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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF WHOLE PLANT OF *TRIDAX PROCUMBENS* **LINN.**

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ABSTRACT

Antioxidants serve a critical role in preventing oxidative stress-related damage. Plants with high phenolic content have been shown to have antioxidant effects. The goal of this study was to look into the phytochemistry and antioxidant properties of ethanolic extracts from *Tridex procumbens* (whole plant) in order to establish a statistical link between isolated component concentration and antioxidant capability. TLC, HPTLC, Column chromatography, and an *in vitro* standard technique employing a spectrophotometer were used to assess the phytochemistry and antioxidant properties of ethanolic extractives. DPPH (l, l-diphenyl-2-piciylhydrazine) radical scavenging assay, Nitric oxide (NO) Radical Inhibition Assay, Reducing Power and hydroxyl radical scavenging assay techniques were used to assess antioxidant activity. TLC plate with various numbers of 5 spots were implemented. The HPTLC report shows the existence of ten spots with distinct Rf values, indicating that they occupy separate areas with varying Rf values. The extracted chemicals from the ethanolic extract of *Tridex procumbens* were column chromatographed using hexane (80ml) and benzene (20 ml) and a stationary phase silica gel. The maximum antioxidant activity was found in extracts. The *Tridax procumbens* extract was the most efficient in terms of DPPH and hydroxyl radical scavenging activities, with IC_{50} values of 0.15, 0.12, 0.008 and 42.39 g/ml, respectively. The findings show a strong link between separated chemical content and extract antioxidant capability, suggesting that *Tridex procumbens* might be a promising option for medicinal plant-based products as a free radical inhibitor or scavenger. However, further research is needed to ensure that it can be used effectively in both modern and traditional medicine.

Keywords: *Tridax procumbens*, Phytochemical analysis, Antioxidant activity.

1. INTRODUCTION

The imbalance between the production of reactive oxygen species (ROS) and the ability of cells to scavenge them is known as oxidative stress (OS). OS has been implicated in the aetiology of a number of disorders, including cancer, diabetes, and heart disease [1]. Many cellular components, such as lipids, proteins, and nucleic acids, such as DNA, are damaged by ROS, resulting in cellular death by necrosis or apoptosis [2]. Due to decreased cellular antioxidant defence systems, the damage might become more extensive. Antioxidant defence mechanisms exist in all biological systems, which protect against oxidative damage and repair enzymes to remove damaged molecules. However, because this natural antioxidant system might be ineffective,

antioxidant chemicals must be included in the diet. The presence of phytochemicals such as polyphenols, carotenoids, and vitamin E and C in fruits and vegetables is known to reduce the risk of several diseases caused by OS, including cancer, cardiovascular disease, and stroke [3]. These health benefits are primarily imposed due to the presence of phytochemicals such as polyphenols, carotenoids, and vitamin E and C in fruits and vegetables. Polyphenols are secondary metabolites found in plants that have one or more hydroxyl groups bound to one or more aromatic rings. Higher plants, including edible plants, have been shown to contain thousands of polyphenolic compounds. Flavonoids and non-flavonoids are the two main types of plant polyphenols [4].

Flavonols, anthocyanidins, flavonols, flavanones, flavones, and chalcones are the different types of flavonoids. Stilbene, phenolic acids, saponin, and tannins are examples of non-flavonoids [5]. Plant polyphenols are known for their antioxidant activity, which is one of their most essential biological properties. Plant polyphenols have been proven in a number of studies to be effective antioxidants in the treatment of oxidative stress-related illnesses [6, 7].

Although *Tridax procumbens* L. (Asteraceae) has been described as a weed that has invaded many crops, it has long been used as a traditional drink to treat bronchial catarrh, diarrhoea, dysentery, and liver illnesses [9, 10] in many African, South, and Southeast Asian nations. Many bioactive compounds, such as procumbetin [8], 8,3'-dihydroxy-3,7,4'-trimethoxy-6-O-β-glucopyranosyl flavone, 6,8,3'-trihydroxy-3,7,4'-trimethoxyflavone; puerarin [11], centaurein, and centaureidin, have been successfully isolated from this plant [12]. Lipid constituents of this plant, including methyl 14 oxooctadecanoate, methyl 14- oxononacosanoate, 30 methyl-28-oxodotriacont-29-en-l-oic acid, β-amyrone, β-amyrin; lupeol, and fucosterol, have been identified [13]. Furthermore, phenolic acids, including benzoic, vanillic, benzene acetic acids, and guiacol, from this plant have been determined [14].

