

Studies on Antihelmintic Activity of Methanolic Extract of *Tamilnadia Ualignosa Retz*

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Abstract

Background: *Tamilnadia ulignosa Retz* is the most significant genus in the family Rubiaceae, which is commonly known as *Divine jasmine* in India.

Aim: To study the antihelmintic activity of methanol extract of *Tamilnadia ulignosa Retz*. on Indian adult earthworms, *Pheretima posthuma* (annelid).

Materials and methods: Aerial parts were extracted by using Soxhlet apparatus. Phytochemical screening of crude extracts showed the presence of Alkaloids, Tannins, phenolics, flavonoids, terpenoids, steroids and proteins. Various concentrations (25, 50, 100mg/ml) of crude extracts were tested for anthelmintic activity when involved the determination of the time of paralysis and time of death of worms. The activity was compared with standard piperazine citrate.

Results: The methanolic extract shows significant activity when compared to the standard piperazine citrate. The paralysis and death time is 46, 28, 16 and 75, 51, 32 minutes respectively at concentrations 25, 50 and 100mg/ml. whereas these were 31, 18, 10 and 63, 41, 22 minutes for piperazine citrate. In order to confirm the studies *in vivo* studies have to be conducted.

Conclusion: Methanolic extract of *Tamilnadia ulignosa Retz* may be used as alternative antihelmintic treatment approach.

Key words: *Tamilnadia ulignosa Retz*. Anthelmintic activity, Piperazine citrate

Introduction

Tamilnadia ulignosa, also known as *Randia ulignosa Retz*, *Tamilnadia*, a member genus of *Rubiaceae* family has its name derived from Tamilnadu state in India [1]. The plant is growing in dry deciduous forests, native to India, Bangladesh, Sri Lanka and Thailand. Species of this genus are very rigid, ramos, dry deciduous,

small armed trees with quadrangular branches up to 7.5 m height and 1.2 m in girth, bear one or two pairs of short thorns, delights in bogs, swamps, banks of rivers and other moist places. Trunk is well defined, covered with a dark rust colored scabrous bark. They freely produce root suckers, and are hardly against frost and drought. The rate of growth is moderate, with a mean annual girth increment of 14-28 mm. Branches are erect rigid, quadrangular, thick set with short, rigid round, and diverging branchlets [2 – 6]. Short lateral shoots, each of which terminally produces one or two pairs of short thorns. Leaves opposite on young shoots, or fascicled at the end of branchlets, short-petioles, oblong, shining, entire, 2-3 inches long by 1.5 inch broad [7]. In a study of Kommu *et al* (2019) [7- 10], It was recognized that different species with various phytochemical effects are known in India mythology as a medical plant that contain active gradients like flavonoids, saponin, and steroids [11 - 19].

Materials and methods

Collection and preparation of plant material:

Tamilnadia ulignosa Retz is collected from Tirumala hills, Chittoor (dist), Andhra Pradesh, India. The botanical identification of plant was performed by Dr. K. Madhava Chetty, Professor, and HOD, Dept of Botany, SV University, Tirupathi, A voucher specimen (TUR-503) is being maintained in the department of pharmacognosy, Mother Teresa Pharmacy College, Khammam. The aerial parts were separated, cleaned, air dried, made free from debris and grounded into powder. The dried powder material was passed through a sieve no.24 and stored in air tight container.

Extraction of the plant:

The shade dried (5 days) *Tamilnadia ulignosa Retz* powder (250 g) was extracted with methanol by using Soxhlet apparatus [20]. After extraction, the contents were filtered and concentrated under reduced pressure. The concentrated extract was dried in desicator and packed in a vacuum sealed container. Same protocol of extraction was adopted as that of Hasan Musha *et al* (2018) [17] and Kalpana and Prakash (2018) [18], and the only differences was they were use alcohol extractant, while in our study we used methanol.

Qualitative phytochemical screening:

The qualitative phytochemical screening of plant extracts were carried out to detect the various plant constituents

Preparation of plant extract:

The stored dried plant extracts were re dissolved at concentrations of 25, 50 and 100 mg/ml were suspended in 2% v/v tween80 in normal saline solution and used for screening the anthelmintic activity. Standard piperazine citrate was used with the same concentrations [11–20]. All the solvents are freshly prepared before commencement of the experiment.

