Phytochemical and *In vitro* Anthelmintic Screening of *Butea frondosa* and *Swertia chirata* from Pakistan

A. I. HUSSAIN, F. ANWAR*, H. N. BHATTI AND U. RASHID
Department of Chemistry
University of Agriculture, Faisalabad-38040, Pakistan

(Received 25th May, 2005, revised 3rd December, 2005)

Summary: Seeds of *Butea frondosa* and whole plant of *Swertia chirata* were investigated for phytochemical and anthelmintic screening. *Butea frondosa* gave positive test for the presence of alkaloids, steroids and glycosides but negative for flavonoids and saponins. *Swertia chirata* showed negative results for the presence of steroids and saponins but positive for alkaloids, flavonoids and glycosides. The concentration of alkaloids in *Butea frondosa* and *Swertia chirata* was found to be 3.00 % and 2.00 % respectively. *In vitro* treatment on the live parasites *(Haemonchus contortus and Pheretime posthuma)* with the crude alkaloids solution (5.00 % w/v) of *Swertia chirata* revealed complete immobilization of parasites in 3-4 hours and thus exhibited good anthelmintic activity. However, 2.25 % (w/v) solution of *Swertia chirata* exhibited quite low anthelmintic activity and 1.25 % (w/v) solution did not show any considerable activity. The alkaloid solution of *Butea frondosa* at concentrations of 5.00 %, 2.25 % and 1.25 % caused death of parasites in 1-1.5, 2-4 and 4-5 hours post exposures respectively. The anthelmintic activity of *Butea frondosa* was found to be comparable with piprazine. Parasites exposed to control (Normal saline and Tween 80) did not show any remarkable change in physical activity and remained viable even up to 5 hours. The results of the present study revealed the anthelmintic potential of the investigated medicinal plants indigenous to Pakistan.

Introduction

The traditional medicines hold a great promise as a source of easily available and effective anthelmintic agents, particularly, in the tropical developing countries like Pakistan. The phytochemical screening of different plants has been undertaken by various researchers [1-4]. The ethnopharmacological survey of some medicinally important plants of Galligat areas of NWFP Pakistan revealed that about 41 wild plant species belonging to 40 genera of 33 families including *Swertia chirata* have been in use by the local inhabitants for medicinal purposes [5-6]. A number of plants have been evaluated for their anthelmintic efficacy [7-11]. The phytoconstituents i.e. alkaloids, glycosides, flavonoids and saponins are active principles of plants. Of these phytoconstituents, alkaloid have been reported to exhibit anthelmintic activity.

Economy of agricultural countries like Pakistan greatly depends upon the production, potential and health status of their livestock. Many reports indicate that the poor production and growth of livestock animals is due to the prevalence of infectious diseases that may cause by many parasites. These parasites adversely affect the nutritional status and thus stunted growth, loss of body weight, loss of wool and reduce milk production and even cause the death of the host [12-14].

Regarding the use of synthetic anthelmintic drugs, toxicity and possibility of drug residues in meat and milk is a persistent problem. Long term use of these synthetic anthelmintics caused the loss of natural resistance of animals against nematodes [15-16]. Conventionally, anthelmintic chemotherapy is a priority method for controlling nematodiasis in ruminants [17]. Currently, there is a revival of interest in the use of plants for curing of different ailments. Efforts are being made all over the world to isolate natural anthelmintic agents that could be employed as plant protection measures [18-19]. *Butea frondosa* belonging to the *Papilionaceae* family has been used abundantly in different parts of the world as an anthelmintic drug and phytochemical screening of this plant has been conducted [20]. Mengi [21] investigated the anti-inflammatory properties of *Butea frondosa.* Swertia chirata belongs to the *Gentianaceae* family. Rafatullah *et al.,* [22] reported the use of *Swertia chirata* for the treatment of gastric ulcers in traditional medicine. Brahmachari *et al.,* [23] studied the pharmacological and chemical attributes of *Swertia chirata.*

*To whom all correspondence should be addressed.

Pakistan is rich in medicinally important flora. Wide varieties of folk medicinal plants, growing on the fertile land of Pakistan are unknowingly exploited and have not been scientifically investigated for their biologically active principles [24]. Although a significant numbers of these plants are in focus for drug discovery and a wealth of information is available in the literature about their therapeutic applications. Only a few of them have been collected from Pakistan and studied for their perceived anthelmintic potential. However, no studies have been conducted so far on the anthelmintic activity of locally grown *Butea frondosa* and *Swertia chirata*. So, the present research was planned to screen and investigate *Butea frondosa* (Palas papra) and *Swertia chirata* (Chireta) for their phytochemical and anthelmintic activity against *Haemonchus contortus* and *Pheretima posthuma*.

