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PHYTOCHEMICAL STUDIES AND ANTIHELMINTHIC ACTIVITY OF *GYMNEMA SYLVESTRE*

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ABSTRACT
Helminthes are the parasitic threat to the man. Many synthetic anti helminthic agents have been identified so far, the major disadvantages with these agents are their side effects. Drugs from natural sources are giving an edge to combat parasitic problems with minimal side effects and better pharmacological action than the synthetic agents. Many agents from natural sources have been identified and many are yet to be explored in this area. In the present study, *Gymnema sylvestre* was selected based on ancient literature and are subjected to scientific pharmacological screening on *Pheretima posthuma* for anti helminthic activity. The ethanolic extract of the plant was found to have significant anti helminthic activity. Except Chloroform extract all groups of other extracts i.e., Ethanolic, Methanolic and Petroleum Ether extract have significance with the Standard drug (Albendazole) *($p<0.05$).
KEYWORDS: *Gymnema sylvestre*, Antihelminthic activity, Albendazole.

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INTRODUCTION
Parasitic infections in particular are the infections caused by Helminthes have always been a topic of concern of Human Health Care. The common Helminth Parasitic infections include Round worm, Hook worm, Pin worm, Thread worm, Whip worm and Tape worm infections [1].Antihelminthic drugs are the major stream of drugs used in treatment of these parasitic manifestations. Parasitic worms are classified into three major groups namely Cestodes, Trematodes and Nematodes[2].

Taxonomical Profile

Kingdom : Plantae
Division : Angiospermae
Class : Dicotyledoneae
Order : Contortae
Family : Asclepiadaceae
Genus : Gymnema
Species : sylvestre[3]

MATERIALS AND METHODS
Animals
Pheretima posthuma, the Indian earth worms having a length of 3-5 cm and a width of 0.1-0.2 cm were selected. The animals were divided into different groups containing 6 animals in each group.

Chemicals
Albendazole (One drug), Ethanol (Chanshu yangyan chemical), Methanol (Merk), Chloroform (SD Fines), Petroleum ether (SD Fines), Dimethyl formamide- DMF (SD Fines) and Normal Saline (B.E).

The shade dried plant material was subjected to size reduction by the use of a grinder. It is sieved several times so as to ensure the uniformity of the powdered plant material. *Gymnema Sylvestre* was extracted with various extracts of the powdered drug were prepared using solvents of varying polarity. The solvents used for the various extractions included Ethanol, Methanol, Chloroform and Petroleum ether. The extraction was conducted in a Soxhlet apparatus. The extracts were subjected to the preliminary phytochemical screening to identify the chemical constituents in respective extracts.

Pheretima posthuma worms having a length of 3-5 cm and a width of 0.1-0.2 cm were selected. The animals were divided into different groups containing 6 animals in each group. The solutions were prepared from the extracts to obtain the specific concentrations. Vehicle was prepared by 5% of DMF in saline. 100mg/ml of each extract was prepared in a standard vehicle. From the stock solution the aliquots of 10 mg/ml, 20 mg/ml, 40 mg/ml, 80mg/ml and 100 mg/ml solutions were prepared and the final volume was made to 10ml with the vehicle. In a similar fashion the solutions of various concentrations similar to the extracts was prepared for the standard powdered drug Albendazole. Albendazole was taken as the standard drug as it was found to have broad spectrum of Antihelminthic activity[4]. On addition of these solutions to different petre plates containing six earth worms each, the times taken for the paralysis and death of each worm were observed[5]. Statistical analysis for significance is carried out.

The following pictures are for the standard trial of Albendazole, ethanolic, methanolic, chloroform and petroleum ether extracts of *Gymnema sylvestre*.

The comparative Anti helminthic study of the various extracts of *Gymnema sylvestre* was performed by taking 40mg/ml of the extracts in comparison with the standard drug Albendazole.

