STANDARDIZATION, FINGERPRINTING, AND QUALITY CONTROL OF WOUND HEALER PLANT-*TRIDEX PROCUMBENS* L.

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Abstract: *Tridex procumbens L. (Asteraceae)* is a procumbent herb found throughout India. This plant is commonly known as the 'coat button' in English and 'Jayantiveda' in Sanskrit. This plant is often treated as a weed, and its pharmaceutical potential has been neglected. So, in the present investigation, exomorphic, anatomical, pharmacognostic, physicochemical and chromatographic (fingerprinting) standards had been evolved for *T. procumbens* L. for quality control. This plant has useful pharmaceutical properties viz. anti-diabetic, antioxidant, anti-inflammatory, wound healing, analgesic, hepato-protective, anticancer, and immune-modulatory. *T. procumbens* L. has ethnomedicinal importance. It is widely used in the formulations that cure wounds. *T. procumbens* is a rich source of phytochemicals such as alkaloids, flavonoids, saponins, carotenoids, B-sitosterols, fumaric acid, luteolin, and tannins. Anatomical parameters and quantitative microscopy provide a valuable key for the identification of this plant. The leaves of this plant have a thick cover of both glandular and eglandular trichomes. The presence of anomocytic stomata on both the surfaces of the leaf is a characteristic feature of *T. procumbens* leaf. The physicochemical parameters viz. total ash, water-soluble ash, and acid-insoluble ash values were recorded as 12.60, 10.24, and 2.30% w/w respectively for leaf powder. The extractive values of leaf powder with ethanol, chloroform, acetone, water, and methanol yielded 8.80%, 4.10%, 7.20%, 8.60%, and 9.5% w/w respectively. The fluorescence studies of leaf showed a distinctive pattern. The characteristic TLC pattern of leaf extracts with Chloroform: Methanol (93:7) solvent system serves as the fingerprint for *T. procumbens* L.

Index Terms: Ash values, fingerprinting, Physico-chemical parameters, quality control, *T. procumbens* L., Thin Layer Chromatography, and wound healing.

1.INTRODUCTION

Tridex procumbens L. (Asteraceae) is a procumbent herb commonly known as the 'coat button' due to its typical shape of a flower. This plant is known as 'Jayantiveda' (in Sanskrit) and 'Gamra'(in Hindi). *T. procumbens* L. is widely distributed weed found everywhere in India, Asia, America, Tropical Africa, and Australia [1]. This plant primarily grows in the rainy season. This plant has many useful pharmaceutical properties such as anti-diabetic [2], antioxidant [3] anti-inflammatory, wound healing, analgesic, hepatoprotective [4], anticancer [5], immune-modulatory [6] and anticoagulant [7]. This plant also possesses anti-bacterial, anti-fungal, insecticidal, and parasiticidal properties. This plant is often treated as a weed, and its pharmaceutical potential has been neglected. WHO emphases on standardization of medicinal plants [8]. Qualitative and quantitative studies of this plant-based drug are an important step to establish its botanical quality control. In view of this plant's ethnomedicinal and biotherapeutic potential, exomorphic, anatomical, pharmacognostic, physicochemical, and chromatographic (fingerprinting) standards had been evolved. The evaluation of ash value, extractive value, fluorescence analysis, and fingerprinting ensure the quality and purity of the crude drug. The quantitative microscopy also provides some key parameters for the standardization of this plant. Primarily the standardization establishes identity, quality, and purity of *T. procumbens* L.

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2. MATERIALS AND METHODS

2.1. Plant material: The plant specimens were collected from Western Ghats (Tamhini, Pune). The healthy and fully matured leaves were taken for the investigation. The plant was collected and authenticated using standard methods [9]. The leaves were dried in a semi-shade condition for further use.

2.2 Microscopic studies: Free-hand sections of 10 µm-12 µm thickness were taken, stained with phloroglucinol -hydrochloric acid (1:1), and mounted in glycerin. The Camera lucida drawings of a transverse section of leaf were taken. The permanent slides were made as per the standard procedure. The leaf powder was prepared using sieve mesh 60 (Sixty), and further microscopic investigations of leaf powder were made treating with phloroglucinol-hydrochloric acid (1:1) solution.

2.3 Fluorescence studies: The fluorescence analysis was carried out as per the standard method [10]. The mountant medium such as distilled water, 1 N NaOH, 1 N HCl, 1 N H_2SO_4, and 1 N HNO_3 was used. The fluorescence at ordinary light and at under 254nm and 366nm UV light was recorded. The fluorescence of solvent extracts of water, alcohol, acetone, and chloroform was examined under normal light and UV light. The colour for fluorescence was confirmed from 'A Mycological Colour Chart' of Rayner [11].

2.4 Physico-chemical analysis: The physicochemical studies were carried out as per the WHO guidelines [12]. The parameters viz. total ash, water-soluble ash, acid-insoluble ash, and sulphated values were determined. Water, ethanol, acetone, chloroform, and methanol soluble extractive values were determined. The leaf extractives of *T. procumbens* L. were used for chemical analysis. The standard phytochemical tests were performed to detect the presence of phytochemicals present in the leaf [13].

