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RESEARCH ARTICLE

Pharmacognostic and phytochemical screening of Leaves and Stem of *Tridax procumbens* Linn.

Ranjan, R., Patil, V. N., Joshi. A. R., Kamble, S. R., Chaudhary, V. R. and *Deokule, S. S.

Department of Botany, University of Pune, Pune-411007 (M.S) India

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ABSTRACT

Tridax procumbens Linn. is an important medicinal plant known as 'akdandi' used as an It is used as an antidiabetic, antibacterial, anti-inflammatory. The species is also well known in ayurvedic medicine for wound healing (R.Nia *et al.*).. The species is also well known in ayurvedic medicine for liver disorder. The present paper reveals the botanical standardization on the stem of *T. procumbens*. The pharmacognostic studies includes the phytochemistry, histochemistry, microscopic, macroscopic evaluation, percentage extractives, ash and acid insoluble ash, fluorescence analysis and estimation of polyphenol. The phytochemical and histochemical test includes starch, proteins, saponins, alkaloids, glycosides, sugar and flavonoid.

INTRODUCTION

Tridax procumbens Linn. is an important medicinal plant known as 'akdandi' which belongs to family Compositae which is found throughout India and is employed in the indigenous system of medicines. It also occurs throughout the tropical and subtropical belt of the world and is frequently found in annual crops, roadsides, pastures, fallow land and waste areas and occasionally in lawns, perennial crops and nurseries (Holm *et al*., 1997). The plant body is semi-prostrate perennial herb, taproot slender, wavy with many lateral branches. Stems more or less ascending 30 to 50cm in height, branched, round, sparsely to very hairy, greenish purple, slender, thickened at node, minutely pubescent or nearly glabrous, branched. Literature survey indicated that the wound as a medical problem was first discussed by Maharshi Agnibesha in Agnibesha Samhita (later known as Charaka Samhita) as "Vrana" Maharshi Sushruta in Sushruta Samhita. Kani tribals have a general knowledge of medicinal plants that are used for first aid remedies like wound healings, cough treatment. Review of literature indicates Flavones, glycoside, polysaccharide and monosaccharides have been isolated from the leaves of the plant (Ali *et al*; 2001, Yadawa and Saurabh, 1998). It is used as an antidiabetic (Bhagwat *et al.*) antibacterial (Mahato *et al.*), anti-inflammatory (Nia *et al.*). The species is also well known in ayurvedic medicine for wound healing (Nia *et al.*).

MATERIAL AND METHODS

Collection and Identification of Plant Material

The plant material was collected from in and around Pune district of Maharashtra state. The plant was collected in flowering and fruiting condition for the correct botanical identification. It was identified with the help of Flora of The Presidency of Bombay (Cooke, 1958). The herbarium specimens were prepared and authenticated from Prof. S. S. Deokule, Head, Department of Botany, University of Pune, Pune.

***Corresponding author:** deokule.ss@gmail.com

Microscopic and macroscopic evaluation

Stem was examined macroscopically according to (Wallis, 1967 and Trease and Evans 2002) which includes shape and size, colour (inner and outer), odour and taste. For microscopic evaluation thin (25µ) hand cut sections were taken from fresh stem, permanent double stained and finally mounted in Canada balsam as per the plant micro-techniques method of Johansen (1940) and Krishnamurthy (1988).

Histochemical study

The thin transverse sections of fresh stem were taken (about 25µ). It was treated with respective reagent for the detection and localization of active constituent present in the tissues as per the method of Krishnamurthy (1988).

Phytochemical study

Some material were dried under the shade so as to avoid the decomposition of chemical constituents, powdered in blender and finally stored in dry air tied containers for phytochemical screening. Ash, acid insoluble ash and percentage extractives were accomplished by following standard pharmacopoeia techniques of Anonymous (1955). Fluorescence analysis was carried out as per Chase and Pratt (1949). Qualitative phytochemical tests were carried out by standard method of Harborne (1973) and Trease and Evans (2002). Quantitative phytochemical analysis were determined for proteins, carbohydrates, starch, and flavonoid by the methods of Lowry *et al.,* (1951), Nelson (1944), Boham and Kocipai, (1994). The phytochemical screening is also detected by the High Performance Thin Layer Chromatography (HPTLC). HPTLC study was carried out on instrument comprising of Linomat5 for application using Densitometer- TLC scanner 3 with "WINCATS" software (Camag, Switzerland). These studies were carried out on HPTLC precoated aluminum fluorescent plates (E. Merck). For HPTLC studies, an extract of methanol (25% GR) solvent system was used and after development, plate was scanned at 254 and 366nm (Wagner and Bladt, 1966; Reich and Schibii, 2007).