RESULT

The results of the percentage yield of *Gymnema sylvestre* extracted using various solvents i.e., Ethanol, Methanol, Chloroform and Petroleum ether are shown in Table 1.The paralysis time taken by the standard drug Albendazole is reported in Table 2.The paralysis time taken by each extract is tabulated in the tables (Table 3-Ethanolic extract; Table 4-Methanolic extract; Table 5-Chloroform extract; Table 6- Petroleum Ether extract). The results of the comparative study performed are shown in the Table 7. The Anti Helminthetic profile of various extracts of *Gymnema sylvestre* showed that Ethanolic extract has got greater potency to produce paralysis earlier than the other extracts of the same plant. The standard graphs various concentrations of Albendazole and Ethanolic, Methanolic, Chlororform and Petroleum ether extract of *Gymnema sylvestre* are illustrated as Fig 7- Fig 11. The comparative bar graph from the result is shown in the Fig 12. Except Chloroform extract all groups of other extracts are significant when comparing with the Standard drug (ALBENDAZOLE) *($p<0.05$).

The Fig 1-Fig 6 are the photographs of the paralyzed worms using various extracts.

DISCUSSION

The standard drug Albendazole, that was taken for the study was found to possess anti helminthic activity having a mechanism of action of neuromuscular blockade[6] (Albendazole causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules[7]. There is a higher affinity of Albendazole to the parasite tubulin and so the activity is mediated mainly against the parasite rather than on the host[8]). Under the influence of the vehicle containing DMF and normal saline, the control group showed no death and hence it confirms the safety of the vehicle.

The anti helminthetic profile of various extracts of *Gymnema sylvestre* showed that ethanolic extract has got greater potency to produce paralysis earlier than the other extracts of the same plant. This effect might be as a result of the polar and non polar constituents present in the ethanolic extract of *Gymnema sylvestre*, as ethanol has the property to extract both polar and non polar constituents. The anti helminthic effect is due to both polar and non polar constituents of the plant. The next potent extract was found to be that of methanol followed by petroleum ether followed by chloroform extracts. The activity followed a descending pattern of response of activity with ascending nature of the non polarity of the solvents used, which infers that the main constituents which produce greater anti helminthic activity are the polar constituents. The polar constituents present in *Gymnema sylvestre* are Oleanane type triterpenoid saponins known as Gymnemic acids[9, 10].

Other characteristic response observed in the various extracts of *Gymnema sylvestre* is that they could produce only paralysis in the earthworms similar to that of standard drug Albendazole and faster action when compared to Albendazole except the chloroform extract of *Gymnema sylvestre.*

CONCLUSION

Anti helminthic activity of the various extracts of *Gymnema sylvestre* was performed on Pheretima posthuma, a physiologically similar model for helminthes. The trial showed that that with the variation in polarities, there was a significant change in the activity. The ethanolic extract of the plant showed highest significance of activity, which might be due to the presence of both the polar and non polar constituents in the extract.

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TABLE 1: PERCENTAGE YIELDS OF VARIOUS EXTRACTS OF *GYMNEMA SYLVESTRE*

Gymnema sylvestre		
S.No	Name of the extract	Percentage yield
1	Ethanolic	29.64%
2	Methanolic	27.8%
3	Choloroform	7.1%
4	Petroleum Ether	6.6%

TABLE 2: TIME TAKEN FOR PARALYSIS USING ALBENDAZOLE (AB).

Albendazole powder					
	Time taken for paralysis in minutes				
S.No	Ab(A)	Ab(B)	Ab(C)	Ab(D)	Ab(E)
1	84.38	83.38	41.8	5.91	11.4
2	90.11	85.81	42.2	12.66	11.55
3	97.25	85.91	53.76	21.58	12.41
4	103.61	92.5	72.06	21.63	16.73
5	109.58	103.45	77.53	22.95	21.58
6	147.45	107	78.35	24.05	21.63

TABLE 3: TIME TAKEN FOR PARALYSIS USING *GYMNEMA SYLVESTRE* ETHANOLIC EXTRACT (GSE).