T. procumbens has a diverse range of biological functions. This plant's ethyl acetate extract has high allelopathic and larvicidal properties [15, 16]. Methanol and ethanol extracts were shown to have anti-hyperglycemic [17], anti-fungal [18], anti-leishmanial [19], hepatoprotective [20] properties, whereas ethyl acetate extract had anti-inflammatory, anti-cyclooxygenase, and antioxidant properties [12]. This herb's acetone extract has anticoagulant, anti-hepatic, and antibacterial properties [20]. However, no information on this plant's anti-hyperuricemia properties has been recorded.

2. MATERIAL AND METHODS

2.1. Plant Collection and Authentication

The whole plant of the *Tridax procumbens* was collected from Local Bundelkhand Region of Uttar Pradesh. The whole plant was dried in shade. The whole plant was authenticated by Dr. Gaurav Nigam (Accession No. BU/Bot./M.Pharm II/2021/001) from Department of Botany, Bundelkhand University, Jhansi, Uttar Pradesh, India. Plant materials were then washed separately with fresh water to remove dirty materials and were shade dried for several days with occasional sun drying. The dried materials were ground into coarse powder by

grinding machine and the materials were stored at room temperature for future use.

2.2. Extract Preparation

The whole plant was coarsely powdered and put in a Soxhlet device, where it was continually extracted with ethanol at 60°-80°C until all contents were separated. The amount to which chlorophyll was extracted into the solvent determined the success of the ethanol extraction. When the tissue was totally clear of green colour after many extractions, it was considered that all of the low molecular weight chemicals had been removed [21]. Characteristics of extract were as colour: Dark green, Odour & taste: characteristics.

2.3. Preliminary Phytochemical Investigation

Plant extracts were subjected to phytochemical testing to determine the presence of various chemical elements such as alkaloids, glycosides, flavonoids, essential oils, carbohydrates, proteins, tannins, and other compounds responsible for biological activity. As a result, chemical tests were carried out in an ethanolic extract of *Tridex procumbens* to discover several chemical elements [22].

2.4. Thin Layer Chromatography

A thin layer is placed over a rigid supporting plate, and the mobile phase, a liquid, is allowed to migrate across the plate's surface. Thin layer chromatography investigations were performed on the ethanolic extract of the complete plant of Tridex procumbens to determine the existence of a number of chemicals that were supported by the chemical test. Retention Factor (Rf) = Distance travelled by solute front from origin line/Distance travelled by solvent front from origin line. TLC plate showed the 5 spots with different colour with different Rf values in 0.5% vanillin in dil. H_2SO_4 in solvent system (Benzene: Chloroform) in a ratio of 9: 1.

2.5. Detecting Reagents

0.5% Solution of Vanillin in dil. H_2SO_4 and Ethanol (4:1). 5 spots with different Rf value have been obtained in TLC of Ethanolic extract of *Tridex procumbens.*

2.6. High Performance Thin Layer Chromatography

Mobile Phase used was Toluene: Ethyl Acetate: Formic Acid (7: 3: 0.2 v/v/v), Saturation Time was 20 Minutes. Applied 4µL, 5 µL and 6 µL of test solution on different tracks i.e., T1, T2 and T3 over pre-coated silica gel 60F254 TLC plate (Merck) of uniform thickness (0.2

mm) and developed the plate in the mobile phase upto a distance of 7 cm. The plate was dried and visualized at wavelengths 254 nm and 366 nm. For the derivatization step, diped the plate in Anisaldehyde Sulphuric acid reagent (ASR) and heated at 105°C till the color of the spots/bands appeared. The plate (pre and post derivatization) was observed under different wavelengths *i.e.,* UV-254 nm, UV-366 nm and white light to record the fingerprint profile. This revealed several prominent bands of different colors.

2.7. Column Chromatography

When working on a small scale, column chromatography is one of the most helpful procedures for separating and purifying solids and liquids. Another solid-liquid approach is column chromatography, which involves a solid (stationary phase) and a liquid (flowing phase) (Mobile phase). Adsorption Chromatography is a technique in which the stationary phase is a solid support of adsorptive nature and the mobile phase is a liquid. When utilising a column to carry out chromatographic processes, Column chromatography is the name given to this method.

2.8. Spectral studies

Spectroscopy is a field of science that studies how electromagnetic radiation interacts with materials. The practise of spectroscopy encompasses the methods used to make experimental measurements of radiation frequency (emitted or absorbed) and the energy levels determined from them.

