Comparative Studies of Different Species of Tulsi for Their Anthelmintic Activity

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Abstract—Helminthes or worm infection is one of the most prevalent disease and one of the most serious problems in the world. It contributes to malnutrition anemia, eosinophilia and pneumonia. Various numbers of plants available for anthelmintic activity a new lead for helminth control is much desirable and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be important sources of bioactive substances [10]. Anthelmintics derived from plant source can be an answer to this world wide problem as they form secure and non-toxic with a modified site of action. Ocimum sanctum Linn (Ocimum tenuiflorum) known as Tulsi in India, is a sacred plant for hindus known from centuries being used in Ayurveda for its varied healing properties. In the present study different species of tulsi were studied for their anthelmintic activity using earthworms as they have physiological resemblance to the worms or helminthes using standard drug Albendazole.

Keywords—Helminthiasis, tulsi, anthelmintic, albendazole

INTRODUCTION
The Helminthes or worm infection is one of the most prevalent disease and one of the most serious problems in the world. It contributes to malnutrition anemia, eosinophilia and pneumonia. A number of plants have been tested for their anthelmintic efficacy. Achyranthes aspera linn. is known as uttareni. It belongs to family Amaranthaceae. Due to limited availability and affordability of pharmaceutical medicines, species of higher plants are used for the treatment of helminthiasis. [1-6]. Tulsi is the as sacred and medicinal plant. The name Tulsi is derived from ‘Sanskrit’, which means “matchless one”. This plant belongs to the family Labiatae, characterized by square stem and specific aroma. Botanical name of Tulsi is Ocimum sanctum (Linn). In India, the plant is grown throughout the country from Andaman and Nicobar islands to the Himalayas up to 1800 meters above the sea level. It is also abundantly found in Malaysia, Australia, West Africa and some of the Arab countries.

The sacred, Tulsi is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani system of holistic health and herbalmedicine. In traditional system of medicine, different parts of O. sanctum have been recommended for the treatment of bronchitis, malaria, diarrhoea, dysentery, skin disease, arthritis, eye diseases, insect bites and so on [7-8].

Three varieties of Tulsi are:
- Rama or Light Tulsi (Ocimum Sanctum)
- Shyama or Dark Tulsi (Ocimum Sanctum)
- Vana Tulsi (Ocimum Gratissimum)

Plant Anatomy
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: Ocimum
Species: O.tenuiflorum
Botanical Name: Ocimum Tenuiflorum

The leaf of OS contains 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene [9]. Fresh leaves and stem of OS extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid and appreciable quantities of eugenol [10]. Two flavonoids, viz., orientin and vicenin from aqueous leaf extract of OS have been isolated ursolic acid, apigenin, luteolin, apigenin-7-O-glucuronide, luteolin-7-O glucuronide, orientin and molludistin have also been isolated from the leaf extract[11]. OS also contains a number of sesquiterpenes and monoterpenes viz., bornyl acetate, α-elemene, neral, α and β-pinenes, camphene, campesterol, cholesterol, stigmasterol and β-sitosterol.
OS is also known as "the elixir of life" since it promotes longevity. Different parts of plant are used in ayurveda and siddha systems of medicine for prevention and cure of many illnesses like common cold, headache, cough, flu, earache, fever, colic pain, sore throat, bronchitis, asthma, hepatic diseases, malaria fever, as an antidote for snake bite and scorpion sting, flatulence, migraine headaches, fatigue, skin diseases, wound, insomnia, arthritis, digestive disorders, night blindness, diarrhoea and influenza. The leaves are good for nerves and to sharpen memory. Chewing of Ocimum Sanctum leaves also cures ulcers and infections of mouth.

MATERIALS AND METHOD [12-14]
Collection of plant material
Leaves of Ocimum Sanctum Linn. (Tulsi) Ram tulsi and Krishna tulsi collected from near by area of Tarale, Satara, methanol was used as solvent.

