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ORIGINAL RESEARCH ARTICLE

Phytochemical estimation and antimicrobial activity assessment of different solvent extracts of *Tridax procumbens* L.

JOSHI A • PATRA PK • MISHRA PK • KHODIAR PK • SAHU GK*

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ABSTRACT

This study estimates the pharmacological properties of T. procumbens using qualitative and quantitative analysis of secondary metabolites, antioxidants, and antimicrobial activity analysis. Using standard methods, four aqueous extracts were prepared and screened for alkaloids, flavonoids, saponins, tannins, phenol, and ascorbic acid. The antioxidant profiling was undertaken with the support of 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical, ferric reducing antioxidant power (FRAP) and reducing power assay. The antimicrobial assay of crude fractions was conducted against human pathogens E. coli (MTCC 443) and Aspergillus niger (ITCC 4718) using MIC analysis. Catalase (CAT), peroxidase (POX), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity was performed following standard methods for correlating antimicrobial properties with reactive oxygen species (ROS) scavenging activity. Tridax procumbens contains adequate amounts of carbohydrates and protein with some secondary metabolites. The extract also showed the presence of a significant quantity of antioxidants and satisfactory antimicrobial activity.

INTRODUCTION

India has a rich history of traditional medicines, in which many plants are used to treat human and animal diseases. In India more than 70% of the population are known to use herbal medicines for their health related problems (Samal, 2016). Drug discovery from various natural products and new technological inputs have drawn the pharmaceutical industry's attention, promising better investment returns (Lahlou, 2013). Plants are an essential source of potentially beneficial new compounds for developing therapeutic agents. Medicines derived from different parts of plants are comparatively less harmful than synthetic drugs. Plant-based drugs offer affordable and less expensive treatments (Ajali and Okoye, 2009). Some plants' use and essential values have been discovered and published, but many still need to be explored. *Tridax procumbens* is one such plant whose medicinal values need to be explored. This medicinal herb (Asteraceae) is commonly known as 'Ghamra' in Hindi, and is famous as a coat button in English. It is best known as a widespread weed and pest plant in India (Jain and Jain, 2012). The plant leaves possess phytochemicals such as alkaloids, phenols, flavonoids, tannins, proteins, carbohydrates, saponins and ascorbic acid (Mundada and Shivhare, 2010). The plant is used for the treatment of rheumatism, swelling, itching, wound healing and is known to possess anti-inflammatory and anticancer activities (Ingole *et al*., 2022). The herb's juice is dropped in the eyes to cure conjunctivitis and other eye diseases (Kumari *et al*.,

Department of Biochemistry, Pt. JNM Medical College, Raipur, Chhattisgarh, India-492 001
*Corresponding author; E-mail: sahugk234@gmail.com
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2011). Though *T. procumbens* is known to pose various pharmacological activities like antihepatotoxic, immunomodulatory, antidiabetic, antioxidant and anti-inflammatory, the plant has yet not been biochemically fully characterized. Therefore, it is necessary to explore the plant's pharmacological characteristics to discover its medicinal properties (Jain *et al*., 2015). The present study has evaluated the phytochemical constituents, antioxidant properties and antimicrobial activity of *T. procumbens*.

MATERIALS AND METHODS

Collection of the Plants

Fresh leaves of *T. procumbens* were collected from different localities of Raipur, Chhattisgarh. The leaves were washed and shade-dried at room temperature until all the water molecules evaporated and became well-dried. After drying, the leaves were crushed into small pieces and transferred into airtight containers for future use.

Preparation of Plant Extracts

Before the extract preparation, dried leaves were kept for defatting in petroleum ether overnight. Four different types of extracts were prepared using water as a solvent. The extracts were prepared according to the methods described (Tiwari *et al*., 2011). 15 g of leaves were used in each of the methods. In one of the methods, the crude plant extract was prepared by Soxhlet extraction. The crushed leaves were uniformly packed into a thimble and extracted with 150 ml of distilled water. The extraction process continued till the solvent in the siphon tube of an extractor became colourless. In the decoction method, the fresh leaves of *T. procumbens* were boiled in 150 ml of distilled water on a hot plate for two hours. Dried leaves were dipped in 150 ml of distilled water for 48 h in the maceration extraction. The fourth extraction involves the homogenization of the leaves. 15 g of crushed leaves were soaked in 150 ml of distilled water and kept in a shaker for 24 h. Following filtration, all extracts were kept in a water bath at 50-60 ^{o}C till the complete evaporation of the solvents. Reduced extracts were kept in a rotary evaporator until the extracts were converted into dry powder. The dried extract was kept in the refrigerator at 4 ^{o}C for future analysis.

