



Phytochemical determination and antioxidant potential of *Tridax Procumbens* L.

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Abstract

Medicinal plants serve as an important source of bioactive compounds with significant therapeutic potential. The present study investigates the phytochemical composition, antioxidant activity, and chemical profiling of one medicinal plant, *Tridax procumbens* L. (family: Asteraceae) Leaf extracts were prepared using solvents of varying polarity (n-hexane, chloroform, methanol, and water) and subjected to qualitative phytochemical screening, DPPH free radical scavenging assay, and GC-MS analysis. Phytochemical screening revealed the presence of important bioactive compounds such as flavonoids, phenols, carbohydrates, terpenoids, and phytosterols in this plant species, with methanolic and aqueous extracts showing higher phytochemical diversity. Antioxidant analysis demonstrated that the methanolic extract of *T. procumbens* L. exhibited the highest radical scavenging activity with the lowest IC₅₀ value (42.23 µg/ml), followed by aqueous and chloroform extracts. GC-MS analysis further confirmed the presence of various bioactive compounds responsible for pharmacological properties. The study highlights the significance of solvent polarity in extraction efficiency and supports the potential use of the plant as a natural source of antioxidants and therapeutic agents.

Keywords: *Tridax procumbens* L., Phytochemical screening, Antioxidant activity, GC-MS analysis

Introduction

Medicinal plants have been an integral component of traditional healthcare systems since ancient times and continue to play a crucial role in modern drug discovery. They are rich sources of diverse bioactive compounds, including flavonoids, phenolics, alkaloids, terpenoids, and glycosides, which exhibit a wide range of pharmacological activities. In recent years, there has been a growing interest in plant-based therapeutics due to their efficacy, safety, and reduced side effects compared to synthetic drugs (Harborne, 1998; Sasidharan *et al.*, 2011) [2, 7]. Additionally, the increasing prevalence of oxidative stress-related disorders such as cancer, cardiovascular diseases, and diabetes has intensified the search for natural antioxidants derived from medicinal plants (Kumar & Pandey, 2013) [4]. Free radicals and reactive oxygen species (ROS) are generated as by-products of normal cellular metabolism; however, their excessive accumulation can lead to oxidative damage to biomolecules, including lipids, proteins, and DNA. Antioxidants play a vital role in neutralizing these free radicals and preventing cellular damage. Plant-derived antioxidants, particularly phenolic and flavonoid compounds, are considered highly effective due to their ability to donate hydrogen atoms or electrons and stabilize free radicals (Sasidharan *et al.*, 2011) [7]. Therefore, evaluation of antioxidant potential using reliable methods such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely adopted in phytochemical studies. *Tridax procumbens* L. (family Asteraceae), commonly known as coat buttons, is a widely distributed medicinal herb known for its diverse therapeutic applications, including wound healing, antimicrobial, anti-inflammatory, and antioxidant activities. Previous studies have reported that the plant is rich in flavonoids, carotenoids, tannins, and terpenoids, which contribute to its biological properties (Saxena & Albert, 2005; Ikewuchi *et al.*, 2009) [8, 3].

The biological activities of medicinal plants are strongly influenced by extraction methods and the polarity of solvents used, which determine the type and quantity of phytochemicals extracted. Polar solvents such as methanol and water are generally more effective in extracting phenolic and flavonoid compounds, which are primarily responsible for antioxidant activity (Sasidharan *et al.*, 2011) [7]. In addition to phytochemical screening, advanced analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) provide detailed insights into the chemical composition of plant extracts and help identify compounds responsible for biological activity. In this context, the present study aims to comparatively evaluate the phytochemical composition, antioxidant potential, and GC-MS profiling of *Tridax procumbens* L. in different solvent extracts. The study seeks to establish a scientific basis for their traditional uses and explore their potential as natural sources of bioactive compounds for therapeutic applications.

Materials and Methods

Collection of Plant Materials

Green leaves of *T. procumbens* L. were collected from the Karauli district, Rajasthan. Those were washed with running tap water, shade dried and ground to make coarse powder and stored in a refrigerator for further use.

