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RESEARCH ARTICLE

TRIDAX PROCUMBENS (L.)- A PHARMACOGNOSTIC SCREENING

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ABSTRACT

Tridax procumbens (L.) commonly known as coat buttons or Tridax daisy, is a species of flowering plants in daisy family. It is best known as a wide spread weed and pest plant. It belongs to family compositae commonly known as 'Ghamra' and have been extensively used in Ayurvedic system of medicine for various ailments and is dispensed for 'Bhringraj' by some of the practitioners of ayurveda which is well known medicine for liver disorders. Pharmacognosy is the scientific and systematic study of the structural, physical, chemical and biological characters of crude drugs along with their history, method of cultivation, collection and preparation of the market. Present Pharmacognostic studies in *T. procumbens* (Asteraceae) was done by analysing the morphology, anatomy and physico-chemical studies. Microscopic and macroscopic characters of the plant were studied for the easy identification of the plant. Phytochemical screening tests are done in 2 extracts of whole plant viz methanol and acetone extracts. Methanol extracts of whole plants showed the presence of coumarins, tannins, alkaloids, quinines, flavanoids, resins, Proteins and carbohydrates. Acetone extract of whole plant shows the presence of coumarins, quinines, steroids, alkaloids, tannins, resins, protiens and carbohydrates.

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INTRODUCTION

Compositae is the largest family including 1250-1300 genera and 20000-25000 species distributed all over the world and in almost all habitats. Members are much diversified, may be annual or perennial; xerophytes, succulents or normal mesophytes; herbs, shrubs or less commonly trees or climbers. Normal tap roots are branched and fibrous, root tubers are produced in some members. Stem is soft erect or prostrate rarely climbing, usually hairy, often with milky or coloured sap. Leaves are alternate, rarely opposite generally dorsiventral Stomata variously distributed, and is mostly Ranunculaceous type, sometimes cruciferous. It is a wide herb distributed throughout India. Now this methods have been developed for its ex-situ conservation through micro propagation. Morphological Features:- It is a small perennial herb having short hairy blade like leaves. Corolla is yellow in colour. Stem is ascending 30-50cm height, branched, hairy and rooting at nodes. Leaves are simple, opposite, exstipulate, lanceolate or ovate, 5-7cm long irregularly toothed margin, base wedge shaped, shortly petiolate and hairy on both surfaces. Flowers are tubular, yellow with hairs; inflorescence is capitulum consisting of a large number of sessile flowers arranged on the variously shaped receptacle and surrounded by one or more than one whole of involucral bracts that are protective in function. Fruit is a hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. The plant is invasive in part because it can be carried to some distance. Calyx is reduced to pappus hairs. Seed have pendulous embryo and endosperm is absent. *T. procumbens* (L.) is known for several potential therapeutic activities like antiviral, antioxidant, antibiotic efficiencies, wound healing, insecticidal and anti inflammatory activity. Some reporters from tribal areas of India state that the leaf juice can be used to cure fresh wounds to stop bleeding and as a hair tonic. Despite these known benefits, it is still listed in the United States as a noxious weed and regulated under the federal noxious weed act. Traditionally *T. procumbens* (L.) has been in use in India as anticoagulant, antifungal and as an insect repellent. It is also used in diarrhoea and dysentery. Its leaf extract were known to treat infectious skin diseases in folk medicine. It is a well known ayurvedic medicine for liver disorders besides gastritis and heart burn. It is used in treatments for boils, blisters and cuts by local healers in Nalgonda and Warangal districts of Telangana and Andhra Pradesh in India. Study has found anticancer properties of *T. procumbens* (L.) against human prostrate epithelial cancer cell.

MATERIALS AND METHODS

The plant *T. Procumbens* (L.) of the family Asteraceae was collected from Guruvayoor.

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- **Anatomical studies**

Anatomical features of root and stem were conducted by free hand sectioning and microscope examination cross sections of stem and root about 2.5mm diameter were taken. The sections were observed carefully and photos were taken using digital camera.

- **Palynological studies**

Palynological features of *T. procumbens* (L.) were conducted by microscopic examination of pollen grains dusted on a glass slide. Pollen grains were observed clearly and photos were taken using digital camera.

- **Powder analysis**

Shade dried and coarsely powered raw drug of the whole plant is used for powder analysis which include both macro and micro characterization and behaviour of the drug powder with different chemical reagent or acids. The fine powder was observed under the microscope and the characteristic features were noticed. Behaviour of the drug powder was observed by treating the powder with twelve different reagents and the colour changes were noticed under normal day night.