Estimation of Phytochemicals

Qualitative estimation of different phytochemicals presented in the four extracts prepared above was carried out using standard protocols. The number of alkaloids, carbohydrates, phenols (Yadav and Agrawala, 2011), flavonoids, tannins, saponins (Sawant and Godghate, 2013), and proteins (Bradford, 1976) were estimated as described in the following sections:

Alkaloids

The alkaloid content was determined using 2M phosphate buffer (71.6 g Na_2 HPO_4 in 1 L distilled water) was adjusted to pH 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water) and Bromocresol green (BCG) solution. Both the reagents were dissolved in equal amounts. 500 µl of each extract was added into 500 µl of the above reagent (phosphate buffer and BCG solution). 3 ml of chloroform was added to the above mixture. The upper blue layer was discarded, and the absorbance of the yellow-coloured layer obtained at the bottom of the tube was taken at 470 nm. Atropine was used as standard at different concentrations.

Carbohydrates

The total carbohydrate content was estimated by the anthrone method. Five hundred µl of plant extract was mixed with 2 ml of 0.2% anthrone reagent prepared in 95% ice-cold sulphuric acid. The mixture was heated for 8 min in a boiling water bath, cooled rapidly, and the colour developed was read at 630 nm. One mg ml^{-1} dextrose was used as the standard.

Flavonoids

The aluminium chloride colourimetric method estimated the total flavonoid content with some modifications. 500 µl of plant extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.0 ml of distilled water and kept at room temperature for 30 min. The absorbance of the mixture was measured at 420 nm. 0.5 mg ml^{-1} of quercetin was used as standard.

Phenols

Phenol was estimated by the Folin-Ciocalteu reagent (FCR) method with some modifications. 1.5 ml of 10% FCR and 1 ml of 2% Na_2CO_3 were added to 0.5 ml of plant extract and shaken. The resulting mixture was incubated for 15 min at room temperature. The absorbance of the sample was

measured at 765 nm. 0.05 mg ml^{-1} gallic acid was used as the standard.

Saponins

To estimate total saponin, 500 µl of leaf extract was dissolved in 500 µl of 80% methanol and 1.0 ml of 1% vanillin (in ethanol). The content was mixed thoroughly. To this mixture, 2.5 ml of 0.02 N H_2SO_4 was added, mixed well and incubated on a water bath at 60 ^oC for 10 min. The absorbance of the mixture was measured at 544 nm. Diosgenin dissolved in hot ethanol (1 mg ml^{-1}) was the standard.

Tannins

The estimation of total tannin was determined by the Vanillin-Hydrochloride method. An equal volume of 8% HCl (in methanol) and 4% vanillin (in methanol) was mixed. The solution must be mixed just before use. 500 µl of the extract was treated with 2.5 ml vanillin hydrochloride reagent. The absorbance was taken at 500 nm. 0.33 g ml^{-1} tannic acid was the standard (Katoch, 2011).

Protein

Protein content was estimated using Bradford's (1976) method using bovine serum albumin (BSA) as the standard.

Extraction and Assay of Enzymatic Antioxidants

Enzyme protein extraction

To extract the enzymatic antioxidants, freshly collected leaves were cut into small pieces and homogenized with 100 mM potassium phosphate buffer (pH 7.0), 1% polyvinyl pyrrolidone, and 1 mM EDTA. The extraction buffer for ascorbate peroxidase contained 1 mM ascorbate. The process was carried out under ice-cold conditions in a pre-chilled mortar and pastel. The homogenates were filtered with a muslin cloth, and the filtrate was centrifuged at 12000x*g* for 15 min. The supernatant so obtained was used as a crude enzyme source.

Catalase (CAT) assay

The activity of CAT was measured spectroscopically based on the decrease in absorption of H_2O_2 at 240 nm. The reaction mixture consisted of 2.56 ml of 1 M phosphate buffer, pH 6.5, and 10 µl enzyme extract, followed by the rapid addition of 34 µl of 10 mM H_2O_2. The decrease in absorbance at 240 nm due to the consumption of H_2O_2 was recorded for 1 min. The reaction mixture without H_2O_2 was used as the control. The activity of CAT was calculated as below and was expressed as units of mg^{-1} protein (Ahmad *et al*., 2012; Maksimović and Živanović, 2012).

