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Evaluation of anthelmintic activity of leaves of *Tragia involucrata* Linn

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Abstract

Methanolic extract of the leaves of *Paederia foetida* were screened for its anthelmintic activity against *Pheretima posthuma* and *Tubifex tubifex*. Various concentrations (25, 50, 100 mg/ml) of all extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Also, prepared herbal tablet of *Tragia involucrata*.

Keywords: *Tragia involucrata*, Anthelminthic, methanol, Tubifex tubifex.

Introduction

Ayurvedic medicine *Kolthee churna*, known to be effective in diarrhoea diseases has been standardized by following modern scientific quality control procedures, both for the raw material and the finished product. *Tragia involucrata* commonly known as the Mauritius or Mysore thorn or the cat's claw is a tropical tree species originating in India. It is as a robust, thorny, evergreen shrub 2-4 m high or climber up to 10 m or higher; often forming dense thickets; the stems are covered with minute golden-hair; the stem thorns are straight to hooked, numerous, and not in regular rows or confined to nodes.

Plant derived drug serve as a prototype to develop more effective and less toxic medicines. Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent, particularly in third world countries due to poor management Helminthiasis practices. A number of medicinal plants have been used to treat parasitic infections in man and animals. 5, 6, 7, 8 the plants are known to provide a rich source of botanical anthelmintics.



Local names in India

Marathi - Kolthee, **Hindi** - Barhanta **English** - stinging nettle, **Malayalam** - Kodathooa, Kodithova, Choriydnam. **Sanskrit synonyms** - Dusparsa, Ananta, Yavasa, Duralabha, **Family:** Euphorbiaceae.

Sanskrit Synonyms-- Dusparsa, Duralabha, Yavasa, Ananta, Yavasa

Ayurvedic properties

Rasa : Katu, Tikta, Madhura, Kashaya.
Guna : Lakhu, Snigdha.
Virya : Seeta.
Vipaka : Katu.

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Fig: *Tragia Involucrata*

Plant description

A perennial twinning herb with stinging hairs all over. Leaves simple, alternate, stipulate, oblong-lanceolate or ovate, serrate, base cordate or rounded, acuminate; flowers in axillary racemes, unisexual, female few, in lower part of inflorescence, male many in upper part. Fruits capsules, 3 lobed, containing 3 globose smooth seeds.

Materials and Methods

Plant material

The leaves of *Tragia involucrata* Linn. (EUPHORBIACEAE), was collected from, Koyana Dam, India and was identified with the Herbarium of Botanical Survey of India.

Extraction-*Tragia Involucrata* leaves were collected, shade, dried, powdered mechanically. About 100g of the powdered leaves extracted in methanol by Soxhlet Extraction.

Phytochemical tests

The preliminary phytochemical tests revealed the methanolic extract of the leaves shows the presence of alkaloids, sterol, and fixed oil.

Worms

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil in Bankura. *Tubifex tubifex* (Annelida) were collected from Aquarium of the local market. The average size of *Pheretima posthuma* and *Tubifex tubifex* were 6-8 cm and 1-1.5 cm respectively. They were washed with water to remove dirt.

Procedure

The anthelmintic assay was carried as per the method of Ajayieoba E. O. *et al.* with minor modifications 5. The experiments were done on adult Indian earthworm *Pheretima posthuma* and the aquarium worm, *Tubifex tubifex*, because they belong to same group of Annelida (Mueller, 1774). 20 ml formulations containing three different concentrations, methanolic extract (25, 50 and 100 mg/ml indouble distilled water) were prepared and taken in different petridishes and sixearth worms (same type) were placed in the solutions respectively. Similarly lump of *Tubifex* worms were placed in the test solutions. All the test solution and standard drug solution were prepared freshly before starting the experiments. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50 °C. Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the control. 19, 20, 21 Three sets of experiments were done statistical significance.

