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Research Paper

Qualitative and Quantitative Screening of total Phenolic and total Flavonoid for Different Extracts of *Tridax procumbens* L. and Comparative Study of their Anti-Oxidant Properties

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Abstract
The different solvent extracts such as Acetone extract, Ethyl acetate extract, 70% Alcoholic extract and Aqueous extract were first qualitatively screened for Flavonoids and Phenolics. Flavonoids and Phenol were present in every solvent extract of Tridax procumbens L. except that phenol was absent in acetonic extract. Highest concentration of Flavonoids and Phenolic were present in 70% alcoholic extract and ethyl acetate extract respectively with 398.33 mM equivalent of Quercetin and 343.66 mM equivalent of Gallic acid respectively. The acetonic extract had least quantity of Flavonoids and Phenolics with 38.33 mM equivalent of Quercetin and 160.33 mM equivalent of Gallic acid respectively. However this (acetonic) extract show comparatively higher antioxidant activity with 34.19% of radical scavenging activity followed by 70% alcoholic extract with 9.03% radical scavenging activity. Ethyl acetate and aqueous extract didn't have any measurable radical scavenging activity.
Keywords: *Flavonoids, Phenolics, DPPH assay.*

Introduction

Tridax procumbens L. is a common medicinal herb used by ethno-medical practitioners, belonging to family *Asteraceae*. It is best known as a widespread weed and pest plant. It is native to tropical America but it has been introduced to tropical, subtropical and mid temperate regions worldwide. The plant is a procumbent herb and is valued for its pharmaceutical properties. (Sahoo M. et.al., 1998, The Wealth of India, 1988).

Materials and Methodology

Sample Collection: Plant sample was collected and authenticated from herbarium.

Sample Preparation: The Plant sample was washed properly with distill water, then with 10% Sodium chloride solution and again with distill water. The sample was then shade dried to reduce upto 10% of moisture content. The dried sample was then cold blended to reduce the size of the plant sample.

Crude Extract Preparation: Solvent extraction method was carried out for extraction of different metabolites by using different organic solvents of different polarities. (Sanchez et.al.,1999). Coarsely powdered sample was taken and dissolved in solvent in the ratio 20 part: 1part (sample:solvent). The samples were kept for agitation for 48 hr. After 48 hr of continuous

agitation samples were filtered and the filtrate was used as crude extract. Organic solvents such as Acetone, Ethyl acetate, 70% Propanol, Water were used for the solvent extraction. The crude extract so obtained were S1- Acetone extract, S2- Ethyl acetate extract, S3- 70% Propanol extract and S4-Water extract.

Qualitative Estimation of Flavonoids and Phenolics (Kokate CK et. al.,1995):

Qualitative test for Flavonoids (Alkaline reagent Test): 1ml of extract was taken and treated with 5-10 drops of 20% Ammonium hydroxide solution. Formation of intense yellow color/fluorescence, indicates the presence of flavonoids.

Qualitative test for Phenols (Ferric chloride Test): 1ml of extract was taken and add 5-6 drops of 5% aqueous ferric chloride solution was added Formation of deep blue or black colour indicates the presence of Phenols.

Quantitative Estimation of Flavonoids and Phenolics: The quantitative analysis was for flavonoid (Kumaran et.al., 2006) and phenolic content (Hagerman A.et.al., 2000) was estimated from Quercetin and Gallic acid standard calibration curve respectively. The total Flavonoids and Phenolics was calculated from the Slope equation *(y=mx+c)* of each calibration curve and represented as as mM equivalent of Quercetin and mM equivalent of Gallic acid respectively.

Antioxidant activity: DPPH is stable free radical at room temperature and accepts an electron / hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical is determined by the decrease in its absorbance at 5l7 nm, induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. In-vitro antioxidant activity was calculated by the Percentage of inhibition (%age) carried out by 2,20 - diphenyl-1-picrylhydrazyl (DPPH) assay. (Brand-Williams W et. al., (1995), Bursal E, et. al., (2011)).

Results

The different solvent extracts such as S1- Acetone extract, S2- Ethyl acetate extract, S3- 70% Propanol extract and S4-Water extract were first screened for presence or absence of Flavonoid and Phenolics by alkaline reagent test and Ferric Chloride Test respectively. (Table-1)

Table 1: Qualitative Screening for Presence or Absence of Flavonoids and Phenolics in plant extracts of *Tridax procumbens* L..