Volume activity (Units ml^{-1})= $\frac{\Delta A.V_q}{0.043.6V_s}$, Enzyme activity (Units mg^{-1} protein) = $\frac{\text{Units / ml}}{\text{mg protein / ml}}$ where, Vq = reaction volume in cuvette in ml, 0.0436 = millimolar extinction coefficient of H_2O_2 at 240 nm (mM^{-1} cm^{-1}) and Vs = Volume of sample in ml.

Peroxidase (POX) assay

In the presence of the hydrogen donor pyrogallol, POX converts H_2O_2 to H_2O and O_2. The oxidation of pyrogallol to a coloured product called purpurogallin was analyzed spectrophotometrically at 430 nm. The reaction mixture consisted of 3.0 ml of 0.05 M pyrogallol and 20 µl of 1% H_2O_2, prepared in 0.1 M phosphate buffer pH 6.5. 10 µl leaf extract was added to the reaction mixture, and the change in absorbance was measured at 430 nm at an interval of 10 s for 3 min. The activity of POX was calculated using 2.6 mM^{-1} cm^{-1} as the molar extinction coefficient for purpurogallin (Rani *et al*., 2004).

Ascorbate peroxidase (APX) assay

H_2O_2-dependent ascorbate oxidation is followed by a decrease in the absorbance at 290 nm. The reaction mixture consists of 2.915 ml 100 mM phosphate buffer (pH 7.0), 15 µl 0.5 mM ascorbate, and 50 µl sample. The reduction in ascorbate concentration was recorded by reading the absorbance continuously for 3 min by adding 20 µl of H_2O_2. One unit of ascorbate peroxidase activity is defined as the amount of enzyme that can oxidize 1µmol of ascorbic acid per minute. The peroxidase activity was calculated using the formula and expressed as units mg^{-1} protein (Maksimović and Živanović, 2012).

Volume activity (units ml^{-1}) = $\frac{\Delta A.2.Vq}{2.8 .Vs}$, Enzyme activity (units mg^{-1}) = $\frac{\text{Units / ml}}{\text{mg protein / ml}}$

Where Vq = reaction volume in ml, 2.8 mM^{-1} cm^{-1} is the extinction coefficient of ascorbate at 290 nm and Vs = volume of sample added in ml.

Superoxide dismutase (SOD) assay

The SOD activity was determined through the combination of riboflavin photo reduction and nitrite formation, as described by Das *et al*., (2000). A reaction mixture of 1.4 ml containing 1.11 ml of 50 mM phosphate buffer, pH 7.4, 0.075 ml of 20 mM L-methionine, 0.04 ml of 1% triton X-100, 0.075 ml of 10 mM hydroxylamine hydrochloride, 0.1 ml of 100 µM EDTA was taken in test tubes. The enzyme extracts equivalent to 10 µg of protein were added to the tubes, followed by a brief pre-incubation at 37 C for 5 min. Then 80 µl of 100 µM riboflavin was added to the tubes. The tubes were exposed for 10 minutes to two 20 W fluorescent lamps fitted parallel to each other in an aluminium-coated wooden box. The control tubes contained an equal amount of buffer instead of the sample. After exposure to a fluorescent lamp, 1 ml of Greiss reagent prepared freshly by mixing an equal amount of 0.1% N-(1-Napthyl) ethylenediamine and 1% sulphanilamide in 5% ortho-phosphoric acid was added to each tube. The absorbance of the colour so formed was measured at 543 nm. Corrections were made for the background absorbance at 543 nm without sample and riboflavin. The activity of SOD was calculated using the formula mentioned below and was expressed as unit SOD mg^{-1} protein. One unit of enzyme activity is the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

Percent inhibition of nitrite formation = $(1-A_s/A_c)*100$ where, A_s = absorbance of sample, A_c = absorbance of control.