Traditional Uses

The leaves are sweetish, tonic, carminative, antipyretic, emmenagogue. Used as a medicine to treat many different ailments. The most common use was to use the leaves to make a tea that would be consumed to treat intestinal worms. There are also accounts of this plant being used to treat malaria and it is used for the fever, it also used for UIT track infections.

Chemicals

All the chemicals used were of analytical grade obtained from s.d Fine – Chem. Ltd., Bombay, Toms Baker, Mumbai, Qualigens Fine chemicals, Mumbai and Bengal Chemicals & Pharmaceuticals Ltd., Calcutta. Chemicals- 1. Piperazine Citrate (Glaxo), 2. Double distilled water.

Activities of kolthee plant

The kolthee plant shows various activities such as antimicrobial activity, antibacterial activity, it shows antidiarrhoeal activity, anti inflammatory activity, antipyretics, it also used for the UIT TRACK infections. It also used as antidiabetics. Used in skin diseases so, kolthee plant is multi used plant.

Experimental

The marketed available kolthee churna standardise by using all parameters. Organoleptic evaluation was used for identification of sensory characteristics like colour, odour, taste, shape, size, texture and fracture. Active phytochemical constituents like glycosides, flavonoids, alkaloids, acids, gums, tannins, were identified through qualitative chemical analysis in each of the ingredients (Table 1). Thin layer chromatography (TLC) was performed and R_f values were calculated. Quantitative analysis of the raw material was done for standardization parameters including foreign organic matter, water soluble extractive, methanol soluble extractive, total ash and acid insoluble ash. Their values were calculated and found to be well within the available standard ranges.

Phytochemical Estimation

Qualitative chemical investigation

The ethanolic extract of *Caesalpinia decapetala* were subjected to Qualitative chemical tests

1. Test for carbohydrates

- a) **Molisch's test:** Test solution with few drops of molisch reagent and 2ml of conc. Sulphuric acid is added slowly from the sides of test tube shows a purple ring at the junction of two liquid.
- b) **Barfoed's test:** Test solution treated with barfoed's reagent on boiling on water bath shows brick red precipitate.

2. Test for proteins

- a) **Million's test:** Test solution treated with million's reagent and heated on boiling water bath, protein is stained yellow on warming.
- b) **Xanthoproteic test:** Test solution treated with conc. Nitric acid and on boiling gives yellow precipitate.
- c) **Biuret test:** Test solution treated with 40% sodium hydroxide and dilute copper sulphate solution gives blue color.

3. Test for free amino acid

- a) **Ninhydrin test:** Test solution treated with ninhydrin reagent gives blue colour.

4. Test for fats

- a) **Solubility test:** Oils are soluble in ether, benzene, chloroform, but insoluble in 90% ethanol and in water. (Exception- castor oil soluble in alcohol).
- b) **Filter paper test:** Filter paper gets permanently stained with oils.

5. Test for volatile oils

- a) **Odour test:** Volatile oils have characteristic odour.
- b) **Filter paper test:** Filter paper not permanently stained with oils.

6. Test for Flavonoides -

- a) **Ferric chloride test:** Test solution with few drops of ferric chloride solution shows intense green colour.
- b) **Shinoda test:** Test solution with few fragments of magnesium ribbon and conc. Hydrochloric acid, shows pink to magenta red colour.
- c) **Zinc-Hydrochloric acid reduction test:** Test solution with zinc dust and few drops of HCL shows magenta red colour.
- d) **Alkaline reagent test:** Test solution when treated with sodium hydroxide solution shows increase in intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

7. Test for alkaloids

- a) **Mayer's test:** Test solution treated with Mayer's reagent (Potassium mercuric iodide) gives cream coloured ppt.
- b) **Wagner's test:** The acidic solution treated with Wagner's reagent (iodine in potassium iodide) gives brown ppt.
- c) **Hager's test:** Acidic solution with Hager's reagent (saturated picric acid solution) gives yellow ppt.
- d) **Dragendorff's test:** The acidic solution with Dragendorff's reagent (potassium bismuth iodide) shows reddish brown ppt.