Sl.NO	Metabolite	Observations	S1-Acetone	S2- Ethyl Acetate	S3- 70% alcoholic	S4-Aqueous
1.	Flavonoids (Alkaline reagent Test)	Yellow colouration/fluroscence on addition of Ammonium hydroxide)	Present	Present	Present	Present
			+	+	++	++
2.	Phenols (Ferric Chloride Test)	Deep blue or Black colour on addition of aq.$FeCl_3$	Absent	Present	Present	Present
			-	++	+	++

+ = Slightly positive.

Quantitative estimation for Flavonoids and Phenolics was determined from standard calibration curve for Quercetin (Figure-1) and Gallic acid (Figure-2) respectively.

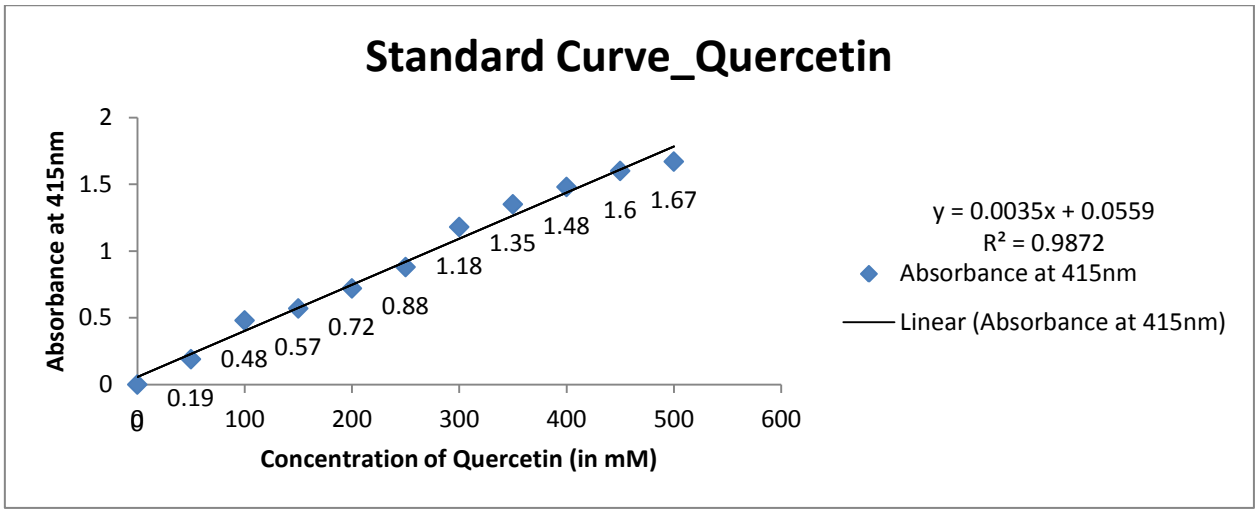

Figure 1: Standard Calibration Curve (Quercetin)

