 1% potassium ferricyanide (w/v) and incubated at 50 degree for 20 min. After incubation, 1ml of 10% tri-chloro acetic acid was added to the mixture, followed by the centrifuge at 3000 rpm for 10 min. The upper layer 1.2ml was mixed with 1ml of deionized water and 0.25ml of 0.1% ferric chloride, and the absorbance of the resultant solution was measured at 700nm. Ascorbic acid was used as reference.

DPPH radical scavenging assay:

The scavenging activity was measured using 2,2-diphenyl-1-picrylhydrazyl(DPPH). The stock solution (500mg/ml) of each sample was diluted to the conc. of 200, 150, 100, 50 mg/ml in ethanol or distilled water and 1ml DPPH and 3.98ml of ethanol (for 20 µl and so on less in value). Incubation for 30 min. at room temperature is required. The decrease in absorbance was measured at 517nm. Ascorbic acid was used as reference.

DPPH scavenged (%) = (A con - A test)/A con x 100
Where, A con = is the absorbance of the control reaction.
A test= is the absorbance in the presence of the sample of the extracts.

STATISTICAL ANALYSIS

Results were expressed as mean±standard deviation using MS excel.

RESULT AND DISCUSSION

The genus *Thunbergiamysorensis* (Wight) T. Anders.exBedd, yellow red trumpetvine is a popular ornamental and medicinal plants belongs to Acanthaceae family, widely distributed in most of the tropical and subtropical region of the world having various biological activities such as anti-diabetic, antifungal, anthelmintic, anti-inflammatory[14,15,16,17,18]. It comes from tropical mountain slopes in south of India and thrives in frost-free conditions similar to that of itshomeland.The evergreen woody-stemmed climber can climb up to about 6m, and has narrow leaves, up to about 15 cm in length and a handsome dark glossy green, with toothed margins.The pendant flower spikes area cheerful combinationofbrownish red and yellow, and appear from springto autumn.. Therefore, Easy availability in the garden and having various biological activities subjected to the estimation of phytoconstituents and antioxidant activity of ethanolic extract of *T. mysorensis*. The findings of the present studies are discussed under the following headings:

Yield and Preliminary Phytochemical Screening

Phytochemicals are the chemicals which derived from the plant sources, generally affect health, but are not established as essential nutrients. Alkaloids, terpenoids, tannin, saponin, flavonoids, glycosides etc. are some classes of phytochemicals [24-26]. They play important roles in plant growth and provide defence against pathogens and predators.They also exhibited various biological activities such as anticancer, antioxidants and anti-inflammatory and are used to cure various ailments. The extract was subjected for qualitative and quantitative phytochemical estimation. The % yield of crude extract was 4.7%.The appearance of ethyl alcoholic extract was dark green in colour.On the qualitative estimation, the crude ethanolic extract showed the presence of alkaloid, phenolic compounds, flavonoids, carbohydrate whereas glycosides were absent (Table 1). Similarly Jeeva *et. al*., (2011)[27], Kabir*et.al., (*2015)[28] reported the presence of Proanthocyanidin, acondensed, tannin compound , phenols, alkaloids and flavonoids in *Thunbergiagrandiflora*. According to the findings in *T. grandiflora* , polyphenols are a large and diverse class of

compounds, many of which occur naturally in a wide range of food and plants. The flavonoids are the largest and best studied group among polyphenols. A range of plant polyphenols is either being actively developed or already currently sold as dietary supplements and/or herbal, derived medicines. Although these compounds play an unknown role in nutrition (non-nutrients), many of them have properties including antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of genome [29].

Table 1
Phytochemical analysis of Thunbergiamysorensis(Wight) T. Anders.exBedd

S.N.	Constituents	Test for confirmation	Result
1.	Alkaloids	Mayer test	+ve
2.	Proteins	Biuret test	+ve
3.	Amino acid	Ninhydrin test	+ve
4.	Carbohydrate	Molisch test	+ve
5.	Flavonoids	Alkaline test	+ve
6.	Phenolic content	Acetic acid test	+ve
7.	Glycosides	Bromine water	-ve

(+) and (-) sign indicate presence or, absence of the compound, respectively.