2.8.1. Infra red spectroscopy

The excitation of a molecule from a lower to a higher vibrational state is caused by the absorption of IR radiations. [24]. Peaks seen in the range of 3415-3426 cm⁻¹ in the IR spectra of the isolated chemical indicate the presence of the OH group. The absorption ribbon in the area of 2854 cm^{-1} is caused by symmetric vibrations of the CH₂ group, which emerge at a wavelength of 1703.46 cm^{-1} . A distinctive ribbon, derived from CH₃ gp and located at 1108.23, occurs in the vicinity of 1378.99.

2.8.2. Nuclear Magnetic Resonance (NMR) Spectroscopy

The signal at 0.9δ (ppm) in the 1-H NMR spectra of an isolated molecule shows the existence of a primary proton R-CH₃. The existence of a tertiary proton RCH₃ was indicated by the signal emerging at 1.5 δ. The presence of Hydroxide is shown by the signal at 4.9 δ ,

which denotes a trisubstituted double bond (unsaturation). The presence of Benzylic proton is shown by the signal at 2-3 δ .

2.8.3. Mass spectroscopy

It is the most precise method for determining a compound's molecular mass and elemental content. MS involves bombarding the component under examination with a beam of electrons, which results in the formation of an ionic molecule or ionic fragments of the original species.

2.9. In-Vitro Antioxidant Activity *2.9.1. DPPH Radical Scavenging Activity*

DPPH was used to test the extract's free radical scavenging activity (25). In 95 percent ethanol, a DPPH solution $(0.004$ percent w/v) was produced. To make the stock solution (5 mg/mL), the extract of Tridax procumbens was combined separately with ethanol. Freshly produced DPPH solution $(0.004$ percent w/v) was placed in test tubes, and the extract was added, followed by serial dilutions $(1 \text{ g to } 500 \text{ µg})$ until the final volume was 3 mL, and the absorbance was measured at 450 nm using a spectrophotometer after 10 minutes. The reference standard was ascorbic acid, which was diluted in distilled water to create a stock solution with the same content (5 mg/mL) . A control sample with the same volume of extract and reference ascorbic acid was created (26). As extract blanks, 95 percent ethanol was used. The percentage of DPPH free radical scavenging was calculated using the following equation:

% Scavenging Activity= $\{$ (Absorbance of the control-Absorbance of the test sample) / Absorbance of the control}X 100

2.9.2. Reducing Power

As previously mentioned, the decreasing power of Tridax procumbens was tested (27). In 1 ml of distilled water, different quantities of the extract (200 to 1000 g) were combined with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K3fe(CN)6] (2.5 ml, 1 percent). For 20 minutes, the mixture was incubated at 50°C. The mixture was then centrifuged at 3000 rpm for 10 minutes after a quantity (2.5 ml) of trichloroacetic acid (10%) was added. The absorbance was measured at 450 nm after the top layer of the solution (2.5 ml) was combined with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1 percent). The reaction mixture's enhanced absorbance indicated greater reducing power. The standard utilised was ascorbic acid. As a blank solution,

phosphate buffer (pH 6.6) was utilised. The absorbance of the final reaction mixture from two separate experiments was calculated and given as mean standard deviation.

2.9.3. Nitric oxide (NO) Radical Inhibition Assay

The Griess Illosvoy reaction can be used to evaluate nitric oxide radical inhibition (28). The Griess-Illosvoy reagent was changed in this study by substituting naphthyl ethylene diamine dihydrochloride (0.1 percent w/v) for 1- napthylamine (5 percent). The combination (3 ml) was incubated at 25°C for 150 minutes with sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) , and Tridax procumbens extract $(10 \text{ to } 320 \text{ µg})$ or standard solution (ascorbic acid, 0.5 ml). Following incubation, 0.5 ml of the reaction mixture was combined with 1 ml of sulfanilic acid reagent (0.33 percent in 20% glacial acetic acid) and left to stand for 5 minutes to complete diazotization. Then 1 mL of naphthyl ethylene diamine dihydrochloride was added, stirred, and set aside at 25°C for 30 minutes. In diffused light, a pink chromophore was generated. At 450 nm, the absorbance of these solutions was compared to that of blank solutions.

