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Investigation on Phytochemicals, Anthelmintic and Analgesic Activities of *Smilax zeylanica* Linn. Leafy Extracts

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The present study involves the preliminary investigation of phytochemical constituents, acute toxicity study and the anthelmintic activity of different extracts of leaves of *S. zeylanica* using petroleum ether, diethyl ether, chloroform and methanol as solvents. The study was also extended to evaluate analgesic activity of different extracts of leaves of *S. zeylanica* using petroleum ether, chloroform and methanol as solvents. The phytochemical studies indicated that the tests for glycosides, alkaloids, tannins, triterpenoids and sterols compounds were positive for all the extracts except chloroform extract, while the tests for carbohydrate, proteins, saponins, lipids and flavonoids were negative for all extracts. The various doses of solvents extracts were evaluated for their anthelmintic activities on adult Indian earthworms, *Pheretima postuma*. All extracts exhibited the anthelmintic activity at 10 mg/mL concentration and the activities were well comparable with the standard drug, piperazine citrate. The analgesic activity of above extracts was evaluated by using tail immersion method in Swiss albino mice. All the extracts showed analgesic activity that was comparable with standard drug (aspirin). Among all the solvent extracts, methanolic extract showed better anthelmintic as well as analgesic activities. The data were verified as statistically significant by using one way ANOVA at 5 % level of significance ($p < 0.05$).

Key Words: *Smilax zeylanica*, Anthelmintics, Analgesics, Piperazine citrate, Aspirin, Swiss albino mice.

INTRODUCTION

Smilax zeylanica Linn (*Smilacaceae*) commonly known as Jangliashbha (Hindi) is widely distributed in Indian forests. It is a brambled, woody vine that grows up to 50 m long. It produces small flowers and black, blue or red berry-like fruits which are eaten frequently by birds[1]. Plants flower in May and June with white/green clustered flowers. If pollination occurs, the plant will produce a bright red to blue-black spherical berry fruit about 5-10 mm in diameter that matures in the fall[2,3].

Helminthiasis or worm infestation, is one of the most prevalent diseases and one of the most serious public health problems in the world. Hundreds of millions of human infections by helminthes exist worldwide and with increased world travel and immigration from the developing countries[4].

Analgesics are defined as the substances which decreases pain sensation by increasing pain threshold to external stimuli. Noxious pain stimuli can be developed by thermal, chemical and physical pressure[5].

The literature survey reveals that various parts of *Smilax zeylanica* have been used as a folklore medicine for curing various ailments like veneral diseases (root and plant); antifertility, analgesic and anthelmintic activity (leaf); as a carminative and in dropsy (plant), for relief in burning sensation in the feet accompanied by vesicular watery eruptions (plant)[6]. The literature survey also reveals that there are no reports on the anthelmintic and analgesic activity of the leafy extracts of *S. zeylanica*. This prompted us to investigate the anthelmintic and analgesic activities of solvent extracts of *S. zeylanica* leaves.

EXPERIMENTAL

Piperazine citrate and aspirin (Micro Lab. Ltd., Goa), petroleum ether AR (60-80 °C, Thomas Baker Chemical Pvt. Ltd.), Chloroform GR and diethyl ether (Loba Chemicals, Mumbai) and methanol AR (Merck Pvt. Ltd., Mumbai) were procured from the chemical suppliers.

The leaves of *S. zeylanica* Linn were collected from local area of Baipariguda (Dt. Koraput) in the month of July-August 2009. The plant was identified and authenticated by the Biju Pattnayak Medicinal Plants Garden and Research Centre, Dr. M.S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Odisha (Letter No. MJO8/DBT/575, date,

12.06.2008). The leaves were shade dried under normal environmental condition. The dried leaves were powered and stored in a closed container for further use.

Preparation of extract: In the extraction procedure, a total amount of 1.5 Kg dried leaves were made coarse powder and were extracted with each solvent (petroleum ether, diethyl ether, chloroform and methanol) by using soxhlet apparatus. For each solvent, 50 cycles were run. Each extract was filtered and concentrated by distilling the solvent to obtain the crude extract. Then each crude extract was dried by rotary evaporator. The successive solvent extraction of leaves of *S. zeylanica* with different solvents resulted in separation of constituents of different polarities. All solvent extracts were stored in refrigerator for further studies.