Gymnema sylvestre ethanolic extract					
	Time taken for paralysis in minutes				
S.No	GSE(A)	GSE(B)	GSE(C)	GSE(D)	GSE(E)
1	19.58	16.17	4.9	2.1	1.06
2	22.5	19.25	6	2.13	1.95
3	24.12	23.67	7.53	3.15	1.98
4	25.83	29.17	8.3	4.33	2.1
5	60.03	31.17	8.67	4.37	5.25
6	61.3	55.62	9	7.3	6

TABLE 4: TIME TAKEN FOR PARALYSIS USING *GYMNEMA SYLVESTRE* METHANOLIC EXTRACT (GSM).

Gymnema sylvestre-Methanolic extract					
	Time taken for paralysis in minutes				
S.No	GSM(A)	GSM(B)	GSM(C)	GSM(D)	GSM(E)
1	23.67	19.17	8.82	6.5	2.73
2	24.08	24.75	14.38	7.98	3.03
3	25.85	36.78	14.8	9.65	3.33
4	28.22	36.8	18.53	12.28	4.17
5	30.35	36.95	20.48	13.52	5.78
6	31.22	37.1	21.82	13.63	10.73

TABLE 5: TIME TAKEN FOR PARALYSIS USING *GYMNEMA SYLVESTRE* CHLOROFORM EXTRACT (GSC).

Gymnema sylvestre-Chloroform extract					
	Time taken for paralysis in minutes				
S.No	GSC(A)	GSC(B)	GSC(C)	GSC(D)	GSC(E)
1	74.45	61.4	60.83	14.77	9.9
2	142.55	67.9	63.6	15.08	10.73
3	157.68	77.55	66.75	16.43	13.48
4	157.82	83.68	67.93	18.3	17.18
5	163.38	87.72	68.92	22.5	18.88
6	169.85	90.55	80.57	23.18	23.73

TABLE 6: TIME TAKEN FOR PARALYSIS USING *GYMNEMA SYLVESTRE* PETROLEUM ETHER EXTRACT (GSET).

Gymnema sylvestre-Petroleum ether extract					
	Time taken for paralysis in minutes				
S.No	GSET(A)	GSET(B)	GSET(C)	GSET(D)	GSET(E)
1	78.25	51.25	23.35	14.87	11.72
2	79.25	58.17	29.02	22.55	13.22
3	79.9	59.17	33.73	22.73	13.58
4	80.48	96	41.83	26.5	21.8
5	90.37	97.35	42.56	41.38	21.8
6	97.2	105.05	43.2	54.5	24.37

TABLE 7: COMPARATIVE STUDY OF PARALYSIS TIMES FOR VARIOUS EXTRACTS OF *GYMNEMA SYLVESTRE*.

Extract	Eth(P)+/- Std.error	Meth(P) +/- Std.error	Chlor(P) +/- Std.error	P.ether(P) +/- Std.error
Time in min	7.4+/- 0.66357	16.47+/-1.9547	68.1+/-2.77565	35.6+/-3.37574