2.9.4. Scavenging of Hydrogen Peroxide

The procedure based on the previously stated method was used to determine the extract's capacity to scavenge hydrogen peroxide (29). In phosphate buffered saline, hydrogen peroxide (43 mM) was produced (pH 7.4). At concentrations ranging from 50 to 250 mM, standards (ascorbic acid) and extract solutions were generated. In a 0.6 mL hydrogen peroxide solution, aliquots of standard or extract solutions (3.4 mL) were added. The reaction mixture was incubated for 10 minutes at room temperature, with the absorbance measured at 450 nm. The scavenging percentage was computed as follows: % H_2O_2 Scavenging = 100 x (Absorbance of Control-Absorbance of Sample)/Absorbance of Control

3. RESULTS AND DISCUSSION

3.1. Extraction of Drug

The *Tridex procumbens* whole plant was extracted with ethanol then Percentage Yield of ethanolic extract of *Tridex procumbens* was calculated. The percentage yield was Found to be 14.87% (Table 1)

3.2. Phytochemical Studies

Ethanolic extract of the *Tridex procumbens* whole plant shows the presence of carbohydrates, proteins, amino acids, steroids, phenolic compounds, flavonoids and saponin glycosides & absence of alkaloids, cardiac glycosides, anthraquinone glycosides and cynogenetic glycosides (Tables 2-4).

Test performed	Results
Mayer's Test	
Dragendroff's test	
Wagner's test	
Hager's test	
Van-urk's for indole alkaloids	
Vitali morin test for tropane alkaloids	
Thalleoquin Test for Quinoline Alkaloids	$\overline{+}$
Modified Born-Trager's Test	
Test For Saponin Glycosides	$^{+}$
Foam Test	$\overline{+}$
Antimony Trichloride Test	
Libermann Burchard Test	$^{+}$
Raymond's Test	
Kedde's Test	$\overline{+}$
Baljet's Test	
Xanthohydrate Test	$\overline{+}$
Tollen's Test	$^{+}$
Test for Coumarin Glycosides	$\, + \,$
Test for Cyanogenetic Glycosides	
Test for hydroxyl anthrax quinines	
Test for Cyanophoric Glycosides	
Legal Test	
Schonteten's Test	
Bromine Test	$^+$
Klunge's Isobar baloin Test	
Test for Flavonoid Glycosides	$^{+}$

Table 3: Preliminary Phytochemical Investigation for Tannin and Flavonoids

Table 4: Preliminary Phytochemical Investigation for Carbohydrate

Resorcinol test for ketones (Selvinoff's test) -

Killer-Kilani test for deoxy sugars -

Test performed Results Fehling's Solution Test + Molisch Test + Osazone formulation:

Test for pentoses $+$

3.4. HPTLC

For the better isolation & identification of the different components of ethanolic extract of *Tridex procumbens*

Linn., the HPTLC of ethanolic extract of *Tridex procumbens* Linn. was also carried out at Vaidhnath Ayurvedic Institute, Jhansi. The report of HPTLC indicates the presence of 10 spots with different Rf values in white light (derivatized in ASR) 9 spots present in different Rf value in 366nm and 6 spot present different Rf value in 254nm Rf value same as in table 6 and Fig. 1 which shows to occupy the different area with different Rf values. It shows that the extract contains about 10 constituents.

Table 5: TLC of ethanolic extract of whole plant of *Tridex Procumbens*

Extract	Solvent System	Spots	No. of Colour of Spots	Rf. value
Ethanolic	Benzene: Chloroform		Brown Green Green	0.11 0.19 0.44
Extract	(9:1)		Pinkish Purple	0.52 0.66

Fig. 1: HPTLC chromatograms of *Tridax procumbens* **(Whole Plant) under UV 254 nm, UV 366 nm, and after derivatization (under white light)**

3.5. Column Chromatography

The TLC studies of ethanolic extract of *Tridex procumbens* shows 5 spots of different colours, with different R_f value in solvent system Benzene: Chloroform (9:1 drop). It indicates the presence of 05 different components in the extract. Detecting reagent used is 0.5% vanillin in dil. H_2SO_4 For the better isolation & identification of the different components of ethanolic extract of *Tridex procumbens*, the column chromatography of isolated compounds from the ethanolic extract of *Tridex procumbens* were carried out with Hexane (80ml) and benzene (20 ml) & stationary phase silica gel. One fraction was collected F_1 on performing the TLC of fraction was showing one spot. Since fraction F_1 showing only one spot so it was further investigated for ¹H-NMR, Mass, FTIR analysis & structure was proposed. From comparison of IR, NMR and MASS spectra of standard with isolated compound, it indicated that this compound may be flavonoidal compound as a proposed structure:

3.6. Spectral studies

3.6.1. IR spectra of isolated compound

Peaks seen in the range of $3415-3426$ cm⁻¹ in the IR spectra of the isolated chemical indicate the presence of the OH group. The absorption ribbon in the area of 2854 cm⁻¹ is caused by symmetric vibrations of the $CH₂$ group, which emerge at a wavelength of 1703.46 cm^{-1} . A distinctive ribbon, derived from CH₃ gp and located at 1108.23, occurs in the vicinity of 1378.99.