Phytochemical investigation: Chemical tests were carried out on all the extracts for the qualitative determination of phytochemical constituents[7,8].

Acute toxicity study: Albino mice of either sex (20-25 g) were used. The animals were kept in the standard polypropylene cages and provided with food and water *ad libitum*. The animals were acclimatized for a period of 14 days prior to performing the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee.

Acute toxicity study was performed as per OECD-423 guidelines[9]. Swiss albino mice of either sex were used. The animals were fasted for 4 h, but allowed free access to water throughout. The fasted mice were divided into different groups of six animals each. Each solvent extract was administered orally at a dose of 5 mg/Kg. The control animals received a similar volume of 2 % (v/v) aqueous Tween 80 solution. Mortality in each group was observed for 7 days. As no mortality was observed, the procedure was repeated at doses 50, 100 and 200 mg/Kg.

Anthelmintic activity

Animals: Healthy adult Indian earthworms, Pheretima posthuma (Annelida, Megascolecidae) were used for evaluating the anthelmintic activity due to this anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[10-12]. All the selected earthworms were of approximately equal size. They were collected from local place, washed and kept in water.

Drugs: The petroleum ether, diethyl ether, chloroform and methanolic extracts of *S. zeylanica* were tested in various doses (10, 50 and 100 mg/mL) in each group. Normal saline water was used as control. Piperazine citrate was used as the standard drug for anthelmintic activity study.

The method of Nargund[13] was followed for the screening of anthelmintic activity. Anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima postuma*. Earthworms were divided into fourteen groups (6 each). The first group (I) served as a control which received saline water only. The second (II) group received the standard drug (piperazine citrate) at a dose level of 10 mg/mL. Groups (III) to (V) received doses of petroleum ether extracts of 10, 50 and 100 mg/mL, respectively. Groups (VI) to (VIII) received doses of diethyl ether extracts of 10, 50 and 100 mg/mL, respectively. Groups (IX) to (XI) received doses of chloroform extracts of 10, 50 and 100 mg/mL, respectively. Groups (XII) to (XIV) received doses of methanol extracts of 10, 50 and 100 mg/mL, respectively. Observations were made for the time taken to cause paralysis and death of individual worms for 2 h. Paralysis was said to occur when the worms do not revive even in normal saline water. Death was concluded when the worms lost their motility followed with fading away of their body colours.

Analgesic activity

Animals: Healthy albino mice of Swiss strain of either sex were used. They were housed in standard conditions of temperature 25 ± 2 ºC, 12 h light per day cycle, relative humidity of 45-55 % in animal house of School of Pharmaceutical Education and Research, Berhampur University, Bhanja Bihar, Berhampur, Ganjam. They were fed with standard pellets of food and water. Animals were kept aseptically and all operations on animals were done in aseptic condition.

Drugs: *S. zeylanica* extracts were tested in one dose in each group of experimental model (100 mg/Kg). Aspirin was used as the standard drug in tail immersion model in a dose of 10 mg/Kg of body weight of mice.

The tail immersion method was used to evaluate the analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water[14].

Albino mice were divided into five groups of six animals each. The animals were fasted for 16 h with water *ad libitum*. The group I was served as solvent control which received the vehicle 0.5 % carboxy methyl cellulose through oral route, the group II was served as reference control which received aspirin (10 mg/Kg) and group III to V were received each solvent extract (petroleum ether, chloroform and methanol) in a dose of 100 mg/Kg. After administration of above drugs, the basal reaction time was measured after in a regular interval of 0.5 h, by immersing the tail tips of the mice (last 1-2 cm) in hot water heated at 55 ± 1 ºC. The actual flick responses of mice *i.e.*, time taken in seconds to withdrawn its tail from hot water source was calculated and the results were compared with control and standard groups.

Statistical analysis: All data were calculated statistically by standard error mean (n = 6) and statistically significant were verified by applying one way ANOVA at 5 % level of significance[15].

RESULTS AND DISCUSSION

Phytochemical studies: Table-1 shows the phytochemicals detected in *S. zeylanica* leafy extracts. The phytochemical studies indicated that the tests for glycosides, alkaloids, tannins, triterpenoids and sterols compounds were positive except chloroform extract, while the tests for carbohydrate, proteins, saponins, lipids and flavonoids were negative for all extracts.

