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Review Article

In-Vitro Evaluation Of Tridax Procumbens In The Treatment Of Urolithiasis

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ABSTRACT

India has been blessed with an abundance of medicinal herbs primarily because of the country's climate and seasons, which promote the growth of various plant species. Tridax procumbens is a typical medicinal herb that is generally found in India as a weed and pest plant. It is classified as part of the Asteraceae family and is commonly referred to as Coat Button, Kansari (Hindi), or Ghamara (local language). The most valuable medicament utilized for the manufacturing of compounds described in Ayurvedic literature is Tridax procumbens. It is mostly used as an anticoagulant, an antifungal, and a wound healing agent insect repellent, dysentery and diarrhea in Indian traditional remedies. And their extract has various pharmacological properties (anti-inflammatory, hepatoprotective, immunomodulating, antimicrobial or antibacterial, antiseptic, anticancer, repellent, hemostatic, antidiabetic, urolithiasis, blood pressure lowering, antioxidant, bradycardia, etc.). The chemical components of the plant showed that its leaves contain various alkaloids, flavonoids, carotenoids, fumaric acid, etc. The synthesis of aromatic substances in the plant results in its primary secondary metabolites, such as the phenol derivative and oxygen oxidation. It is also active against antimicrobial agents such as gram-positive and gram-negative bacteria. It is also used as an adsorbent for chromium. Here we try to focus on the broad phytochemical and pharmacological activities of Tridax Procumbens.

INTRODUCTION

Tridax procumbens could be a species of blossoming plant within the daisy (Asteraceae) family. It is best known as a broad weed and bug plant. The plant is local of tropical America and naturalized in tropical Africa, Asia, and Australia. It could be a wild herb dispersed all through India.

It is yearly or biennial to some degree patently hispid herbs. Stem branched, inching at base, sub erect or trailing over.^[1]

Tridax procumbens is best known as a far reaching weed and bother plant. T. procumbens is additionally known as 'Mexican daisy' (in Mexico), 'Coat button' and 'Tridax daisy' (in

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English), 'Jayanti Veda' (IN Sanskrit), 'Gharma' (in Hindi), 'Dagadipala' (in Marathi), 'Vettukkaaya-thalai' (Tamil/Siddha) and 'Akala kohadi' (in society).^[14] It is frequently establishing at hubs with singular, long stalked, yellow composite, heterogamous, promiscuous blooms with white blooming heads and exceptionally shaggy, coarsely toothed, petislate, praise or lanceolate takes off.^[13] It is found in street sides, squander ground, railroads, riverbanks, glades. This plants wide spread conveyance and significance as a weed due to the spreading steam and inexhaustible seed generation. ^[2,3] *Tridax procumbens* is found to have pharmacological exercises like hepatoprotective impact, immunomodulating property, promising wound recuperating movement, antidiabetic, hypertensive impact, antimicrobial, creepy crawly repellent action, against incendiary and antioxidant, bronchial catarrh, diarrhea, loose bowels moreover anticipate falling of hairs and leads to hair development advancement.^[1] Flavonoids,

fundamental oils, saponins, tannins, steroids, alkaloids, carbohydrates, carotenoids, terpenoids, and other chemical substances have been separated and recognized from this species.^[14]

Scientific classification of *Tridax procumbens*:
[3,16]

Table 1: Classification Of *Tridax Procumbens*

Kingdom	Plantae
Division	Angiosperms
Arrange	Asterales
Family	Asteraceae
Genus	<i>Tridax</i>
Species	<i>Procumbens</i>
Scientific title	<i>Tridax Procumbens</i> Linn

The complete plant and seed being utilized to treat an assortment of afflictions the clears out are cooked and eaten as a vegetable. But no Pharmacognostical work has been done so distant. Subsequently, an endeavor has been made to consider the Pharmacognostic parameters on the takes off of *Tridax procumbens* L.^[13]