Extraction

The collected parts were extracted in various solvents. For this, 1 g of plant material was dipped into 10 ml of solvent (water, methanol, chloroform and n-hexane). Those were kept in a sonicator for 10 minutes at 40°C temperature. Those were filtered and solvents were evaporated. Dry extract yield was noted down.

Qualitative determination of phytochemicals

The extracts were determined for the presence of various primary and secondary metabolites using various standard methods such as-

Carbohydrate test (Fehling's test)

The extract was treated with Fehling's solution; the formation of brick-red precipitate indicated the presence of carbohydrates.

Protein test (Ninhydrin test)

The extract was heated with the ninhydrin reagent; the development of violet/purple colour confirmed proteins.

Glycosides test (Sulphuric acid test)

The extract was treated with concentrated H₂SO₄; colour change indicated the presence of glycosides.

Flavonoid test (Lead acetate test)

The addition of lead acetate produced a yellow precipitate, confirming flavonoids.

Phenol test (FeCl₃ test)

Ferric chloride was added to the extract; the formation of a blue/green colour indicated phenolic compounds.

Alkaloid test (Mayer's and Dragendorff's tests)

The addition of Mayer's or Dragendorff's reagent resulted in precipitate formation, indicating alkaloids.

Saponin test (Foam test)

The extract was shaken with water; stable foam formation indicated the presence of saponins.

Terpenoid test (Salkowski's test)

The extract was treated with chloroform and H₂SO₄; reddish-brown coloration indicated terpenoids.

Phytosterol test (Libermann-Burchard test)

The extract was treated with acetic anhydride and H₂SO₄; green/blue colour confirmed phytosterols.

GC-MS Study

The ethanolic samples of the plant was subjected to determine phytochemical constituents. The samples were sent to Accuphysem Analytics, Jaipur, for characterization.

DPPH Free Radical Scavenging Activity

For the determination of DPPH radical scavenging potential of the extracted samples, the 1,1-diphenyl 2-picryl-hydrazil (DPPH) method was applied. The mixing of the 100 µl sample was done in 3.9 ml taken from a 0.1 mM DPPH (methanolic) solution. The blend was subjected to vortex and left for incubation in the dark for 30 min. Its OD was calculated at 515 nm while methanol was used as a blank.

The radical scavenging activity was determined by the ratio = $(Ab_{control} - Ab_{sample} / Ab_{control}) \times 100$

Where $Ab_{control}$ is the absorbance of the DPPH solution and the absorbance of the DPPH solution with the sample is denoted by Ab_{sample} .

A linear plot of concentration versus % inhibition was plotted and by this IC₅₀ values were determined. The antioxidant potential of each extract was shown in the form of IC₅₀ (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), found with the help of an inhibition curve.

Results

Extraction of the Plant Material

The extraction of leaf samples of *Tridax procumbens* L. using solvents of varying polarity revealed significant differences in extraction yield and consistency. In *Tridax procumbens* L., the aqueous extract showed the highest yield (0.612 g/5 g), followed by methanol (0.133 g/5 g), chloroform (0.063 g/5 g), and n-hexane (0.039 g/5 g). The aqueous extract appeared dry, whereas extracts obtained using organic solvents exhibited a sticky consistency. These findings indicate that polar solvents are more efficient in extracting phytoconstituents from this plant species.



Fig 1: Collection, processing and extraction of the plant material

Table 1: Properties of the extracts

Sample	solvent	Extractive value (mg/g. dw)	Consistency
<i>Tridax procumbens</i> L.	N hexane	0.0078	Sticky
	Chloroform	0.0126	Sticky
	Methanol	0.0266	Sticky
	Water	0.1224	Dry

