Preliminary Phytochemical Screening and Anthelmintic Activity of
Chloroxylon swietenia Root Extract

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Abstract

The preliminary phytochemical investigation was carried out on the crude extracts of the roots of Chloroxylon swietenia. The crude extracts were investigated for their anthelmintic activity against Indian earthworms (Pheretima posthuma). Three concentrations (25, 50 and 100 mg/mL) of each extract were performed in activity, which involved the evaluation of paralysis and death period of the worm. Chloroform and methanol extracts exhibited significant anthelmintic activity at highest concentration of 100 mg/mL. Piperazine citrate was selected as standard compound and Di-methyl formamide as control. The anthelmintic activity of Chloroform and methanol of roots of Chloroxylon swietenia has therefore been evaluated for the first time.

Keywords: Anthelmintic, Chloroxylon swietenia, methanolic, chloroform, Pheretima posthuma.

Introduction

Parasitic diseases are a major infestation in the human beings like helminthiasis. The disease is caused by round worm, hook worm, thread worm, tape worm and filarial, guinea worm are found in intestine [1]. The worm is responsible for many type of disease; they harm the host by depriving him of food, causing blood loss in stool, injury to organs, intestinal or lymphatic obstruction and by secreting the toxins [2]. Helminthiasis is really fatal, but it is a major cause of ill health [3]. Number of synthetic drugs used to control and prevent the infestation related to worms like mebendazole, albendazole, piperazine and pyrantel, almost mebendazole used as broad spectrum anthelmintic drug [4]. Adverse effect like tolerance, resistdance, nausea, vomiting drowsiness, dizziness, and abdominal pain occurred at long term used of synthetic medicine [5,6]. Therefore, overcome the problem associated with synthetic medicine, the natural compound are selected. Naturally produced medicinal products offer as an alternate anthelmintic and therapeutic agents so as to overcome some of these infestation and subsequently may be sustainable and environmentally acceptable because the natural or herbal compounds are free from adverse effect [7]. The objective of studies are to improve the living standard of human beings, avoiding the problem created with synthetic origin of medicine
which disturb the pathological, physiological and anatomical function of body. *Chloroxylon swietenia* belonging to family *Rutaceae* is a medicinal and aromatic tree of dry deciduous forests [8]. It is commonly known as Yellow wood, East Indian satin wood, Ceylon satin wood, Bhirra and Bharhul [9]. The whole part of this tree has long been used in the indigenous system of medicine such as the bark is used as an astringent [10]; leaves are applied to worm infested wound of animals, fungal infection of skin, and for the treatment of inflammation related disorder like pain and rheumatism [11]. Previous phytochemical investigation and isolation revealed the presence of alkaloid (furoquinoline, skimmiamine), coumarin (xanthyletin, xanthoxyletin and 7-demethylsuberosin), lignan (swietenone), germacrene sesquiterpene, pregeijerene and geijerene [12-14]. Earlier studies have shown that the extract of plant posses antifeedant, antifertility, larvicidal, mosquito repellent, anti-inflammatory, antimicrobial, hepatoprotective and antioxidant activity [15]. Traditionally; the roots of *C. swietenia* produced astringent properties as well as to treat worm infestation in animals. However, no scientific data are available regarding its usefulness as anthelmintic agent. Keeping the above information in view, the present study was an endeavor to ratify the anthelmintic activity of the methanolic extract of the roots of *C. swietenia* (MCS) on Indian earthworm (*Pheretima posthuma*).

**Phytochemical investigation and parameters**

The preliminary phytochemical investigation the crude extract was fractioned successively by using methanol, chloroform, petroleum ether, hexane and ethyl acetate successively and ready to use for further study. The presence or absence of different phytoconstituents viz amino acid, alkaloids, carbohydrates, glycosides, tannins, flavonoids and phytosterols were detected by usual prescribed methods [16]; were represented in table1. Fluorescence analysis of the crude extract was carried out by standard methods [17, 18]; were depicted in table2.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Petroleum ether</th>
<th>Hexane</th>
<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amino acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Present, - Absent

**Table2:** Consistency and fluorescence analysis of various extracts of root of *C. swietenia*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Consistency</th>
<th>Day light</th>
<th>UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Solid</td>
<td>Yellowish</td>
<td>Yellow fluorescence</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Resinous</td>
<td>Brown</td>
<td>Brown fluorescence</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>Sticky mass</td>
<td>Dark green</td>
<td>Green fluorescence</td>
</tr>
<tr>
<td>Hexane</td>
<td>Semisolid</td>
<td>Dark green</td>
<td>Green fluorescence</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Semisolid</td>
<td>Dark green</td>
<td>Green fluorescence</td>
</tr>
</tbody>
</table>

**Materials and methods**

**Extraction of Plant Material**

The fresh and dried roots of *C. swietenia* were collected in the month of August from the Bilaspur region, Chhattisgarh state, India. They were identified by characters mentioned in the literature of various florias [19] and were authenticated by Dr. Pankaj Oudhia, Agriculture scientist, Raipur; Chhattisgarh. The crude roots were converted in to coarse powdered through size reduction. The powdered drug was then extracted with methanol for 3-4 days, kept in refrigerator. The methanol extract was filtered.
and concentrated to a semisolid mass by vacuum evaporation. The yellow residue was fractioned successively by using methanol (MCS), chloroform (CCS), petroleum ether (PCS), hexane (HCS), and ethyl acetate (ECS); were used for phytochemical investigations. The residues obtained were prepared as 25, 50 and 100 mg/mL solutions in DMF and investigated for their anthelmintic activity. Further, the percentage of yield of MCS, CCS, PCS, HCS and ECS were found to be 11.87, 9.81, 6.56, 8.50 and 7.80 respectively.