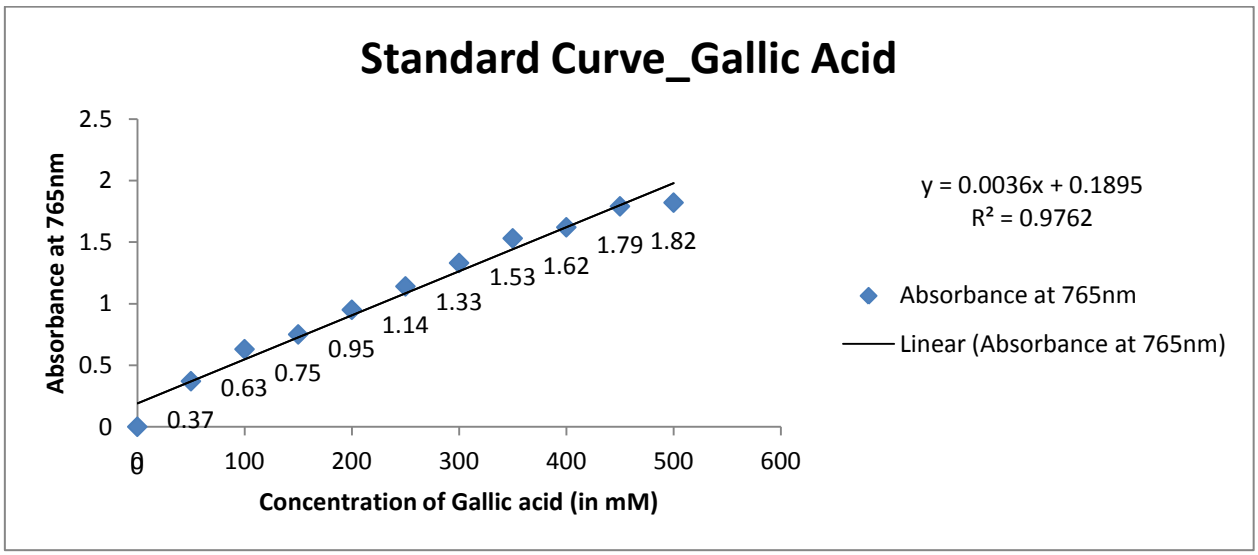

Figure 2: Standard Calibration Curve (Gallic acid)

The Unknown concentration of Flavonoids (Table 2, Figure 3) and Phenolics (Table 3, Figure 4) was determined from the Standard calibration curve.

Table 2: Unknown concentration (Flavonoids) of different Plant Extracts of *Tridax procumbens.*

Sample Extracts	Absorbance at 415nm			Mean OD	Concentration (equivalent to mM Quercetin)
	O.D. 1	O.D. 2	O.D. 3		
S1-Acetone	0.23	0.22	0.24	**0.23**	**58.33**
S2-Ethyl Acetate	0.67	0.67	0.69	**0.68**	**208.33**
S3-70% Alcoholic	1.23	1.28	1.24	**1.25**	**398.33**
S4-Aqueous	0.96	0.93	0.93	**0.94**	**295.00**

Table 3: Unknown concentration (Phenolics) of different Plant Extracts *Tridax procumbens* L..

Sample Extracts	Absorbance at 765nm			Mean OD	Concentration (equivalent to mM Gallic acid)
	O.D. 1	O.D. 2	O.D. 3		
S1-Acetone	0.67	0.66	0.68	**0.67**	**160.33**
S2-Ethyl Acetate	0.56	1.54	1.55	**1.22**	**343.66**
S3-70% Alcoholic	0.99	0.97	0.96	**0.97**	**260.33**
S4-Aqueous	1.18	1.17	1.16	**1.17**	**327.00**

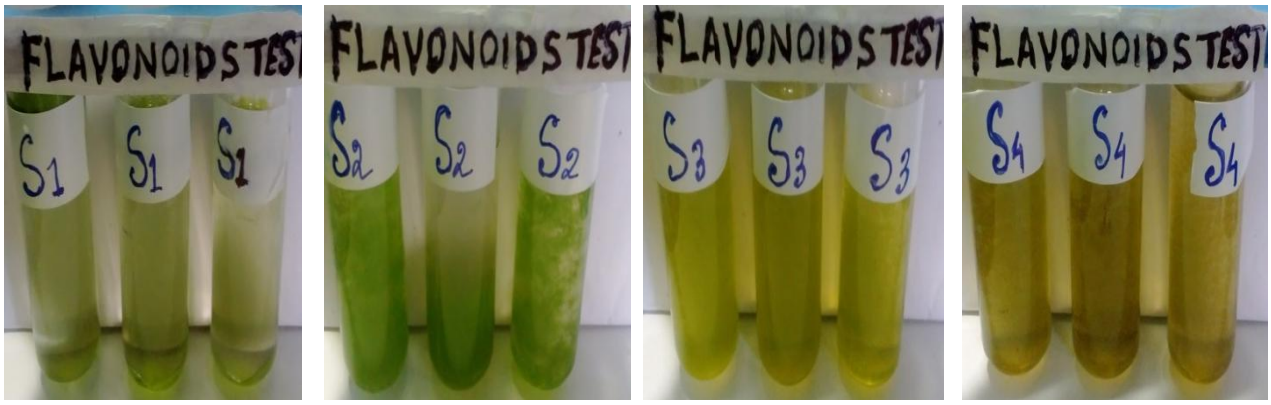

Figure 3: Quantitative Estimation for Flavonoids.