Phytochemical test

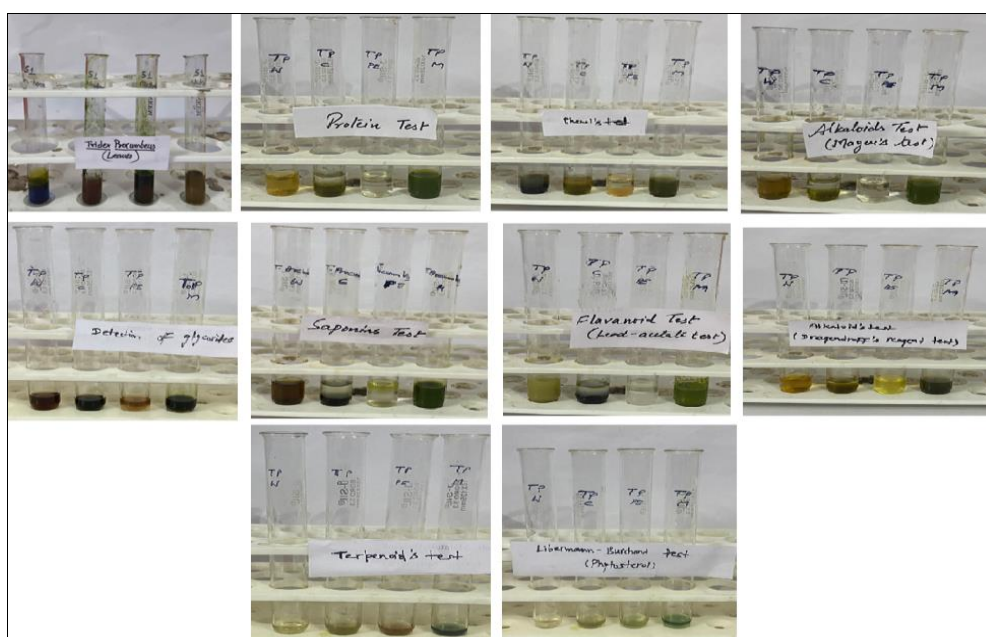
Qualitative phytochemical screening of *Tridax procumbens* L. revealed the presence of several important secondary metabolites.

Carbohydrates were detected in all solvent extracts, indicating their widespread distribution. Flavonoids and phenolic compounds were predominantly present in aqueous, chloroform, and methanolic extracts, while they were absent in the hexane extract. Proteins were detected only in the aqueous extract. Terpenoids were mainly observed in chloroform extract, whereas phytosterols were present in chloroform, n-hexane, and methanol extracts. However, alkaloids, glycosides, and saponins were not detected in any of the extracts. Overall, methanolic and aqueous extracts showed greater phytochemical richness compared to other solvents.

Table 2: Qualitative phytochemical test for *Tridax procumbens* L

Phytochemical test	Water		Chloroform		n-Hexane		Methanol	
	O	R	Observation	R	Observation	R	Observation	R
Protein	yellow	+	Green transparent	-	No Colour Transparent	-	Green	-
Glycosides	Brown	-	Green	-	Brown	-	Green	-
Carbohydrate	Blue (ketose sugar)	+	Red brown (Aldose sugar)	+	Blue (ketose sugar)	+	Red brown (Aldose sugar)	+
Flavonoid	Creamy green	+	Blue	+	White transparent	-	Green	+
Phenol	Green	+	Green	+	Yellow	-	Green	+
Alkaloids	Yellow	-	Green transparent	-	No Colour Transparent	-	Green	-
	Yellow transparent	-	Green	-	Yellow	-	Green	-
Saponin	Brown	-	Blue transparent	-	No Colour transparent	-	Green	-
Terpenoids	Yellow transparent	-	Grey	+	Brown	-	Green	-
Phytosterol test	Transparent	-	Green ring	+	Green ring	+	Green ring	+

*O- Observation; R- Result

**Fig 2:** Qualitative phytochemical test of *T. procumbens* L**GC-MS studies**

GC-MS analysis of the extracts of *Tridax procumbens* L. revealed the presence of a diverse range of bioactive compounds, including fatty acids, terpenoids, alcohols, esters, and phytosterols, which are known to possess significant pharmacological properties.

In *Tridax procumbens* L., a total of several compounds was identified, with major constituents including diethyl phthalate (17.98% and 10.55%), benzenopropanoic acid derivatives (11.08%), and decanedioic acid, bis(2-ethylhexyl) ester (11.07%), indicating their dominance in the extract.