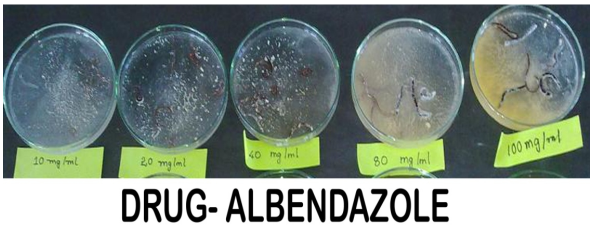

Figure 1: Antihelminthic activity of Albendazole

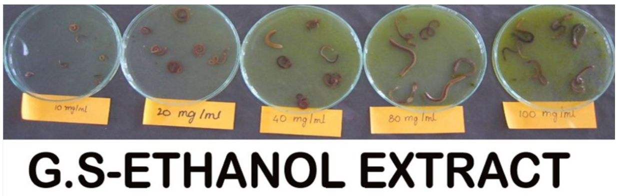

Figure 2: Antihelminthic activity of *Gymnema sylvestre* ethanolic extract

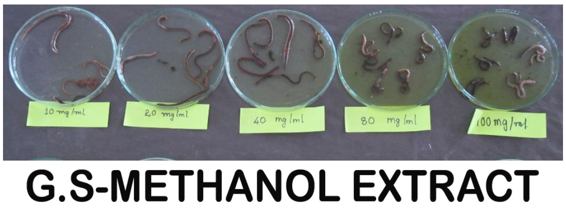

Figure 3: Antihelminthic activity of *Gymnema sylvestre* methanolic extract

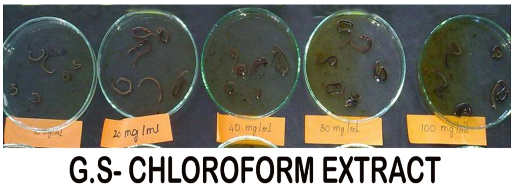

Figure 4: Antihelminthic activity of *Gymnema sylvestre* chloroform extract

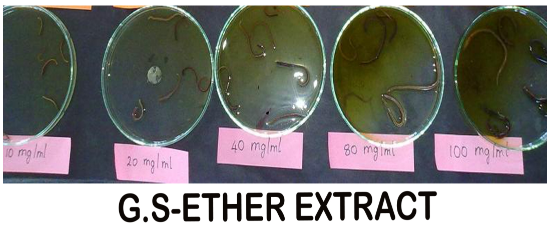

Figure 5: Antihelminthic activity of *Gymnema sylvestre* petroleum ether extract

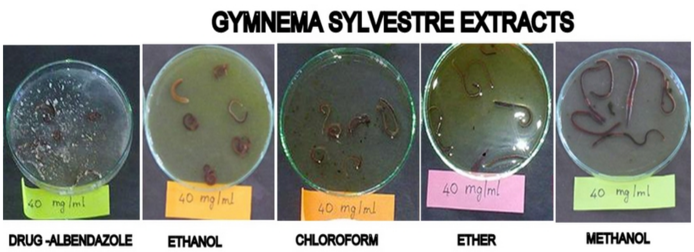

Figure 6: Comparative study of Antihelminthic activity of *Gymnema sylvestre* extracts

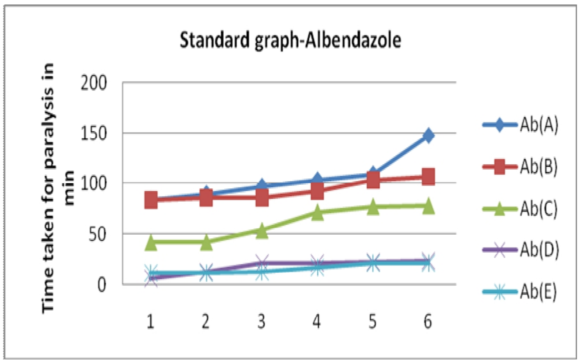

Figure 7: Standard graph - Paralysis time for Albendazole (Ab).
(A)-10mg/ml; (B)-20mg/ml; (C)-40mg/ml ;(D)-80mg/ml; (E)-100mg/ml.

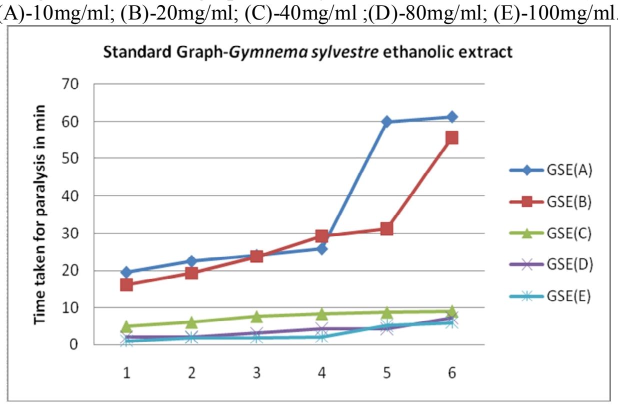

Figure 8: Standard graph of paralysis time for *Gymnema sylvestre* ethanolic extract (GSE).
(A)-10mg/ml; (B)-20mg/ml; (C)-40mg/ml ;(D)-80mg/ml; (E)-100mg/ml