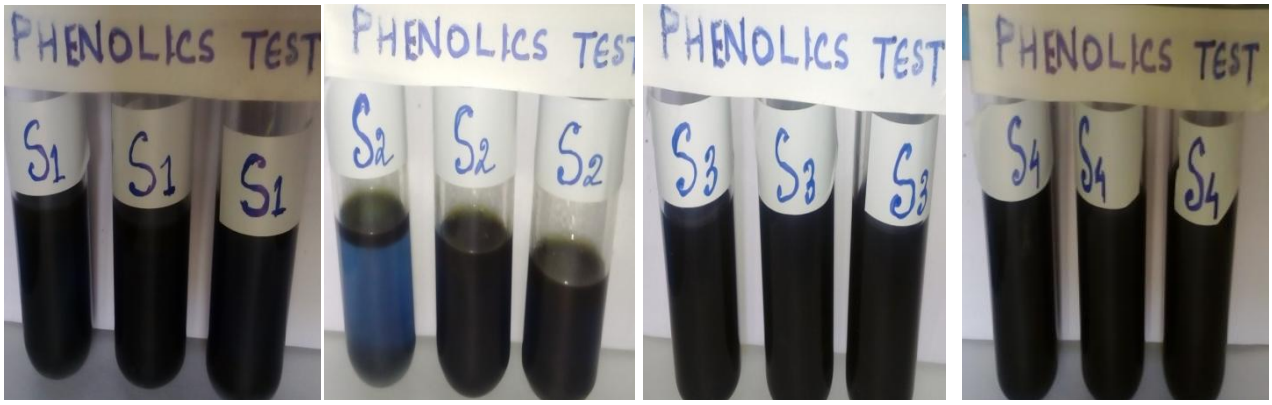

Figure 4: Quantitative Estimation for Phenols.

The Radical scavenging activity was determined by DPPH assay was determined by the absorbance recorded at 517nm for Control and Test sample. (Table 4, Figure 5).

$$\text{Radical Scavenging activity (\%age)} = \frac{Absorbance\ (Control) - Absorbance\ (sample)}{Absorbance\ (Control)} * 100$$

Table 4: Anti-oxidant Activity of different extracts of Sample. (DPPH Method)

Sample Extracts	Absorbance at 517nm	Radical scavenging Activity (%)
Negative Control	**1.55**	-
Positive Control (0.1M Ascorbic Acid)	**0.18**	**88.38%**
S1-Acetone	**1.02**	**34.19%**
S2-Ethyl Acetate	**1.71**	**NA**
S3-70% Alcoholic	**1.41**	**9.03%**
S4-Aqueous	**1.89**	**NA**

Figure 5: DPPH assay for different extracts of *Tridax procumbens L.*

Discussion

Tridax plant is present throughout India and is employed as indigenous medicine for variety of ailments. It has been found to possess significant medicinal properties against blood pressure, bronchial catarrh, malaria, dysentery, diarrhea, stomach ache, headache, wound healing, it also prevents hair fall and check hemorrhage from cuts and bruises (Ali M. et.al., 2001). Its flowers and leaves possess antiseptic, insecticidal and parasiticidal properties (Pathak et.al., 1991, Sahoo M. et.al., 1998). The plant also shows various pharmacological activities like Immunomo-dulatory, Antidiabetic, Anti hepatotoxic & Anti-oxidant, Anti-inflammatory, Analgesic, and marked depressant action on respiration (Ravikumar V. et.al., 2005, Vyas P Suresh et.al., 2004, Bhagwat Durgacharan A. et.al., 2010, Reddipalli Hemalatha et.al., 2008, Diwan Prakash V. et.al., 1989). Two new flavones, 8,3′-dihydroxy- 3,7,4′-trimethoxy-6-O-β-D-glucopyr-anosyl flavone (1) and 6,8,3′-trihydroxy-3,7,4′-trimethoxyflavone (2) were isolated from *Tridax procumbens Linn.* The antioxidant activity of the two new flavones were evaluated by two methods, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) assays, and the data showed that compounds 1 and 2 have certain antioxidant activity, with the antioxidant activity of compound 2 being stronger than that of compound 1 (Runsheg Xu. et.al., 2010).

Conclusion

The current studies show a good antioxidant property *Tridax procumbens L.* Amongst extracts such as Acetone extract, Ethyl acetate extract, 70% Propanol extract and Water extract. The Ethyl acetate and 70% alcoholic proved to be a good solvent system for extraction of Phenolic and Flavonoid compounds in quantity wherein the Acetonic extract showed a highest degree of scavenging activity in DPPH assay. It provides further opportunities for study of some other bioactive compounds (except flavonoids or phenolics) in the acetonic extract or some peculiar Flavonoid and Phenolic compound (quantitatively estimated in this experiment= 38.33 mM equivalent of Quercetin and 160.33 mM equivalent of Gallic acid) that that have relatively good anti-oxidant property at its low concentration.

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