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Research Article

Secondary metabolite estimation and antioxidant potential assessment of purple bell *Thunbergia erecta* (Benth.) T. Anderson

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Abstract: To quantify the aqueous and methanolic extracts for primary and secondary metabolites, and the antioxidant potential of leaf extracts of the *Thunbergia erecta* plant and to adopt them in Ayurvedic medications for various illnesses. Primary metabolites like carbohydrates, proteins, and secondary metabolites such as flavonoids, alkaloids, total phenols, and tannins were estimated using standard procedures. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), hydrogen peroxide, and phosphomolybdenum free radical scavenging activities were employed to evaluate the antioxidative potential. The phytochemical examination of *T. erecta* exhibited the presence of carbohydrates, proteins, amino acids, alkaloids, total polyphenolics, flavonoids, and tannins in significant quantity. The *in vitro* antioxidant potential of the species, clearly verifies that methanolic extract shows prominent antioxidant properties followed by the hydroalcoholic extract. From this study, it can be concluded that this species is effective in scavenging free radicals and may be a powerful antioxidant. The *T. erecta* leaf extract showed the existence of bioactive components which are known to exhibit medicinal activities. The findings of this study suggest that these plant leaves could be a potential source of natural antioxidants that could have great importance as therapeutic agents in preventing various diseases.

1. INTRODUCTION

Oxygen, the most crucial component for the survival of living beings, is extremely reactive and can harm the body's healthy cells as a free radical. Free radicals are produced by oxidation, which can start chain reactions that cause cell damage and even cell death (Moonmun *et al.*, 2017). An antioxidant is a molecule (or an ion, or a relatively stable radical) that is capable of slowing or even preventing the oxidation of other molecules (Pinchuk *et al.*, 2012). The characteristic feature of an antioxidant is the ability to scavenge free radicals due to their redox hydrogen donators and singlet oxygen quencher (Senguttuvan *et al.*, 2014). Both free and non-free radical species are produced by reactive oxygen species (ROS), resulting in hazardous and

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lethal diseases such as diabetes, atherosclerosis, ischemic heart disease, inflammation, immune suppression, neurodegenerative disorders, cancer, and so on. Free radicals such as superoxide and hydroxyl radicals are formed as by-products of the cell's energy production. When the concentration of ROS in the cell rises, it causes oxidative stress. Antioxidants, which are found in nature or as food supplements, counteract these effects in live cells. Several research supports the fact that flavonoids and phenolic compounds are potent antioxidants. Antioxidants scavenge free radicals and mitigate the effects of reactive oxygen species (Ganguly *et al.*, 2021). The continued expansion of the antioxidant market reflects the expectation of curing a wide range of ailments thought to be caused or exacerbated by oxidative stress. Medicinal plants provide a variety of alternative medicines with exciting possibilities. Several sources also say that dietary phytometabolites such as flavonoids, phenols, and tannins have been employed in the prevention and treatment of diseases for millennia (Ganguly *et al.*, 2021).

Thunbergia erecta belongs to the family Acanthaceae, native to the tropical part of Madagascar, Australia, Africa, India, and South Asia. The whole plant is said to be medicinally important by having anxiolytic, sedative (Begum *et al.*, 2019), anticholinesterase, antiaging activity (Refaey *et al.*, 2021), antidiabetic activity, antimicrobial screening (Kusimo *et al.*, 2019), antiurolithiatic (Chandel *et al.*, 2020) and neuroprotective activity (El-Din *et al.*, 2023). Investigations of the *T. erecta* plant uncovered various phytochemical characteristics, the chemical composition of the ethyl acetate fraction of *T. erecta* leaf alcohol extract had significant activity on DOX and Cyclo-induced cognitive impairment in rats. (El-Din *et al.*, 2023). Our review has already covered the various bioactivities *T. erecta* plant (Kochar *et al.*, 2023).

However, much scientific validation has been needed for this species for its medicinal uses. To address this lacuna, the present study was carried out for qualitative and quantitative phytochemical analysis and *in vitro* antioxidant activities of the leaves of *T. erecta* using petroleum ether, methanol, and hydroalcoholic extracts.

2. MATERIAL and METHODS

2.1. Chemicals and Reagents

The chemicals utilized included acetone, ammonia, aluminium chloride, ascorbic acid, ammonium molybdate, anhydrous sodium carbonate, *β*-D-glucose, bovine serum albumin, butylated hydroxytoluene (BHT), chloroform, 1,1- diphenyl-2-picrylhydrazyl radical (DPPH), diethyl ether, ethanol, ethyl acetate, ferric chloride, Folin-Ciocalteu reagent, gallic acid, glacial acetic acid, hydrochloric acid, hydrogen peroxide, methanol, *n*-butanol, *n*-propanol, petroleum ether, phenol, phosphate buffer, potassium acetate, potassium ferricyanide, quercetin, rutin, sodium chloride, sodium nitrate, sodium hydroxide, sodium phosphate, and sodium nitroprusside were of analytical grade and purchased from Merck.