RESULTS AND DISCUSSION

Macroscopic evaluation

Herb: 2-2.5 ft in height.

Roots: tap root slender, wavy with many lateral branches measuring about 16-23cm long, 4-9 in numbers, 0.5-1cm diameter.

Leaves: 15-20 in number simple, opposite, lanceolate to ovate, 3-7cm long, 1-4cm wide with irregularly toothed margins; base wedge shaped short-petiole, hairy on both surfaces.

Inflorescence: a terminal involucrate flower head or capitulum, 1 to 2 cm across, solitary on erect peduncle 10 to 25cm long.

Flower: involucres 3-seriate, ovate, acute to shortly acuminate, 5 to 6mm long; receptacle with oblong, hairy scales; ray flowers 3-dentate, few, pale yellow; disk flowers 5-dentate, tubular, yellow to brownish-yellow, with recurved hairy segments.

Fruit: a black achene covered with fine, pale hairs giving a grayish-brown appearance, 2mm long, 1mm wide at apex, base narrow.

Microscopic evaluation

T. S. of stem in circular outline is differentiated into epidermis, cortex and vascular bundles. The outermost is a single layer epidermal cell. Epidermis consists of uniseriate trichomes which is having bulbous base and pointed end. Epidermis is followed by cortex. The cortex is followed by a 6-7 rows of parenchymatous cells, some cells contain starch grain. The stele represents the closely arranged vascular bundles. Xylem vessels are polygonal to oblong, mostly found in groups of 2-8. Phloem surrounds the xylem vessels and occurs crescent shaped patches. Vascular bundle radially arranged.

Histochemical screening

Histochemical screening showed the presence of starch, protein, fat, saponins, tannin, sugar and alkaloids (Table 1).

Table 1: Histochemical study of Stem and Leaf of *Tridax procumbens*

Sr. No.	Test	Reagents	Results For Stem and Leaves	Tissue localization
1.	Starch	I_2KI	++	Lower epidermis, V.B
2.	Protein	Millan's reagent	++	Trichomes, Mesophyll cell
3.	Tannin	Acidic $FeCl_3$	++	Cortex
4.	Saponin	Conc. H_2SO_4	++	Trichomes, cortex, V.B
7.	Glycoside	benzene	++	Trichomes, cortex, V.B
8.	Alkaloids	Mayer's reagent	++	Trichomes, cortex
		Wagner's reagent	++	Mesophyll cell, cortex.
		Dragendorff's reagent	++	Lower epidermis, cortex, V.B.
		Tannic acid	++	Cortex, Trichomes
		Hager's Reagent.	++	Cortex, Trichomes

++ (Positive sign) = denotes the presence of chemicals.

Phytochemical study

The plant *T. Procumbens* contains the total ash13.5% and acid insoluble ash 13% (Table 2). The value of percentage extractive is higher in absolute alcohol and lower in distilled water (Table 3). Fluorescence analysis was carried out to check the purity and potency of the drugs. The powdered drug was then observed in ultraviolet light, it was treated with reagents like Nitrocellulose, 1N NaOH, 1N NaOH in methanol, 1N NaOH in methanol dry it for 30 min + Nitrocellulose and observed under UV light to emits the color as shown in Table 4. Qualitative analysis of the stem drug indicated the presence of proteins, reducing and non reducing sugars, saponins, fats, tannin, glycoside and alkaloids in the plant (Table 5). The quantity of proteins, carbohydrate, starch and flavonoid are mentioned in Table 6. In HPTLC study, the methanolic extract is sonicated for 5 min and then centrifuged at 10,000 rpm for 5 min. The extract was filter and filtrate is used as an application for quantifaction of flavonoid. For each application 10µl extracts were used and loaded on instrument comprising of Linomat5 for application using densinometer-TLC scanner3 with "WINCATS" software (Camag, Switzerland). These studies were carried out on precoated aluminum fluorescent plates (E. Merck). The plates were scanned at 254 and at 366nm (Wagner and Bladt, 1996; Reich and Schibii, 2007) (Graph Iand II; Table-7and8).

Table 2: Ash and Acid insoluble ash of *Tridax procumbens* stem

Sample	Total ash %	Acid insoluble %
Stem	13.5%	13%
Leaf	13.5%	1.4%

Table 3: Percentage extractives of *Tridax procumbens* Stem and Leaf

Sr. No.	Solvent Used	% extractive (Dry Wt.) for stem	% extractive (Dry Wt.) for leaf
1	Acetone	22.6%	21.2%
2	Benzene	4.4%	7.8%
3	Chloroform	3.4%	6.8%
4	Petroleum ether	3.6%	5.6%
5	Abs. Alcohol	8.8%	8.8%
6	Distilled water	27.8%	33.2%

