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# EVALUATION OF ANTHELMINTIC ACTIVITY OF STEM BARK OF *HOLOPTELEA INTEGRIFOLIA* (ROXB) PLANCH

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**ABSTRACT**
*Holoptelea integrifolia* (Roxb) Planch (Ulmaceae) is commonly known as Indian elm, kanju. It is widely distributed throughout India in deciduous forests. In traditional system of medicine, bark and leaves are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism. The present study was carried out to investigate the anthelmintic activities of different extracts of benzene, chloroform, methanol and aqueous extracts of the stem bark of *Holoptelea integrifolia* against adult earth worm *Pheretima posthuma*. The time taken for each worm for paralysis and death were determined. The results were compared with the results of standard i.e. Piperazine citrate. Methanolic and aqueous extracts both were found to possess significant anthelmintic activity in comparison to the standard drug. Both the extract showed dose dependent anthelmintic activity.

**KEYWORDS:** *Holoptelea integrifolia,* anthelmintic activity, *Pheretima posthuma,* piperazine citrate, stem bark.

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## INTRODUCTION

*Holoptelea integrifolia* (Roxb) Planch (Ulmaceae) is commonly known as Indian elm, kanju. It is a large deciduous tree, commonly found throughout the greater part of India up to an altitude of 660 m, lower ranges of Himalaya from Jammu to Oudh, Rohilkhand, forest of Dehra Dun, Saharanpur, Orissa, Chota Nagpur, Bihar, West Bengal, Hill of Deccan, Eastern slopes of Western Ghats and North Circas[1,2]. In traditional system of medicine, bark and leaves are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism[3].

The phytoconstituents isolated so far from stem bark are holoptelin A and B[4], 2-aminonapthaquinone,friedelin, epifriedelin , β-sitosterol and its β-D-glucose[5,6].

Helminthes are recognized as major constraints to livestock production throughout the tropics. The economic impact of parasitic gastroenteritis (PGE), which is caused by mixed infection with several species of stomach and intestinal round worms, as a production disease in ruminants lies not only in direct losses such as a mortality associated with the clinical form of the disease but also indirect insidious losses as a result of weaknesses, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity[7].

The traditional system of medicine strongly suggests the anthelmintic activity of the parts of *Holoptelea integrifolia* which is not proved scientifically. Hence the present study was carried out to determine the anthelmintic activity of the *Holoptelea integrifolia.*

## MATERIALS AND METHODS

### Drugs and Chemicals

The drug, Piperazine citrate was procured from SD Fine Chemicals Ltd., Mumbai. All organic solvents and chemicals were purchased from SD Fine Chemical Ltd., Mumbai and were of analytical grade.

### Plant material

Stem bark of *Holoptelea integrifolia* from various parts of Tirupati were collected and authenticated by Mr.Madhava Chetty (S V University, Tirupati, Andhra Pradesh). A voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore. The stem bark was dried under normal environmental conditions. The dried stem bark were powdered and stored in a closed container for further use.

### Preparation of extracts

The dried stem bark powder were coarsely powdered and subjected to successive extraction by soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, benzene, chloroform, methanol and distilled water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 0.15, 0.26, 0.21, 4.77 and 7.85 % w/w respectively with reference to the air dried plant material.

### Preliminary phytochemical screening

The coarse powder stem bark of *Holoptelea integrifolia* (20 g) was subjected to successive extraction with different solvents in their increasing order of polarity from petroleum ether, benzene, chloroform, methanol and distilled water. The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents[8].

### Earthworms collection and authentication

Healthy adult Indian earthworm (*Pheretima posthuma*; Annelida; Megascolecidae) were collected from Microbial Resources Division, Gandhi Krushi Vijnana Kendra (GKVK), Government of Karnataka, Bangalore. Earthworms in moist soil were washed with normal saline and used for the study. The

earthworm of 3 -5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[9,10].