8. Test for saponin

- a) **Foam test:** Saponin when mixed with water and shaken shows formation of foam

9. Test for sterols

- a) **Salkowski test:** when few drops of conc. sulphuric acid is added to the test solution shaken and allow to stand, lower layer turns red indicating presence of sterols.
- b) **Liebermann Burchard test:** The test solution treated with few drops of acetic anhydride and mix well. When conc. Sulphuric acid is added from side of test tube, it shows a brown ring at junction of two layers and upper layer turns green.
- c) **Sulphur test:** Sulphur when added in to test solution, it sinks in it.

9. Test for triterpenoids

- a) **Salkowski test:** when few drops of conc. sulphuric acid is added to the test solution shaken and allow to stand, lower layer turns yellow.

10. Test for Glycosides

- a) **Baljet's test:** Test solution treated with sodium picrate gives yellow to orange colour.
- b) **Keller-Killiani test:** The test solution with few drops glacial acetic acid in 2ml of ferric chloride and conc. Sulphuric acid added from the sides of test tube which

shows separation between two layer, lower layer shows reddish brown and upper layer turns bluish green.

- c) **Raymond's test:** Test solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.
- d) **Bromine water test:** Test solution dissolve in Bromine water gives yellow ppt

1. Test for Tannins

Ferric-chloride test: Test solution with a few drops of ferric chloride solution gives dark colour.

Gelatin test: Test solution treated with gelatin solution gives white ppt.

Observations:

Sr.no.	Name of the test	Ethanol
1.	Test for carbohydrate	
	a. Fehling's test	-
	b. Benedict's test	+
	c. Molisch test	+
2	Test for volatile oils	
	a. Odour test	+
	b. Filter paper test	+
3	Test for Flavonoides	
	a. Ferric chloride test	+
	b. Shinoda test	+
	c. Alkaline reagent test	+
	a. Salkowski test	-
	b. Liebermann Burchadt test	+
6	Test for sterols	
	a. Liebermann Burchadt test	+
	b. Sulphur test	+
7	Test for Glycosides	
	a. Baljet's test	+
	b. Keller-Killiani test	+
	c. Legal's test	+
8	Test for Tannins	
	a. Ferric-chloride test	+
	b. Gelatin test	+

2. Microscopic Test

Sr.no	reagents	observations	characteristics
1	Phloroglucinol+conc.Hcl	Pink colour	Lignified epidermal trichomes, unicellular trichomes present. xylem, phloem fibers also present. cork cells, stone cells present.
2	Dil.Iodine+conc.sulphuric acid	Blue colour	Endospermic wall-emicellular
3	Sulphuric acid 60 percent	Crystal soluble	Calcium oxalate crystals present
4	Acetic acid	Insoluble	Calcium oxalate crystals present
5	Alcoholic picric acid	Yellow colour	Aleurone grains present
7	Strong KOH solution	crystals	Needle shaped potassium eugenate crystals

Thin layer chromatography



Mobile Phase = 1.Methanol: Ethyl acetate: Benzene (1:2:3).

R.F. Value = Distance Travelled by solute / Distance Travelled by solvent

1. $0.5/0.8=0.62$.

2. **Benzene: Pet Ether: Methanol** (2:3:4).
 $0.6/0.9= 0.66$.

Quantitative analysis

Quantitative analysis of the raw material was done for standardization parameters including foreign organic matter, water soluble extractive, ethanol soluble extractive, total ash and acid insoluble ash.