Table 4: Fluorescence analysis of *Tridax procumbens* stem and leaf

Sr. No.	Test	Color Emits (For Stem)	Color Emits (For Leaf)
1	Powder as such	Greenish yellow	Greenish yellow
2	Powder as such U. V. light	Grayish green	Grayish yellow
3	Powder + Nitrocellulose	Grayish yellow	Grayish black
4	Powder + 1N NaOH in Methanol	Greenish yellow	Grayish green
5	Powder + 1N NaOH in Methanol dry it for 30 min. + Nitrocellulose	Blackish green	Grayish black

Table 5: Phytochemical study of *Tridax procumbens* stem and leaf

Sr. No.	Reagents	Test for	Result for Stem	Result for Leaf
Alcohal extract	Wagner's	Alkaloid	+ ve	+ ve
	Mayer's	Alkaloid	+ ve	+ ve
	Dragendorff's	Alkaloid	+ ve	+ ve
	Hager's	Alkaloid	+ ve	+ ve
	Conc. HCL + Mg turning	Flavonoid	+ ve	+ ve
	Benzene	Glycoside	+ ve	+ ve
Water extract	I_2KI	Starch	+ ve	+ ve
	Millans	Protein	+ ve	+ ve
	Conc H_2SO_4	Saponin	+ ve	+ ve

Table 6: Quantitative estimation of *Tridax procumbens* stem and leaf

Quantitative estimation	Protein (mg/gm)	Carbohydrate (mg/gm)	Starch (mg/gm)	Flavonoid (mg/gm)
Stem	1.29650	1.60012	1.98672	1.11213
Leaf	1.32440	1.23654	1.99254	1.13242

Table 7: Showing the peak values for Flavonoid for 10µl plant extract (Stem)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area%
1	0.01	1.1	0.13	100.3	11.65	0.14	96.2	4529.4	8.73
2	0.16	94.7	0.34	228.1	26.52	0.36	221.9	19651.4	37.89
3	0.37	221.6	0.44	276.4	32.14	0.52	0.1	17058.0	32.89
4	0.55	2.6	0.67	92.0	10.70	0.70	79.9	5537.4	10.68
5	0.71	81.5	0.74	92.0	10.70	0.79	50.4	3841.7	7.41
6	0.93	38.6	0.94	44.5	5.17	0.95	21.4	602.6	1.16
7	1.02	25.4	1.04	26.9	3.12	1.06	21.2	644.8	1.24

Table 8: Showing the peak values for Flavonoid for 10µl plant extract (Leaf)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area%
1	0.02	6.8	0.15	102.7	17.92	0.17	93.3	5635.7	12.85
2	0.27	146.1	0.45	268.0	46.78	0.53	2.8	28804.2	65.68
3	0.57	1.0	0.69	88.6	15.47	0.71	83.9	5363.3	12.23
4	0.72	84.8	0.74	92.0	16.05	0.82	29.8	3885.0	8.86