**Anthelmintic activity**

The anthelmintic activity of stem bark extracts of *Holoptelea integrifolia* was evaluated as per the method reported by Dash et al[11]. The extracts were suspended in Tween 80 (0.1 %) in normal saline. All the drugs and extracts were freshly before starting the experiment. Twelve groups of six earthworms each were released into 20 ml of desired formulation as follows; vehicle (0.1 % Tween 80 in normal saline), piperazine citrate (40, 60 mg/ml), benzene extract (20 mg/ml), chloroform extract (20 mg/ml), methanol extract (20, 40, 60, 80 mg/ml) and aqueous extract (40, 60, 80 mg/ml). As the percentage yield of benzene and chloroform extract were less, so only few concentrations could be prepared. Observation were made for the time (in minutes) taken to paralysis and death of individual worms up to 4 hrs of the test period. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body color[12].

## RESULTS AND DISCUSSION

The results of preliminary phytochemical screening the stem bark of *Holoptelea integrifolia* are tabulated in **Table 1**.Petroleum ether extract showed the presence of steroids, flavonoids, tannin; benzene and chloroform extracts contain steroids; methanol extract contains steroids, alkaloids, cardiac glycosides, tannins, saponins and carbohydrates and aqueous extract contains alkaloids, tannins and saponins.

From the **Table 2** and **Fig 1**, it is very clear that methanol extract at the concentration of 20, 40, 60 and 80 mg/ ml produced anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D). Methanol extract at 80 mg/ml (9 ±0.89 min & 35.66 ± 0.816 min), 60mg/ml (10.66 ± 0.33 min & 45.83 ± 0.307 min), 40 mg/ml (12.33 ± 0.33 min & 48.5 ± 0.50 min) and 20 mg/ml (32.83 ± 0.47 min & 172 ± 0.365 min) showed shortest time of paralysis (P) and death (D) when compared with piperazine citrate at 40 mg/ml (42.0 ± 1.26 min & 59.4 ± 0.40 min) and 60 mg/ml (33.4 ± 0.60 min & 55.6 ± 0.24 min) concentrations respectively. Benzene extract at 20 mg/ml (113.16 ± 0.477 min & 132 ± 0.365 min) and chloroform extract at 20 mg/ml (102.83 ± 0.401 min & 122.75 ± 0.224 min) did not show good anthelmintic activity in comparison to piperazine citrate even at 40 mg/ml concentration. Aqueous extract at 80 mg/ml (27 ± 0.614 min & 57.83 ± 0.401 min) and 60 mg/ml (41.33 ± 0.614 min & 115.33 ± 0.615 min) showed shortest time of paralysis (P) and death (D) when compared with piperazine citrate. Whereas, in control group, worms were observed for 24 hours and no paralysis or death was found during that period. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis[13]. The stem bark of *Holoptelea integrifolia* extracts not only demonstrated paralysis, but also causes death of worms especially at higher concentration of 20, 40, 60 and 80 mg/ml [methanol], in shorter time as compare to reference drug piperazine citrate.

## CONCLUSION

Among all the extract tested, methanol and aqueous extracts showed dose dependent anthelmintic and better activity in comparison with reference standard. Chloroform and benzene extracts at 20 mg/ml concentration did not show any activity in comparison with piperazine citrate at dose of 40 and 60 mg/ml. On the basis of these investigations, we may partially conclude that *Holoptelea integrifolia* could be a potent anthelmintic agent for next generation. Further studies are required on phytochemical profiling as well as isolation and identification of bioactive component responsible for anthelmintic activity.

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**Table 1: Preliminary phytochemical screening of the stem bark of *Holoptelea integrifolia* (Roxb) Planch**

| Phytoconstituents | Petroleum ether Extract | Benzene extract | Chloroform extract | Methanol extract | Aqueous extract |
|---|---|---|---|---|---|
| Carbohydrates | - | - | - | + | + |
| Cardiac glycosides | - | - | - | + | - |
| Steroids | + | + | + | + | - |
| Alkaloids | - | - | - | + | + |
| Flavonoids | + | - | - | - | - |
| Tannins | + | - | - | + | + |
| saponins | - | - | - | + | + |