Results and Discussion

Phytochemical analysis

The qualitative analysis of the seeds of *Butea frondosa* and whole plant of *Swertia chirata* gave positive results with Mayer's reagent and Dragendroff reagent thus indicated the presence of alkaloids in these plants. The quantitative analysis of *Butea frondosa* and *Swertia chirata* showed the contents of alkaloids to be 3.00 % and 2.00 % respectively (Table-1). Saponins, as determined by froth test, were absent in both of these plants (Table-1). *Butea frondosa* gave positive results for steroids with Liberman Berchard's test and Salkovaski test thus reflected the presence of steroids but *Swertia chirata* gave negative results (Table-1). *Swertia chirata* showed the presence of flavonoids as indicated by Ammonia and Shinoda tests. Whereas, *Butea frondosa* gave negative tests for the occurrence of flavonoids (Table-1). Both the *Butea frondosa* as well as *Swertia chirata* gave positive results with Keller Killiani and Legal tests thus showing the presence of glycosides. Literature also revealed the presence of alkaloids and glycosides in these plants [25-29].

Table 1: Phytochemical screening of *Butea frondosa* and *Swertia chirata*

Phytochemical Constituents	Butea frondosa		Swertia chirata	
	+Ve/-Ve	%	+Ve/-Ve	%
Alkaloids	+ Ve	3.00 %	+ Ve	2.00 %
Glycosides	+ Ve	-	+ Ve	-
Flavonoids	- Ve	-	+ Ve	-
Saponins	- Ve	-	- Ve	-
Steroids	+ Ve	-	- Ve	-

In vitro Anthelmintic activity of crude alkaloids

Anthelmintic activity of alkaloids solutions of *Butea frondosa, Swertia chirata* and piprazine, in normal saline, against *Pheretima posthuma* have been shown in Fig. 1a, 1b and 1c respectively. Alkaloid solutions of 5.00 and 2.25 % (w/v) concentration of *Butea frondosa* showed almost comparable anthelmintic activity to that of standard piprazine against *Pheretima posthuma* and caused the death of worms after 1.00 and 1.50 hours post exposure respectively. The anthelmintic activity of *Butea frondosa* might be due to the presence of alkaloids. The anthelmintic activity of 1.25 % solution of *Butea frondosa* was found to be lower than piprazine. Similar results have been reported by Lal [30], who studied the *in vitro* anthelmintic activity of *Butea frondosa* against *Ascardia galli* worms. The alkaloids solution of

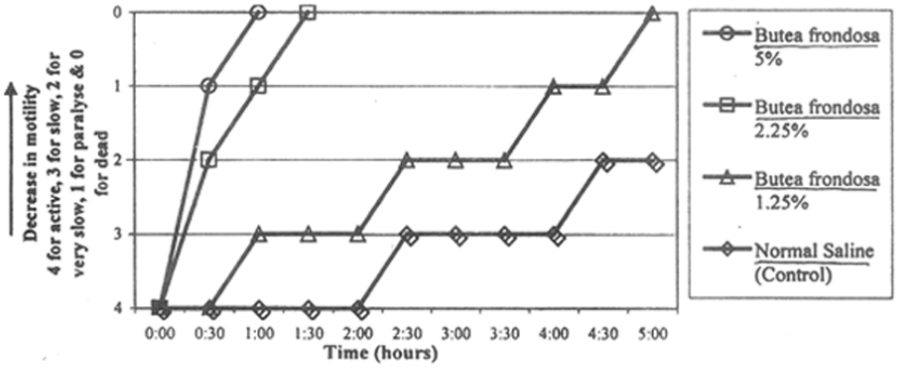

Fig. 1a: Anthelmintic activity of alkaloids solution of *Butea frondosa* in normal saline against *Pheretima posthume.*

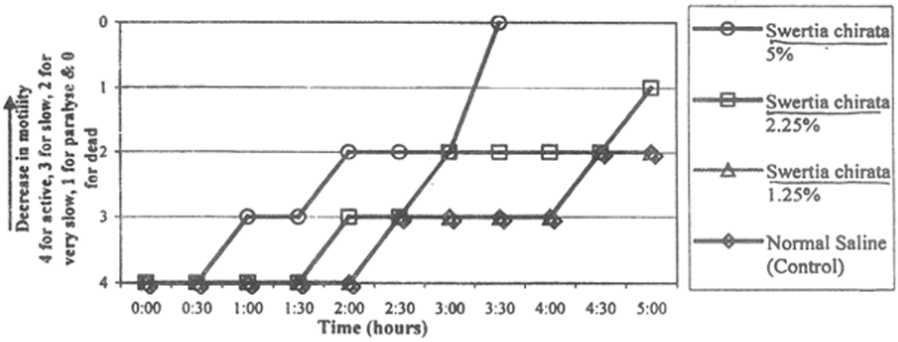

Fig. 1b: Anthelmintic activity of alkaloids solution of *Swertia chirata* in normal saline against *Pheretima posthume.*