Estimation of Non-enzymatic Antioxidants

For non-enzymatic antioxidant profiling, four different extracts of *T. procumbens* were used, i.e. soxhlet extract, decoction extract, maceration extract and homogenization extract. The following assays were performed:

Scavenging activity of DPPH radical

The DPPH free radical scavenging assay was carried out to determine antioxidant activity. The reaction mixture consisted of 1.5 ml of diluted extract and 0.5 ml of 0.1M DPPH in ethanol. The mixture was incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm against blank. The following equation was used to determine each extract's radical scavenging activity percentage. 100, 200, 300, 400 and 500 µl from each extract were taken to determine the scavenging activity by DPPH. Ascorbic acid was used as the antioxidant.

$$\text{Radical scavenging activity (\%)} = \frac{[\text{OD control} - \text{OD sample}] \times 100}{\text{OD control}}$$

The IC_{50} value (µg ml^{-1}) is the effective concentration at which DPPH radicals were scavenged by 50%, and the value was obtained by interpretation from linear regression analysis (Pauline *et al*., 2013).

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the method of Beara *et al*., 2009) with some modifications. 1.0 ml of extract solution, 0.5 ml of 7.5 mM ortho-phenanthroline, 2.0 ml of 0.2 M phosphate buffer pH 6.6, 0.5 ml of ferrous sulphate and 0.5 ml of 0.1% H_2O_2 were mixed and diluted with 1.0 ml of distilled water. After incubation at room temperature for 30 min, absorbance was measured at 510 nm. 100, 200, 300, 400 and 500 µl from each extract were taken to determine the hydroxyl radical scavenging activity. The scavenging percentage (P%) was calculated as P% = [(A-A1)/(A2-A1)] x 100. A is the absorbance of the solution, including H_2O_2 and the extract, A1 is the system without extract solution, and A2 is the system without H_2O_2 and extract solution (Li *et al*., 2011).

Reducing power assay

One ml of the extract was mixed with 1.5 ml of phosphate buffer (0.2 M, pH 6.6) and 1.5 ml of potassium ferrocyanide (1%). The mixture was incubated at 50 ^oC for 20 min. 1.5 ml of aliquots of trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution was transferred to another tube and diluted with distilled water and 0.5 ml of $FeCl_3$ (0.1%). 100, 200, 300, 400 and 500 µl of different extracts were taken to determine the reducing ability. The increase in absorbance was measured at 700 nm against the blank (Pauline *et al*., 2013).

Total antioxidant activity by FRAP assay

The ferric-reducing antioxidant power (FRAP) method was used to estimate the total antioxidant

activity, which measures the reduction of ferric ions to the ferrous form in the presence of antioxidant compounds. The fresh FRAP reagent consists of 500 µl of 300 mM acetate buffer pH 3.6, 50 of 10 mM 2, 4, 6-Tri(2-pyridyl)-s-triazine (TPTZ) and 50 ml of 50 mM $FeCl_{3.}$ $6H_2O$. For the assay, 75 µl of each extract was mixed with 2.0 ml of FRAP reagent and the optical density was read after 2 min at 593 nm against blank (Pauline *et al*., 2013).

FRAP value = (OD of standard/OD of sample) x FRAP value of standard

All the quantitative tests were performed with the help of a UV-visible spectrophotometer (ELICO, SL-152).

Antimicrobial Assay

Two pathogenic strains of microorganism were used in this study, one human pathogenic bacteria *E. coli* (MTCC 443), procured from MTCC, Chandigarh and other fungi, *Aspergillus niger* (ITCC 4718), was procured from ITCC, Division of Plant Pathology, IARI, New Delhi. Soy agar media was used for *E. coli* culture and potato dextrose agar (PDA) was prepared for *A. niger*.

Preparation of Inoculum

The absorbance of both the *E. coli* and *A. niger* cultures was measured at 650 and 595 nm, respectively. The absorbance of both cultures was maintained at 0.3-0.2 for proper growth and further study on the inoculum. After that, 100 µl of inoculum was spread on solidified plates.

Antimicrobial Assessment

The antimicrobial activity of the extracts was evaluated by the disc diffusion method (Chandrasekaran and Venkatesalu 2004). Four 5 mm sterilized paper discs of Whatman No.1 were impregnated with the desired concentration of each extract dissolved in dimethyl sulfoxide (DMSO) and placed on the surface of the agar plates. The plates were kept for two minutes at room temperature for the diffusion of the extract, and thereafter the plates were incubated at 37 ^{o}C for 24 h for *E.coli* and at 30 ^{o}C for 48 h for *A. niger* colony. The disc with DMSO served as a negative control. Streptomycin was taken as standard for *E. coli* and Ketoconazole for *A. niger*. Each assay in this experiment was replicated three times.