Fig 1: Habit of *T. procumbens*

Fig. 2: Trichomes

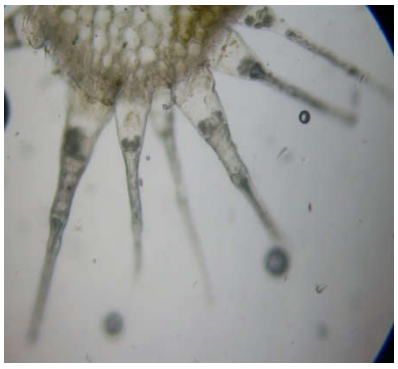


Fig. 3: Trichomes

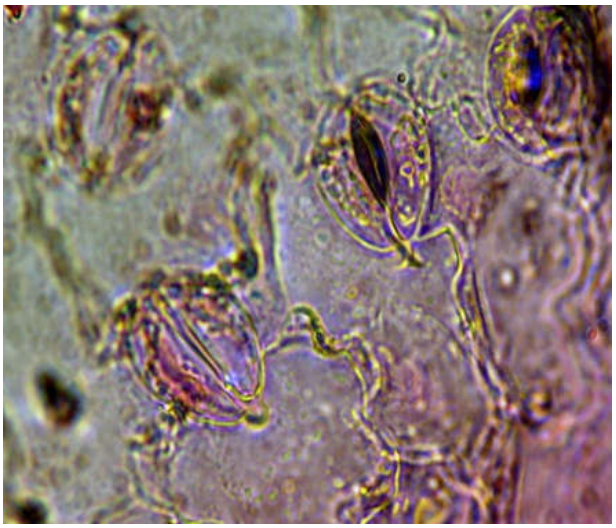


Fig. 4: Anatomy of Stomata

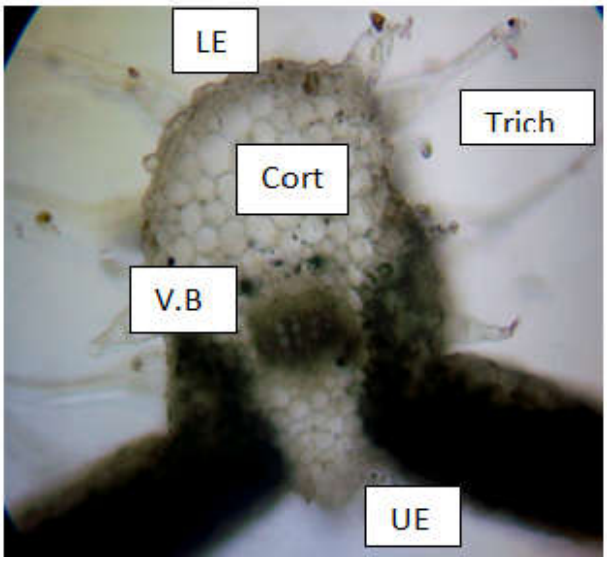
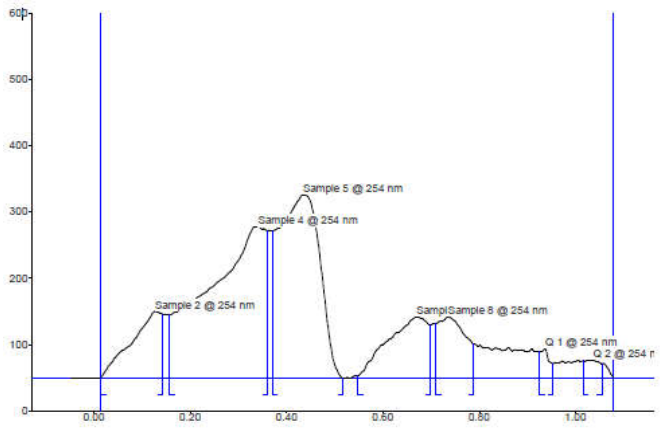
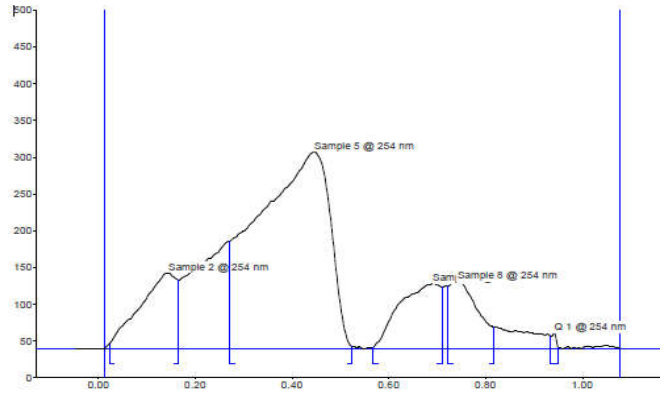


Fig. 5: Microscopic observation of Leaf of *T.procumbens*
UE- upper epidermis, LE- lower epidermis, Cort- Cortex, Trich- Trichome, V.B.- Vascular bundle



Stem



Leaf

Estimation of Phytoconstituents by HPTLC Analysis

Fig 6: For visible light

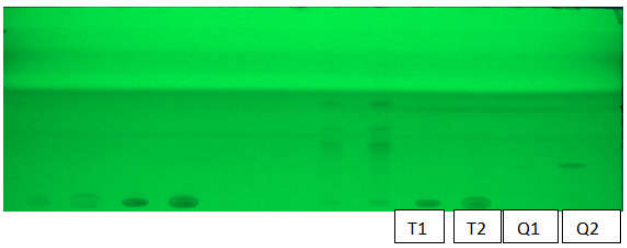


Fig 7: For 254nm

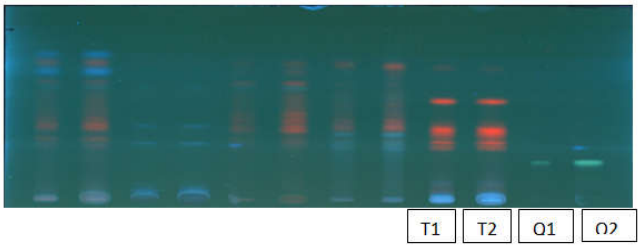


Fig 8: For 366nm

T1 (5µl) & T2 (10µl)-*T.procumbens* (Stem and Leaf)
Q1 (5µl) & Q2 (10µl) - Std. Quercetin

Conclusions

The plant *T. procumbens* showed the correct taxonomy which is helpful for the standardization of drug. Findings of the present investigation will be useful for the correct botanical identification and authentication of the drug.

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