**Evaluation of Anthelmintic activity**

Indian adult earthworms (*Pheretima posthuma*) were collected from moist soil of the field and washed with normal saline solution to remove all faecal matter were used for further investigation. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were selected for all the experimental parameters. The anthelmintic activity was evaluated on adult Indian earthworm (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal round worm of human beings. Five different concentrations, each of crude extract of MCS, CCS, PCS, HCS and ECS (25, 50, 100 mg/mL) were prepared in DMF and six worms (identical to each other) were placed in it. Observations were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis in min was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; time of death in min was recorded after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm water (50°C) and Piperazine citrate (PC; 10 mg/mL) [20, 21] was included as reference compound. Treatment with normal saline served as control. Three replicates of each experiment were performed to estimate any sources of error. Paralysis is assumed to occur when they do not revive even in saline solution. Potency is inversely proportional to time taken for paralysis and/or death of parasite. Observation were shown in table3 regarding the anthelmintic activity of *C. swietenia* against Indian earthworm.

**Table3. Anthelmintic activity of different extracts of roots of *C. swietenia* on *Pheretima posthuma***

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration (mg/mL)</th>
<th>Time taken for Paralysis (Min)</th>
<th>Time taken for death (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>25</td>
<td>80±0.6</td>
<td>110±0.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>45±0.2</td>
<td>69±0.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>40±0.2</td>
<td>60±0.2</td>
</tr>
<tr>
<td>Chloroform</td>
<td>25</td>
<td>68±0.4</td>
<td>98±0.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>47±0.1</td>
<td>62±0.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>33±0.3</td>
<td>51±0.1</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>25</td>
<td>98±0.5</td>
<td>125±0.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>57±0.2</td>
<td>109±0.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>53±0.1</td>
<td>90±0.4</td>
</tr>
<tr>
<td>Hexane</td>
<td>25</td>
<td>118±0.9</td>
<td>143±0.1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>72±0.5</td>
<td>104±0.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>64±0.4</td>
<td>89±0.6</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>25</td>
<td>134±0.2</td>
<td>145±0.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>98±0.6</td>
<td>132±0.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>78±0.8</td>
<td>97±0.2</td>
</tr>
<tr>
<td>PC</td>
<td>25</td>
<td>23±0.4</td>
<td>55±0.6</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All the values are Mean±SEM (n = 6). P<0.05 compared to PC

**Statistical analysis**
The data were analyzed with GraphPad Prism 4 (San Diego, CA). Statistical analysis of data was done by One-way ANOVA, followed by Newman Keuls test. Data are expressed as Mean ± Standard error of mean (S.E.M.). A level of P<0.05 was accepted as statistically significant.

**Results and Discussions**
Table3 illustrates the effect of different extracts of root of *C. swietenia* (25, 50 and 100 mg/mL) in time for paralysis and death of *Pheretima*
Statistical analysis by One-way ANOVA showed that there was significant difference in time taken for paralysis (P<0.05) of Indian earthworm among groups. Post-hoc test revealed that MCS (100 mg/mL), CCS (50 mg/mL) and CCS (100 mg/mL) groups were not significantly different compared to PC in time taken for paralysis of Indian earthworm, indicating equivalence in potency. Further, all the treated groups except MCS (100 mg/ml), CCS (50 mg/ml) and CCS (100 mg/ml) groups showed significant difference compared to PC (10 mg/ml) in time taken for paralysis of earthworm. Furthermore, statistical analysis by One-way ANOVA showed that there was significant difference in time taken for death (P<0.05) of Indian earthworm among groups. The post-hoc test indicated that the time taken for death of Pheretima posthuma was similar to that of the effect observed in time taken for paralysis of earthworm, indicating equivalent potency while compared to PC. The present study revealed that the MCS (100 mg/ml) and CCS (50 and 100 mg/ml) have equivalent potency compared to PC (10 mg/ml) in time taken for both paralysis and death of Pheretima posthuma. Preliminary phytochemical screening of the extracts revealed that the presence of terpenoids, flavonoids, alkaloids, tannins, saponins and steroids. It has been well established that PC by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis [22,23] thus, our drug may have the similar profile of mechanism of action. Therefore, standardization of each extracts and isolation of phytoconstituents in each extracts for anthelmintic activity is required in the future. Furthermore, the pharmacological studies for anthelmintic activity should be undertaken in other parasites to mimic the exact human helminthesis.

Conclusions
The methanolic and chloroform extract of root of C. swietenia showed anthelmintic activity on Pheretima posthuma. Therefore, standardization of each extracts and isolation of phytoconstituents in each extracts for anthelmintic activity is required in the future. Furthermore, the pharmacological studies for anthelmintic activity should be undertaken in other parasites to mimic the exact human helminthesis.

References
17. Pratt RT, Chess ER. Fluorescence of powdered vegetable drugs in particular to develop system of identification. J Am Pharm Ass. 1949; 38:324-331