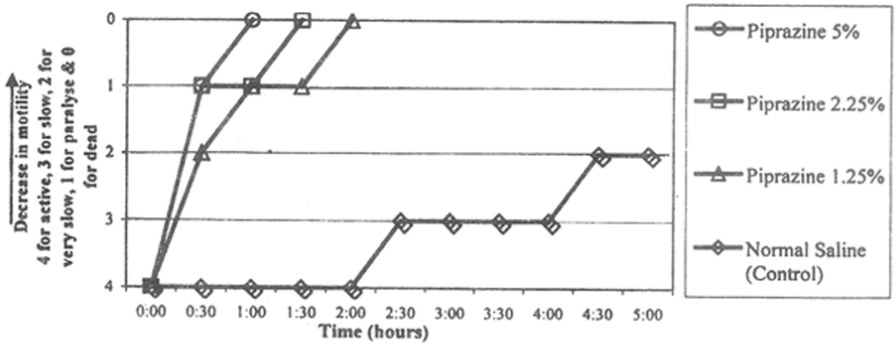

Fig. 1c: Anthelmintic activity of standard piprazine diluted in normal saline against *Pheretima posthume.*

Swertia chirata at 5.00 % concentration gradually inhibited the motility of *Pheretima posthuma* and resulted in the death of worms after three to four hours post exposure. However, more diluted solution did not show any mortality (Fig. 1b). Chowdhury [31], Das [32] and Islam [33] also screened *Swertia chirata* for its bioactivity.

Anthelmintic activity of alkaloids solution of 5.00, 2.25 and 1.25 % concentration of *Butea frondosa, Swertia chirata* and piprazine, in normal saline, against *Haemonchus contortus* have been shown in Fig. 2a, 2b and 2c respectively. A 5.00 % solution of alkaloids of *Butea frondosa, Swertia chirata* and piprazine caused the death of *Haemonchus contortus* after 1.5, 2.0 and 1.0 hours respectively. A solution of *Butea frondosa* and piprazine of 2.25 % concentration caused the death of *Haemonchus contortus* after 4.50 hours and thus showed comparable anthelmintic activity. However,

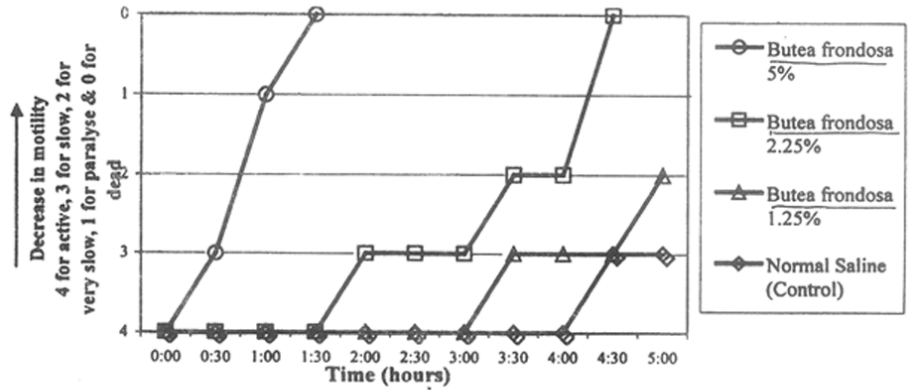

Fig. 2a: Anthelmintic activity of alkaloids solution of *Butea frondosa* in normal saline against *Haemonchus contortus.*

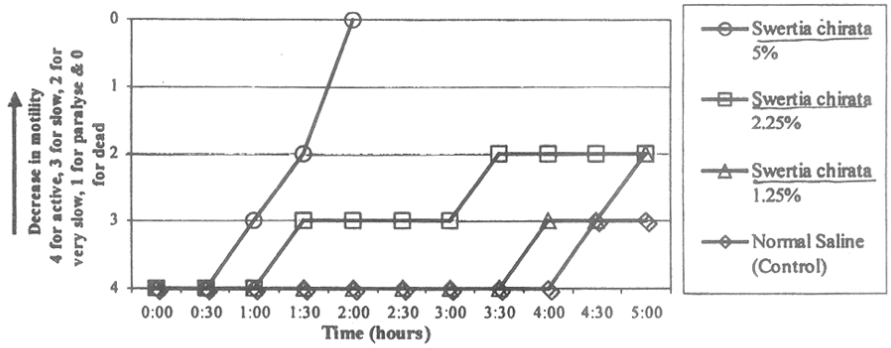

Fig. 2b: Anthelmintic activity of alkaloids solution of *Swertia chirata* in normal saline against *Haemonchus contortus.*

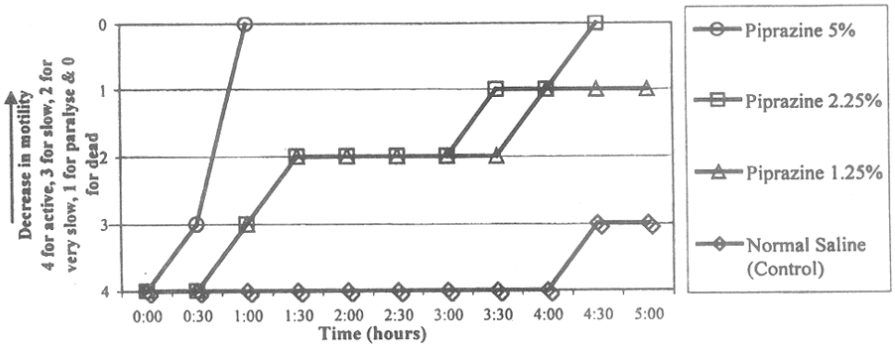

Fig. 2c: Anthelmintic activity of standard piprazine diluted in normal saline against *Haemonchus contortus.*

