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Evaluation of Natural Antioxidant Properties of *Tridax Procumbens L.* Through Phytochemical and FRP Analysis.

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ABSTRACT

The present study was undertaken to evaluate the phytochemical composition and antioxidant potential of *Tridax procumbens L*. (Asteraceae), a traditionally used medicinal plant. Ethanolic extract of the plant leaves was prepared and subjected to qualitative phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity assessment using the Ferric Reducing Power (FRP) assay. Preliminary phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolics, tannins, terpenoids, and carbohydrates. Quantitative estimation revealed a high concentration of phenolics and flavonoids with TPC of 84.03 ± 2.21 mg GAE/g extract and TFC of 76.68 ± 2.27 mg QE/g extract, respectively. The FRP assay demonstrated a dose-dependent increase in reducing power, with maximum absorbance (0.953 ± 0.038 at 1000 µg/mL) comparable to that of the standard antioxidant ascorbic acid (0.910 ± 0.035). These findings indicate the antioxidant potential of *T. procumbens*, attributed to its rich content of polyphenols and flavonoids. The results validate its traditional medicinal use and suggest its applicability in the development of natural antioxidant formulations and nutraceutical products.

Keywords: *Tridax procumbens*, phytochemicals, total phenolics, total flavonoids, medicinal plants

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INTRODUCTION

Antioxidants are compounds that prevent the oxidation of other substances and are commonly utilized in dietary supplements [1]. Some of them inhibit the generation of ROS, and mend oxidative damage by activating a signaling pathway [2]. These compounds can function in both chemical and biochemical systems through various mechanisms, including neutralizing reactive oxygen and nitrogen species, binding transition metals, donating hydrogen atoms, blocking enzymes associated with oxidative stress, and enhancing or safeguarding the body's natural defense systems [3]. The body's natural antioxidant system typically maintains a balance between oxidation and anti-oxidation, but exposure to stressors can lead to excessive Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), disrupting this balance and contributing to chronic and degenerative diseases [4]. The recent advancement of functional foods and pharmaceutical products derived from medicinal plants has enhanced various aspects of life, including easing physical ailments, reducing reliance on synthetic antibiotics, and increasing life expectancy [5]. These plants have long served as safe, effective, and sustainable sources of natural antioxidants particularly due to vast number of bioactive compounds present which contributes a wide range of biological activities like antioxidant, antimicrobial, anti-inflammatory, antitumor, anti aging and other degenerative diseases [6, 7]. Plant bioactive compound not only combat free radical-induced oxidative stress but also mitigate the side effects associated with synthetic antioxidants. Given this background and the rich source of unique active components found in plants, the present study focused on the medicinal plant *Tridax procumbens* L. (Family: Asteraceae), referred to as "coat buttons" or "daisy weed," is a small, creeper that grows in tropical and subtropical areas which possess significant ethnopharmacological value and are utilized in the treatment of various ailments. *Tridax procumbens* is widely used by both formal (Ayurvedic, Unani) and informal (folk, tribal) traditional medicine practitioners to treat various ailments. It exhibits hemostatic, anti-inflammatory, anti-arthritic, anti-diabetic, antihypertensive, and antimicrobial activities [8]. Research suggests that these compounds may activate Nrf 2, leading to the over expression of antioxidant enzymes like CAT, GPx, GST, and GR, and thus enhancing the adaptive response to oxidative stress [9]. These therapeutic effects are attributed to its rich phytochemical profile, particularly the presence of flavonoids, phenolic acids, carotenoids, and other secondary metabolites known for their redox-modulating properties. Although it has been used in many traditional ways and is known to be important for pharmacology, scientific validation through systematic phytochemical screening and antioxidant assessment is still not well understood. There is a need for a more in-depth study that links its phytoconstituent profile to its antioxidant activity, which would back up its possible use as a natural medicine. This study explores the leaves of *Tridax Procumbens* through phytochemical analysis, quantification of phenolic and flavonoid content, and evaluation of antioxidant activity via the FRP assay, trying to validate its traditional medicinal applications and potential in natural antioxidant-based therapies.

MATERIALS AND METHODS

Collection of Plant Sample

The plant material (*Tridax procumbens* Linn.) was collected from local medicinal flora near district Katol, Nagpur, Maharashtra, India, from the month of August. The plant material were washed with water and dried in a shade for several days, dried material were grind in grinding machine and powder were kept in a labeled airtight container at room temperature for further use.

Extract Preparation

Plant material was extracted with ethanol with countinuous stirring for 48-72 hrs and then filtered through Whatman filter paper No. 42 to remove all non-extractable matter, including cellular material and other constituents that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. Final dried samples were stored in labeled sterile bottles at −20°C. The extraction yield was expressed in percentage. [10, 11].