Other notable compounds detected were cis, cis, cis-7,10,13-hexadecatrienal (7.01%), epoxyanthralene derivatives (6.15%), and n-hexadecanoic acid (4.50%). Additionally, biologically important compounds such as phytol (2.34%), 9,12-octadecadienoic acid (2.78%), octadecanoic acid (3.90%), oleyl oleate (4.06%), and squalene (0.75%) were identified. Minor compounds, including acetone, toluene, neophytadiene, and various phthalate derivatives, were also present. The abundance of fatty acids, terpenoids, and phenolic derivatives suggests a strong antioxidant and antimicrobial potential of *T. procumbens* L.

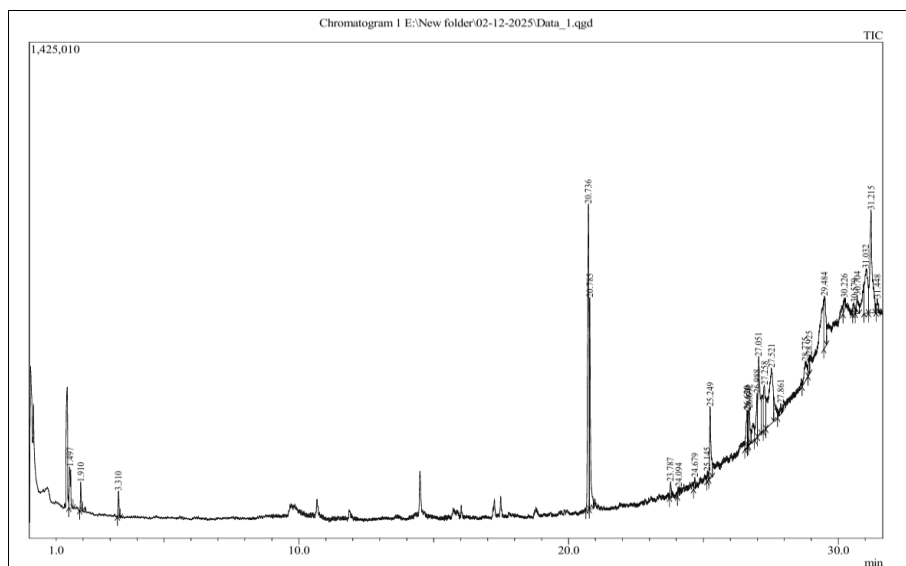


Fig 3: GC-MS Chromatograph of *T. procumbens* L

Table 3: Phytoconstituents identified in leaves of *T. procumbens* L. by GC-MS study