2.25 % solution of *Swertia chirata* only inhibited the motility of *Haemonchus contortus* even up to exposure of 5.00 hours and did not cause any death and thus showed relatively less anthelmintic activity. Whereas, 1.25 % solutions of *Butea frondosa* and *Swertia chirata* were only able to decrease the motility of *Haemonchus contortus* even up to 5.0 hours and thus exhibited least anthelmintic activity. While, 1.25 % solution of piprazine was found to paralyzed *Haemonchus contortus* even up to 3 hours exposure and thus exhibited more anthelmintic activity as compared with that of 1.25 % solution of both *Butea frondosa and Swertia chirata.* These results indicated that both plants and standard drug showed more anthelmintic activity against *Pheretima posthuma* than Haemonchus *contortus.*

Anthelmintic activity of alkaloids solution of *Butea frondosa, Swertia chirata* and piprazine, in 1% Tween 80, against *Pheretima posthuma* have been represented in Fig. 3a, 3b and 3c respectively. It was noted that the anthelmintic activity of the investigated plants in Tween 80 was varied to those of normal saline. The anthelmintic activity of *Butea frondosa* against *Pheretima posthuma* slightly decreased whereas, that of *Swertia chirata* and piprazine was found to be slightly increased. *Butea frondosa* at concentration of 5.00, 2.25 and 1.25 % completely immobilized the *Pheretima posthuma* after 1.50, 3.50 and 4.50 hours respectively. The solution of *Swertia chirata* at concentration of 5.00 and 2.25 % caused the death of *Pheretima posthuma* after 3.00 and 4.00 hours respectively. While, a solution of 1.25% concentration paralyzed the worms after five an hours post exposure. Whereas, piprazine immediately killed the *Pheretima posthuma.*

Anthelmintic activities of alkaloids solution of *Butea frondosa, Swertia chirata* and piprazine, in 1 % Tween 80, against *Haemonchus contortus* have been shown in Fig. 4a, 4b and 4c respectively. It could be understandable from Fig. 4a, that alkaloids solutions of *Butea frondosa* at concentration of 5.00, 2.25 and 1.25 % showed the mortality of *Haemonchus contortus* and resulted in the death of worms 1.50, 3.00 and 4.50 hours exposure respecti-

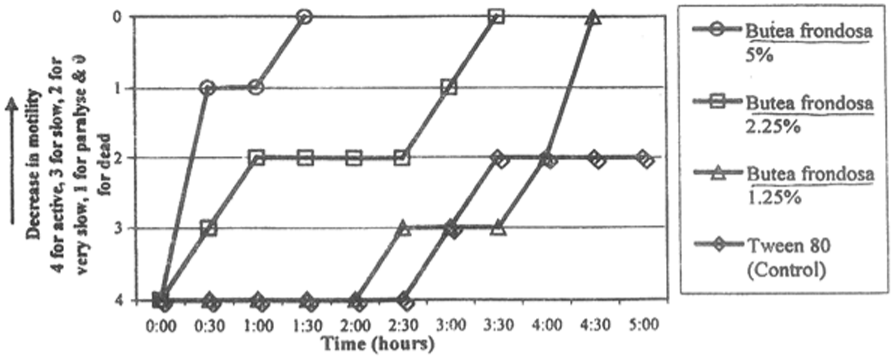

Fig. 3a: Anthelmintic activity of alkaloids solution of *Butea frondosa* diluted in 1 % Tween 80 against *Pheretime posthuma.*

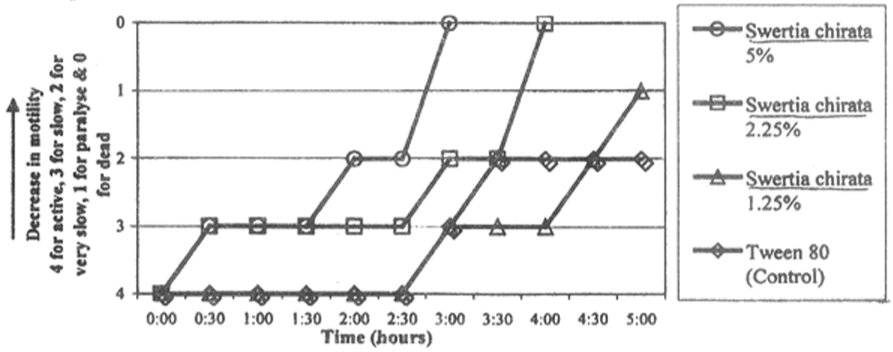

Fig. 3b: Anthelmintic activity of alkaloids solution of *Swertia chirata* diluted in 1 % Tween 80 against *Pheretime posthuma.*