Animals:

Adult Indian earthworms, *Pheretima posthuma* resemble the intestinal round worm parasites of human beings both anatomically and physiologically and hence were used to study the anthelmintic activity [21]. Healthy adult Indian earthworms *Pheretima posthuma* were used for evaluating the anthelmintic activity. All healthy earthworms were of approximately 5-7cms in size and 0.1-0.2 cm in width. They were

collected from local place, washed and kept in water until they were used for screening of activity.

Anthelmintic Activity:

The anthelmintic activity was evaluated on adult Indian earthworms by Mathew *et.al* method [22]. For preliminary evaluation of anthelmintic activity test samples of the extract was prepared at the concentration of 25, 50 and 100 mg/ml in 2% v/v tween80 in normal saline solution, 6 worms *Pheretima posthuma* of 5-7cm were placed in Petri dish containing 30 ml of above test solutions of extracts. Piperazine citrate (25, 50 and 100mg/ml) was used as reference standard and normal saline with Tween80 (2%) is used as negative control. All the test solutions and standard solutions were prepared freshly before starting the experiment. Observations are made for the time taken for paralysis when movement was lost or no movement. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water at 50^oc and fading of color of worms [23–27].

Results and discussion

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

- a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.
- b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
- c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
- d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides. Modified Borntrager's test was used. Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

Detection of Saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of Saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of Saponins.

Detection of phytosterols

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

b) Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of tannins Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Detection of proteins and amino acids

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

Detection of diterpenes Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes [28-30].

Table 1 shows that preliminary phytochemical screening of the methanolic extract of *Tamilnadia ulignosa Retz* reveals the presence of Alkaloids, tannins, Saponins, Carbohydrates, Amino acids, Flavonoids and Glycosides. Different doses of the extracts were screened for their activity mainly due to the presence of flavonoids respectively.

Methanolic extract has significant anthelmintic activity when compared to standard Piperazine citrate. The paralysis time of methanolic extract was 46, 28 and 16 min at 25, 50 and 100 mg/ml concentrations respectively. The death time is 75, 51 and 32 min at 25, 50 and 100 mg/ml. Whereas these values when compared to standard

Piperazine citrate is as follows, 31, 18 and 10 min for paralysis and 63, 41 and 22 min respectively.

Table.1. Phytochemical screening

Name of phytoconstituent	Methanolic extract
<i>Alkaloids</i>	+
<i>Carbohydrates</i>	+
<i>Amino acids</i>	+
<i>Tannins</i>	+
<i>Steroids</i>	+
<i>Saponins</i>	+
<i>Flavonoids</i>	+
<i>Glycosides</i>	+
<i>Mucilage's</i>	-
<i>Proteins</i>	+

Table. 2. Antihelmintic activity (Paralysis) of *Tamilnadiaulignosa* Retz methanolic extract:

Type of extract	Dose (mg/ml)	Time taken (min)
Methanol	25	46
Methanol	50	28
Methanol	100	16
Piperazine citrate	25	31
Piperazine citrate	50	18
Piperazine citrate	100	10
Control	---	---

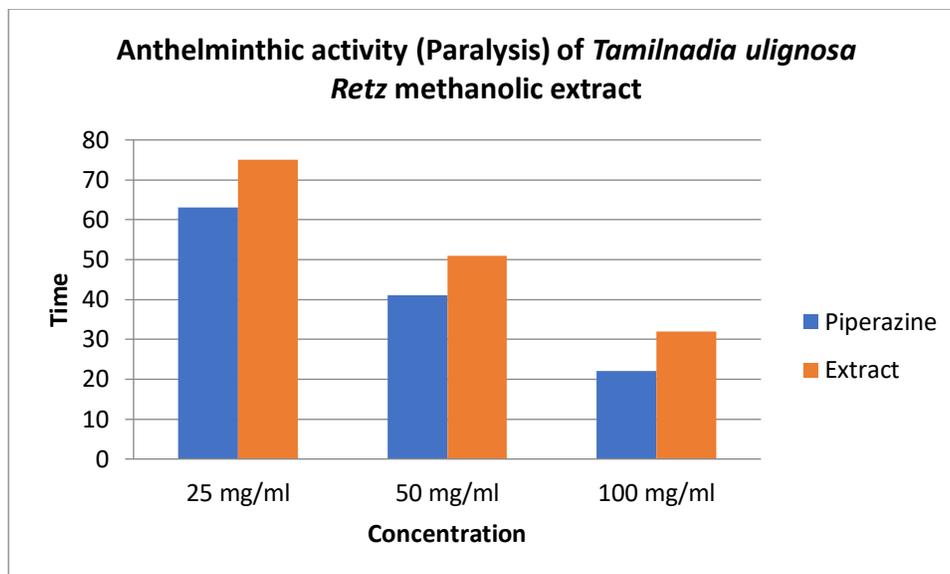


Fig – 1: Anthelmintic activity (paralysis) of *Tamilnadiaulignosa* Retz methanolic extract.