Peak#	R. Time	I. Time	F. Time	Area	Area%	Height	Height%	A/H	Compound Name
1	1.497	1.465	1.625	436062	2.63	117475	2.99	3.71	Acetone
2	1.910	1.870	1.985	169232	1.02	81816	2.08	2.07	Trichloromethane
3	3.310	3.270	3.380	174027	1.05	74340	1.89	2.34	Toluene
4	20.736	20.645	20.765	2975865	17.98	877418	22.32	3.39	Diethyl Phthalate
5	20.785	20.765	20.930	1746755	10.55	607184	15.45	2.88	Diethyl Phthalate
6	23.787	23.740	23.815	121552	0.73	45713	1.16	2.66	Neophytadiene
7	24.094	24.035	24.115	82731	0.50	24711	0.63	3.35	1,2-Benzenedicarboxylic acid derivative
8	24.679	24.630	24.700	83050	0.50	33143	0.84	2.51	7,9-Di-tert-butyl-1-oxaspiro compound
9	25.145	25.130	25.200	80258	0.48	27174	0.69	2.95	Dibutyl phthalate
10	25.249	25.200	25.335	744809	4.50	206855	5.26	3.60	n-Hexadecanoic acid
11	26.620	26.535	26.635	383749	2.32	107144	2.73	3.58	Bis(2-ethylhexyl) phthalate
12	26.650	26.635	26.670	198491	1.20	101719	2.59	1.95	Bis(2-ethylhexyl) phthalate
13	26.692	26.670	26.765	386500	2.34	105217	2.68	3.67	Phytol
14	26.988	26.895	27.005	460371	2.78	126234	3.21	3.65	9,12-Octadecadienoic acid (Z, Z)
15	27.051	27.005	27.140	1160107	7.01	227406	5.79	5.10	cis, cis, cis-7,10,13-Hexadecatrienal
16	27.258	27.215	27.315	645101	3.90	129723	3.30	4.97	Octadecanoic acid
17	27.521	27.315	27.605	1834535	11.08	159261	4.05	11.52	Benzenepropanoic acid derivative
18	27.861	27.745	27.905	133472	0.81	31914	0.81	4.18	2-Oleoylglycerol
19	28.775	28.655	28.880	424421	2.56	56505	1.44	7.51	2-Oxo-5-acetyl-6-methyl compound
20	28.925	28.880	28.940	158648	0.96	55189	1.40	2.87	Bis(2-ethylhexyl) methylphosphonate
21	29.484	29.465	29.565	672771	4.06	149697	3.81	4.49	Oleyle oleate
22	30.226	30.175	30.275	147835	0.89	35966	0.92	4.11	10-Methylnundec-2-en-4-olide
23	30.579	30.535	30.630	120113	0.73	32387	0.82	3.71	Bis(2-ethylhexyl) phthalate
24	30.704	30.630	30.780	237867	1.44	52561	1.34	4.53	1,2-Oxathiane derivative
25	31.032	30.950	31.115	1017377	6.15	127580	3.25	7.97	Epoxynaphthalene derivative
26	31.215	31.115	31.370	1831809	11.07	295681	7.52	6.20	Decanedioic acid, bis(2-ethylhexyl) ester
27	31.448	31.405	31.495	124641	0.75	40432	1.03	3.08	Squalene

Antioxidant assay

The antioxidant activity evaluated using the DPPH free radical scavenging assay showed a clear concentration-dependent increase in activity for all extracts of the plant. In *Tridax procumbens* L., the methanolic extract exhibited the highest antioxidant activity, with radical scavenging increasing from 43.98% at 20 µg/ml to 67.41% at 100

µg/ml, and the lowest IC₅₀ value of 42.23 µg/ml, indicating strong antioxidant potential. The aqueous extract showed moderate activity (IC₅₀ = 115.90 µg/ml), followed by the chloroform extract (IC₅₀ = 128.96 µg/ml).

The hexane extract exhibited the least activity with a high IC₅₀ value of 379.72 µg/ml, indicating poor radical scavenging ability.

Table 4: DPPH free radicals scavenging activity of *T. procumbens* L. extracts.

Solvent	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	IC ₅₀ µg/ml
Methanol	43.98±0.08	49.47±0.07	53.77±0.11	61.53±0.15	67.41±0.11	42.23±0.20
Chloroform	27.89±0.02	29.54±0.21	39.47±0.08	40.95±0.26	42.41±0.18	128.96±0.26
Water	20.18±0.01	25.14±0.30	31.57±0.15	40.09±0.16	44.41±0.16	115.90±0.69
Hexane	2.86±0.17	6.57±0.28	10.08±0.07	10.72±0.30	13.68±0.22	379.72±3.57