Statistical Analyses

All tests were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). IC_{50} of DPPH was calculated by linear regression analysis.

RESULTS

Phytochemical Screening

The phytochemical screening results revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, carbohydrates and proteins in all four leaf extracts of *T. procumbens*. The quantity of phytochemicals present in extracts is shown in Table 1.

Table 1: Quantity of phytochemicals in different extracts of *T. procumbens*. The letters D, S, H and M denote decoction, soxhlet, homogenization and maceration extracts, respectively. '-' indicated the absence of the particular phytochemicals. The values presented are the mean of three independent experiments and ± SD

Phytochemicals	Quantity (µg)			
	D	S	H	M
Alkaloid	-	115±3.627	90±4.023	110±5.29
Carbohydrate	195±7.93	335±9.21	215±6.081	105±4.321
Flavanoid	105±4.023	80±4.821	130±4.026	75±3.294
Phenol	315±7.265	115±3.239	275±5.124	145±4.248
Protein	34±2.64	33±1.987	21±1.254	18±1.967
Saponin	135±3.215	105±4.287	160±3.316	125±2.985
Tanin	110±2.365	220±4.897	90±3.256	175±4.234

Enzymatic Antioxidants

The activities of enzymatic antioxidants found in *T. procumbens* are shown in Table 2. Fig 1 represents the change in absorbance at various time intervals for the assay of CAT, POX and APX.

Table 2: Activity of enzymatic antioxidants of *T. procumben*

Enzymatic antioxidants	Activity (units mg^{-1} protein)*
CAT	169.74± 3.214
POX	36.26 ± 2.513
APX	30.98 ±1.943
SOD	87.38 ±2.684

**Mean of three different experiments with ± SD*

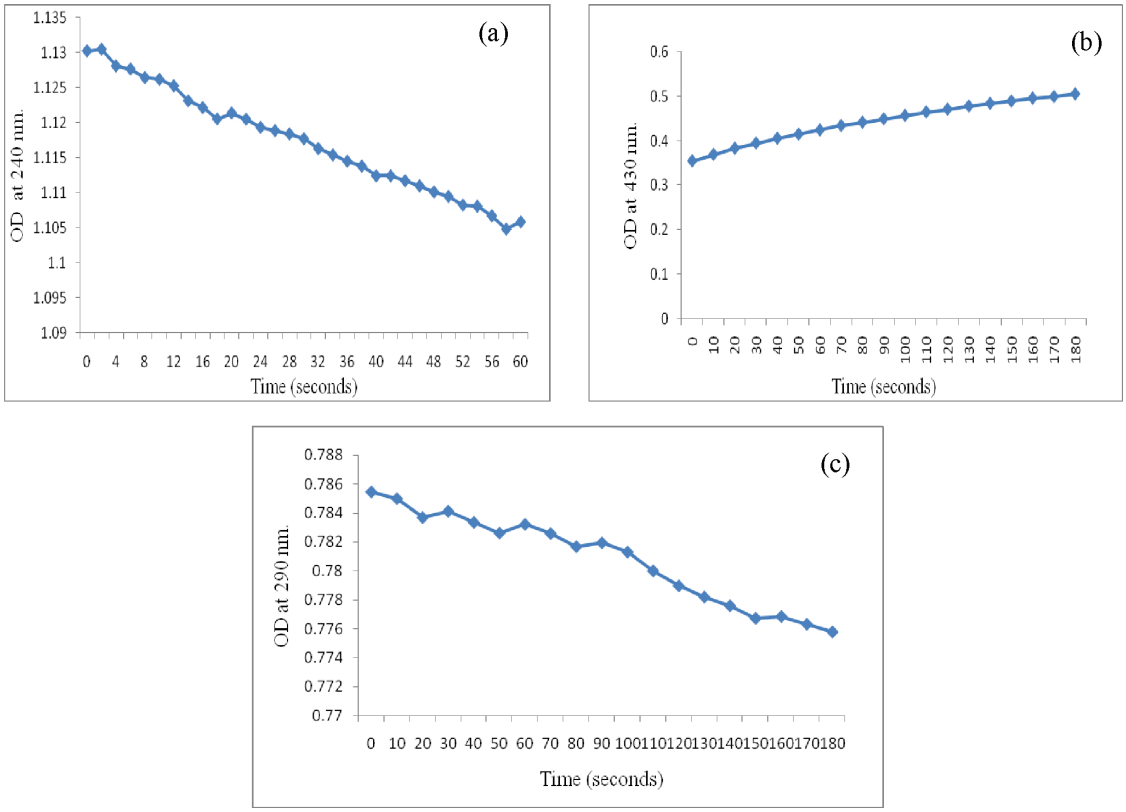

Figure 1: Change in absorbance at various time intervals for assay of CAT (a), POX (b) and APX (c)