Table. 3. Anthelmintic activity (Death) of *Tamilnadiaulignosa* Retz methanolic extract:

Type of extract	Dose (mg/ml)	Time taken (min)
Methanol	25	75
Methanol	50	51
Methanol	100	32
Piperazine citrate	25	63
Piperazine citrate	50	41
Piperazine citrate	100	22
Control	---	---

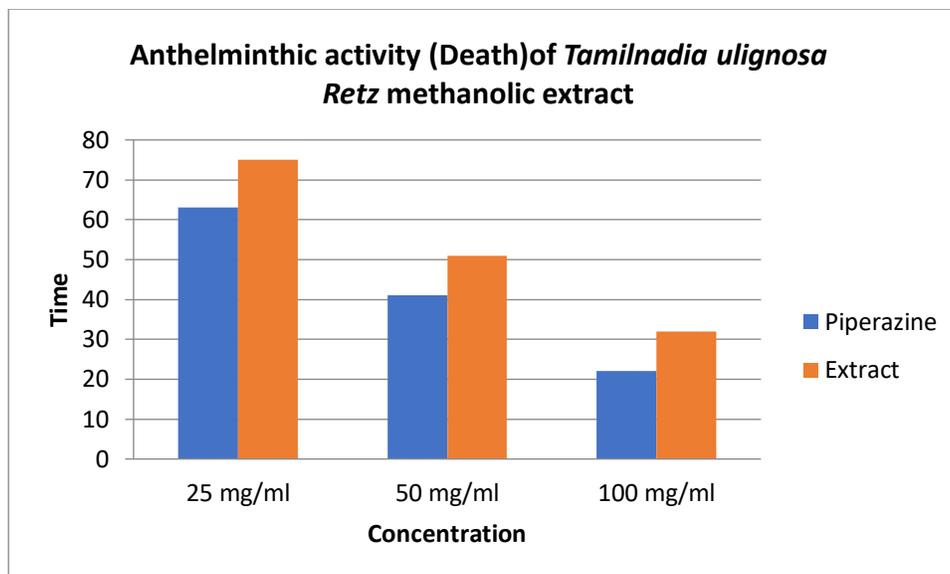


Fig – 2: Anthelmintic activity (Death) of *Tamilnadia uliginosa* Retz methanolic extract.

Conclusion

The work states that the presence of flavonoids, carbohydrates, glycosides, Tannins, Saponins and steroids in the extract of *Tamilnadia uliginosa* Retz was responsible for its anthelmintic activity. Methanolic extract was shown significant values with respect to paralysis and death time of earth worms. It is interesting to observe the results of anthelmintic effect of methanolic extract. But further investigations on the isolation of active compounds present in the extracts and *in vivo* studies are necessary to identify a potential chemical entity for clinical use.

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References

1. Newman DJ, Cragg GM, Sander KM. Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 2003; 66: 1022-1037.
2. Haines HH, 1921-25. *The Botany of Bihar and Orissa I-VI vols.* London.
3. Jain M. Chemical examination of *Randia uliginosa* DC. *Curr Sci* 1965; 17:505.
4. Nadkarni AK and Nadkarni KM. *The Indian Materia Medica*, Popular Prakashan. Bombay, 1976; PP: 1047-1048.
5. Naik VN. *The flora of Marathwada* Amrut Prakashan, Aurangabad. Vol. I; 1998; PP: 79 -89.
6. Neerugatti D, Rao Battu G and Jangiti RK. Anti-inflammatory effect of Methanolic extract of *Tamilnadia uliginosa* (Retz.). *International Journal of Phytopharmacology*, 2014; 5(1): 34-37.
7. Santapau H. *Botanical collector's Manual Bot.* Sury. India, Calcutta. 1995; PP: 342 – 348.

