

Antifungal activity of acetone and Ethanolic extract of Leaves of *Tridax Procumbens*

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Abstract: In the present study the ethanol and acetone extract of *Tridax procumbens* leaves were extracted by soxhlet apparatus and were screened for the presence of phytochemical components and tested for antifungal activity against *Candida albicans*, *Aspergillus niger*. Results revealed the presence of steroids, alkaloids, saponins, tannins, glycosides and the effective extraction with acetone/ethanol extract with 1:3 ratios of *tridax procumbens* has wide range of antifungal activity against fungal pathogens. *Tridax procumbens*, soxhlet apparatus, anti-fungal activity.

Key words: *Tridax procumbens*, Soxhlation, Antifungal activity.

Introduction

Plant monograph:

Botanical classification; Kingdom: plantae Division: Magnoliophyta; Family: asteraceae; Species: *Tridax procumbens* linn; Synonyms: Vernacular names *tridax procumbens*; English: coat button and *tridax daisy*; Sanskrit: *Jayanthi veda*; Hindi: *ghara*; Telugu: *gaddi chemanthi*.

Description: The plant bears daisy like yellow-centered white or yellow flowers with three-toothed ray with stiff hairs and having a feathery, plume like white pappus at one end. Calyx is represented by scales or reduced to pappus. The plant is invasive in part because it produces so many of these achenes, up to 1500 per plant, and each achene can catch the wind in its pappus and be carried some distance. This plant can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides in areas with tropical or semi-tropical climates. Plant occupies tropics and subtropics throughout the world. Native to Central America and tropical South America. Habitat: Coarse textured soils of tropical regions, Sunny, dry locations, especially sandy and rocky sites like roadsides, railways, dunes, and waste places.

Morphological Characteristics: Leaves are opposite, simple, carried by a petiole, 1 to 2 cm long. They are thick, soft and dark green. The lamina is oval to lanceolate, 2 to 6 cm long and 2 to 4 cm wide, base attenuate in the corner and with strongly and irregularly serrated margin. Both sides are hispid, with tuberculate based bristles. Pubescence is most abundant on the underside. Inflorescences in solitary capitulum, held by a peduncle, 12 to 32 cm long, abundantly hispid. The bracts of the involucre are arranged in 2 rows. They are oval to lanceolate; 6 mm long, pubescent and green. Flowers are Capitulum formed of 3 to 8 ligulate daisy like female flowers, creamy white on the periphery of capitulum, tridentate. In the center of capitulum, flowers are yellow, tubulate bisexual. The tube, 6mm long, with five short lines at the top. This plant has two types of the flower as ray florets and disc florets with basal placentation. The fruit is a conical achene, 3.5 mm high, pubescent and brown to black at maturity. It is surrounded by a pappus of feathery bristles, horizontally prostrate at maturity. Stem and Root Stem is cylindrical, hispid, covered with multi-cellular hairs of 1 mm; tuberculation at the base. The root is a strong taproot. Whole plant has medicinal values. List of various pharmacological activity of parts of plant *Tridax procumbens* Whole plant can have Antimicrobial activity against both gram-positive and gram-negative bacteria¹ anti-coagulant, anti-inflammatory

Flowers-Anti septic, Insecticidal, analgesic² and Parasiticidal³.

Aerial parts-Hepato protective.

Leaves-Wound healing, to check hemorrhage from cuts, bruises and wounds Hypotensive activity, anti-diabetic activity⁴, dysentery, Diarrhea to prevent falling of hair and promotes the growth of hair. Immunomodulation property, anti-oxidative,^{5,6} insect repellent activity.

General information: *Tridax procumbens* is a perennial herb that has a creeping stem which can reach from to 8-30 inches (20-75 cm) long. The leaves of *Tridax procumbens* are opposite, pinnate, oblong to ovate, and 1-2 inches (2.5-5 cm) long with cuneate bases, coarsely serrate margins, and acute apices.

Chemical constituents: The chemical constituents present are alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins. Mineral composition present in leaves is calcium, magnesium, potassium, sodium and selenium. Leaf mainly

contains crude proteins 26%, crude fiber 17% soluble carbohydrates 39% calcium oxide 5%, Luteolin, glucoluteolin, quercetin and isoquercetin. Whereas the oleanolic acid, fumeric acid, fl-sitosterol and tannin is present in good amounts.