Non-enzymatic Antioxidants

DPPH

The percent in inhibition activity of *T. procumbens* has been provided in Table-3. the overall IC_{50} of *T. procumbens* extract was 92.38 µg ml^{-1}. Ascorbic acid was taken as standard, where IC_{50} is 57.61 µg ml^{-1}.

Table 3: DPPH per cent inhibition activity of *T. procumbens*. D, S, H and M are decoction, soxhlet, homogenization and maceration extract, respectively. The values presented are the mean of three independent experiments and ± SD

Extract volume (µl)	Percent inhibition activity (%)			
	D	S	H	M
100	81.71±3.516	82.56±4.321	81.59±4.065	83.12±3.125
200	70.97±2.961	73.14±2.321	72.45±3.021	74.26±4.162
300	59.76±1.852	66.50±3.210	63.80±2.564	64.15±3.276
400	52.72±1.021	51.27±1.254	61.89±3.282	60.67±4.531
500	34.57±1.245	37.72±1.025	50.76±1.864	49.66±3.216

Hydroxyl radical scavenging activity

Percent inhibition scavenging activity of the hydroxyl radical increases with the increasing concentration. The results showed that the extracts obtained have shown significant scavenging activities for the hydroxyl radicals (Fig 2).

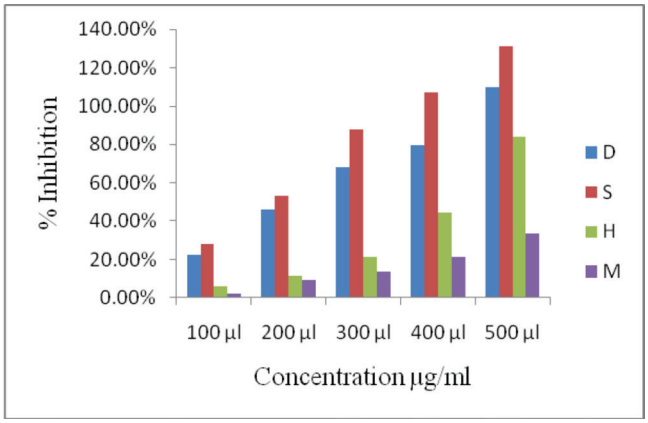

Figure 2: Hydroxyl radical scavenging activity of *T. procumbens*. D, S, H and M stand for decoction, soxhlet, homogenization, and maceration extract, respectively

Reducing Power Assay

The reducing power ability four different extracts has been shown in Fig 3. In the reducing power assay, the conversion of Fe^{3+} to Fe^{2+} in the presence of the extract was analyzed. In this study, the reducing ability of extracts showed a linear relationship with increased concentrations of the leaf extracts.

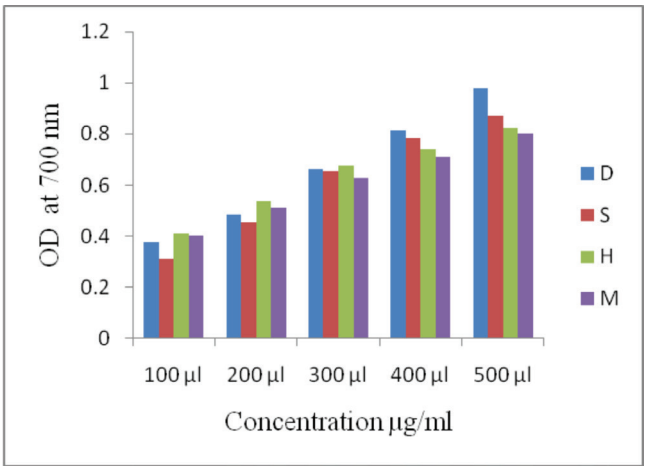

Figure 3: Reducing power assay of *T. procumbens*. D, S, H and M stand for decoction, soxhlet, homogenization, and maceration extract, respectively

Total Antioxidant Activity by FRAP

The total antioxidant activity by FRAP was found to be a maximum of 8.93 µg ml^{-1} in soxlet extraction and a minimum value of 2.9 µg ml^{-1} in decoction extract (Fig 4).