- **Preliminary phytocheminal screening**

Test for flavanoids

Shinodas Test (Mg/HCl):- Dissolved a small amount of the extract in methanol or ethanol, a few magnesium turnings and a few drops of 5m HCl were also added. Development of deep red or magenta colour indicated the presence of flavanones and dihydroflavanols.

Test for coumarins

A little amount of extract was dissolved in methanol or ethanol and 3-4 ml alcoholic KOH or NaOH was added. Formation of a yellow colour which disappeared on adding conc. HCl indicated the presence of coumarins.

Test for tannins

Ferric chloride test:- A few drops of ferric chloride were added to a little amount of the extract. The development of green colour revealed the presence of tannin.

Test for alkaloids

Mayer's Test:- One or two drops of Mayes's reagent was added to the acidified plant extract. A white precipitate indicated the presence of alkaloids. Mayer's reagent:- HgCl2 (1.36g) was dissolved in 60ml distilled water and mixed with a solution of 5g of KI in 10ml water. As this reagent reacts only with the salts of the alkaloids, the solution made distinctly acidic with HCl or H2SO4. Wagner's test:- Alkaloids gave brown flocculent precipitate with wagners reagent. Wagner's reagent:- 1.27g of ioidine and 2g of KI were dissolved in 5 ml of distilled water and the solution was made up to 100ml with distilled water.

Detection of steroids / terpenoids

Salkowski Test:- A few drops of con. H_2SO_4 were added to a little amount of the extract and was shaken for a few minutes. The development of a red or brown colour indicated the presence of sterols.

Test for saponins

Shaken an aqueous/ alcoholic plant extract in a test tube and a persistent foam indicates the presence of Saponins.

Test for quinines

To the test sample, few drops of NaOH was added. Formation of blue, green or red colour indicated the presence of quinines.

Test for anthraquinines

Born trager's test:- The extract was shaken with aqueous NH_3 or castic soda. Formation of pink red or violet colour aqueous layer indicated the presence of anthraquinens

Test for phenols

A few drops of alcoholic ferric chloride solution were added to the sample dissolved in alcohol or water. Formation of violet, bluish green or bluish black colour indicated the presence of phenol.

Test for resin

A little amount of extract was dissolved in 5 ml of alcohol and added 2 ml of distilled water and petroleum ether both respectively. The development of white turbidity indicated the presence of Resin.

Test for the detection of glycoside / reducing sugar:

Benedict's test

The extract was mixed added with Benedict's reagent in equal amount and the mixture was heated for 2 minutes. The appearance of brown to red colour indicated the presence of glycoside.

Test for protein

xanthoprotein test:- A small amount of the extract was mixed with 0.5 ml of concentrated HNO_3, appearance of white or yellow precipitate revealed the presence of the protein. Biuret

Test:- A small amount of the extract was added to 0.5ml of 4% sodium hydroxide solution followed by a drop of 1% copper sulphate solution. The development of violet to pink colour indicated the presence of protein.

Test for carbohydrate

Molish's Test: 100mg of the substance was dissolved in 1ml water and 2 drops of 1% alcohols solution of alpha-naphthol was added to it. 1ml of con.H_2SO_3 was added along the sides of the test tube, so that it formed a heavy layer at the bottom. A deep violet ring at liquid junction indicated the presence of carbohydrate. Phytochemical screening tests are done in 2 extracts of whole plant viz acetone and methanol extracts. Acetone extract of whole plant shows the presence of coumarins, quinines, steroids, alkaloids, tannins, resins, protiens and carbohydrates.

Table 1. The results of Phytochemical properties in Methanol extracts of the whole plant

S. No.	Test	Reagent	On Acetone Extract
1	Flavanoids	Alkaline reagent	Negative
2	Coumarins	Methanol +KOH	Positive
3	Tannins	Ferrichloride	Positive
4	Alkaloids	Mayr's reagent	Positive
5	Steroids	Conc.H_2SO_4	Positive
6	Saponines	Water+ Shake	Negative
7	Quinines	NaOH	Positive
8	Anthraquinones	Aqueous ammonia or Caustic soda	Negative
9	Phenols	Alcoholic Ferrichloride	Negative
10	Resins	Water+Petroleum ether	Positive
11	Proteins	Conc.HNO_3	Positive
		4% NaOH +1% $CuSO_4$	Positive
12	Glycosides	Benedict's reagent	Negative
13	Carbohydrates	Water +Alcoholic alpha-naphthol+ Conc.H_2SO_4	Positive

Methanol extracts of whole plants showed the presence of coumarins, tannins, alkaloids, quinines, flavanoids, resins, Proteins and carbohydrates.