Table 2: Plant Information Of *Tridax Procumbens* Linn

Parts	Observation
Whole Plant	Semi prostrate, annual, creeper herb with stem ascending to 30-50 cm in height, branched, sparsely hairy and rooted at nodes. ^[16]
Leaves	Arrangement Simple, Opposite, Size 3-7 cm long, 1- 4 cm wide, Colour Green, Odour Characteristic, Taste Acrid, Appearance Rough & Scabrous, Margin Irregularly toothed, Apex Acute, Base wedge shaped, Petiole Short, Texture Short, Hairy on both surface, Fracture Easy. ^[11,16,17]
Upper epidermis	Appears single layered, multicellular covering trichome and lower epidermis is single layered, stretched cell and closely organized. ^[16]
Xylem vessel	Appears the nearness of calcium oxalate precious stones. ^[16]
Flowers	This has two sorts of blooms: beam florets and plate florets with basal placentation Blooms are tubular in nature, yellow in color with hairs having a capitulum inflorescence. ^[16]
Stems	Green, woody, erect, and round and hollow, up to 40 cm in tallness bearing various branches and 3-6 mm thickness, smell was characteristic and taste was harsh ^[18]
Fruits	Natural product may be a difficult achene secured with hardened hairs and having a fluffy, plume-like white pappus at one conclusion, which helps in ethereal dispersal. ^[16]





Fig 1: A. Whole Plant



B. Tridax Leaves

MATERIAL AND METHODS:

Plant material :-The *Tridax procumbens* linn plant [figure 1(B)] by uprooting the whole plant and their leaves were removed very carefully. The cleaned leaves were shade dried for one week.

Extraction of plant material: -The cleaned *T. procumbens* leaves were allowed for the complete shade drying and then made the fine powder with mechanical grinder and store in an airtight container. A powdered plant parts were extracted successfully with the organic solvent ethanol by using Soxhlet apparatus

Apparatus and chemicals used in extraction process:

Apparatus: Soxhlet extractor, heating mantle, Round bottomed flask, reflux condenser, stand.

Chemicals: ethanol.

Extraction:

It is dried powder material then obtained is seep with polar solvent (ethanol) for 6 hours in Soxhlet extractor followed by polar solvent ethanol.



Fig 2: Soxhlet Extraction Of Tridax Procumbens

Procedure of extraction: 30gm powdered form of *T. procumbens* is poured into Soxhlet extractor and its level is maintained. Cotton is used to cover connectors to prevent flow of fluids. After setting Soxhlet extractor electricity is being supplied through heating mantle and temperature is maintained at 100°C. Then Soxhlet extractor is being connected to a cooler to provide chilling. This process is continued for 6 hours. In such 6 hours process it involved step by step process in which firstly solid matrix is placed in Soxhlet thimble, solvent is heated under reflux. Then condensation and extraction with fresh solvent. Solute are transferred from extractor chamber into reservoir. Exhaustive extraction is complete.

Phytochemical Screening (Covering) Of Tridax Procumbens: [3,9,10]

Table 3: Qualitative Test For T. Procumbens Linn

Chemical constituents	Chemical Test	Observations	Results (positive)/(Negative)
1. Alkaloids	Dragendorff's test or Mayer test	Orange/red accelerates (Precipitate) and yellowish precipitation.	Positive

2. Flavonoids	Soluble (Alkaline) reagent	Strongly yellow color	Positive
3. Glycosides	Bronberg's test	Pink color (Alkali layer's) (Ammonia layer's)	Positive
4. Tannin	Fecl3 test	Green of blue-black coloration.	Negative
5. Saponins	Foaming test	Froth (Foam)	Negative
6. Terpenoids	Nollers test	Purple color to red (ruddy red)	Positive

Preliminary Phytochemical Screening (Covering) Of T. Procumbens:

The distinctive organic dissolvable extracts of *Tridax procumbens* were utilized to screen the taking after phytochemicals like sugar, alkaloids, phenolic compounds, Flavonoids, tannins, saponins, amino acids and ascorbic acids. Chemical tests were carried out on the watery extricate (Extract) on the powdered examples utilizing standard methods. [7]

Thin Layered Chromatography: [1] Thin layer chromatography is done as employing a thin, uniform layer of silica gel coated onto a piece of glass. The stationary stage for thin layer chromatography too frequently contains the mobile stage could be a appropriate fluid dissolvable or mixture of solvents. The RF value for each compound is at that point worked out utilizing the equation. (Mobile Phase: 7ml Hexane: 3ml Acetone) [8].