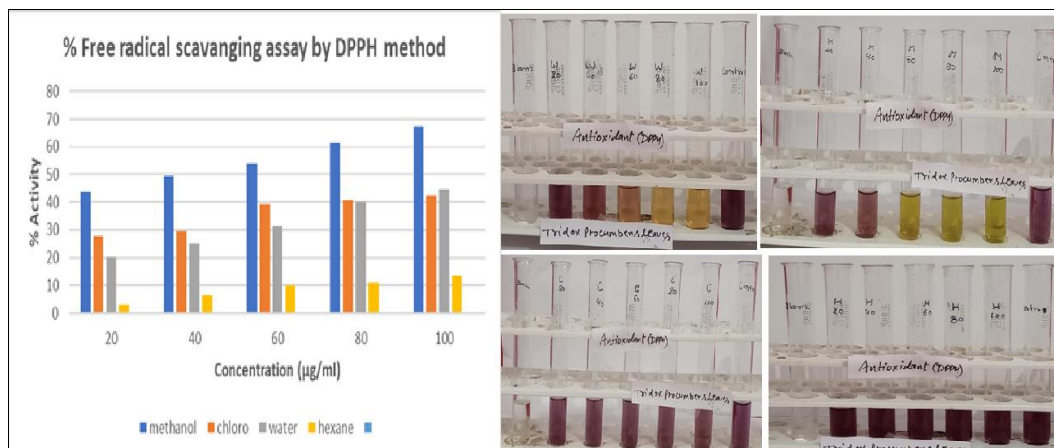


Fig 4: Antioxidant potential of leaves of *T. procumbens* L. by DPPH assay

Discussion

The present study demonstrates that *Tridax procumbens* L. possesses significant phytochemical constituents and antioxidant potential, which are strongly influenced by solvent polarity and extraction efficiency. The higher extraction yields observed in polar solvents (methanol and water) in the plant species indicate that the majority of bioactive compounds are polar in nature. This observation is consistent with earlier studies by Harborne (1998) and Sasidharan *et al.* (2011) [2, 7], which reported that phenolics and flavonoids are more efficiently extracted using polar solvents due to their chemical structure and solubility. Qualitative phytochemical screening in the present study revealed the presence of carbohydrates, flavonoids, phenols, terpenoids, and phytosterols, with methanolic and aqueous extracts showing greater phytochemical diversity. The absence of alkaloids and saponins in most extracts suggests species-specific phytochemical variation. Similar findings have been reported in previous studies on *Tridax procumbens* L., where flavonoids, phenolics, and terpenoids were identified as dominant constituents responsible for biological activity (Saxena and Albert, 2005; Ikewuchi *et al.*, 2009) [8, 3].

The DPPH assay results clearly indicate that antioxidant activity is concentration-dependent and strongly correlated with the presence of phenolic and flavonoid compounds. Among all extracts, the methanolic extract of *Tridax procumbens* L. exhibited the highest antioxidant activity ($IC_{50} = 42.23 \mu\text{g/ml}$), suggesting its strong free radical scavenging ability. This aligns with previous findings by Sahoo *et al.* (2011) and Kumar *et al.* (2012) [6, 4], who reported high antioxidant activity in methanolic extracts of *T. procumbens* L. due to elevated phenolic content. In contrast, non-polar hexane extracts showed minimal activity, reinforcing the idea that antioxidant compounds are predominantly polar. GC-MS analysis further substantiated the presence of several biologically active compounds such as n-hexadecenoic acid, phytol, octadecanoic acid, squalene, stigmaterol, and various fatty acid derivatives. These compounds are well documented for their pharmacological properties. For instance, n-hexadecenoic acid (palmitic acid) has been reported to exhibit antioxidant and antimicrobial activity, while phytol is known for its anti-inflammatory and anticancer potential (de Morais *et al.*, 2014) [1]. Similarly, stigmaterol and squalene are recognized for their antioxidant, hypocholesterolemic, and anticancer properties (Rao and Gurfinkel, 2000) [5]. The presence of these compounds provides a biochemical basis for the observed antioxidant activity in this plant.

Overall, the findings of the present study are in strong agreement with previous research and further validate the traditional use of the plants in herbal medicine. The study also highlights the importance of solvent selection, as it significantly affects the extraction of bioactive compounds and their biological activity.

Conclusion

The present study highlights that leaves of *Tridax procumbens* L. are rich sources of bioactive phytochemicals with significant antioxidant potential. Among all extracts, the methanolic extract of *T. procumbens* L. showed the highest antioxidant activity, indicating its potential as a natural antioxidant source.

The findings emphasize the importance of solvent selection in phytochemical extraction and validate the traditional medicinal uses of the plant. Further studies involving compound isolation, mechanism of action, and clinical evaluation are recommended to explore their full therapeutic potential.

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