8. Sarma TK, Sarkar AK. Flora of Palamu District, Jharkhand.2002; PP: 98 – 109.
9. Sudhakar K, Eshwariah MC, Eshwaradu MM, Kumar PK and Nagarju K. An updated review on *Tamilnadia uliginosa*. Int. Res. J. Pharm. App. Sci 2012; 2(5): 185-189.
10. Yadav SR, Sardesai MM. Flora of Kolhapur district, Shivajji University Kolhapur Publication. 2002; PP: 367 – 372.
11. Avato P, Bucci R, Tava A, Vitali C, Rosato A, et al. Antimicrobial activity of Saponins from *Medicago* sp.: structure-activity relationship. Phytother Res 2006; 20:454-457.
12. Kommu SK, Eswaraiah MC, Reddy DP, and Illendulas S. In-vivo Anthelmintic Activity of *Tamilandia uliginosa* fruits. Research J Pharm Technol 2019; 12(10): 321 – 323.
13. Hossain S, Ai-Amin, Hossain A, and Rana S. Phytochemical, Antimicrobial, Anthelmintic and Anti-diarrhoeal Activity of Traditional Plant *Randia uliginosa*. Nat Prod Chem Res 2016; 4(6): 245 – 248.
14. Taleb-Contini SH, Salvador MJ, Watanabe E. Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species. Braz J PharmaSci 2003; 39: 403-408.
15. Abu-Shanab BG, Adwn D, Safiya A, Jarrar N, Adwan K. Anti-microbial activities of some plant extracts utilized in popular medicine in Palestine. Turkish Journal of Biology 2004; 28: 99-102.
16. Maiti A, Dewanjee S, Mandal SC. In Vivo Evaluation of Anti-diarrhoeal Activity of the Seed of *Swietenia acrophylla* King (Meliaceae). Tropical J Pharmaceut Res 2007; 6: 711-716.
17. Hasan Musha M, Shulor Juraimi K, and Anwar P. Exploring 55 tropical medicinal plant species available in Bangladesh for their possible allelopathic potentiality', Anal of Agriculture sciences,2018; 63(1): 99 – 107.
18. Kalpana B and Prakash M. Anti-inflammatory Activity of Ethanolic Leaf Extracts of *Euphorbia heterophylla* L. and *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre. Int J Curr Res Biosci Plant Biol. 2018; 5(3): 32-37.
19. Ghani A. Medicinal Plants of Bangladesh with chemical constituent's and uses. 2nd edn. Asiatic Society of Bangladesh, 5 old Secretariat Road, Nimtali, Dhaka, Bangladesh. 2003.
20. Yadav P, Singh R. A review on anthelmintic drugs and their future scope. Int J Pharm Pharmaceut Sci 2011; 3: 17-21.
21. Bhattacharjee C, Debjit B, Tiwari P, Tripathi KK, Dutta AS (2010) In Vitro anthelmintic activity of *Benincasahispida* (Petha) thumb leaves. International Journal of Pharma and Bio Sciences 1: 9.
22. Mathew AS, Patel KW, Shah BK. Investigation on antifeedant and antihelmintic potential of *Adhatoda vasica* Nees. Indian J Nat Prod 1995; 14(1):11.
23. Wadkar GH, Kane SR, Matapati SS, Hogade MG. In vitro anthelmintic activity of *Caesalpinia bonducella* (Linn) Flem Leaves. J Pharm Res 2010; 3: 926-927.
24. Iqbal Z, Nadeem QK, Khan MN, Akhtar MS, Waraich FN. In Vitro Anthelmintic Activity of *Allium sativum*, *Zingiber officinale*, *Curcubita mexicana* and *Ficus religiosa*. Int J Agric Biol 2001; 3: 454-457.

25. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In-vitro anthelmintic properties of *Buchholzia coiaceae* and *Gynandropsis gynandra* extract. *Pharm Biol* 2001; 39: 217-220.
26. Athnosiadou S, Kyriazakis I, Jackson F, Coop RL. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: In vivo studies. *Vet Parasitol* 2001; 99: 205-219.
27. Thompson DP, Geary TG. The structure and function of helminthes surfaces. *Biochemistry and Molecular Biology of Parasites* 1995; 1: 203-232.
28. Roopashree TS, Dang R, Rani SRH, Narendra C. Antibacterial activity of anti-psoriatic herbs: *Cassipourea*, *Momordica charantia* and *Calendula officinalis*. *Int J Appl Res Nat Products* 2008; 1(3): 20-28.
29. Obasi NL, Egbuonu ACC, Ukoha PO, Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. *African J Pure and Applied Chem* 2010; 4(9): 206-212.
30. Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of *Lophiral anceolata* (Ochanaceae). *Life Sci J* 2007; 4(4): 75-79.