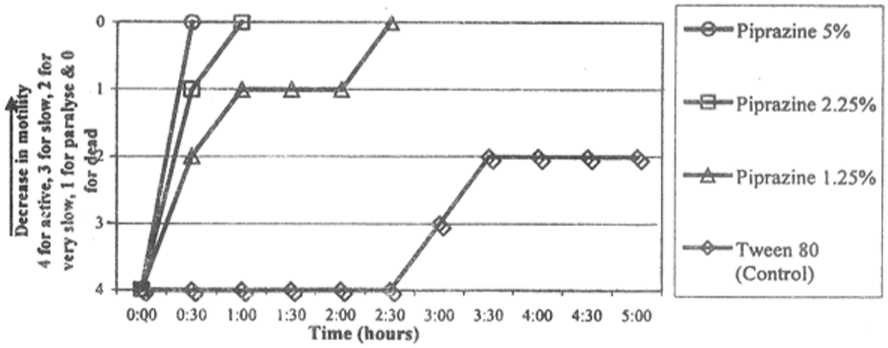

Fig. 3c: Anthelmintic activity of standard piprazine diluted in 1 % Tween 80 against *Pheretime posthuma.*

vely. Whereas, the solution of piprazine at concentration of 5.00, 2.25 and 1.25 % showed strong activity against *Haemonchus contortus* and resulted in the death of worms within half to two hour's exposure. A solution of *Swertia chirata* at a concentration of 5.00 and 2.25 % showed poor anthelmintic activity. However, solution of 1.25% concentration did not show any anthelmintic activity as shown in Fig. 4b.

It could be predicted from the results of activity based assays in the present analysis that seeds of *Butea frondosa* had comparable activity with piprazine which might be attributed to the presence of alkaloids and glycosides. Garg [34], and Kaleysaraj and Kurup [20] studied the anthelmintic effect of different parts of *Butea frondosa* alkaloids. The seeds work effectively against *Ascaris lumbricoides in vitro* at level similar to piprazine. Rajapurkar *et al.* [35]; Raj and Kurup [36] also reported pharmacological properties of *Butea frondosa* seeds.

The Anthelmintic activity of *Swertia chirata* was found to be lower than *Butea frondosa* and

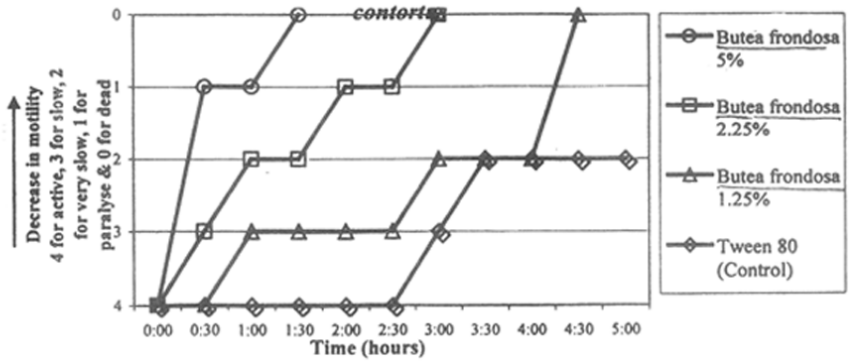

Fig. 4a: Anthelmintic activity of alkaloids solution of *Butea frondosa* diluted in 1 % Tween 80 against *Haemonchus contortus.*

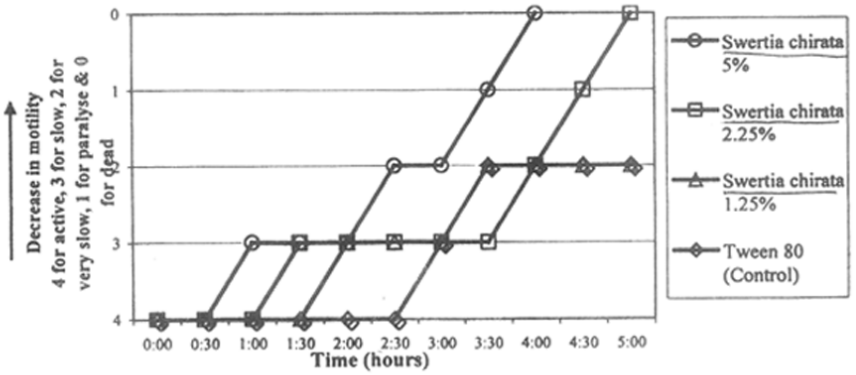

Fig. 4b: Anthelmintic activity of alkaloids solution of *Swertia chirata* diluted in 1 % Tween 80 agains *Haemonchus contortus.*