Total Phenol and flavonoid Content

Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new high in recent years[30].. In this study, the total phenol content was determined by using Folin- Ciocalteu method, with the Gallic acid as a standard. The content of phenol was evaluated from the regression equation of the calibration curve (y=0.77x+0.064, R^2= 0.832), expressed in GAE as milligram per gram of extract (mg GAE/g extract).The value of plant sample was 26.4±1.36mgGAE/g extract . (Figure1).Whereas Chan and Lim (2006)[31] reported that the total phenolic content in the methanol extract of leaves of *T. laurifolia*was 2720mg GAE/100g.This study revealed that total phenolic content was less in *T. mysorensis*than *T. laurifolia,*one of the beneficial species commonly used for preparation of green tea. The value of total phenolic content in the other members of Acanthaceae family like *Justiciaspicigera, Diclipteraverticillata* was 5.01gGAE/100g, 2.82g GAE/100g, respectively which was less than the value reported for the first time in *T. mysorensis*[32].

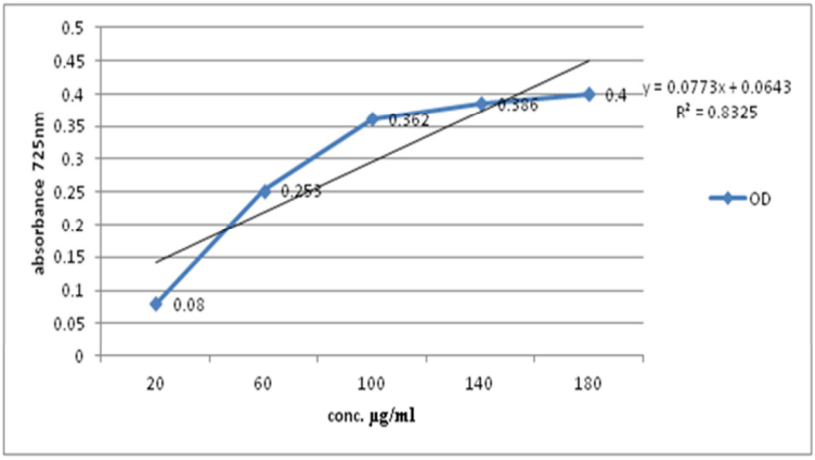

Figure 1
Standard curve represent concentration of gallic acid (µg/ml) against absorbance

Flavonoids are well known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties.The antioxidant can donate an electron to free radicals, which leads to the neutralization of the radical. The total flavonoid content was evaluated from the calibration curve (y=0.051x+0.080, R^2=0.981) expressed in QE in milligrams per gram of extract (mg/g extract). The value of the plant sample was 25.9±1.24 mg QE/g extract (Figure2). . Similarly,Total flavonoid content in *Justiciaspicigera* was between 0.18 and 1.30 g Catechin/100 g d.wt. which was less than the value reported for the first time in *T. mysorensis*[32].Detailed studies on the extraction and quantification of phenolic and flavonoids in *T. mysorensis* are required for understanding their contribution to the antioxidant activity as present in their natural form.

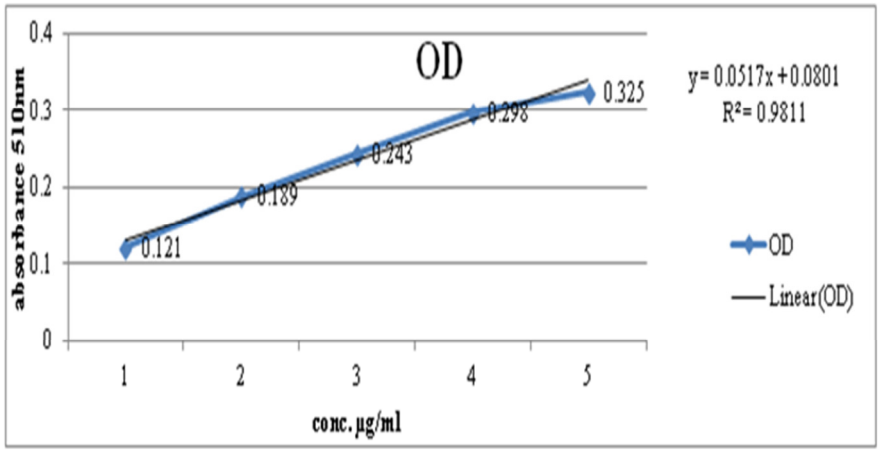

Figure 2
Standard curve represent concentration of Quercetin (µg/ml) against absorbance

Antioxidant Activity

An antioxidant is a molecule that inhibits the oxidation of molecules in biological system have multiple purposes including defending against oxidative damage. Natural antioxidants along with synthetic onces can be used to act against free radicals in the food and biological systems. [5, 6].As the synthetic antioxidants have several side effect, therefore the natural antioxidants are replacing the synthetic antioxidants. In the present study, Reducing power activity and DPPH radical scavenging assay was used for the determination of antioxidant potential of *T. mysorensis*.