2.2. Collection and Authentication of Plant Material

Fresh leaves of the *Thunbergia erecta* plant were collected from the local area of Wardha, Maharashtra, India. The authentication of plant material was carried out by the Botanical Department of RTMNU Nagpur University, Nagpur. The voucher specimen number is 10421. The collected plant material was washed in tap water to get rid of any contaminant, then dried under shade at room temperature for seven days, and then ground to obtain a coarse powder.

2.3. Organoleptic Study

Organoleptic properties like color, odor, shape, size, taste, and texture were studied (Table 1).

2.4. Physicochemical Investigation

The physicochemical studies were performed on *T. erecta* leaf powder, such as different ash values, extractive values, extractive matter, fiber content, foaming index, and moisture content.

2.4.1. *Determination of ash value*

The plant powdered was analyzed for total ash, sulphated ash, acid-insoluble ash, and water-soluble ash as per standard techniques of WHO quality control methods for herbal materials (Maduka *et al.*, 2020; Singh *et al.*, 2019).

2.4.2. Determination of extractable matter

The extractive value was done on different solvents such as petroleum ether, ethyl acetate, chloroform, acetone, ethanol, methanol, hydroethanolic extract in the ratio (80:20, 60:40, 50:50). A glass stoppered conical flask was filled with approximately 5 g of coarsely powdered air-dried material. 100 mL of the above-mentioned solvents were added. The conical flask was corked and left for 24 hours with continuous shaking. The solvents were then filtered using dry filter paper to ensure that no solvent was lost, and 25 mL of the filtrate was transferred to a tarred flat bottom dish and evaporated to dryness on a water bath. The solvent was dried for 6 hours at 105°C and chilled for 30 minutes in a desiccator before being weighed. The extractable matter quantity in mg/g of air-dried material was calculated (Singh *et al.*, 2019).

2.4.3. Determination of foaming index

Approximately 1 g of coarse powder was precisely weighed and put in a 500 mL conical flask holding 100 mL boiling water. The mixture was kept at a moderate boil for 30 minutes, then cooled and filtered into a 100 mL volumetric flask, with enough water added through the filter to dilute the volume. In ten test tubes with stoppers (diameter: 16 mm; height: 16 cm), the decoction was added in portions of 1 mL, 2 mL, 3 mL, etc., up to 10 mL, and the liquid volume in each tube was adjusted to 10 mL using water. The tubes were stopped and shaken lengthwise for 15 seconds at two shakes per second. The foam was Allowed 15 minutes to rise before measuring its height (Mishra *et al.*, 2016).

The foaming index was calculated using the following formula:

Foaming index = 1000/a

Where a = the volume in mL of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

2.4.4. Determination of moisture content

Moisture content was determined using the standard method described by Maduka (2020), a 1 g sample was precisely weighed and placed in a dry crucible. The samples were dried in an oven at 100-110° C for 2 hours, removed, cooled in a desiccator, and weighed. This process continued until consistent weights were achieved (Maduka *et al.*, 2020). The moisture content of the samples was calculated using the following formula:

$$\% \text{ moisture} = (ab-ac) * 100/ab$$

Where ab = weight of dish + weight of sample before drying (g)

ac = weight of dish + weight of sample after drying (g)

2.4.5. Estimation of crude fiber

A crude sample (2 g) was extracted with petroleum ether to remove fat. After filtration, the material was dried and heated with 200 mL of 0.3M H_2SO_4 for 30 minutes. When the washings were acid-free, it was filtered and washed again with hot water. After heating in 200 mL NaOH for 30 minutes, the residue was filtered and washed in 25 mL boiling H_2SO_4. The residue was placed on a pre-weighed ashtray ("W1", g). After cooling in a desiccator and drying at 130° C for 2 hours, the residue was weighed ("W2", g).

It was burned at 600°C for 30 minutes, cooled in a desiccator, and reweighed ("W3", g). (Maduka *et al.*, 2020; Mishra *et al.*, 2016)

Calculation: Loss in weight = (W2 - W1) - (W3 - W1)

2.5. Extract Preparation

The coarse powder of the leaves of the *T. erecta* plant was defatted with petroleum ether. After that, the defatted plant extract was macerated for 7 days in MeOH and thereafter hydroethanolic solvent (80:20). It was then filtered and concentrated to obtain a dried powdered form of MeOH extract of *T. erecta* (TEME) and hydroalcoholic extract of *T. erecta* (TEHE).

2.6. Preliminary Phytochemical Analysis

The bioactivity of herbal constituents was determined by the phytoconstituent present in it. The petroleum ether, methanolic, and hydroalcoholic extract of *T. erecta* leaves were screened to ascertain the presence of phytoconstituents by using different chemical tests as per standard procedures (Mandal *et al.*, 2013).