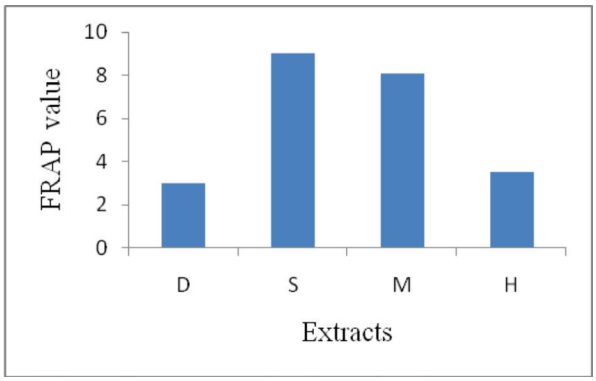

Figure 4: FRAP antioxidant activity (µg ml^{-1}) of *T. procumbens*. D, S, H and M stand for decoction, soxhlet, homogenization, and maceration extract, respectively

Antimicrobial Activity

In vitro, antimicrobial assessments for *E. coli* and *A. niger* were performed by measuring the zone of inhibition, and the results have been presented in Tables 4 and 5 and in Fig 5. The zone of inhibition test results showed that soxhlet and maceration extract show better antimicrobial activity than decoction and homogenization extract.

Table 4: Antibacterial activity of four different extracts of *T. procumbens* against *E. coli*. The data presented are the mean of three independent experiments with ±SD. The values presented are the mean of three independent plates and ± SD. No **activity was observed around the control (DMSO-impregnated) disc. 30 µg ml^{-1} streptomycin was taken as a positive control, which showed a 9.3±0.28 mm zone of inhibition. D, S, H and M stand for decoction, soxhlet, homogenization, and maceration extract, respectively**

Concentration of extracts (mg ml^{-1})	Zone of inhibition (mm)			
	D	S	H	M
5	7.16±0.57	8.16±0.28	7±0.5	7.16±0.28
2.5	6.66±0.57	7.66±0.28	6.8±0.28	6.9±0.17
1.25	6.66±0.28	6.66±0.57	6.63±0.51	6.93±0.81
0.625	6.8±0.28	6.5±0.86	6.6±0.28	7.0±0.5
0.312	6.4±0.45	6.3±1.1	6.3±0.76	6.33±0.28
0.156	6.3±0.26	6.3±0.76	6.1±0.76	6.2±0.42
0.078	6.1±0.28	6.3±0.28	6.05±0.07	5.83±0.28
0.039	5.9±0.1	6.1±0.28	5.8±0.28	5.73±0.40
0.019	5.6±0.28	5.9±0.11	5.75±0.35	5.6±0.28

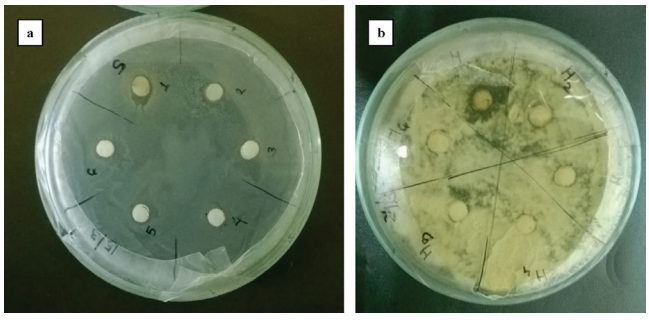

Figure 5: Zone of inhibition test of *T. procumbens* extract for *E. coli* (a) and *A. niger* (b) culture

Table 5. Antifungal activity of four different extracts of *T. procumbens* against *A. niger*. The data presented are the mean of three independent experiments with ± SD. No activity was observed around the control (DMSO-impregnated) disc. Symbol '-' indicated the absence of inhibition. 30 µg ml^{-1} ketoconazole was taken as a positive control, which showed a 12.7 ± 0.28 mm zone of inhibition. D, S, H and M represent decoction, soxhlet, homogenization, and maceration extract, respectively