3.6.2. NMR spectra of isolated compound

The signal at 0.9δ (ppm) in the 1-H NMR spectra of an isolated molecule shows the existence of a primary proton R-CH₃. The existence of a tertiary proton RCH₃ was indicated by the signal emerging at 1.5δ . The presence of Hydroxide is shown by the signal at 4.9 δ , which denotes a trisubstituted double bond (unsaturation). The presence of Benzylic proton is shown by the signal at $2-3\delta$.

3.6.3. MASS spectra of isolated compound

The mass spectrum of an isolated chemical from the complete Tridex procumbens plant was 149.03, 205.09, 206.09, 207.15, 279.15, 280.15, 281.20, and 391.27, with further peaks at m/z 149.03, 205.09, 206.09, 207.15, 279.15, 280.15, 281.20, and 391.27.

3.7. In-Vitro Antioxidant Activity

3.7.1. DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The DPPH (1, l- diphenyl-2-piciylhydrazyl) radical scavenging activity of the extract of Tridex procumbens (Table 7). The IC50 values of the extract was found to be 0.15 μ g/ml respectively whereas IC₅₀ for ascorbic acid was found to be $0.12 \mu g/ml$, which is a wellknown antioxidant.

Table 7: DPPH scavenging activity (% inhibition) antioxidant activities of ethanolic extract of *Tridex procumbens*

DPPH scavenging activity (% inhibition)				
Concentration in mg/ml	Treatments			
	Ascorbic	Tridex		
	acid	procumbens		
0.2	1.65 ± 0.03	1.59 ± 0.01		
0.4	1.78 ± 0.02	1.79 ± 0.02		
0.6	2.43 ± 0.02	1.81 ± 0.01		
0.8	2.67 ± 0.02	2.07 ± 0.02		
	2.72 ± 0.01	2.49 ± 0.02		
mp/	0.12	0.15		

Values are expressed as mean \pm *in standard deviation (N=6).*

3.7.2. Reducing activity

The reducing power of the tested extract suggests low properties. Like other antioxidant assays, the reducing power of *Tridex procumbens* extract increased with increasing the amount of samples. The IC_{50} value of the extract was 0.12µg/ml, whereas that for ascorbic acid was 0.009 µg/ml (Table 8).

3.7.3. NO. Scavenging Activity

The scavenging of nitric oxide by the plant extract was increased prominently in a dose-dependent manner. The IC₅₀ value of the extract was $0.008063\mu\text{g/ml}$, respectively whereas the IC_{50} value of ascorbic acid was 0.005 µg/ml (Table 9).

3.7.4. H2O² scavenging activity

The ethanolic extract showed almost similar H_2O_2 scavenging activity with that of ascorbic acid at similar concentrations. The IC_{50} value of the extract was 42.39 μ g/ml, whereas that for ascorbic acid was 0.007 μ g/ml. ([Table 10]). Upon the presence of iron ions hydrogen peroxide may give rise to hydroxyl ions which can tum toxic to the cells.

Table 8: Reducing power of antioxidant activity of ethanolic extract of *Tridex procumbens*

Values are expressed as mean \pm *in standard deviation (N=6)*

Table 9: Nitric oxide (NO) of antioxidant activity of ethanolic extract of *Tridex procumbens*

Values are expressed as mean \pm *in standard deviation (N=6).*

Table 10: Scavenging of Hydrogen Peroxide of antioxidant activity of ethanolic extract of *Tridex procumbens*

Values are expressed as mean \pm *in standard deviation (N=6).*

4. CONCLUSION

The extract of *Tridex procumbens* demonstrated adequate antioxidant activity *In vitro,* when compared to conventional compounds. The ability of the extract to scavenge nitric oxide and hydrogen peroxide free radicals might be due to a variety of antioxidant processes. Though a respectable TLC, HPTLC, and Column profile was produced, it is hoped that a more refined study would be able to identify and quantify the actual chemicals responsible for the *Tridex procumbens* extract's antioxidant activity. Furthermore, more research employing *Tridex procumbens* extracts should be done to evaluate its in-vivo antioxidant capabilities.

Conflict of interest

Authors have no conflict of interest

Source of funding

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