2.6.1. The qualitative and quantitative examination of phytoconstituents

T. erecta plant extracts were screened for primary and secondary metabolites such as carbohydrates, proteins, total polyphenols, flavonoids, and alkaloids.

2.6.1.1. Total carbohydrate content. Total carbohydrate content was estimated using the phenol sulfuric acid method by using *β*-D-glucose as standard. 0.1 g of plant sample was hydrolyzed with 5 mL of 2.5 N HCl in a water bath. After cooling to room temperature, the solution was neutralized by adding Na_2CO_3 till the effervescence ceased. The solution was filtered, and the volume was made up to 100 mL with distilled water. Various concentrations of working standard glucose were taken (0.2, 0.4, 0.6, 0.8, and 1 mL). Blank was set with all reagents except the sample. One milliliter of a 5 % phenol solution was added to each test tube. 5 mL of 96 % H_2SO_4 was added and thoroughly mixed, and the combination was maintained at 25-30 ^{0}C for 20 min. At 490 nm, the color change was measured (Jain *et al.*, 2017).

2.6.1.2. Quantification of total protein content. Bovin Serum Albumin working standard solution was prepared by adding BSA in distilled water. The dilutions (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg) were taken in triplicates. 5 mL of reagent mixture (50 mL of 2% $NaCO_3$ in 0.1 N NaOH + 1mL of 1% sodium potassium tartrate + 1mL of 0.5% copper sulphate) was pipetted to all test tubes and incubated for 10 min. 0.5 mL of Folin-Ciocalteu phenol reagent (5 mL of 2 N Folin-Ciocalteu + 6 mL of distilled water) was pipette to each test tube and mixed well immediately. The absorbance of the reacting mixture was taken at 660 nm and the amount of protein was estimated as BSAE/g of fresh weight (Daniel G. & Krishnakumari S., 2014).

2.6.1.3. Determination of total polyphenols. The estimation of total polyphenols of *T. erecta* leaf extract was carried out by using the Folin-Ciocalteu method. Gallic acid was used as standard in a concentration range (50-500 µg/mL). 500 µL of Folin-Ciocalteu reagent (10 %) was mixed with 100 µL of plant extract (1 mg/mL) and incubated in the dark for 20 min. After adding 2 mL of 20 % w/v sodium carbonate and 60 min incubation, the absorbance was taken at 765 nm by UV spectrophotometer. All the determinations were performed in triplicate. The TPC was expressed in mg gallic acid equivalent (GAE)/g dry extract with reference to gallic acid as a standard (Das *et al.*, 2018; Hayat *et al.*, 2020; Kaur *et al.*, 2015).

2.6.1.4. Determination of total flavonoid content. The estimation of total Flavonoid content was done as per the method described by (Hayat *et al.*, 2020). The aluminium chloride method was performed for the estimation of flavonoids. Plant extract (500 µL) and varied concentrations (10-100 µg/mL) of standard (Rutin) were mixed with 1500 µL of methanol, 100 µL of aluminium chloride solution, 100 µL of potassium acetate, and 2800 µL of distilled water. It was held at room temperature for 30 minutes. At 510 nm, the absorbance was measured against methanol as a blank. Using rutin as a standard, the total content of flavonoid compounds was estimated using the equation below (Hayat *et al.*, 2020).

$$C= (c \times V) / m$$

Where C: Total flavonoid content of compounds

c: concentration of rutin

V: volume of extract in mL

m: weight of crude plant extract

2.6.1.5. Determination of alkaloids. Different extracts of the *T. erecta* plant were dissolved in 2 N HCl and filtered. 1 mL of filtrate which was treated thrice with 10 mL of chloroform in a separating funnel. The neutral pH was adjusted by using 0.1 N NaOH solution. 5 mL of BCG (0.1 M) solution and 5 mL of phosphate buffer (pH 4.7) were added. The mixture was shaken thoroughly and was extracted with 1, 2, 3, and 4 mL of chloroform in a 10 mL volumetric flask, and volume was adjusted with chloroform. The absorbance of the formed complex was taken at 470 nm by a UV spectrophotometer. Total alkaloid content was measured from the atropine calibration curve concentration range of 10 to 100 µg/mL and expressed as µg of atropine equivalent/g of dry weight. All determinations were performed in triplicates (Darwish *et al.,* 2021; Das *et al*., 2018; Sulekha Rani & Priti, 2019).

2.7. Antioxidant Assays

2.7.1. *DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay*

The free radical scavenging activity of different extracts of the *T. erecta* plant was determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay described by Jeremiah Oshiomame Unuofin *et al.*, Jelita Rahma Hidayati with slight modification (Hidayati *et al.,* 2020; Unuofin *et al.,* 2017).