Thin Layer Chromatography (TLC): It has been carried out as per the standard methods [14].

Solvent system (s): For leaf extract in chloroform, Benzene: Ethyl acetate (4:1) solvent while for ethanol leaf extract Chloroform: Methanol (93:7) solvent system were used. The TLC was carried out on precoated E. Merk silica gel plates of 0.30 mm thickness. After air-drying, the plate was visualized in UV 254 nm, 366 nm, and iodine vapour. The Rf values recorded. For the final outputs of TLC plates, the standardized conditions were maintained.

2.5 Photomicrographs: Photographs of different magnifications were taken with Canon Microscopic unit, and the microscopic descriptions of selected tissues were supplemented with micrographs.

3.RESULTS AND DISCUSSION

3.1Macroscopical characteristics: This plant is a procumbent herb with daisy-like yellow-centered white or yellow flowers with three-toothed ray florets. (Fig.1a).

Figure 1a Habit of T. procumbens plant 1b Leaf of T. procumbens

The details of the macroscopic characteristics of *T. procumbens* leaf have been tabulated in table no.1.

Table no.1 Characteristics features *T. procumbens leaf*

Characteristics	**Observations**
Type	Opposite
Shape	Lanceolate to ovate
Margin of leaf	Irregular toothed
Dimensions of leaf (cm)	3.1-7.2 X 0.5-2.5 cm
Colour of leaf	Dark green
Odour	Characteristic
Taste	Acrid
Texture	Rough
Petiole(long)	0.7-1.3 cm

Microscopic study of leaf: Anatomical peculiarities of *T. procumbens* has been tabulated in Table no. 2.

Fig 2. T.S. *T. procumbens* leaf (X100)

Table no.2. Anatomical peculiarities of *T. procumbens* leaf.

Anatomical parameters	Important peculiarities
Cuticle	Thick
Epidermis	Single layered
Trichomes	Simple, multi-celled (3-5), basal cells of trichomes are swollen, and it looks like a claw. Glandular and non-glandular type of trichomes present
Stomata	Anomocytic stomata are present on the upper and lower surface of the leaf
Palisade cells	Single layered when T. S. passing through the laminar region
Mesophyll cells	5-8 celled when T. S. Passing through the laminar region
Parenchyma cells	Compactly arranged and devoid of intercellular spaces
Cell contents	Starch grains are simple and round.
Vascular bundle	Single centrally located, and it is surrounded by parenchymatous cells.

Powder microscopy: It showed the presence of fragments of thin-walled parenchyma cells, palisade cells, mesophyll cells, simple starch grains, elongated spiral xylem vessels, phloem fibres, glandular and e-glandular trichomes. In view of the importance of quantitative microscopy, the details of leaf cells and leaf constants are tabulated in Table no. 3a and 3b.

Table no.3. a) Quantitative microscopic studies leaf powder

Cells and cell contents	Dimensions
Trichomes (length)	185-240 µm
Palisade cells	58-75 X15-32
Vessels (length)	160-210 µm
Phloem fibres (length)	160-175µ
Starch grains	8-40 µ

Table no.3. b) Quantitative microscopic studies of leaf (constants)

Parameters	Leaf constants
Palisade ratio	1:9
Veinislets number	8-14
Vein termination number	2-4
Stomatal number (Upper)	13.3-22.2
Stomatal number (Lower)	30-40
Stomatal index (Upper)	15.0-23.3
Stomatal index (Lower)	22.0-26.5

The earlier researchers have recorded higher values of the stomatal index, veinislet number, and vein termination number [15].

Physico-chemical characteristics of leaf: The constant physical evaluation of the drugs is an important parameter in detecting adulteration of the drugs. Total ash is important in the evaluation of the purity of drugs. This value indicates the amount of minerals and earthy materials attached to the plant material. The results of ash values and extractives have been tabulated in Table no. 4.

Table 4. Physico-chemical parameters of *T. procumbens* L. leaf

Sr.no.	Parameters	Values (with range)
1	Foreign matter	< 3.0 %
2	Total ash	12.60% w/w (10.40-14.20)
3	Water-soluble ash	10.24% w/w (8.2-12.2)
3	Acid-insoluble ash	2.30 w/w (1.92-2.60)
5	Sulphated ash	3.6 % w/w (2.24-4.41)
6	Ethanol extractives	8.80 w/v (7.2-9.2)
7	Chloroform extractives	4.1% w/v (3.70-4.90)
8	Acetone extractives	7.20 w/v (5.8-9.2)
9	Water extractives	8.60 w/v (7.80-9.20)
10	Methanol extractives	9.5 w/v (8.8-10.20)

The earlier workers reported % of higher values of total ash value, acid insoluble ash, water-soluble ash, water extractive, and methanol extractives 14.0, 2.9,2.1, 16.26, and 10.40, respectively [15].