Acute toxicity study: From the acute toxicity study it was concluded that no mortality was observed up to the dose of 200 mg/Kg for each solvent extract. Hence the extracts can be considered as non-toxic.

Anthelmintic activity: The extracts of *S. zeylanica* produced a significant anthelmintic activity in dose dependent manner as shown in Table-2. The anthelmintic activities of all extracts were comparable with that of standard drug. The normal saline water was used as a control. The extent of activity shown

TABLE-1
PHYTOCHEMICAL CONSTITUENTS OF DIFFERENT SOLVENT EXTRACTS OF LEAVES OF *Smilax zeylanica* LINN.

Phytochemicals	P.E. extract	D.E. extract	Chl extract	MeOH extract
Carbohydrate	–	–	–	–
Glycosides	+	+	–	+
Proteins	–	–	–	–
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Triterpenoids	+	+	+	+
Sterols	+	+	+	+
Saponins	–	–	–	–
Lipids	–	–	–	–
Flavonoids	–	–	–	–

+: Present and –: Absent; P.E.: Petroleum ether, D.E.: Diethyl ether; Chl: Chloroform, MeOH: Methanol.

by the crude extracts was found to be better than that of the control and less than the standard drug piperazine citrate which justifies their activities (Fig. 1). From the above results, it was concluded that the methanolic extract was more potent than

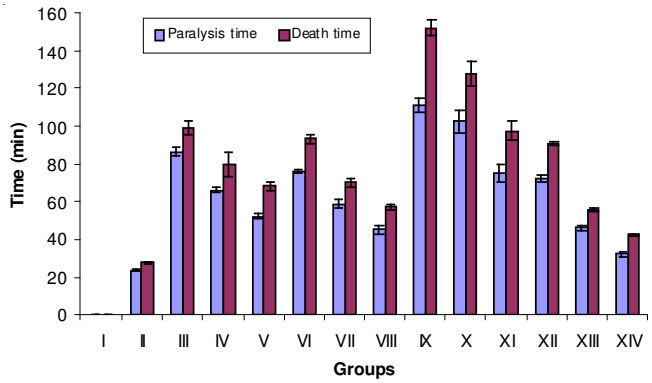

Fig. 1. Anthelmintic activities of various leafy extracts of *Smilax zeylanica* Linn and standard drug. Each bar is represented as mean ± standard error mean (n = 6). Group I: Control (normal saline water). Group II: Standard (piperazine citrate-10 mg/mL). Groups III-V: Petroleum ether extract 10, 50 and 100 mg/mL, respectively. Groups VI-VIII: Diethyl ether extract 10, 50 and 100 mg/mL, respectively. Groups IX-XI: Chloroform extract 10, 50 and 100 mg/mL, respectively. Groups XII-XIV: Methanolic extract 10, 50 and 100 mg/mL, respectively

TABLE-2
ANTHELMINTIC ACTIVITY OF VARIOUS LEAFY EXTRACTS OF *Smilax zeylanica* LINN.

Groups	Treatments	Dose (mg/mL)	Paralysis time (min) (X ± SEM)	Death time (min) (X ± SEM)
I	Vehicle (NSW)	–	–	–
II	Piperazine citrate	10	23.3 ± 0.6	27.3 ± 0.5
III	Petroleum ether extract	10	86.3 ± 2.5	98.9 ± 3.5
IV	Petroleum ether extract	50	66.22 ± 1.5	79.4 ± 6.2
V	Petroleum ether extract	100	52.12 ± 1.0	68.18 ± 2.1
VI	Diethyl ether extract	10	75.7 ± 1.0	93.08 ± 1.9
VII	Diethyl ether extract	50	58.7 ± 2.0	70.11 ± 2.0
VIII	Diethyl ether extract	100	45.25 ± 2.5	57.23 ± 1.5
IX	Chloroform extract	10	111.12 ± 3.5	152.11 ± 4.3
X	Chloroform extract	50	102.44 ± 6.0	128.14 ± 6.2
XI	Chloroform extract	100	75.0 ± 4.4	97.48 ± 5.1
XII	Methanolic extract	10	72.17 ± 1.2	90.76 ± 1.0
XIII	Methanolic extract	50	45.89 ± 1.3	55.47 ± 1.0
XIV	Methanolic extract	100	31.75 ± 1.5	42.13 ± 0.9

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1766.821112	1	1766.821112	1.92220938	0.0178359	4.25967721
Within groups	22059.8792	24	919.1616333	–	–	–
Total	23826.70031	25	–	–	–	–

NSW: Normal saline water. Each value is represented as mean ± standard error mean (n = 6). Data are found to be significant by testing through one way ANOVA at 5 % level of significance as F < F crit ($p < 0.05$).