The different concentrations of ascorbic acid standard solution (10-500 µg/mL) and test were prepared by diluting the stock solution of DPPH 0.04 mg/mL in methanol. 1 mL of DPPH standard solution was mixed with 3 mL of extracts and kept at room temperature for 30 min. Absorbance was measured at 517 nm. The percentage scavenging of DPPH was measured at different concentrations of extract and standard using the following equation.

$$\text{Inhibition percentage} = (\text{Absorbance of DPPH} - \text{Absorbance of DPPH + Extract}) / \text{Absorbance of DPPH} \times 100\ \%$$

The radical scavenging/inhibition activity of *T. erecta* plant extracts was expressed as an inhibition percentage of DPPH. The inhibition percentage data were plotted to construct the linear regression equation and determine the IC_{50} value. All measurements were performed in triplicate (Khorasani Esmaeili *et al.,* 2015).

2.7.2. *Hydrogen peroxide radical scavenging activity*

The approach described by Atere *et al.*, (2018) was used to evaluate hydrogen peroxide radical scavenging activity. A 1 mL of plant extract at different concentrations (0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL) was combined with 2 mL of hydrogen peroxide solution (20 mM) prepared in 0.1 M phosphate buffer (pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against a blank that contained extracts in sodium phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a working standard (Atere *et al.,* 2018; Saeed *et al.,* 2012). The following formula was used to determine hydrogen peroxide's capacity to scavenge free radicals:

$$\%\ \text{Scavenging activity} = [1- (\text{Absorbance of test} / \text{Absorbance of control})] \times 100$$

2.7.3. *Ferric Reducing antioxidant power assay (assay of reductive potential)*

The potassium ferricyanide ferric chloride technique was used to calculate the ferric reducing antioxidant capacity. 1 mL of plant extract solution in various strengths was mixed with 2.5 mL

of phosphate buffer (0.2 M, pH 6.6) 2.5 and mL of 1% potassium ferricyanide solution was added to the aforesaid mixture, and the solution was incubated at 50 °C for 20 minutes. After completion of incubation 2.5 mL of 10 % (w/v) of trichloroacetic acid was added and the solution was centrifuged at 1000 rpm for 10 minutes. To the 2.5 mL of supernatant solution, 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride solution were added. It was mixed thoroughly, and the bluish color complex formed was read at 700 nm against blank with reference to standard (ascorbic acid) on a UV spectrophotometer. The reducing power of the samples was compared with the reference standard (Chan & Lim, 2006; Li *et al.*, 2020; Rahim *et al.*, 2017).

2.7.4. *Assessment of total antioxidant potential by Phosphomolybdenum method*

The TACs of various extracts of the *T. erecta* plant were calculated using the phosphomolybdenum method as per the method described by Maswada (2013). 1 mL of phosphomolybdenum reagent (4 mM ammonium molybdate, 0.6 M sulfuric acid, and 28 mM Sodium phosphate) was added to 1 mL of plant extract. The reaction solution was incubated in a hot water bath for 90 minutes. The absorbances were recorded at 695 nm by UV spectrophotometer using methanol as a blank. TAC was expressed as equivalents (GAE)/g dry extract with reference to ascorbic acid as a standard. The standard calibration curve was generated by mixing ascorbic acid (20, 40, 60, 80, 100, 120, 140, 160, 180, 200, and 400 µg/mL) with methanol (Doraiswamy & Saminathan, 2020; Maswada, 2013; Moonmun *et al.*, 2017).

2.8. Statistical Analysis

All the experiments were carried out in threes. The data were analyzed in Microsoft Excel and are presented as mean ± standard deviation (n = 3). GraphPad Prism 7 and Sigma Plot 15 for Windows 10 were used to calculate the IC_{50} values.

3. RESULTS and DISCUSSION

3.1. Pharmacognostic Study

Shade-dried leaves of the *T. erecta* plant were evaluated for organoleptic, microscopic, and physical characteristics. The organoleptic characteristics of the *T. erecta* plant are given in Table 1.

Table 1. Organoleptic characters of *T. erecta* leaves.

Organoleptic Characters	Descriptions
Color	Dark green
Odor	No Fragrance
Texture	Glossy
Taste	Bitter
Shape	ovate leaves with entire
Size	Less than 5 cm

3.2. Physicochemical Studies

The ash values, extractive values, moisture content, crude fiber content, and foaming index of dried powdered leaves were investigated. The information congregated from the previous studies is presented in Table 2. The physicochemical analysis performed in this study will help to identify plant adulteration with other species.

Table 2. Physicochemical parameters.