Materials and Methods

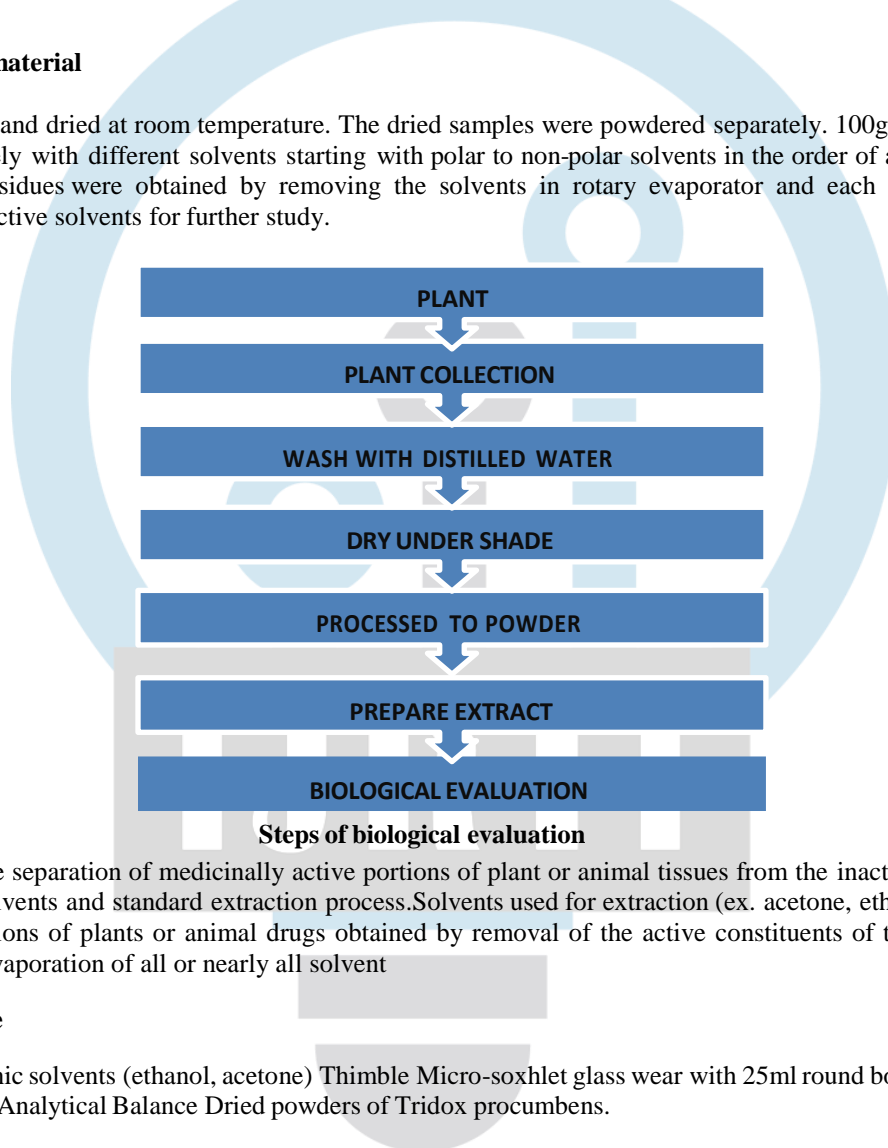
Sample Collection

Tridax procumbens and leaves were collected near Chilkur Balaji College of Pharmacy, Aziz nagar, Moinabad in the month of April.

Materials Required: Soxhlet apparatus, leaves powder, acetone, ethanol, whatsmann filter paper, weighing machine, beakers, round bottom flask and funnel. Authentication: *Tridax procumbens* exhibits various biological activities, such as anti-hepatotoxic, antioxidant, anti-inflammatory, analgesic, wound-healing, antimicrobial, immunomodulatory and anti-cancerous properties. *Tridax procumbens* L. is a medicinal plant and used as a drink to treat bronchial catarrh, diarrhea, dysentery and liver diseases. In this study, we evaluated the potential use of *T. procumbens* to treat hyperuricemia, oxidative stress, and bacterial infection, anti-fungal infection.

Preparation of plant material

Leaves were collected and dried at room temperature. The dried samples were powdered separately. 100gm each of the sample was extracted separately with different solvents starting with polar to non-polar solvents in the order of aqueous, ethanol, and acetone. The crude residues were obtained by removing the solvents in rotary evaporator and each of the extracts were suspended in the respective solvents for further study.



