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Research Article

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Investigation of *In Vitro* Anthelmintic Activity of Phenolic Acid Fraction From *Thespesia populnea* (Stem Bark)

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Abstract: Phenolic acid fraction of *Thespesia populnea* was investigated for their anthelmintic activity against *Pheretima posthuma* (Earth worms). Two concentrations of ethyl acetate fraction (10, and 20 mg/ml) and phenolic acid fraction (5 and 10 mg/ml) were studied for activity, which involved the determination of time of paralysis and time of death of the worm. Albendazole (40 mg/ml) was used as reference standard and 0.5% DMSO in saline solution as control. The result shows that the fractions possess modest anthelmintic activity. The anthelmintic activity of phenolic acid fraction of *Thespesia populnea* therefore been demonstrated for the first time.

Key words: Anthelmintic, *Thespesia populnea, Pheretima posthuma,* Albendazole.

INTRODUCTION:

Anthelmintics or antihelminthics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing) [1]. Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics [2- 4].

Thespesia populnea Soland ex. Correa, a plant of the Malvaceae family, is used in folk-medicine in India for the treatment of liver diseases and dermatitis. The decoction of the bark is used by Ayurvedic physicians for the treatment of skin and liver diseases [5]. The bark and flower of *T. populnea* possess pharmacological properties such as hepato-protection, antioxidant activity, anti-inflammation, memory enhancement property and hypo-cholesterolemic activity [6-12]. *T. populnea* fruit have anthelmintic activity and antidiarrheal activity [13-14]. The plant bark has not been explored for its anthelmintic activity so far. The present study was therefore aimed at investigating the anthelmintic activity of the stem bark phenolic acid fraction with a view to justifying the use of the plant bark in the treatment of helminthes.

MATERIALS AND METHODS:

Chemicals: All the chemicals used in this investigation were of analytical reagent (AR) grade and were purchased from Sisco research laboratory and Merck. Albendazole was taken as a reference standard and the concentration of the standard drugs were prepared in 40 mg/ml DMSO in normal saline.

Plant materials, preparation of extract and fractions: *T. populnea* stem bark was collected from Thodupuzha, Kerala, India in the month of March. The specimens were identified and voucher specimens were deposited in the Herbarium (TTP-876) of Nagarjuna Herbal Concentrates Ltd, Kerala, India. The dried and coarsely powdered stem bark of *T. populnea* was macerated with ethanol for 4 h by continuous stirring at room temperature and then filtered and evaporated the filtrate to dryness under reduced pressure. The yield of ethanol extract was 23% of the stem barks powder. The dried ethanol extract was then suspended in distilled water (1:50, w/v) and fractionated through successive extractions (twice each) with chloroform (1:1, v:v), ethyl acetate (1:1, v:v), n-butanol (1:1, v:v)) saturated with water. Ethyl acetate fraction was concentrated to dryness under reduced pressure and below 45° C on a rotary vacuum evaporator. The yield of ethyl acetate fraction of ethanol extract was 14 percentages.

Fractionation of ethyl acetate fraction and isolation of phenolic acid fraction : The ethyl acetate fraction was subjected to silica gel 60-120 mesh size (Merck, Mumbai) column chromatography (column 15X400 mm, 20 g silica gel and 1g sample) and eluted with Hexane : Ethyl acetate (100:0), (90:10), (80:20), (70:30), (60:40), (50:50), (40:60), (30:70), (20:80) and (10:90), successively; each fraction was extracted in a volume of 10 ml of the solvent mixture. Eluents were combined according to TLC (NP-Silica Gel 60 F_{254} plates) behaviour using solvent system: Hexane-Ethyl acetate-Acetic acid (5:5:0.1, v:v:v). The developed plates were allowed to air dry and chromatograms were observed under visible and UV light (k = 254 nm).

Chemical analysis of active component/fraction: In the column chromatography, fraction 25-50 showed a single band on TLC with an Rf value of 0.8 [6]. The plates were sprayed with Folin – Ciocalteu's phenol reagent and fumigated ammonia vapour, the plate developed dark blue colour band [15]. The yield of active component was 53% of ethyl acetate fraction and 1.7 percentages of plant stem bark powder. This active component was used for further studies. The phytochemical identification was carried out by the methods of Trease and Evans [16].

High performance liquid chromatographic (HPLC) analysis of phenolic fraction: The active component, isolated by column chromatography was subjected to HPLC analysis [6].

Animal: Healthy adult Indian earthworms (*Pheretima posthuma*), due to their anatomical and physiological resemblance with intestinal roundworm parasites of human beings were used in the present study [17-19]. All earthworms were of approximately equal size (10 cm). They were collected from Kerala Agricultural University, Mannuthy, washed and kept in normal saline water.

Anthelmintic Activity: five groups of six earthworms were released into separate 20 ml solutions of Albendazole (40 mg/ml), phenolic acid fraction (5 and 10 mg/ml) and ethyl acetate fraction (10 and 20

mg/ml) in 0.5% DMSO make up with saline solution. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken to paralyse and cause death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colours [20].

RESULTS AND DISCUSSION:

The stem bark fraction of the *T. populnea* showed a significant anthelmintic activity in dose dependent manner as shown in Table 1. The ethyl acetate fraction (10 and 20 mg/ml) and phenolic acid fraction (5 and 10 mg/ml) concentration showed paralysis and death of earth worms and compared with standard drug Albendazole (40 mg/ml). Fractions of *T. populnea* show the paralysis as well as death of worm in lesser time as compared to Albendazole especially at higher concentration (40 mg / ml). Some synthetic phenolic anthelmintics, e.g. niclosamide, oxyclozanide, bithionol, nitroxynil, etc, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [21]. It is possible that phenolic acid contained in the fractions of *T. populnea* produced similar effects. Our privies studies highly active phenolic acid-fraction has been isolated for the first time from *T. populnea* stem bark. In HPLC analysis the component was further separated into 3 closely related compounds [6]. Structures of these phenolic compounds remain to be elucidated.

CONCLUSION:

In conclusion, the traditional use of stem bark of *T. populnea* an anthelmintic have been confirmed as the fractions displayed activity against the worms used in the study. Further studies need to establish the mechanisms of action are required.

Table – 1: Anthelmintic activity of *T. populnea* stem bark ethyl acetate and phenolic acid fraction

Groups	Concentration (mg/ml)	*Pheretima posthuma* (Earthworms)	Time taken for paralysis in minute	Time taken for death in minute
Thespesia populnea **(Phenolic fraction)**	5	n=6	45.3 ± 3.3	55.6 ± 2.7
	10	n=6	26.1 ± 2.4	38.1 ± 3.2
Thespesia populnea **(Ethyl acetate fraction)**	10	n=6	45.1 ± 2.9	55.3 ± 1.9
	20	n=6	26.1 ± 2.4	36.0 ± 2.5
Albendazole	40	n=6	30.6 ± 2.5	40.5 ± 1.5
Control	--	n=6	--	--

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