Equation for Thin layer chromatography:

RF Value (Retention Factor): Distance travelled by solute / Distance travelled by solvent

Experimental Work

In vitro study:

Evaluation for anti-urolithiatic activity:

Step1: preparation of experimental kidney stones (calcium oxalate stones)by homogeneous precipitation :

Exactly 1.47 g of calcium chloride dihydrate was dissolved in 100 ml distilled water and 1.34 g of sodium oxalate was dissolved in 100 ml of 2 N sulfuric acid. Equimolar prepared solutions of

calcium chloride dihydrate and sodium oxalate were allowed to react in a beaker to precipitate out calcium oxalate with stirring. The resultant calcium oxalate precipitate was freed from traces of sulfuric acid by ammonia solutions, washed with distilled water, and dried at 60°C for 2 hour.

Step2: preparation of semipermeable membrane from farm eggs;

The semi – permeable membrane of eggs lies in between the outer calcified shell & the inner contents like albumin & yolk ,shell was removed chemically by placing the eggs in 2M HCL for an overnight, which caused complete decalcification .further, washed with distilled water, & carefully with a sharp pointer a hole is made on the top & the contents squeezed out completely from the decalcified egg, then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution ,in the moistened conditioned for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7-7.4.



Fig 3: Preparation of semipermeable membrane from egg

Step3: Estimation of calcium oxalate by titrimetry:

Weighed accurately 1 mg of the calcium oxalate and 10 mg of the extract /compound / and packed it together in semi permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer .one group served as negative control (contained only 1 mg of calcium oxalate). place the conical flask of all groups in an incubator,

preheated to 37°C for 2 hours, for about 7-8 hours. Removed the contents of semipermeable memberane from each group into a test tube added 2 ml of 1N sulphuric acid and titrated with 0.9494 N $Kmno_4$ equivalents to 0.1898 mg of 4 calcium. The amount of undisclosed calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substances could dissolved.



Fig 4(a): Initial phase

Analytical Determination of Bioactive Compounds UV-Visible Infrared Spectroscopy
UV-visible infrared (IR) spectroscopy is qualitative and an analytical screening approach together with chemometric pattern recognition for the identification of bioactive components from the plants and plant products. Naturally found compounds and phenolic compounds such as tannins, phlobatannins, dyes,anthocyanins, and phenols form complexes with iron which can be easily detected. Typically, ultraviolet (UV) region range extends from wavelength 190 to 350 nm, visible range is 350 to 800 nm, and infrared range is from 800 to 2500 nm. UV visible spectroscopy mostly preferably used for the quantitative analysis of aromatic molecules, as they have strengthened chromophores in the range of UV.

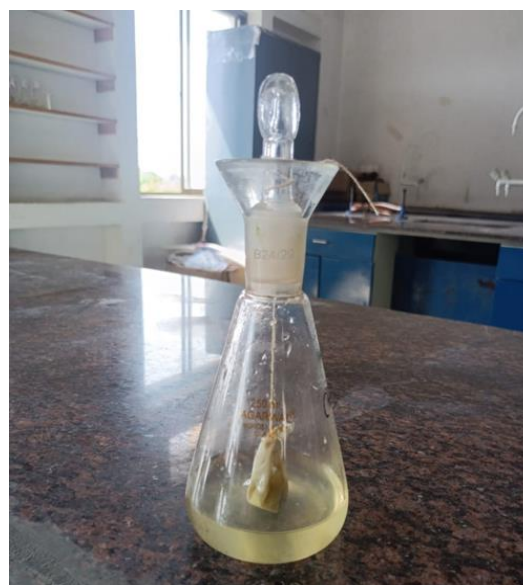


Fig 4(b): End Phase

This technique is cost-effective and not time consuming compared to other techniques for the determination of bioactive compounds. Antinutritional factor especially phytic acid is present in horse gram sprouts; IR and MS are used to characterize the bioactive compounds in the sprout extract.

Titration against 0.9494N $Kmno_4$: -

The invitro Anti-Urolithiatic activity was performed by using extracts of *Tridax procumbens* leaves in Calcium oxalate are as follows:

Table 4: Titration Against 0.9494n $Kmno_4$

Sr. No	Burette reading	In ml
1)	Blank	0.00
2)	Ethanolic extract	14

CONCLUSION:

The result expected from the experimental work suggests that this investigation would provide encouragement for further exploration into new drugs for the prevention and treatment of urolithiasis. The present investigation provides useful information on antiurolithiatic activity of leaves of *tridax procumbens*. The extract showed dissolution of stones (calcium oxalate). Further in-vivo pharmacological and clinical studies with suitable experimental models are required to understand the mechanism and the actual efficacy of the plant *butea monosperma* in treating urolithiasis.

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