Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 3(6): 268-271
Received: 01-01-2015
Accepted: 12-02-2015

Varsha Tiwari
Department of Pharmacognosy, Devsthali Vidyapeeth College of Pharmacy, Rudrapur, Uttarakhand, India

Manish Kumar Pandey
Department of Pharmacognosy, Devsthali Vidyapeeth College of Pharmacy, Rudrapur, Uttarakhand, India

Abhishek Tiwari
Department of Pharmaceutical Chemistry, Devsthali Vidyapeeth College of Pharmacy, Rudrapur, Uttarakhand, India

Correspondence:
Abhishek Tiwari
Department of Pharmaceutical Chemistry, Devsthali Vidyapeeth College of Pharmacy, Rudrapur, Uttarakhand, India

Comparative evaluation of anthelmintic potential of *Bauhinia variegata*

Varsha Tiwari, Manish Kumar Pandey, Abhishek Tiwari

Abstract
Objective: In the present study *in vitro* anthelmintic potential of petroleum ether, benzene, chloroform, ethanolic and aqueous extracts of *Bauhinia variegata* stem and leaf were evaluated using *Pheretima posthuma* model.
Method: Petroleum ether, benzene, chloroform, alcohol and aqueous extracts at the concentration of 10, 20, 30 and 40 mg/ml were tested in the bioassay, which involved determination of the time of paralysis (P) and time of death (D) of the worms. In anthelmintic activity, the parameters like-time of paralysis was noted when no movement of any short could be observed except when the worms when shaken vigorously. Time of death was determined by observing no movement when the worms were shaken vigorously or dipped in warm water (50 ºC). Albendazole (40 mg/ml) was used as standard drug and normal saline as control.
Result: The chloroform, ethanol and aqueous extracts exhibited significant anthelmintic activity. The death of worms were dose dependent and compared with standard drug albendazole. Further petroleum ether and benzene extracts failed to show anthelmintic effect. Further studies are in process to isolate the active principles responsible for the activity.
Conclusion: Present investigation shows that chloroform, ethanol and aqueous extracts of *Bauhinia variegata* stem and leaf possess significant anthelmintic potential. When compared among the parts and the extracts alcohol and chloroform extracts of leaf were found to possess maximum anthelmintic activity. Further research is going on regarding the isolation of phyto-constituents responsible for the activity.

Keywords: *Bauhinia variegata*, anthelmintic activity, *Pheretima posthuma*, albendazole, time of paralysis, death time.

1. Introduction

The increasing prevalence of helminth parasites that are resistant to conventional anthelmintics has been the spur for different research programs exploring alternative approaches to parasite control [1]. For much of our past history forages, plant parts or extracts have been used to combat parasitism, and in many parts of the world such natural products are still in use for this purpose. Some of the potential that undoubtedly lies within these phyto-therapeutics has been revealed in studies in New Zealand and Europe which have shown that some plant species, when grazed or fed as conserved material, may reduce the degree of internal parasite infestation in sheep [2-4].

Bauhinia variegata Linn. (Caesalpiniaceae) is a medium-sized, deciduous tree, found throughout India, ascending to an altitude of 1300 m in the Himalayas. It is commonly known as Kanchnar in Sanskrit and Mountain Ebony in English [5]. In Sanskrit, the word Kanchnar means “A glowing beautiful lady.” The various parts of the plant, viz., flower buds, flowers, stem, stem bark, leaves, seeds, and roots, are utilized in various indigenous systems of medicine and are popular among the various ethnic groups in India for curing a variety of ailments. The bark is used in fever, as tonic and astringent, as anti-leprotic, in skin diseases and wound healing, anti-goitrogenic, and as antitumor [6–9]. The bark is also documented as astringent to the bowels, tonic to the liver, and beneficial for the cure of dysmenorrhea, menorrhagia, tuberculosis, asthma, and wounds [10]. The stem bark of *B. variegata* (BV) is reported to possess hepato-protective, anthelmintic, and anti-diabetic activities [11–13]. In the present investigation, we have evaluated comparative anthelmintic effect of petroleum ether, benzene, chloroform, acetone, alcohol and aqueous extracts of leaf and stem of *B. variegata.*

Although there are number synthetic drugs are available like albendazole, piperazine citrate etc but patient has to face common and serious side effects like headache, nausea, and vomiting, abdominal pain, agranulocytopenia, hepato-toxicity, leucopenia etc. Hence there is a need of an anthelmintic agent from the natural origin. Hence an attempt has been taken to explore the

anthelmintic potential of *B. variegata* stem bark of *which* is reported to possess anthelmintic potential [12].

2. Material and Methods

2.1 Plant material

Stem and leaf of the plant was collected from the local nursery of Rudrapur (Uttarakhand). The specimen was identified and authenticated from CMAP, Nagala, Udam Singh Nagar (Uttarakhand). A herbarium specimen has been prepared (Varsha No.09) and deposited in the herbarium of Devsthali Vidyapeeth College of Pharmacy, Rudrapur.