Sr. No.	Parameter	% Values (w/w)
A.	Ash Value	
1	Total ash value	13.7
2	Acid insoluble ash value	3.96
3	Water soluble ash value	7.92
4	Sulphated ash value	18.0
B.	Extractive value	
5	Petroleum ether	2.70
6	Chloroform	4.60
7	Acetone	3.40
8	Methanol	7.30
9	Ethanol	4.20
10	Hydroalcoholic extract (50:50)	28.2
11	Hydroalcoholic extract (70:30)	29.3
12	Hydroalcoholic extract (80:20)	31.5
C.	Moisture content	5.64
D.	Crude fibre content	27.3
E.	Foaming index	166.67

(% w/w = Percent weight by weight)

3.3. Extraction Yield

The extraction yields of extracts of the *T. erecta* plant were determined for petroleum ether, methanolic, and hydroalcoholic extract. The extraction yield was calculated by applying the equation of the weight of the extract to the dry plant's weight. The hydroalcoholic extracts of leaves exhibited a higher yield (22.18 %w/w) followed by methanolic extract (21.65 % w/w). Petroleum ether extract shows the lowest yield (8.82 % w/w).

3.4. Phytochemical Analysis of Leaf Extracts of *T. erecta*

3.4.1 Qualitative analysis (phytochemical screening)

The phytochemical analysis of the extracts was assessed based on a well-established method reported by the literature. (Khandelwal, 2008) The results of the phytochemical study are presented in Table 3. Phytocompounds are highly present in the methanol extract rather than in the hydroalcoholic and petroleum extracts. Among all the phytocompounds alkaloids, flavonoids, polyphenols, saponin, steroids, and tannin show higher concentrations in the methanol extract. The hydroalcoholic extract shows the presence of amino acids, carbohydrates, glycosides, proteins, polyphenols, and tannins.

Table 3. Phytochemical screening of *T.erecta.*

Sr. No.	Phytoconstituents	Petroleum ether extract	Methanolic extract	Hydroalcoholic extract
1	Carbohydrates	-	-	+
2	Amino acids	-	-	+
3	Proteins	-	-	+
4	Tannins	-	-	+
5	Flavonoids	-	+	-
6	Terpenoids	-	+	-
7	Triterpenoids	-	+	-
8	Alkaloids	-	+	-
9	Saponin	-	+	-
10	Polyphenols	-	+	+
11	Glycosides	-	-	+
12	Fatty components	+	-	-
13	sterols	-	+	-

(-) = Negative test; (+) = Positive test

3.4.2. Quantitative analysis of phytochemicals

The carbohydrate and protein content (mg/g of extract) was found to be high in the hydroalcoholic extract followed by the methanolic extract at 560.50 ± 0.0151 and 155.167 ± 0.001, respectively. The plant had significant levels of both carbohydrates and proteins (Figure 1). Phenolic compounds are secondary metabolites that have been extensively investigated in the medicinal plant leaf extract. Phenolic compounds are associated with color, sensory qualities, and nutritional and antioxidant properties. The total phenolic contents of different extracts of *T. erecta* leaves were evaluated using the Folin-Ciocalteu method. The content of phenolic compounds in petroleum ether, methanolic, and hydroalcoholic extracts ranged from 5.174 ± 0.0246, 70.857 ± 0.0469, and 164.492 ± 0.122 GAE/g of extracts, respectively. The hydroalcoholic extract was shown to have a high total polyphenol concentration (Figure 2).

The total flavonoid content was estimated using spectrophotometry and findings were expressed in mg of Ru/g. The highest flavonoid concentration was noted in methanolic extract (Table 4). The number of total alkaloids in plant extracts was calculated and expressed as mg of AE/g of extract based on atropine equivalents. The highest alkaloid concentration was measured in methanol extract at 141.416 ± 0.001 mg/g (Table 4) (Figure 3).

Table 4. Phytoconstituent estimation (mg/g GAE) of various extracts of leaves of *T. erecta.*

Phytoconstituents	Petroleum ether extract	Methanolic extract	Hydroalcoholic extract
Carbohydrates	121.167 ± 0.0287	269.670 ± 0.0035	560.500 ± 0.0151
Proteins	21.667 ± 0.0014	98.583 ± 0.0021	155.167 ± 0.0010
Total polyphenols	5.174 ± 0.0246	70.857 ± 0.0469	164.492 ± 0.1220
Alkaloids	35.291 ± 0.0025	141.416 ± 0.0010	82.625 ± 0.0009
Flavonoids	215.833 ± 0.0233	577.500 ± 0.0272	498.166 ± 0.1070

The values represent the means of three measurements ± standard deviation.