Selection of worms
Indian adult earthworms were used to carry out the anthelmintic evaluation. The earthworms were collected from the moist soil and washed with saline water to remove the faecal matter. Worms were of about 6 cm to 10 cm length and 0.3 to 0.4 cm wide was selected for the experiment. Indian adult earthworms (Pheretima posthuma) were collected from the local Vermicompost shop and washed with normal saline to remove the faecal matter. Experiments were carried out in adult earthworm due to its structural resemblance with the intestinal roundworm parasites of human beings. Indian adult earthworms of about 5-7 cms long were used to evaluate the anthelmintic activity.

Drugs and chemicals
Albendazole, Glaxo Smithkline Pharmaceuticals Ltd., Bangalore used as standard anthelmintic drug and ethanol [Loba chemie pvt. Ltd, Mumbai] and DMSO were used during the experimental protocol.

Procedure of extraction using soxhlet apparatus[7-12]
Extraction methods for Tulsi Leaves
A. By Soxhlation method
- Dry the leaves of different species of tulsi about 100 gm of dried leaves.
- Fill the dried leaves powder in the soxhlation apparatus with ethanol about 250 ml in BF and boil it at 50-60°C temperature for 5-6 hrs.
- Collect the crud syrup mass of extract at the bottom of flask.

B. By Maceration
The collected, cleaned leaves of ocimum were used for the extraction process. 200g of powder of Leaves were macerated with Methanol, shaking frequently during first 6 hours and allowing stand for 18 hours. The extracts were filtered through what Mann filter paper to remove any impurities if present. The extracts were concentrated by vacuum distillation to reduce the volume 1/10. The concentrated extracts were transferred to 100 ml beaker and to removing solvent were evaporated on the water bath. The dried extracts were packed and labeled in air tight container for the further studies.

Anthelmintic Activity
The anthelmintic assay was carried out as per the method. The assay was performed in vitro using adult earthworm as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings for preliminary evaluation of anthelmintic activity. Test samples of the extract was prepared at the concentrations, 10, 20, 30, 40 and 50 mg/ml in ethanol and six worms of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Albendazole (10 mg /ml) was used as reference standard was advocated earlier. Test samples of the extract was prepared at the concentrations, 10, 20, 30, 40, 50, 60 and 70 mg/ml in distilled water and six worms of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Albendazole (10mg /ml) was used as reference standard and distilled water as control. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were collected and kept in normal saline solution. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). All the results were shown in table and expressed as a mean ± SEM of six worms in each group.

Experimental Work
The extract of Ram Tulsi and Krishna Tulsi obtained by Maceration Process of Extraction using methanol as a solvent. Two separated green coloured extract formed, in that solvent is used the methanol so do not need to evaporate that extract. The extract is ready for to testing or studies anthelmintic activity.

The activity is tested with standard drug was Albendazole and for the comparative study used Ram and Krishna tulsi extract as well as the marketed commercial preparation.

Commercial preparation: (Jolly) Tulsi 51

Those are the known Jolly Tulasi 51 Drops called natural immunity booster.

Each 10 ml contains having combinations of the following tulsi extract- 
1. Vishnu priya
   (Ocimum sanctum)
2. Rama tulsi
   (Ocimum gratissimum)
3. kala tulsi
   (Ocimum canum)
4. Bisva tulsi
   (Ocimum basilicum)
5. Basil sweet lemon
   (Ocimum citiodorum)
6. Base-q.s.

These concentrated liquid extract contains above five types of rare tulsi.
Jolly Tulsi 51 drops is Anti Oxidant, Anti Aging, Anti Viral, Anti Septic, Stimulant, Demulsant, Diuretic and Carminative.

That commercial preparation obtained in Medical ShopAdress- Shaniwar peth, Tarale, Tal-Patan, Dist-Satara.