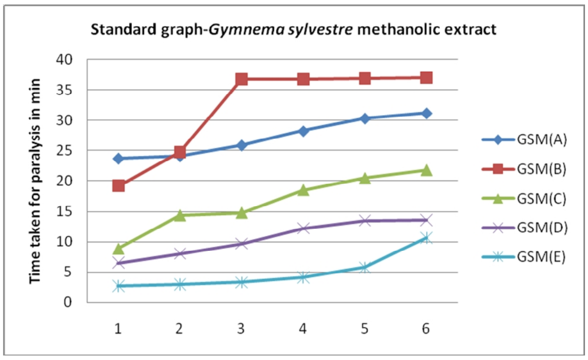

Figure 9: Standard graph of paralysis time for *Gymnema sylvestre* methanolic extract (GSM).
(A)-10mg/ml; (B)-20mg/ml; (C)-40mg/ml ;(D)-80mg/ml; (E)-100mg/ml.

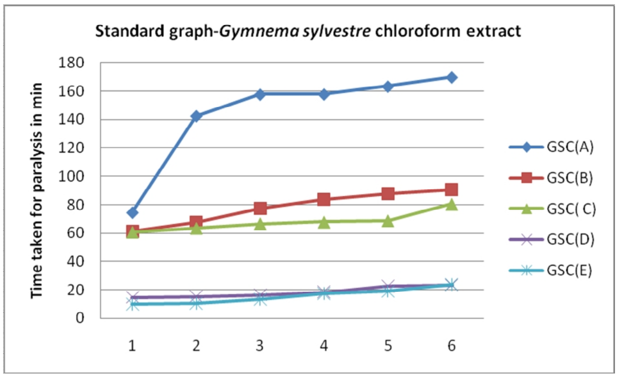

Figure 10: Standard graph of paralysis time for *Gymnema sylvestre* chloroform extract (GSC).
(A)-10mg/ml; (B)-20mg/ml; (C)-40mg/ml ;(D)-80mg/ml; (E)-100mg/ml.

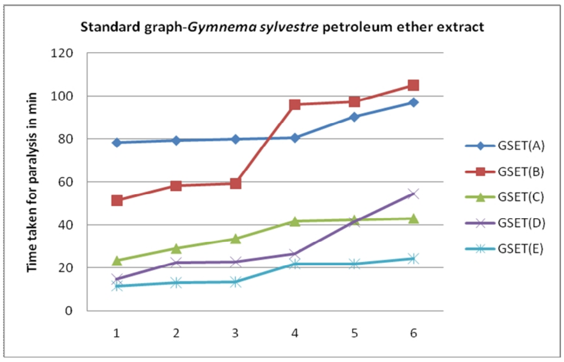

Figure 11: Standard graph of paralysis time for *Gymnema sylvestre* petroleum ether extract (GSET).
(A)-10mg/ml; (B)-20mg/ml; (C)-40mg/ml ;(D)-80mg/ml; (E)-100mg/ml

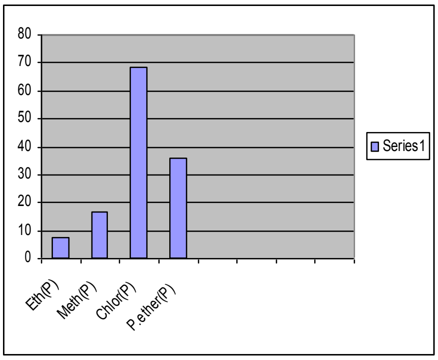

Figure 12: Comparative study of paralysis times of various extracts of *Gymnema sylvestre*
(X-Axis-Name of extract; Y-Axis-Time taken for paralysis in minutes) ;(P): Paralysis time
Except Chloroform extract all groups of other extracts are significant when comparing with the Standard drug (ALBENDAZOLE) *(p<0.05).

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