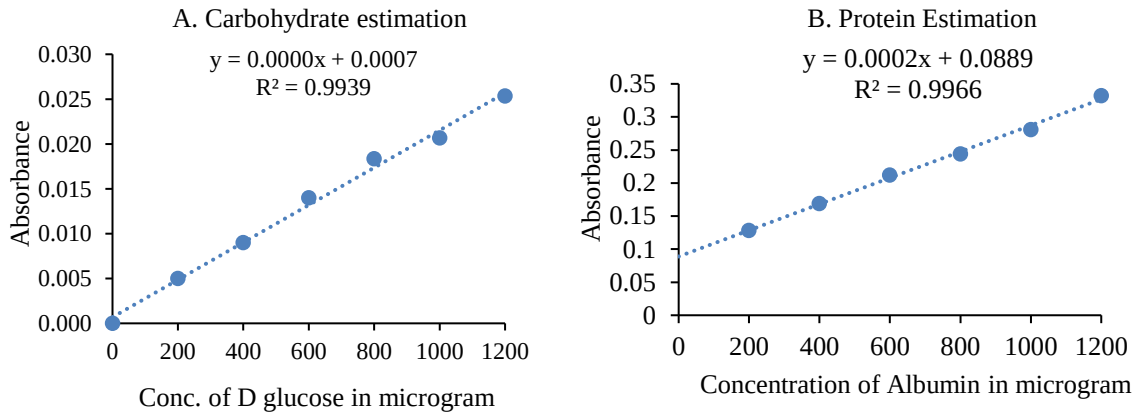

Figure 1. Calibration curve of D-glucose and albumin for primary metabolite A. carbohydrate and B. protein estimation.

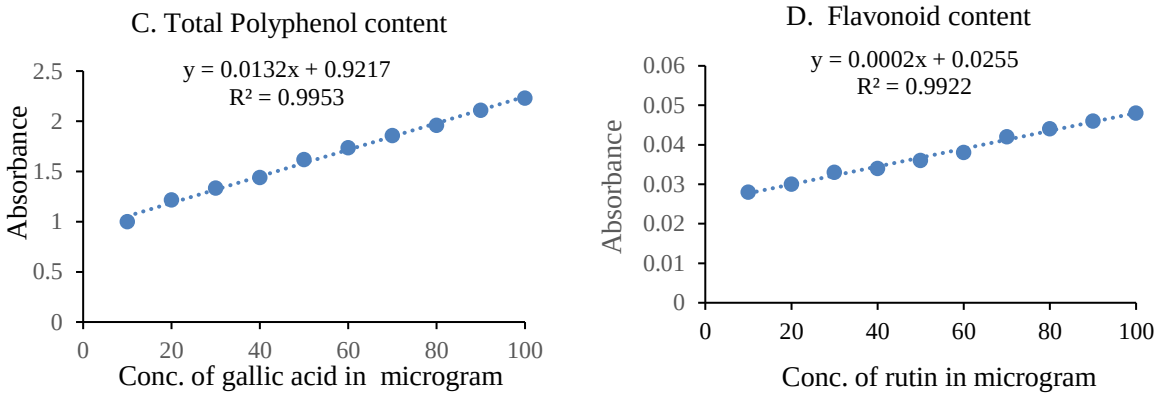

Figure 2. Calibration curve of gallic acid and rutin for C. total polyphenol and D. flavonoid estimation.

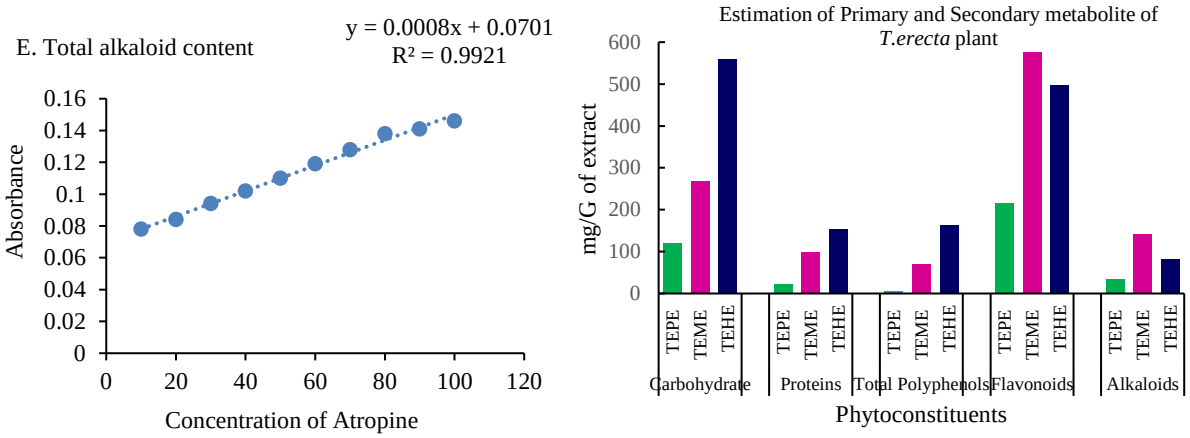

Figure 3. Calibration curve of Atropine for E. Total alkaloid estimation, and Primary and Secondary metabolite estimation of different extracts of *T. erecta*. Results are expressed as the mean ± standard deviation.