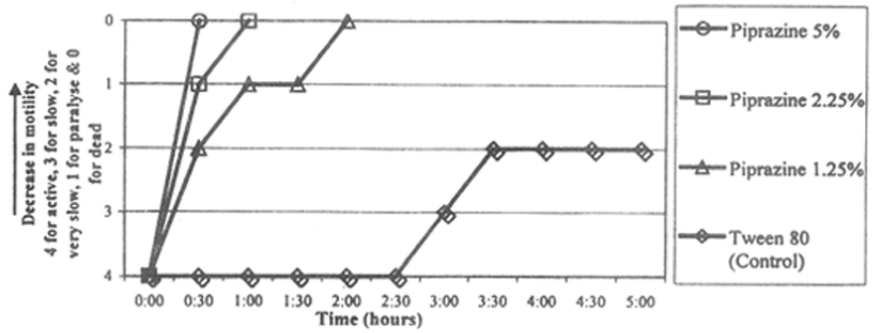

Fig. 4c: Anthelmintic activity of standard piprazine diluted in 1 % Tween 80 against *Haemonchus contortus.*

piprazine. There are no earlier reports available in the literature regarding the anthelmintic activity of *Swertia chirata* to compare the activity with our present analysis. However, different reports are available in literature to deal with the pharmacological properties of this plant. Shah [37] reported the anti-carcinogenic activity of *Swertia chirata.* Kirtikar and Basu [28] used *Swertia chirata,* as a digestive, febrifuge and laxative in gastrointestinal disorders, like dyspepsia/anorexia. Dutt *et al.* [38] reported *Swertia chirata* as a bitter compound for medicina uses. Reen *et al.* [39] reported anti-hepatotoxi activity of *Swertia chirata.* Mandal *et al.* [40 reported anti-inflammatory activity of *Swerti chirata.*

Experimental

Whole plant of *Swertia chirata* and seeds o *Butea frondosa* were collected from the forests in th vicinity of Galligat in Northern Areas, N.W.F.P an

Jhelum, Punjab of Pakistan, respectively and got identified by Professor Dr. M. Ashraf, Department of Botany, University of Agriculture, Faisalabad. The plant materials were washed thoroughly with distilled water to remove dust and other extraneous materials. The washed materials were ambient-dried and then pulverized to get a fine powder.

Phytochemical analysis

Ground plant material (50g) was extracted for four hours with 250 mL of methanol by using electrical shaker. After extraction the contents of the flask were filtered. The filtrated extracts were evaporated to dryness under reduced pressure at 45°C by using a rotary evaporator (ELELA, Rotary Vacuum Evaporator.N.N.Series equipped with an Aspirator and a Digital Water Bath SB-651, Japan) [41]. The extracts were tested for different phyto-chemical constituents after making 20% solution of extract in methanol [42-43].

Detection of Alkaloids

a) Mayer's reagent: To 5 mL methanol solution of the extract, Mayer's reagent (2 mL) was added. Appearance of white precipitate was noted [44].

b) Dragendroff reagent

1mL of Dragendroff reagent was added into 3mL methanol solution of the extract. Appearance of any precipitate or change in color was noted [43].

Detection of Saponins

A small quantity of powdered plant material was shaken with distilled water. Appearance of permanent froth showed the presence of saponins [45].

Detection of Steroids

a) Salkowski test: To 1 mL methanol solution of the extract, concentrated sulfuric acid (2 mL) was added. Appearance of any precipitate or change in color was noted [46].

b) Libermann-Burchard test

To 1mL of the plant extract, added conc. sulfuric acid (1 mL) and few drops of acetic anhydride. Any color change was noted [43].

Detection of Flavonoids

a) Ammonia test: A filter paper dipped in methanolic solution of the plant extract was exposed to vapor of ammonia solution. Appearance of a yellow color showed the presence of flavonoids [42].

b) Shinoda test:

To the methanolic solution of the extract, few chips of magnesium and dilute hydrochloric acid (2 mL) were added. Formation of yellow color showed the presence of flavonoids [47].

Detection of Glycosides

a) Keller-Killiani test: To the plant extract (5 mL) few drops of ferric chloride and concentrated sulfuric acid were added. Formation of color showed the presence of glycosides [46].

b) Legal test

Appearance of blood red color on mixing methanolic solution of the extract with sodium nitro-prusside followed by the addition of few drops of sodium hydroxide showed the presence of glycosides [48].

Extraction and quantitative analysis of crude alkaloids

The extraction of alkaloids was executed according to the method of Brain and Turner [42]. Powdered sample (10g) was shaken with 90 mL of distilled water and 10 mL of ammonium hydroxide in a shaker. The shaking was continued until the plant material gave negative test for alkaloids with Mayer's test [44]. The contents of the flask were then filtered. The filterate was than extracted with 50 mL of chloroform. Separated the chloroform layer and added 50 mL of 50 % commercial HCl. The acidic layer was neutralized with ammonium hydroxide and then again added 50 mL of chloroform. Separated the chloroform layer, evaporated on water bath and percentage of alkaloids was determined by gravime-trically as under;

$$\% \text{ age of alkaloids} = \frac{\text{Wt. of the extract}}{\text{Wt. of plant material}} \times 100$$