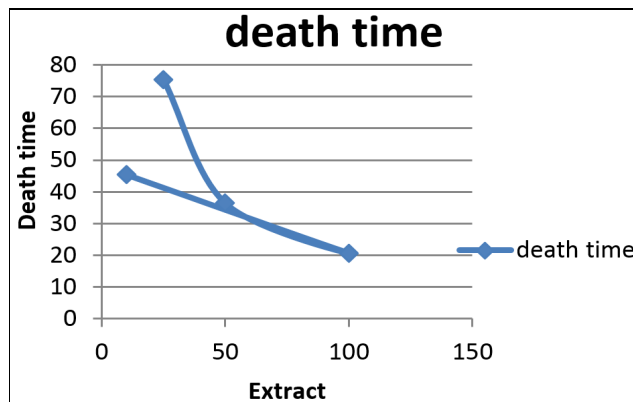
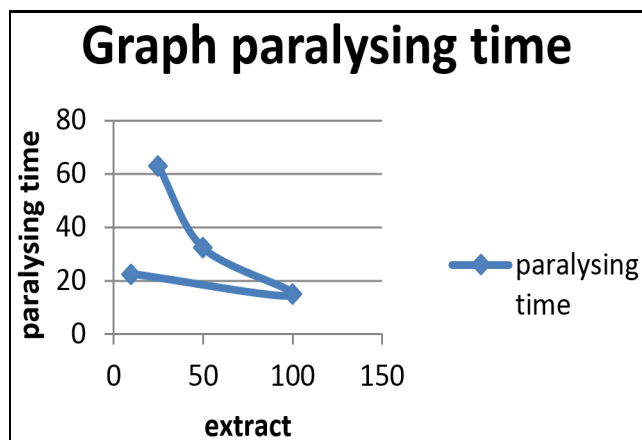
Type of Extractive Percentage (w/w)

- Water soluble extractive: 4.08
- Ethanol soluble extractive 2.16

Table 1: Anthelmintic activity of methanolic extract of *Tragia involucrata*

Groups Concentration(mg/ml)	<i>Tragia involucrata</i>				
Paralysing time Death time Paralysing time Death Time					
Distilled Water					
Leaf Extract (ALCOHOLIC)	25	62.87	83.76	63.00	75.39
	50	35.33	63.33	32.33	36.33
	100	19.33	40.00	14.66	20.66
Tragia involucrata	10	25	64	22.66	45.36

Graph 1



Formulation of *Tragia involucrata* plant containing Herbal Tablet-

Formulation Table as follow-

Ingredients	D1	D2	D3	D4	D5
Herbal (TRAGIA INVOLUCATA PLANT LEAVES)	500	500	500	500	500
Avicel PH102	137	117.5	117.5	117.5	117.6
Povidone30	-	-	-	-	-
Sodium starch g	-	19.5	19.5	-	-
Croscarmellose	-	-	19.5	-	-
Crospovidone	-	19.5	-	19.5	-
Colloidal silicon Dioxide	6.5	6.5	6.5	6.5	6.5
Magnesium stearate	6.5	6.5	6.5	6.5	6.5

Result and Discussion

The marketed koltee chuna were standardized. Organoleptic test, preliminary test, all phyto chemical test like carbohydrate, sterol, saponin, glycoside, alkaloid, flavanoid etc and microscopical test also performed. Quantitative analysis of the raw material was done for standardization parameters including foreign organic matter, water soluble extractive, ethanol soluble extractive, total ash and acid insoluble ash. were studied. From the above study it was seen that the methanolic extract showed dose dependent to be 25, 50, 100 and 10 minutes respectively. In the meantime *Tragia involucrata* at a dose of 10 mg/ml cause paralysis in the above helminth in 25 minutes.

Conclusion

The results obtained could be used to lay down a set of new pharmacopoeial standards for the preparation of *kolthee churna*, to obtain optimal efficacy of the medicine. In this investigation the methanolic extract of *Tragia involucrata* Linn were used to evaluate anthelmintic activity by using the above models. The preliminary phytochemical tests revealed the presence of alkaloids, sterol and fixed oil. Thin layer chromatogram indicated that alkaloids were prevalent in the ethanolic extract because the Rf value was close to the reported value (0.77).

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