Phtochemical analysis

This plant extracts were subjected to phytochemical testing to determine the presence of different classes of phychemical constituents such as alkaloids, flavonoids, terpenoids, saponine, tannins, using the methods as described by [12]. These secondary bioactive metabolites are responsible for the various biological activities.

Estimation of Total Phenolic Content

The total phenolic content (TPC) of *Tridax procumbens* extract was measured using the Folin-Ciocalteu method, a widely used technique for assessing the presence of phenolic compounds in plant extracts. The gallic acid is used as a standard and total phenolic content was estimated as method described by [13] results are expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract), providing an estimate of the extract's antioxidant potential. A standard curve was prepared using gallic acid solutions at concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. 0.5 mL of the *Tridax procumbens* extract was taken and diluted appropriately. 2.5 mL of Folin-Ciocalteu reagent was added to the sample. After addition of 7.5% sodium carbonate solution, the reaction mixture was incubated for 30 minutes at room temperature in the dark. The absorbance was recorded at 765 nm using a UV-Vis spectrophotometer. The total phenolic content was determined expressed as mg GAE/g extract.

Estimation of Total flavonoid Content

The flavonoid content of medicinal plants may be responsible for their antioxidant activity. Flavonoids function as quenchers of singlet oxygen and scavengers of different oxidizing species, such as superoxide anion, hydroxyl radical, or per-oxy-radicals. Using quercetin as a standard, the total flavonoid content of the *Tridax procumbens* plant extract was determined using the aluminum chloride colorimetric method. The method of Aryal S, 2019 was used to estimate the content of total flavonoids [14]. In brief, 2.8 ml of distilled water, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 1.5 ml of methanol were mixed with 0.5 ml of extract in methanol and kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. Quercetin solutions at concentrations ranging from 10 to 100 µg/ml in methanol were prepared in order to construct the calibration curve.

***Invitro* Antioxidant activity**

The antioxidant potential of the plant extract was evaluated with the help Ferric reducing power assay.

Ferric Reducing Power (FRP) Assay

Substances with reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex, which has an absorption maximum at 700 nm. A compound reducing capacity may be a significant indicator of its potential antioxidant activity. As explained by [15], the reducing power assay was performed on a sample of *Tridax procumbens* plant extract. The ethanolic extract of plant and its varying concentrations (200µg–1000µg/ml) were combined with Potassium ferricyanide (2.5 ml, 1%) and phosphate buffer (2.5 ml, 0.2M, pH 6.6). For 20 minutes, the mixture was incubated at 50°C. After adding 2.5 ml of 10% trichloroacetic acid to the mixture, it was centrifuged for 10 minutes at 3000 rpm. Ferric chloride (0.5 ml, 0.1%) and distilled water (2.5 ml) were added to the solution's top layer (2.5 ml). The absorbance was then measured at 700 nm and compared to ascorbic acid as a standard. Higher the absorbance of reaction mixture suggested a higher reducing power.

Statistical Analysis

The mean±standard deviation (SD) of three replicates was used to express the experimental results. Differences among means were considered as to be significant at $P<0.05$.

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RESULTS AND DISCUSSION

Extraction of crude drug

Extraction yield is determined by the plant species, the section of the plant used and the polarity of the extracting solvent, and the extraction process [16], which dictates how many bioactive chemicals are extracted from a given amount of plant material. The *Tridax procumbens* plant was extracted with ethanol and percentage yield of *T. procumbens* ethanolic extract was found to be 11.32% (Table 1). The crude extract was further used to analyze phytochemical properties of plant.

Table 1: Extraction yield of whole *Tridax Procumbens* plant.

Sr. No.	Solvent Used	Appearance	Percentage Yield (%)
1.	*Ethanol*	Yellowish Green And Sticky	11.32

Phytochemical Screening

Phytochemical studies of *Tridax Procumbens* shows the presence of Alkaloids, Phenol, flavonoids, Terpenoids, Tannins and carbohydrates.

Table 2: Preliminary Phytochemical screening of ethanolic extract of *Tridax procumbens*.