Extraction involves the separation of medicinally active portions of plant or animal tissues from the inactive components through the use of selective solvents and standard extraction process. Solvents used for extraction (ex. acetone, ethanol) Extractives are the Concentrated preparations of plants or animal drugs obtained by removal of the active constituents of the respective drug with suitable menstruum, evaporation of all or nearly all solvent

Preparation of sample

Materials used: Organic solvents (ethanol, acetone) Thimble Micro-soxhlet glass wear with 25ml round bottomed flask Heating Mantle Rubber tubing Analytical Balance Dried powders of *Tridax procumbens*.

Soxhlet extraction method

Weigh a 100gm of *Tridax procumbens* and leaves dried powder. Leaves were extracted with aqueous, ethanol, and acetone using soxhlet apparatus for 48 hrs. The collected extracts were filtered with Whatsmann No.1 filter paper and used for estimation of phytochemicals and antifungal activity. The drug to be extracted is packed in a paper cylinder made from a filter paper and it is placed in the body of soxhlet extractor. The solvent is placed in the flask. The apparatus is then fitted. When solvent is boiled on heating the flask it gets converted into vapours. These vapours enter into the condenser through the side tube and get condensed into hot liquid which falls on the column of the drug. When the extractor gets filled with the solvent the level of the syphon tube also raises up to its top. The solvent containing active constituents of the drug in the syphon tube syphon over and run into the flask, thus emptying the body of extractor. This alteration of filling and emptying the body of extractor goes on continuously. The soluble active constituents of the drug remain in the flask while the solvent is repeatedly volatilized. The process of filling and emptying of the extractor is repeated until the drug is exhausted. Normally the process is repeated about 15 times for

complete extraction. Advantages of Soxhlet apparatus, Simple and clear design, Production process continuity, Ease of visual monitoring of the process, a low flow of solvent and the possibility of its reuse after stripping and distillation.

Choice of solvent: Ethanol and methanol contain both polar and nonpolar groups which make it able to extract both polar and nonpolar compounds. The solvents of low polarity were more effective in the extraction of material indicating that the extracted material were of low polarity or have non-polar character. Ethanol (AKA alcohol) when used as a solvent, is one of the oldest forms of botanical extraction and has played this role for thousands of years. In fact, alcohol is one of the oldest recreational drugs used by humans and is relatively easy to manufacture. Ethanol is Safe and Easy to Use, Ethanol is less explosive and toxic, and therefore largely considered safer to operate than hydrocarbon extraction systems. Polar compounds will mix, or dissolve, with other polar compounds. Non-polar compounds will mix, or dissolve, with other non-polar compounds.

Acetone is a good solvent due to its ability to dissolve both polar and nonpolar substances, while other solvents can only dissolve one or the other. Acetone's chemical makeup includes elements that are both polar and nonpolar which means acetone can be used with both organic and inorganic substances. The carbonyl group in acetone is polar, which is due to the difference in electronegativity (a chemical property that describes how strongly an atom will pull electrons toward itself) between the carbon and oxygen atoms. Because of this polarity, acetone enjoys mingling with polar solvents like water. For example, polar solvents such as methanol, ethanol and acetone are miscible with water, thus not suitable for liquid-liquid extraction.

preliminary phytochemical investigation

Test for Steroids (Salkowski's test): Five drops of concentrated sulphuric acid (H_2SO_4) was added to 2 ml of each extract and observed for red coloration.

Test for Alkaloids: To 4 ml of extract filtrate, a drop of Mayer's reagent was added along the sides of test tube. Creamy yellow or white precipitate indicates that the test is +ve.

Test for Glycosides: To 4 ml of extract solution and add few drops of glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of 2 layers and bluish green colour in upper layer.

Test for Flavonoids: A volume of 1.5 ml of 50 % methanol was added to 4 ml of the extracts. The solution and magnesium metal was added and warmed. Then, 5 to 6 drops of concentrated hydrochloric acid was added to the solution and observed for red coloration.

Test for Tannins: To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added, observed for blue or green black coloration.

Test for Saponins: Two ml of distilled water was added to 2ml of the test solution shaken well and observed for frothing.