TABLE-3
ANALGESIC ACTIVITY OF VARIOUS LEAFY EXTRACTS OF *Smilax zeylanica* LINN BY TAIL IMMERSION RESPONSE

Treatments	Dose (mg/Kg)	Tail flicking latency (s) (X ± SEM)				
		30 min	60 min	90 min	120 min	180 min
Control	10	1.97 ± 0.36	1.84 ± 0.3	1.7 ± 0.5	1.98 ± 0.6	1.84 ± 0.3
Aspirin	10	80.2 ± 6.3	83.1 ± 5.9	82.7 ± 6.5	44.82 ± 7.3	83.1 ± 5.9
Pet ether extract	100	29.3 ± 2.6	32.7 ± 2.55	25.5 ± 2.7	21.3 ± 2.8	32.7 ± 2.6
Chloroform extract	100	24.9 ± 3.56	26.5 ± 8.7	23.6 ± 2.2	20.7 ± 1.56	26.5 ± 8.8
Methanol extract	100	64.2 ± 4.4	8.4 ± 1.15	54.4 ± 7.5	35.2 ± 9.5	8.4 ± 1.2

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	754.00752	4	188.5019	0.219297	0.924526	2.866081
Within groups	17191.44	20	859.5724	–	–	–
Total	17945.45	24	–	–	–	–

Control is carboxy methyl cellulose (0.5 % w/v). Each value is represented as mean ± standard error mean (n = 6). All data were found to be significant at 5 % level of significance as F < F crit ($p < 0.05$).

the other three extracts (petroleum ether, diethyl ether and chloroform) even though all the four extracts were endowed with anthelmintic property. The order of activity was methanol extract > petroleum ether extract > diethyl ether extract > chloroform extract. The activities revealed the concentration dependence nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms[16].

Analgesic activity: The results on analgesic activity are presented in Table-3. The data indicates that the extracts produced a significant analgesia after 1.5 h at 100 mg/Kg dose (Table-3). These activities were well comparable with the standard drug (aspirin). It will be worth to mentioning that although different constituents were extracted in different solvents as per polarity but methanolic extract is more effective as compared to other three extracts. The activity showed by this extract is of considerable importance and justified its use for pain relieving as suggested in the folklore medicines. The extent of activity shown by the crude extracts is less than that of the standard drug (aspirin) but many fold more than that of the control group, which justifies its activity[17]. This can be clearly analyzed by the graphical representation as shown in Fig. 2.

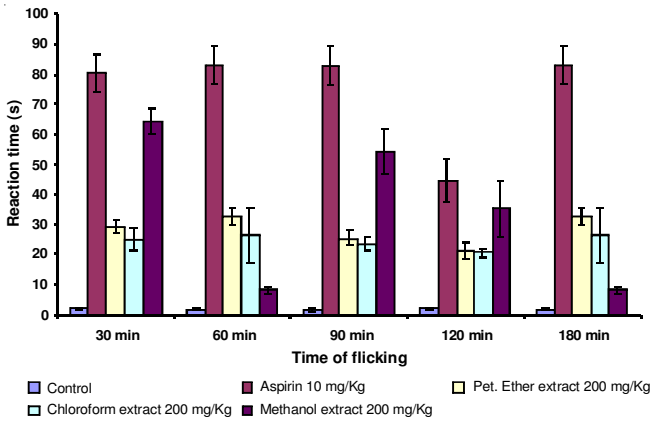

Fig. 2. Comparative study of analgesic activities of various leafy extracts of *Smilax zeylanica* Linn and standard drug. Each bar is represented as mean ± standard error mean (n = 6). Control is carboxy methyl cellulose (0.5 % w/v)

Conclusion

It could be concluded that the leaves of *S. zeylanica* is having anthelmintic, analgesic activities as suggested in the folklore medicines. It is also observed that among various solvent extracts, better results were obtained from methanolic extract. However, further study is required to identify the chemical constituents (at molecular level) present in extracts of this herb and responsible for the biological activity.

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