3.5. Antioxidant Activity

3.5.1. DPPH free scavenging assay

Figure 4 depicts the effects of *T. erecta* extract on the DPPH free radical in the following order: methanolic extract > hydroalcoholic extract > petroleum ether extract (Table 5). Although the study revealed that the antioxidant potential of the extract was lower than that of ascorbic acid ($p < 0.05$), it also revealed that methanol and hydroalcoholic extracts had a significant amount of antioxidant activity. A substantial amount of polyphenols and flavonoids found in the methanolic extract of plants could be attributed to the observed high antiradical properties of these fractions.

3.5.2. H_2O_2 scavenging activity

The various *T. erecta* plant extracts were evaluated and shown in Figure 4. The ability to scavenge free radicals increases with concentration in both hydroalcoholic and methanolic extracts. Both extracts showed the highest levels of radical scavenging activity, as measured by the IC_{50} value. All data were compared with the IC_{50} value of standard ascorbic acid as shown in Table 5.

3.5.3. Phosphomolybdate assay

Standard ascorbic acid equivalents were used to evaluate antioxidant activity using the phosphomolybdate technique. The methanol extract of *T. erecta* was discovered to have higher antioxidant potential (Table 5). The results demonstrated dose-dependent antioxidant activity

at doses of 10 to 100 g/mL. The methanol extract has a higher IC_{50} value for antioxidant capability than hydroalcoholic and petroleum ether extracts. Strong antioxidants are present in this fraction, which may be due to the presence of phenolic and flavonoid components, as evidenced by the methanol extract's strong antioxidant activity being statistically similar to that of ascorbic acid (Figure 5).

3.5.4. Ferric Reducing antioxidant power assay

In this study, the reducing capacity of the extracts and fractions was performed using Fe^{3+} to Fe^{2+} reduction assay. The flavonoids and phenolics compounds were present in considerable amounts in the extract of plant *T. erecta*. The reducing capacity is shown by all extracts in a concentration-dependent manner (Figure 6).

Table 5. Correlation coefficients ($R^{2)}$ for antioxidant activity relationship of different extracts of *T. erecta*.

Extract/Standard	*DPPH free radical scavenging activity IC_{50} (µg/mL)*		*Phosphomolybdate method*	*H_2O_2 scavenging activity IC_{50} (µg/mL)*	
	IC_{50}	R^2	mg/g	IC_{50}	R^2
Pet. ether	232.98	0.9628	25.12 ± 0.017	131.0	0.9977
Methanolic	44.134	0.9913	111.10 ± 0.047	89.75	0.9954
Hydroalcoholic	81.6885	0.9774	96.158 ± 0.017	99.40	0.9991
Ascorbic acid	33.7115	0.9968	--	62.17	0.9993

Results of triplicate tests, each value represents mean ± SD (n=3).

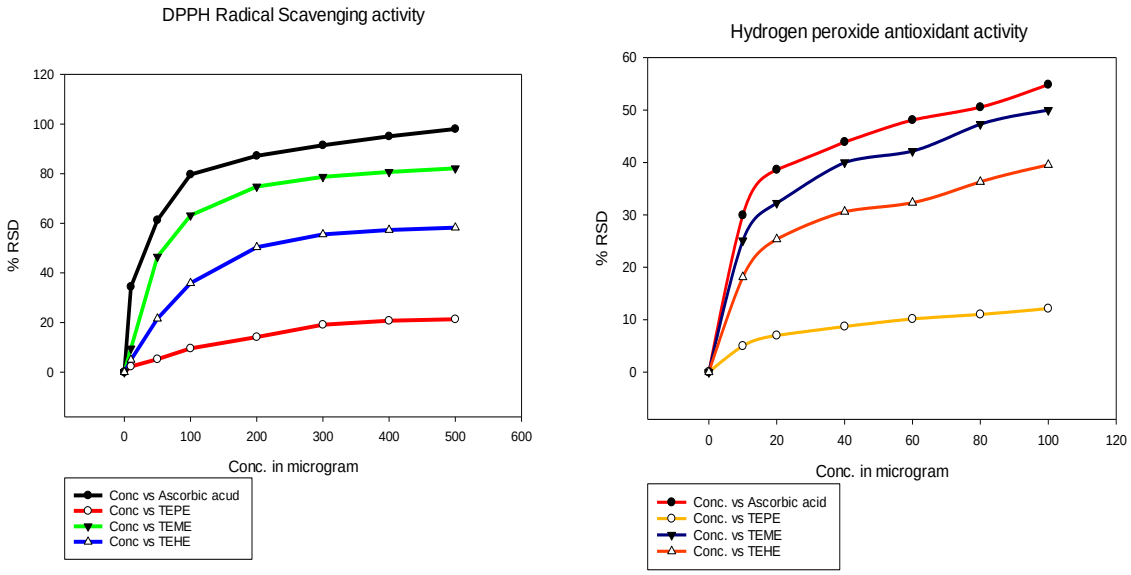

Figure 4. DPPH and H_2O_2 radical scavenging activity of ascorbic acid and leaves extract of *T. erecta* plant. Results were triplicate, each value representing mean ± SD (n=3).