Reducing Power Activity

Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species [27].Reducing power was measured by direct electron donation in the reduction of Fe3+ (CN-)6–Fe2+(CN-)6 [33]. The product was visualized by forming the intense Prussian blue color complex and then measured at λ700nm. The antioxidant activity of the sample of *Thunbergiamysorensis* (Wight) T. Anders.exBedd was determined from distinct color changes (i.e. from green to dark green). The value of the plant sample was 0.311µg/ml, depending on the reducing power of the sample concentration. The high absorbance of the reaction mixture indicates high reducing power (Table-2 and 3).

Table 2
Reducing power of the plantThunbergiamysorensis(Wight) T. Anders. exBedd

Sample	Concentration(µg/ml)	Absorbance (700nm)
Thunbergia sample	250	0.119 ±0.006
	500	0.183± 0.007
	750	0.213±0.005
	1000	0.259±0.001

**** Results are Mean±SD (n=3).***

Table 3
Reducing power of the standard (Ascorbic acid)

Sample	Concentration (µg/ml)	Absorbance (700nm)
Ascorbic Acid	250	0.219±0.004
	500	0.261±0.005
	750	0.299±0.001
	1000	0.321±0.001

**** Results are Mean±SD (n=3).***

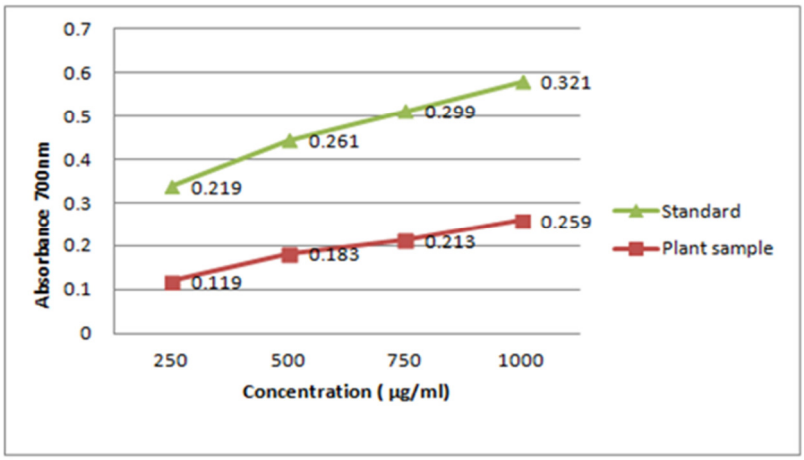

Figure 3
Standard curve represent of reducing power concentration of the plant T. mysorensis(Wight) T. Anders. exBeddagainst standard (Ascorbic acid)

DPPH Radical Scavenging Assay

DPPH scavenging activity of the sample of *T. mysorensis* (Wight) T. Anders.exBedd was compared with standard (ascorbic acid) by evaluating antioxidant efficiencies, known as IC50. IC50 is the concentration of an antioxidant at which 50% inhibition of free radical activity is observed. The study showed that the plant sample of *T. mysorensis* (Wight) T. Anders. eBeddshowed a good antioxidant activity in DPPH assay. The value of the plant sample was measured at 517nm. The IC50 value of *Thunbergiamysorensis* (Wight) T. Anders.exBedd is 125µg/ml. as compare to the standard ascorbic acid (237.5µg/ml). According to the result of the DPPH radical scavenging, the value of the plant sample is lowerthan the standard. The graph shows the comparison between plant sample and standard in Figure 4.Similarly, Chan and Lim(2006)[32] reported that IC50 value in the methanol extract of leaves of *T. laurifolia was* 0.16 mg/ml. IC50 value in other members of Acanthaceae family such as *J. spicigera* was 48.86 µg mL^{-1}, was higher but lower than that evaluated for whole plants of *Diclipteraverticillata* (785.67 µg mL^{-1}).The present studies revealed that phenolic and flavonoids play a significant role for the determination of antioxidative compounds present in plant species. Similarly positive correlations have been reported between total phenolic content and antioxidative activity of the extracts of other members of Acanthaceae and about 70 plant species in folk medicine[34,35,36].