Table 2. The results of physichochemical parameters of the plant

S. No.	Test	Reagent	On Methanol Extract
1	Flavanoids	Alkaline reagent	Positive
2	Coumarins	Methanol + koh	Positive
3	Tannins	Ferric chloride	Positive
4	Alkaloids	Mayer's reagent	Positive
5	Steroids	Conc.h_2so_4	Negative
6	Saponines	Water + shakes	Negative
7	Quinines	Naoh	Positive
8	Anthraquinone	Aqueous ammonia /castic soda	Negative
9	Phenols	Alcoholic ferric chloride	Negative
10	Resin	Water + petroleum ether	Positive
11	Protein	Conc. Hno_3	Positive
		4% naoh + 1% $cuso_4$	Positive
12	Glycoside	Benedict's reagent	Negative
13	Carbohydrate	Water + alcohol alpha naphthol + con. H_2so_4	Positive

- **Biochemical studies**

Contents for total protein and carbohydrates were estimated based on standard procedures.

Estimtion of protein (lowry's method):

100 (0.1 g) mg of the plant sample was homogenized with a few drops of water and the proteins were precipitated using chilled 5% trichloro acetic acid. The solution was centrifuged for10 minutes at 3000 rpm and the precipitate was again washed with cold 5% TCA and centrifuged. The residue was dissolved in 0.1 N NaOH and made up to a known volume. 1 ml of the solution was pipetted out and made up to 4 ml. Alkaline mix of 5.5 ml was added and mixed well and allowed to stand for 10-15 minutes. 0.5 ml of the Folincto caiteau-reagent was added and mixed rapidly. The solution was kept for a 30 minutes and the blue colour developed was read at 650nm.

Alkaline mix

Solution A:- 2% Na_2CO_3 in 0.1N NaOH
Solution B:- 0.5% $CUSO_4$. $5H_2O$ solution in 1% sodium potassium.

- **Physicochemical studies**

pH value, moisture content, ash value, extraction values of whole plant are calculated from powdered root, stem & leaf of plant.

Parameters	Value Observed
pH Value	
1% solution	6.6
10% solution	6.3
Moisture content	75.58%
Extraction values	
Water soluble matters	12.47%
Alcohol soluble matters	15.50%
Ash Content	1.23g

DISCUSSION

Acetone and methanol extracts of the whole plant of Tridax in the present study shows the presence of various metabolites-alkaloids, flavanoids, coumarins, tannins, quinines, carbohydrates and proteins in methanol extract and presence of coumarins, tannins, alkaloids, steroids, quinines, resins, proteins, and carbohydrates were analysed in acetone extract. Anatomic or microscopic characterization of stem, root are studied. In root, single layered epidermis is present. Inner to it, eight to ten layered cortex is present. Cortical cells contain numerous starch grains and tannins. Inside the stelar region vascular bundles and highly reduced. Pith is present. Xylem arrangement is exarch. In stem, single layered epidermis is present which possess a number of multicellular epidermal hairs and a thin cuticle. Collenchymatous hypodermis is followed by chlorenchymatous and parenchymatous cortical region. A ring of vascular bundles and a large pith are present in stelar region. Each vascular bundle is conjoint, collateral and open type with few layers of cambium in between xylem and phloem patches. Xylem is endarch in nature. Pollen grain is simple, tricolporate and show spheroidal shape. Aperture consist of a colpus and a pore. Colpus (furrow) is short membrane and is with small granules. Exine is covered with long pointed spines. Physico chemical analysis shows the total ash value of *Tridax procumbens* (L.) whole plant is 1.23. In the present study the sample were collected freshly and the water content of whole plant was calculated and is very high. The moisture content of whole plant is 75.58%. Physico chemical characteristics are important parameters in the standardization of plant constituents. pH value of both 1% and 10% solutions were observed. pH value of 1% solution of plant was 6.6 and that of 10% solution is 6.3 which indicate the whole plant content is slightly acidic in nature. Ash value of

whole plant is 1.23. Water soluble extractive percentage of plant were 12.47% and alcohol soluble extractive is 15.5%. Moisture content of plant is 75.58%.

Conclusion

The present study on Pharmacognostic investigation of Tridax whole plant provide useful information in regard to it's correct identity and help to differentiate from the closely related other species of Tridax. The results obtained from the present study helps to the easy identification of the plant from co-existing weeds and adulterants. These observations are helpful in identification of our plant species *Tridax procumbens* (L.) It also reveals the medicinal importance of this plant and its scope in ayurveda. Tridax is an important medicinal plant as it can be used for the treatment of many life style diseases such as diabetis, cancer etc. Due to such studies using more advanced and sophisticated techniques, it is possible to give a complete and accurate physico-chemical value of any herbs. This can provide not only a scientific basis and credibility to ayurvedic drugs, but also help in the globalization of Ayurveda.

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