Concentration of extract (mg ml^{-1})	Zone of inhibition (mm)			
	D	S	H	M
10	6.75±0.35	7.75±0.35	7.80±0.28	7.80±0.21
5	-	7.25±0.35	6.95±0.07	7.20±0.28
2.5	-	6.25±0.35	6.25±0.35	6.75±0.35
1.25	-	-	-	6.65±0.22
0.625	-	-	-	-
0.312	-	-	-	-
0.156	-	-	-	-
0.078	-	-	-	-
0.039	-	-	-	-

DISCUSSION

In the present study, the phytochemical screening of *T. procumbens* revealed the presence of flavonoid, phenol, saponin, tannin, protein and carbohydrate in all four extracts. Alkaloids were found in soxlet, homogenization, and maceration extracts. The alkaloids were not found in the decoction extract. Das *et al*., (2009) determined the presence of four secondary metabolites, tannins, flavonoids, saponins and alkaloids, from the aqueous and methanolic leaf extract. Uzuegbu *et al*., (2016) revealed the presence of twelve bioactive compounds in ethanolic and aqueous extract, which included phenols, tannins, flavonoids, saponins, steroids, terpenoids, anthraquinones, cardiac glycosides, alkaloids, phlorotannins, phytosterol and triterpenoids. Five phytochemicals such as phenol, saponin, tannin, steroid, and carbohydrate from aqueous extract of *T. procumbens* have also been observed (Kuldeep and Pathak, 2013).

The crude extract of *T. procumbens* was investigated for enzymatic and non-enzymatic antioxidants. Enzymatic antioxidant assay of *T. procumbens* showed the presence of a significant amount of SOD (87.38 units mg^{-1} protein), CAT (169.74 units mg^{-1} protein), POX (36.26 units mg^{-1} protein) and APX (30.98 units mg^{-1} protein). The hydro-alcoholic extracts showed better superoxide radical scavenging activity than aqueous and petroleum ether extracts (Nair *et al*., 2016). Similarly, non-enzymatic antioxidants were found to be present in the crude extract. The overall aqueous extract showed notable DPPH activity, the IC_{50} value of which was measured as 92.38 µg ml^{-1}. The IC_{50} value of *T. procumbens* collected from the wetland has been estimated as 98.38 µg ml^{-1} (Gnanasekaran *et al*., 2017). In the present study, the hydroxyl radical scavenging activity of *T. procumbens* was observed to be 131.35%. Kumar *et al*., (2016) found that the aqueous extract showed maximum hydroxyl scavenging activity of 27.32±0.98%. In this study, the reducing ability of the extracts showed a linear relationship with the concentration. It has been reported that alcoholic extracts possess better activity than aqueous extracts (Patel *et al*., 2014). An increase in absorbance shows the potent reducing power ability. The total antioxidant activity by FRAP showed a maximum of 8.93 µ ml^{-1} in the present study.

A few researchers have reported antimicrobial activities of different extracts of *T. procumbens* (Naquash and Nazeer, 2011; Andriana *et al*., 2019). In the present study, the antimicrobial activity of *T. procumbens* leaf extracts was investigated against the human pathogenic strains of *E. coli* and *A. niger* in a dose-dependent manner. Very little antimicrobial activity of aqueous extracts of *T. procumbens against E. coli* has been shown (Das *et al*., 2009). In another investigation, Pai *et al*., (2011) found the absence of antimicrobial activity in the aqueous extract of *T. procumbens.* Taddei and Rosas-Romero (2000) reported no antimicrobial activity of the aqueous extract against *A. niger and E.coli.* The crude extract of *T. procumbens* showed good antifungal activity against seed-borne *A. niger* (Rao, 2015). Thus, based on the present study and other supporting investigations as discussed above, *T. procumbens* can be used as an antimicrobial agent. It can also be used as a generous source of secondary metabolites and antioxidants.

CONCLUSION

In conclusion, the analysis showed that *T. procumbens* possess secondary metabolites such as

alkaloid, flavonoid, tannin, phenol and saponin. The plant also contains an adequate amount of carbohydrates and proteins. The plant contains a significant amount of antioxidants; the study reveals the presence of enzymatic antioxidants CAT, POX, APX and SOD. The four extracts showed satisfactory antimicrobial activity against *E. coli* and *A. niger*. The study mentioned above is helpful in the mass production of bioactive and antimicrobial compounds from *T. procumbens*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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