Table 3
DPPH radical scavenging of Thunbergiamysorensis(Wight) T. Anders.exBeddplant sample

S. No.	Conc. (µg/ml)	Sample	Control	% inhibition	IC 50(µg/ml)
1.	50	0.461	0.521	11.51±0.02	125±0.03
2.	100	0.413	0.521	20.72±0.01	
3.	150	0.388	0.521	25.52±0.03	
4.	200	0.341	0.521	34.54±0.03	
5.	250	0.298	0.521	42.80±0.04	

**** Results are Mean±SD (n=3).***

Table 4
DPPH radical scavenging of standard (Ascorbic acid)

S. No.	Conc. (µg/ml)	Sample	Control	% inhibition	IC 50(µg/ml)
1.	50	0.431	0.521	17.27±0.03	237.5±0.02
2.	100	0.400	0.521	23.22±0.01	
3.	150	0.356	0.521	31.66±0.05	
4.	200	0.301	0.521	42.22±0.02	
5.	250	0.214	0.521	58.92±0.01	

**** Results are Mean±SD (n=3).***

Result of the DPPH radical scavenging, the value of the plant sample is low than the standard. The graph shows the comparison between plant sample and standard

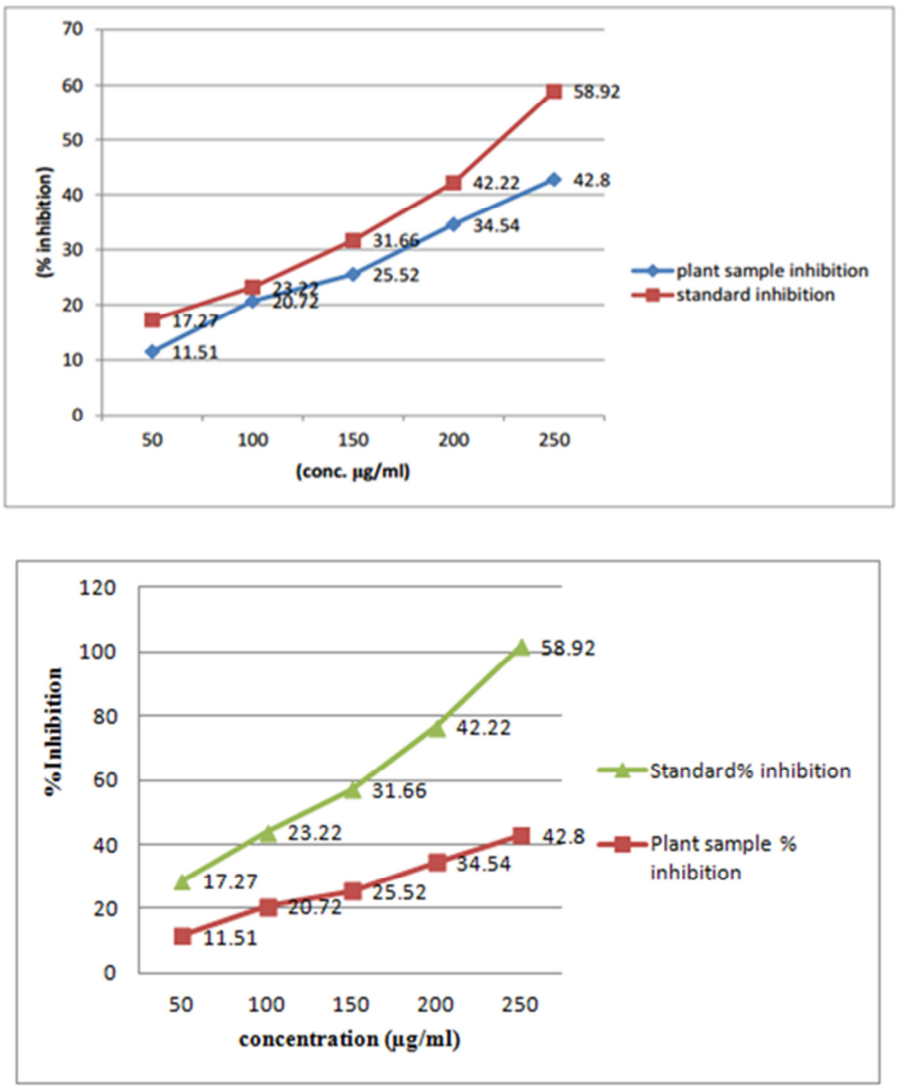

Figure 4
Comparison on percentage inhibition of plant sample with standard (Ascorbic acid)

CONCLUSION

The present finding suggests for the first time that*Thunbergiamysorensis* (Wight) T. Anders. ExBedd cultivation should be promoted as medicinal plant along with its ornamental values as it was rich in phenolic and flavonoids which will be positively correlated with antioxidative potential.s. Thus in future, the plant extract could be used to control various diseases such as cough, jaundice, liver diseases, fever, arthritis etc

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AUTHOR CONTRIBUTION STATEMENT

Dr. M. Singh has designed the experiment and Mrs.GunjanKimothi has performed the experiment under the guidance of Dr. M. Singh.

CONFLICT OF INTEREST

Conflict of interest declared none.

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