The extracts were concentrated by rotary evaporator and used for testing anthelmintic activity. Albendazole was used as standard reference drug and its 20 mg/ml concentration was prepared by as per the prescribed method. The suspension of aqueous extract of leaves of Ocimum sanctum Linn, concentration 100 mg/ml was prepared and final volume was made up to 50 ml for relevant concentration. Groups of approximately equal size worms consisting of five earthworms individually in each group were released into in each 20 ml of desired concentration of drug and extracts in the petridish. The anthelmintic activity was performed according to standard screening methods. Five Indian earth worms (adult) positioned in petridish containing 50 ml contained 100 mg/ml of aqueous extract of leaves of Ocimum sanctum Linn. Every petridish was placed with 5 earth worms and studied for paralysis or death. The mean time for paralysis was recorded when no movement of any sort could be observed, the time to death of worm (min) was recorded after ascertaining that worms not moved even with external physical stimuli. The test results were compared with reference compound Albendazole (20 mg/ml) treated samples.

1. Earthworm in standard drug solution Albendazole
The drug Albendazole or that tablet Albendazole Bandy it is triturate and make fine powder that are dissolved in distilled water and formed solution of standard drug for the comparative study of tulsi anthelmintic activity. In that solution taken in petri plate and placed or deep the worm and record their paralysis time or death.

2. Earthworm in solvent used in extraction process methanol
Worm present in solvent methanol and to record death and paralysis time.

3. Earthworm in extract of Ram Tulsi

4. Earthworm in extract of five rare types of tulsi that is marketed preparation of Jolly Tulsi 51

5. Earthworm in dilute aqueous extract of tulsi
These are dilute extracts containing different concentrations like 2, 4, 6, 8, 10mg/dl with help of water.
RESULT AND DISCUSSION

Antihelminthic Activity of Ocimum Sanctum

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Antihelminthic substance</th>
<th>Concentration (mg/ml)</th>
<th>Time taken for paralysis (sec)</th>
<th>Time taken for death (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Albendazole</td>
<td>0.1</td>
<td>128</td>
<td>138</td>
</tr>
<tr>
<td>3.</td>
<td>Aqueous extract of Krishna tulsi</td>
<td>0.1</td>
<td>144</td>
<td>144</td>
</tr>
<tr>
<td>4.</td>
<td>Extract of commercial preparation</td>
<td>0.2</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>5.</td>
<td>Solvent extract of Ram tulsi</td>
<td>0.3</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>6.</td>
<td>Solvent extract of Krishna tulsi</td>
<td>0.4</td>
<td>65</td>
<td>65</td>
</tr>
</tbody>
</table>

Comparative study of different extract of tulsi and commercial preparation of jolly tulsi against standard drug Albendazole for antihelminthic activity (time taken for the death of worms).

Discussion

The aqueous extract of leaves of Ocimum sanctum showed good activity against Phereetema posthuma at the tested concentrations. Eugenol was the active constituent present in Ocimum sanctum L., has been found to be accountable for the therapeutic potentials of Tulsi [16]. Our data was also correlating with studies of D. J. Taur et al. and Kamlesh Chandra Joshi et al. and L. S. Verma et al. study has proved their antihelminthic activity of Ocimum sanctum L. in Syphacia muris in Mice to be taken into consideration for strong antihelminthic activity. The aqueous extract of Krishna tulsi showed good antihelminthic activity comparable to the standard. While the marketed preparation jolly tulsi was less effective as compared to aqueous extract of Krishna tulsi.

CONCLUSION

Aqueous extract of Ocimum sanctum Linn and their species like commercial preparation, Ram and Krishna Tulsi is more potent than control and lesser antihelminthic activity than albendazole is noted for AEKT but more than the marketed preparation of jolly tulsi. Additional studies using in vivo models and to isolate active constituents from extract is required and also its probable mechanism of antihelminthic activity needs to be investigated and rationale for the use of Ocimum sanctum as an antihelminthic drug need to be explored.

Conflicts of interest

There were no conflicts of interest.

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REFERENCE

[8] www.goggle.com