Table1: Phytochemical analysis of leaves of *Tridax Procumbens*

S. No.	PHYTOCHEMICALS	OBSERVATIONS	RESULTS
A	Steroids	Reddish colour	+
B	Alkaloids	Creamy colour	+
C	Glycosides	Reddish brown/ bluish green	+
D	Flavonoids	Yellow orange	+
E	Tannins	Brownish green/ black	+
F	Saponins	Stable persistent/ Frothing	+

Antifungal activity using disc diffusion method

Disc diffusion method: The disc diffusion method⁷ test is used to determine the susceptibility of clinical isolates of bacteria to different antibiotics.

Apparatus required: Balance machine, incubator, autoclave, millimeter scale, scissor, cotton swab stick, agar, forceps

Steps involved:

Media preparation

Dissolve 20gms of dehydrated PDA medium into 1 liter distilled water shake and heat to dissolve completely. Now autoclave the medium at 121°C temp and 15 lb pressure for 15 mins. Pour the medium into sterile petri dish cool to solidification. Check the contamination before use. Fungal culture preparation Bring a pure culture plate of fungus, in this test, we will use *Candida albicans* and *Aspergillus niger* because we want to examine the antifungal activity of our sample. Bring a fresh (Crapex Dox Agar) CDB tube culture will be prepared. Burn an inoculating loop to red hot & cool inside the Biosafety cabinet after 5 min of cooling. Pick one loop full of fungal colony from the pure on culture plate using the sterile inoculating loop. Inoculate the fresh CDB & fungi attach the cap of the inoculated CDB and rotate several times to mix. Incubate the CDB at 36°C for 24 hr. Check the contamination

before use.

Inoculation of culture plate

At this point, it is better to match the fungal concentration with 0.5 McFarland standard using UV spectrophotometer, but without matching the concentration you can conduct this experiment and there will be no major problem with it. We matched the bacterial concentration with 0.5 McFarland std. Bring sterile cotton swab sticks. Rotate the both culture tube several times to mix the culture. Take a sterile cotton swab stick. Insert the cotton swab stick into the broth culture and hold for 5 sec. Now touch the moistened cotton swab stick on the glass tube, surface several times to remove the surplus culture from the swab. Now take out cotton the swab sticks from the culture tube. Now, inoculate the MHA plate using the swab in zigzag style. Inoculation should be done by rotating the MHA- plate occasionally of each edges of MHA plate. Cover the inoculated plate.

Sample preparation

Wet a scissor with 70% ethanol and Burn the scissor to make it sterile and cool the scissor inside the Biosafety cabinet. Now wet forceps with 70% ethanol and Burn the forceps to make it sterile. Cool the forceps inside the Biosafety Cabinet and bring the sample and Sanitize with your hands. Cut a square block of sample (6mm by 6mm) using sterile scissor. Place the sample on inoculated MHA plate using the forceps. Press on every side of the sample to ensure the proper placement and pick a disk of Fluconazole with the forceps. Place the disk on MHA surface keeping a distance from the sample. Now cover the plate with lid but do not invert it. Incubate the plate at 35°C for 24hrs. After incubation take out the plate from the incubator after the incubation.

Results and discussion

Place the plate on a black background by inverting it. Observe the zone of inhibition around the antibiotic and sample. Fungal cultures used in study.

Table 2: Fungal strains used

S.NO.	Fungal strain
1.	Candida albicans
2.	Aspergillus niger

Table 3: Determining the value of zone of inhibition with fungal strains

Medicinal plant	Solvent extract	Candida albicans (mm)	Aspergillus niger (mm)
Tridax procumbens	Acetone & Ethanol	17mm	19mm
Fluconazole	Acetone & Ethanol	24mm	25mm

Table 4: Antifungal activity of standard drug on different fungal agents

S. No.	Name of standard drug	Name of microbes	Zone of inhibition
			Leaves (ethanolic and acetone extract)
1.	Fluconazole	Aspergillus niger	24mm
		Candida albicans	25mm

Conclusion:

In this present work extraction of *Tridax Procumbens* leaves has done by using ethanol, acetone 75:25 ratio of solvents. This ratio of solvent has given effective extraction of leaves constituents of *Tridax Procumbens* and proved with of simple ethanolic extraction. And it has evaluated for its antifungal activity using *Aspergillus niger* and *Candida albicans* as fungal strains. And the results of antifungal activity were found to be effective.

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