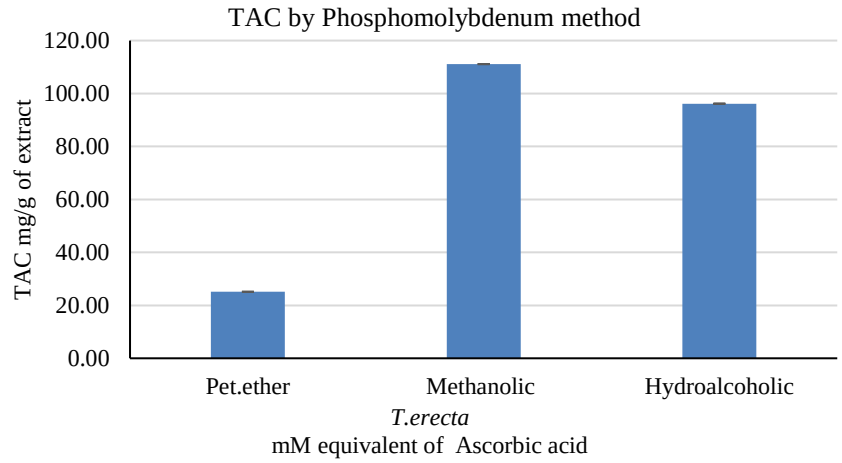

Figure 5. Phosphomolybdate assay of leaves extract of *T. erecta* plant. Results were triplicate, each value represent mean ± SD (n=3).

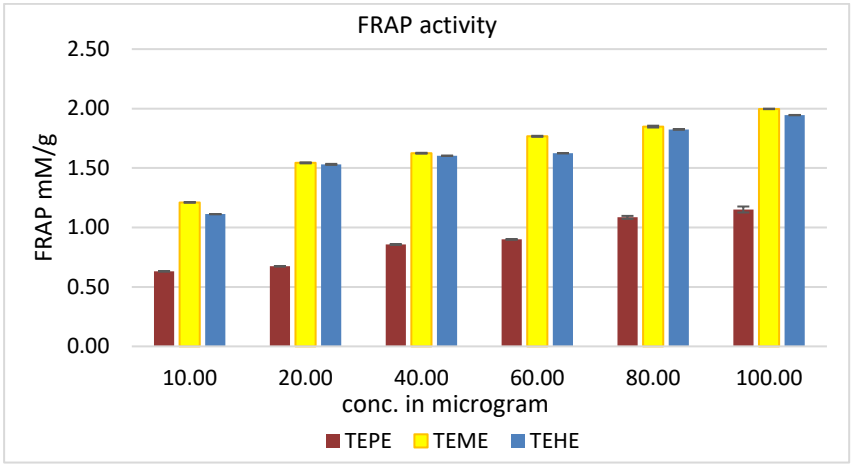

Figure 6. Ferric Reducing antioxidant power assay of leaf extract of *T. erecta* plant. Results were triplicate, each value represent mean ± SD (n=3). Values are mean ± S.D., n = 3, values indicate no significant difference at a level of 5% ($p < 0.05$).

4. CONCLUSION

This study aimed to screen various bioactive compounds for the presence of primary and secondary metabolites and to assess their antioxidant activity. *T. erecta* species is rich in carbohydrates proteins, alkaloids, flavonoids, and polyphenols. The present investigation showed that methanol extract shows a high percentage of flavonoids, polyphenols, and alkaloids whereas the hydroalcoholic extract shows the presence of carbohydrates, proteins, flavonoids, and total polyphenols. The radical scavenging activity showed that the extracts possess potential antioxidant activity when evaluated through various methods. The *in vitro* assays demonstrate that the plant extracts could be a valuable source of an intrinsic antioxidant, which may help in preventing the development of diverse oxidative stress-related diseases. These results support the notion that a diet rich in herbs and plants can aid in the possible reduction of free radicals and could act as a defense against associated disorders. The results of these studies provide scientific development of natural bioactives that have the potential to act as antioxidants for various diseases of the human body.

Further research to isolate individual compounds and their *in vivo* antioxidant activities with different mechanisms is needed. Further studies investigating the isolation and identification of the causative antioxidant components and their mechanisms of action are needed to better understand their ability to combat diseases that significantly influence quality of life.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Nitin Kochar: Methodology, Supervision, Validation, statistical analysis, and manuscript review. **Jayshree C. Vyas:** Formal Analysis, Writing - original draft, experimental studies, manuscript review. **Khushbu Vyas:** Data Analysis, Writing - original draft, experimental studies, manuscript review. **Anil Chandewar:** Definition of intellectual content, data analysis, manuscript review. **Dharmendra Mundhada:** Definition of intellectual content, data analysis, manuscript review.

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