In vitro anthelmintic activity of crude alkaloids

For nematodal activity, procedure of Tandon [49] and Lal [30] was followed with little modification as described below;

The *in vitro* trials of the alkaloids solution, extracted from these two plants were conducted on

mature live *Pheretima posthuma* and *Haemonchus contortus*. The piprazine (gamma amino butyric acid) was used as a standard anthelmintic drug. Two types of solutions; normal saline and 1 % Tween 80 were used as a control. The *Haemonchus contortus* were collected in physiological buffered saline (PBS) from the gastro-intestinal tract of freshly slaughtered sheep in a local abattoir and *Pheretima posthuma* were collected from fresh water channels. Ten petri dishes (Three sets of three petri dishes and a single) were placed on a laboratory bench at 30 ± 2 °C. Using a metal loop, 5 worms were transferred into each of the labeled ten petri dishes. 5.00 %, 2.25 % and 1.25% solutions (In normal saline) of *Butea frondosa*, *Swertia chirata* and piprazine were added into the 1st, 2nd and 3rd sets of petri dishes respectively. A control was also run. The decline in motility was observed qualitatively after every half an hour [50-52]. and gave rating 4 for active, 3 for slow motion, 2 for very slow motion, 1 for paralyze and 0 for dead. Finally the treated worms were washed three times with fresh warm normal saline solution. The worms were kept for thirty minutes in the warm fresh normal saline to observe any revival of the motility. Five replicates were used for each concentration. The same experiment was repeated with Tween 80 as a control.

Similar set of experiment was conducted for Pheretima posthuma as described by Siddique and Gorge [52].

Conclusions

It could be concluded from the findings of the present analysis that the seeds of *Butea frondosa* showed anthelmintic activity quite comparable to standard piprazine, whereas, *Swertia chirata* showed less anthelmintic activity than *Butea frondosa* and piprazine. The results of the present comprehensive study demonstrated that *Swertia chirata* and *Butea frondosa* indigenous to Pakistan might be exploited for their pharmacological applications and medicinal attributes.

Acknowledgment

We acknowledge the guidance of Dr. Riaz Ahmad (ex-Dean), Faculty of Basic Sciences, University of Agriculture, Faisalabad for his kind help and support in this research work.

References

1. G. Badar, M. Seibold, K. Tintelnot and K. Hiller, *Pharmazie,* **55**, 72 (2000).
2. K. Nishimuta, T. Miyase and H. Noguchi, *J. Natural Products*, **62**, 1128 (1999).
3. B. Rastrelli, A. Caceres, F. De-Simone and R. Aquino, *J. Agric. Food Chem.,* **47**, 1537 (1999).
4. X.C. Li, H.N. Elsohly, A.C. Nimrod and A.M. Clark, *J. Natural Products*, **62**, 674 (1999).
5. A. Ejaz, M. Arshad, M. Ahmad, M. Saeed and M. Ishaque, *Asian Journal of Plant Sciences*, **3** (4), 410 (2004).
6. S.H. Zaidi, Quarterly Progress Report on "Medicinal Plant Survey" under Biodiversity Project IUCN, Islamabad (1996).
7. V. Tangpu and A.K. Yadav, *Pharmaceutical Biol.*, **42**, 1 (2004).
8. V. Paolini, P. Dorchies and H. Hoste, *Veterinary Record*, **152**, 600 (2003).
9. J.B. Githiori, J. Hoglund, P.L. Waller and R.L. Baker, *Journal of Ethnopharmacology,* **80**, 187 (2002).
10. M.K. Asha, D. Prashanth, B. Murali, R. Padmaja and A. Amit, *Fitoterapia,* **72**, 669 (2001).
11. J.A. Hammond, D. Fielding and S.C. Bishop, *Veterinary Research Communication*, **21**, 123 (1997).
12. S. Gross, W.G. Rayan and H.W. Ploeger, *Vet. Rec.*, **144**, in Press (1999).
13. M. Forbes, *Oikos.*, **67**, 444 (1993).
14. L. Dennis, Murray, B. Lloyd, Kath, R. John and Cary, *Ecological Society of America,* **79**(4), 1209 (1998).
15. A. Bluthgen and W.H. Heeschen, Parasiticides. International Dairy Federation. Special Issue, 9701, 35 (1997).
16. P.J. Waller, FAO Animal Health Paper 141. FAO. Rome. pp 1 (1998).
17. S. Ranjan and R. Delay, *Vet. Parasit.*, **120**, 315 (2004).
18. I.J. Kuppasta, Vasudeva and Nayak, *India J. Natl. Prod.*, **19**, 27 (2003).
19. V. Lakshmi, R. Kumar, P. Gupta, V. Varshney, M.N. Srivastava, M. Dikshit, P.K. Murthy and S. Misra-Bhattacharya, *Parasit. Res.*, **93**, 468 (2004).
20. R. Kaleysaraj and P.A. Kurup, *Indian Journal of Medical Research,* **56**, 1818 (1968).
21. S.A. Mengi and S.G. Deshpande, *Indian Journal of Pharmacology,* **27**, 116 (1995).
22. S. Rafatullah, M. Tariq, J.S. Mossa, M.A. Al-Yahya, M.S. Al-Said and A.M. Ageel, *Drugs Exp. Clin. Res.*, **19**(2), 69 (1993).
23. G. Brahmachari, S. Mondal, A. Gangopadhyay, D. Gorai, B. Mukhopadhyay, S. Saha and A.K. Brahmachari, *Chemistry and Biodiversity,* **1**(11), 1627 (2004).