Fluorescence analysis: It is an easy and effective identification tool for the detection of adulterants and to check the purity of powders.

Table no.5 Fluorescence characteristics of leaf powder of *T. procumbens*

Sr no	Mountant medium	254nm	366nm	Natural daylight
1	Dry powder	Vinaceous buff	Vinaceous buff	Dark vinaceous
2	P + D.W.	Dark herbage green	Sepia	Isabelline
3	P+1N HCl	Dark herbage green	Umber	Olivaceous
4	P+1N HNO_3	Livid vinaceous	Brown vinaceous	Fawn
5	P+1N H_2SO_4	Dark green herbage	Darkvinaceous	Fiscuous black
6	P+1N HNO_3	Dull green	Greenish black	Gray olivaceous

Table no.6. Fluorescence characteristics of *T. procumbens* leaf extracts

Sr no	Extracts	256nm	366nm	Natural daylight
1	Water	Umber	Fiscuous black	Chestnut
2	Chloroform	Greenish black	Yellowish green	Vinaceous grey
3	Acetone	Greenish grey	Olivaceous black	Herbage green
4	Ethanol	Sepia	Dark brick	Dark vinaceous
5	Methanol	Brown vinaceous	Sepia	Chestnut

Preliminary phytochemical analysis: The activity of the drug is due to the presence of a particular phytochemical in appropriate concentration. The phytochemical and chromatographic studies are of paramount importance in the standardization of the plant-based drugs. In view of this, phytochemical and TLC studies have been carried out. The results of phytochemical tests have been recorded in table no.7.

Table no. 7. Preliminary phytochemical tests T. *procumbens* L.

Sr no	Chemicals	Test Performed	Extracts				
			Water	Chloroform	Acetone	Ethanol	Methanol
1	Alkaloids	Dragendorff's test	-	-	-	+	+
2	Flavone	Shinoda test	+	+	+	-	-
3	Steroid	Liebermann-Burchard reagent	+	-	+	-	-
4	Tannins	$FeCl_3$	+	-	-	+	-
5	Sugar	Molisch's test	+	-	-	+	-
6	Terpenes	Noller's test	+	+	-	+	-
7	Glycosides	Berlin-blue reaction	-	+	+	-	-

'+' sign indicates the presence of a particular phytochemical while '-'indicates that the particular phytochemical is not detected.

Phytochemically leaf exhibited alkaloids, flavone, steroids, tannins, sugars, terpenes, and glycosides. The earlier workers reported the presence of alkaloids, glycosides, phenols, flavonoids, steroids, tannins, and saponins in the methanol extract while in water extract only glycosides [16]. *T. procumbens* is the rich source of valuable phytochemicals such as alkaloids, flavonoids, saponins, carotenoids, B-sitosterols, fumaric acid, luteolin, and tannins.

Thin Layer Chromatography (TLC) analysis: TLC technique has applications in standardization, determination of the ingredients of formulations, and detection of adulterants or substitutes. TLC is a major qualitative tool for the study of admixtures. In the present investigation, TLC was performed using Benzene: Ethyl acetate (4:1) and Chloroform: Methanol (93:7) solvent system.

Fig. 3 T. procumbens leaf TLC pattern (chloroform extract)

Table no. 8. TLC pattern of various extracts of *T. procumbens* leaf

Sr	Extractives	Adsorbent	Solvent system	Viewing	Rf Values (Retention factor)
1	Chloroform	Precoated silicagel	Benzene: Ethyl acetate (4:1)	Iodine vapor	0.41,0.55,0.70, 0.90
2	Distilled water	Precoated silicagel	Chloroform: Methanol (93:7)	Iodine vapor	0.30, 0.55
3	Ethanol	Precoated silicagel	Chloroform: Methanol (93:7)	Iodine vapour	0.17, 0.29, 0.35, 0.82, 0.94

Table no. 10. TLC Fingerprint of *T. procumbens* leaf ethanol extract (Solvent system- Chloroform: methanol (93:7)

No. of spots	Rf value	254nm	366 nm	Iodine developer
1	0.17	Faint blue	Intense blue	Faint yellow
2	0.29	Faint blue	Faint blue	Yellow
3	0.35	Faint blue	Intense blue	Yellow
4	0.82	Intense blue	Intense blue	Faint yellow
5	0.94	Intense blue	Intense blue	Yellow

In the present investigation, a unique fingerprinting pattern for *T. procumbens* leaf has been worked out using two solvent systems viz. chloroform: methanol (93:7) and Benzene: ethyl acetate (4:1).

CONCLUSION: *T. procumbens* contains various bioactive phytochemicals that provide a therapeutic cure for many health problems. The multidisciplinary approach ensures reproducible quality of herbal products. Exomorphic, microscopic, quantitative microscopy, pharmacognostic, preliminary phytochemical, and TLC studies are useful in authentication, detection of adulteration, and to evolve standards for quality control of *T. procumbens* L.

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