Studies on Antihelmintic Activity of Methanolic Extract of Tamilnadia Ulignosa 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, ramous, 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 anthelminthic 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 anatomicaly 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°C 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

<table>
<thead>
<tr>
<th>Name of phytoconstituent</th>
<th>Methanolic extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Mucilage's</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Dose (mg/ml)</th>
<th>Time taken (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>25</td>
<td>46</td>
</tr>
<tr>
<td>Methanol</td>
<td>50</td>
<td>28</td>
</tr>
<tr>
<td>Methanol</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>100</td>
<td>10</td>
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<tr>
<td>Control</td>
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</tbody>
</table>
Fig - 1: Anthelminthic activity (paralysis) of *Tamilnadia ulignosa* Retz methanolic extract.

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

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Dose (mg/ml)</th>
<th>Time taken (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>Methanol</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>Methanol</td>
<td>100</td>
<td>32</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>25</td>
<td>63</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>50</td>
<td>41</td>
</tr>
<tr>
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<td>100</td>
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<tr>
<td>Control</td>
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</tbody>
</table>
Conclusion

The work states that the presence of flavonoids, carbohydrates, glycosides, Tannins, Saponins and steroids in the extract of *Tamilnadia ulignosa* Retz was responsible for its anthelmintic activity. Methanolic extract was shown significant values with respective 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.

Acknowledgement

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References


Fig – 2: Anthelminthic activity (Death) of *Tamilnadia ulignosa* Retz methanolic extract.