24. A. Saleem, M. Ahotupa, and K. Pihlaja, *Z. Naturforsch.*, **56**, 973 (2001).
25. A.K. Chahravarty, S. Mukhopadyay and B. Das, *Phytochemistry,* **30,** 4087 (1991).
26. R.K. Asthana, N.K. Sharma, D.K. Kulshreshtha and S.K. Chatterjee, *Phytochemistry*, **30**, 1037 (1991).
27. A.K. Nadkarni, Indian Materia Medica, 3rd edition, reprint in 2000. Popular Book Depot, Bombay (1954).
28. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants 2nd Edition (Edited by: Kirtikar KR, Basu BD). Allahabad, India, Lalit Mohan Basu Publications 1935 2, 1090 (1935).
29. R.N. Chopra, K.L. Handa and L.D. Kapoor, Indigenous Drugs of India 2nd edition . Dhar and Sons, Calcutta (1958).
30. J. Lal, S. Chandia, V. Raviprakash and V.P. Sabir, *Indian Journal of Physiological Pharmacology*, **20**, 64 (1976).
31. N.I. Chowdhury, S.K. Bandyopadhyay, S.N. Banerjee, M.K. Dutta and P.C. Das, *Indian Journal of Pharmacology*, **27**, 1, 37 (1995).
32. P.C. Das, 5th International Society of Horticulture Science Symp. *Med. Aromat. and Spice Plants*, Feb., 23-26, Abstr., F-7 (1985).
33. C.N. Islam, Satellite Symposium of Traditional Medicine as Adjunct to Asian Congress of Pharmacology, Dept. Siddha Med. Tamil. Univ. Tanjavur, Jan.,22-23, Abstr., P-24 (1985).
34. L.C. Garg, and R.K. Mehta, *J. Vet. Husb. Research India*, **3**, 28 (1958).
35. M.V. Rajapurkar, K.S. Sachdov, M.H. Panjwani and A.J. Baxi, *Indian Journal of Medical Science*, **15,** 353 (1961).
36. R.K. Raj and P.A. Kurup, *Indian Journal of Medical Research*, **56**, 1818 (1968).
37. P. Shah, S. Mandal, A. Das and P.C. Das, *Phytotherapy Research,* **18**, 373 (2004).
38. D. Bhupender, L.J. Srivastava and J.M. Singh, *Ancient Science of Life*, **15**, 226 (1996).
39. R.K. Reen, M. Karan, K. Singh, N. Karan, R.K. Johri and J. Singh, *Journal of Ethnopharmacology*, **75**, 239 (2001).
40. S. Mandal, P.C. Das, P.C. Joshi, A. Chatterjee, C.N. Islam, M.K. Dutta, B.B. Patra and S. Sikdar, *Fitoterapia* ,**63,** 122 (1992).
41. P. Siddhuvaj and K. Becker, *Journal of Agriculture and Food Chemistry*, **51**, 2144 (2003).
42. K.R. Brain and T.D. Turner, The Practical Valuation of Phytopharmaceuticals, 1st edition, Bristol. Wright Scientechnia, 105 (1975).
43. J.B. Harborne, Methods of plant analysis in Phytochemical Methods, London Chapman and Hall, 132 (1973).
44. B.N. Mayer N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, *Planta Med.*, **45**, 312 (1982).
45. M. Feroz, R. Ahmad, S.T.A.K. Sindhu and A.M. Shahbaz, *Pakistan Veterinary Journal,* **13**, 4 (1993).
46. A. Sofowora, Medicinal plants and traditional medicine in Africa. Spectrum books 2nd ed. pp. 150-153 (1993).
47. W.S. Woo, Experimental methods for Phytochemistry, Seoul National University Press, Seoul, pp. 1 (2001).
48. G.E. Trease, W.C. Evans, *A Text-book of Parmacognosy*. Bailliere Tinall Ltd, London, pp. 53 (1989).
49. V. Tandon, P. Pal, B. Roy, H.S.P. Rao and K.S. Reddy, *India Parasitol Res.*, **83**, 492 (1997).
50. C. Goto, S. Kasuya, K. Koga, H. Ohtomo and N. Kagel, *Parasitol Res.*, **76**, 653 (1990).
51. J. Togo, M.T. Santamarina, D. Reris, F.M. Uberia, S.L. Leiro and M.L. Sanmartin, *Japanese Journal Parasitol.*, **41**, 473 (1992).
52. N. Siddique and S.C. Gorg, *Pak. Journal of Industrial Research,* **33**, 536 (1990).