SCREENING OF *CHLOROXYLON SWIETENIA* DC ROOT FOR ANTIBACTERIAL AND ANTHELMINTIC ACTIVITIES

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Summary

The methanolic and Chloroform extracts of root of *Chloroxylon swietenia* DC were screened for antibacterial and anthelmintic activities. All the extracts were tested against certain gram positive and gram negative organisms by well diffusion method. In the methodology, azithromycin and DMF were used as standard and control respectively. The anthelmintic activity was investigated against adult Indian earthworm (*Pheretima posthuma)* using Piperazine citrate as a standard compound, which involved the evaluation of paralysis and death period of the parasite. Both the extracts exhibited significant antibacterial and anthelmintic activity at higher concentration of 100mg/ mL.

Keywords: Antibacterial, Methanolic, *Pheretima posthuma*, Chloroform, Anthelmintic

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Introduction

The plant *Chloroxylon swietenia* DC (Rutaceae) is a medicinal and aromatic tree of dry deciduous forests [1]. It is commonly known as Yellow wood, East Indian satin wood, Ceylon satin wood, Bhirra and Bharhul [2]. 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 [3]; 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 [4]. 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 [5], [6], [7]. Earlier studies have shown that the extract of plant posses antifeedant, antifertility, larvicidal, mosquito repellent, anti-inflammatory, antimicrobial, hepatoprotective and antioxidant activity [8]. 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 antibacterial and anthelmintic agent. Keeping the above information in view, the present study was an endeavor to ratify the antibacterial and 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, glycoside, tannins, flavonoids and phytosterols were detected by usual prescribed methods [9]; were represented in table1. Fluorescence analysis of the crude extract was carried out by standard methods [10, 11]; were depicted in table2.

Table 1: Phytochemical investigation on methanolic extract fractions of root of *C. swietenia*

Phytoconstituents	Methanol	Chloroform	Petroleum ether	Hexane	Ethyl acetate
Glycosides	+	+	-	-	-
Flavanoids	-	+	+	+	-
Saponins	-	+	-	-	-
Triterpenoids	-	+	+	-	-
phytosterols	+	+	+	-	-
Tannins	-	+	-	-	-
Amino acid	-	+	-	-	-
Alkaloids	-	+	-	+	-
Carbohydrate	-	-	-	-	+

+ Present, - Absent

Table 2: Consistency and fluorescence analysis of various extracts of root of *C. swietenia*

Extracts	Consistency	Day light	UV light
Methanol	Solid	Yellowish Brown	Yellow fluorescence
Chloroform	Resinous	Brown green	Brown fluorescence
Petroleum ether	Sticky mass	Dark green	Green fluorescence
Hexane	Semisolid	Dark green	Green fluorescence
Ethyl acetate	Semisolid	Dark green	Green fluorescence

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 floras [12] 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 and phytochemical investigations. The residues obtained were prepared as 25, 50 and 100 mg/mL solutions in DMF and investigated for antibacterial and 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.

Antibacterial Activity

The *in-vitro* antibacterial activity of MCS, CCS chloroform, PCS, HCS and ECS at different concentrations of 25, 50 and 100 mg/ml were studied by agar well diffusion method [13, 14], against *Staphylococcus aureus* NCTC 8530, *Escherichia coli* ATCC 2457T, *Klebsiella*

pneumoniae UC 564 and *Pseudomonas aerugenosa* 25619 organisms obtained from stock cultures of Indian institute of cholera and Enteric diseases, Kolkata, India. Stock cultures were maintained on nutrient agar medium at 40^{o}C, then subcultures in nutrient Broth at 37^{o}C, prior to each antimicrobial test. The antimicrobial activity of all the extracts was compared with standard antimicrobial agent azithromycin. The zone of inhibitions was determined by measuring scale as per standard procedure [15]. The results are recorded in Table 3.

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) [16, 17] 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. Observations were shown in table 4 regarding the anthelmintic activity of c. swietenia against Indian earth worm.

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 (SEM). A level of $P<0.05$ was accepted as statistically significant.

Results and Discussions

All the extracts have shown antibacterial activity against the all microorganisms. Methanolic and chloroform extract has shown significant effect against all organisms but mainly on *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aerugenosa*. Whereas petroleum ether, hexane and ethyl acetate extract has shown not significant effect against all organisms. Methanolic and Chloroform extracts were shown moderate activity against gram positive and gram negative bacteria in comparison to the standard azithromycin. However, whether the antimicrobial activity is due to the individual or combined effect of the chemical groups presents in the plant is to be ascertained. Results were shown in table 3.

Table 3: Antibacterial activity of different extracts of *C. swietenia* root

Groups	Concentration (mg/mL)	Zone of Inhibition (diameter in mm)			
		E. coli	S. aureus	Klebsiella	Pseudomonas
Methanol	25	13±2	12±4	20±3	16±7
	50	12±4	19±3	23.7±2	22±1
	100	15±5	28±5**	28.4±3**	28.8±4***
Chloroform	25	14±1	13±3	14±1	13.5±6
	50	15.8±3	15±1	18.9±5	14.6±2
	100	18±4	27±2*	25.9±6*	29.7±1**
Petroleum ether	25	9.9±6	11±4	9.7±3	13±3
	50	11.3±7	13±5	14±4	15±5
	100	17.5±2	14.6±2	17±2	21.8±3
Hexane	25	11.9±3	11±1	13±5	16±2
	50	18.5±4	12.6±3	15±2	18.5±4
	100	19.6±1	23±5	20.7±1	22.6±5
Ethyl acetate	25	8.7±4	9±7	10±3	12.3±6
	50	10.8±5	14.2±2	16±6	13.9±3
	100	15.8±3	22.5±3	18.4±3	22.5±1
Azithromycin	1	30±3	31.5±1	29.7±4	32±2
Control	-	-			-

*Results are expressed as Mean ± SEM (n = 6) *p<0.05, **p<0.01, ***p<0.001 compared to standard*

Table 4 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 posthuma*. 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*.

Table 4: Anthelmintic activity of different extracts of roots of *C. swietenia* on *Pheretima posthuma*

Groups	Concentration (mg/mL)	Time taken for Paralysis (Min)	Time taken for death (Min)
Methanol	25	80±0.6	110±0.3
	50	45±0.2	69±0.6
	100	40±0.2*	60±0.2**
Chloroform	25	68±0.4	98±.06
	50	47±0.1	62±0.4
	100	33±0.3	51±0.1***
Petroleum ether	25	98±.05	125±0.2
	50	57±0.2	109±0.5
	100	53±0.1	90±0.4
Hexane	25	118±0.9	143±0.1
	50	72±0.5	104±0.3
	100	64±0.4*	89±0.6
Ethyl acetate	25	134±0.2	145±0.6
	50	98±0.6	132±0.3
	100	78±0.8	97±0.2
PC	10	23±0.4	55±0.6
Control	-	-	-

*All the values are expressed as Mean±SEM (n = 6) *p<0.05, **p<0.01, ***p<0.001 compared to PC*

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 [18, 19] thus, our drug may have the similar profile of mechanism of action.

Conclusion

The methanolic and chloroform extract of root of C. swietenia have shown antibacterial and anthelmintic activity on pathogenic microorganism and *Pheretima posthuma* respectively. 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.

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