Phytochemical test										
Terpenoid	Steroid and terpenoids	Phenols	flavanoids	Alkaloids				Tannin	Saponin	Carbohydrate
salkowski test	Lieberman Burchard test			Mayers reagent	Dragendroff's reagent	Hager' reagent	Wagner's reagent			
+	+	++	++	+	+	+	+	+	+	+

Total Phenolic (TPC) and Flavonoid (TFC) content of plant extract

Tridax procumbens ethanolic extract showed the presence of phytoconstituents having antioxidant properties. The Total Phenolic Content (TPC) was 84.03 ± 2.21mg GAE/g extract and the Total Flavonoid Content (TFC) was 76.68 ± 2.27 mg QE/g extract (Table 3). These results demonstrate a high content of polyphenolic and flavonoid substances in the extract, and these are known to exert antioxidant and free radical scavenging activity. The high content of phenolics and flavonoids is liable for the antioxidant activity of *T. procumbens*; because both phenolics and flavonoids can scavenge free radicals by hydrogen donating, metal ion chelating and by the inhibition of oxidative enzymes [17]. The action of the phenolic compounds, in particular of those derived from gallic acid, blocking the oxidative reaction chain caused by their end has been reported being nuclei of oxygen reduction [18]. According to [19], flavonoids like quercetin can also stabilize lipid membranes and scavenge superoxide and hydroxyl radicals, which helps protect cells from oxidative stress. According to previous studies on *T. procumbens* and other medicinal plants, the values shown here are comparable. The methanolic extract of *T. procumbens*, for example, had a TPC of 81.50 mg GAE/g and a TFC of 74.20 mg QE/g, according to [20], supporting the consistency of the bioactive content across various extraction procedures. Overall, the results support the traditional use of *Tridax procumbens* in herbal medicine and its potential for use in nutraceutical formulations by indicating that its ethanolic extract is a viable natural source of antioxidants.

Table 3: Total phenolics and flavonoids content in Ethanolic extract of plant *Tridax Procumbens.*

TPC(mg GAE/g extract)	TFC(mg QE/g extract)
84.03±2.21	76.68±2.27

TPC=total phenolic content; TFC=total flavonoid content; GAE= Gallic acid equivalent; QE =Quercetin equivalent

Antioxidant Activity

Ferric reducing power (FRP) assay

The Ferric Reducing Power (FRP) assay is a widely used method for determine the electron-donating capacity of bioactive compounds, indicating their potential as antioxidants. Antioxidants donate electrons that convert Fe^{3+} (ferric) to Fe^{2+} (ferrous), and the amount of color change (as measured at 700 nm) corresponds with antioxidant capability. This method enables the screening of natural extracts for therapeutic efficacy, particularly in treating conditions related to oxidative stress [21]. The FRP assay was used in this study to evaluate the sample's antioxidant capacity in a concentration range of 200–1000 µg/mL. A concentration-dependent increase in reducing power was observed in the results, as absorbance increased from 0.45 at 200 µg/mL to 0.95 at 1000 µg/mL. This results suggests that the sample has a high ability to donate electrons, which reduces Fe^{2+} ions to Fe^{2+} ions, which indicate the potent antioxidant activity. The test sample had the similar reducing power to the reference ascorbic acid at higher concentrations. At 1000 µg/mL, the absorbance of the sample (0.953 ± 0.039) was slightly higher than that of ascorbic acid (0.910 ± 0.036), showing significant antioxidant activity, which could be due to polyphenol or flavonoid contents in the extract. The low standard deviation values for all concentrations imply good reproducibility and consistent antioxidant activity. These results are in line with the recent reported data where the plant extracts acted as strong reducing agents [22]. Therefore, the FRP assay endorses the antioxidant capacity of the extract, indicating its potential in production of natural antioxidant drugs or functional foods.

Table 4: Reducing ability of plant extract of *Tridax Procumbens* with Ascorbic acid as positive control.

Concentration (ug/ml)	200	400	600	800	1000
Plant Extract	0.450± 0.0245	0.530± 0.0163	0.603± 0.0125	0.680± 0.008	0.953± 0.038
Ascorbic acid	0.463± 0.0125	0.557± 0.0249	0.620± 0.0245	0.707± 0.024	0.910± 0.035

Each value represents mean±SD. (n=3) with significant difference at $P<0.05$.

CONCLUSION

The current study evaluates the phytochemical content and antioxidant activity of *T. procumbens* leaf extract. The early phytochemical screening revealed the presence of various bioactive chemicals, including flavonoids, phenolics, alkaloids, tannins, and terpenoids, all of which are known to have important biological functions. The ethanolic extract has a high total phenolic content (84.03 ± 2.21 mg GAE/g) and flavonoid content (76.68 ± 2.27 mg QE/g), indicating strong antioxidant effects. The Ferric Reducing Power (FRP) experiment showed concentration-dependent antioxidant activity, with reducing power comparable to ascorbic acid at higher concentrations. These findings indicate that *T. procumbens* has significant electron-donating ability, which supports its ethnomedicinal applications and potential as a natural source of antioxidants. Overall, the study supports *T. procumbens'* traditional therapeutic use as well as its potential for future development into natural antioxidant formulations or nutraceuticals. Further study, including in vivo evaluations and compound identification, is needed to determine its therapeutic significance and formulation potential.

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