'+': Present; '-': Absent

**Table 2: Anthelmintic activities of different extracts of *Holoptelea integrifolia* stem bark**

| Treatment group | Concentration (mg/ml) | Paralysis time (min) | Death time (min) |
|---|---|---|---|
| Vehicle (0.1 % Tween 80 in normal saline | ------ | ------ | ------ |
| Piperazine citrate | 40 | 42.0 ± 1.26 | 59.4 ± 0.40 |
| | 60 | 33.4 ± 0.60 | 55.6 ± 0.24 |
| Benzene extract | 20 | 113.16 ± 0.477 | 132 ± 0.365 |
| Chloroform extract | 20 | 102.83 ± 0.401 | 122.75 ± 0.224 |
| Methanol extract | 20 | 32.83 ± 0.47 | 172 ± 0.365 |
| | 40 | 12.33 ± 0.33 | 48.5 ± 0.50 |
| | 60 | 10.66 ± 0.33 | 45.83 ± 0.307 |
| | 80 | 09.0 ± 0.89 | 35.66 ± 0.816 |
| Aqueous extract | 20 | ----- | ----- |
| | 40 | 67.16 ± 0.401 | ----- |
| | 60 | 41.33 ± 0.614 | 115.33 ± 0.615 |
| | 80 | 27.0 ± 0.614 | 57.83 ± 0.401 |

Results are expressed as mean ± SD of six determinations; vehicle worms were alive up to 24 hrs of observation. -----: worms were alive.

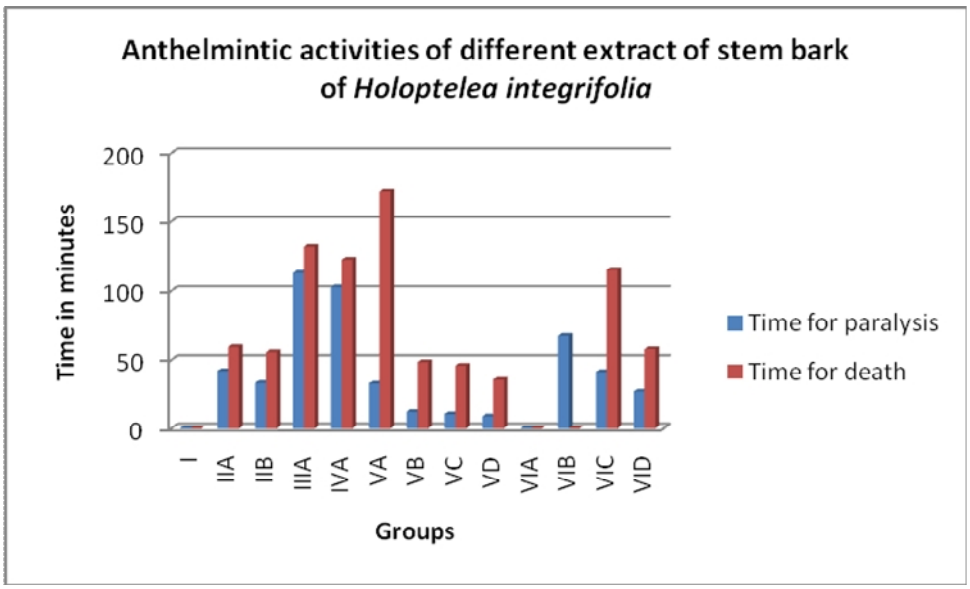


**Figure 1: Anthelmintic activities of different extract of stem bark of *Holoptelea integrifolia* on *Pheretima postuma*.**

Group I: Vehicle (Normal saline); group IIA, IIB:standard Piperazine citrate at 40 and 60 mg/ml);group IIIA : Benzene extract at 20 mg/ml; group IVA: chloroform extract at 20 mg/ ml; group VA,VB,VC,VD: Methanol extract at dose of 20,40,60,80 mg/ ml; group VIA,VIB,VIC,VID: Aqueous extract at 20,40,60,80 mg/ml respectively.

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