2.2 Animals

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

2.3 Drugs and chemicals

Albendazole (Pfizer Ltd., Bangalore), Petroleum ether, Chloroform, Benzene, Acetone, alcohol (chensynth lab) (PCL, Pune), distilled water.

2.4 Preparation of extract

The stem and leaf of *Bauhinia variegata* were collected, washed and dried at room temperature. After complete drying it was powdered and passed through a sieve no. (60-40 #) and stored in a tight container. Dried powdered drug was used to prepare the extract.

2.4.1 Successive solvent extraction

About 50 g of the air dried powdered plant material was extracted successively with Petroleum ether ($60\text{-}80^0$c), followed by benzene, chloroform and ethanol (70%) in a soxhlet apparatus. Each time before extracting with the next solvent, the marc was dried below 50 ^{o}C. Finally, the marc was macerated with chloroform water for 24 hours to obtain an aqueous extract. The extracts were filtered, the solvent was evaporated and accurate weight of the extracts was taken. The extractive value (%) was calculated with reference to air dried drug. The color and consistency of the extracts were noted down.

2.5 Physicochemical constants:

Physical constants were determined following *Indian Pharmacopoeia* (1996) [14].

2.6 Phyto-chemical analysis:

Various extracts prepared by soxhelation were subjected to the phyto-chemical analysis [15].

2.7 Experimental methods:

The anthelmintic assay was carried out as per the method of described with minor modification [16-17]. The assay was performed on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. 1%, 2%, 3% and 4% dilutions of petroleum ether, benzene, chloroform, ethanol extracts prepared in propylene glycol and aqueous extract were prepared in normal saline. Albendazole with normal saline to obtain 1%, 2%, 3% and 4% solution used as standard. Normal saline and propylene glycol served as control. All dilutions of test, standard, and control were prepared into different Petri dishes. Six earthworm of nearly equal size were placed in each of three petri dishes at room temperature. The time taken for complete paralysis and death was recorded. External stimuli were applied to ascertain the paralysis time. The time taken by worm to become motionless was considered as paralysis time and lethal time was ascertained by death of motionless worm.

2.8 Statistical Analysis

Statistical analysis for animal experiment was carried out using one way analysis of variance (ANOVA) followed by Tukey-kramer multiple comparison test. The results obtained were compared with the vehicle control group. p-values <0.05 were considered to be statistically significant.

3. Results

3.1 Physicochemical parameters

Moisture content, extractive value and percentage yield has been calculated and given in Table 2, 3, 4, 5.

3.2 Phyto-chemical analysis

Presence or absence of phyto-constituents has been determined and shown in the Table 1, 6.

Table 1: It shows preliminary phyto-chemical analysis of *Bauhinia variegata* leaves

S.N	Test	Extracts					
		Pet. Ether	Benzene	Chloroform	Acetone	Ethanol	Aqueous
1.	Alkaloids	-	-	+	-	+	+
2.	Carbohydrates and Glycosides	-	-	+	-	+	+
3.	Proteins and amino acids	+	-	-	-	+	+
4.	Phenolic compounds and tannins	-	-	+	-	+	-
5.	Saponins	-	-	-	-	-	+
6.	Flavonoids	-	-	+	-	+	+
7.	Gums and mucilage	-	-	-	-	-	-
8.	Volatile oils	-	-	-	-	-	+

+ = present, - = absent

Table 2: It shows moisture content of *Bauhinia variegata* leaves

Fresh weight (g)	Dry weight (g)	Loss on drying (g)
2.00	1.64	0.36

Table 3: It shows extractive values of *Bauhinia variegata* leaves

S.N	Extractives	Extractive values (% w/w)
1.	Alcohol soluble	8.5
2.	Water soluble	12.75

Table 4: It shows moisture content of *Bauhinia variegata* stem

Fresh weight (g)	Dry weight (g)	Loss on drying (g)
2.00	1.21	0.79

Table 5: It shows extractive values of *Bauhinia variegata* stem

S.N	Extractives	Extractive values (% w/w)
1.	Alcohol soluble	6.56
2.	Water soluble	13.65

Table 6: It shows preliminary phyto-chemical analysis of *Bauhinia variegata* stem

S.N	Test	Extracts					
		Pet. Ether	Benzene	Chloroform	Acetone	Ethanol	Aqueous
1.	Alkaloids	-	-	+	-	+	+
2.	Carbohydrates and Glycosides	-	-	+	-	+	+
3.	Proteins and amino acids	+	-	-	-	+	+
4.	Phenolic compounds and tannins	-	-	-	-	-	-
5.	Saponins	-	-	-	-	-	++
6.	Flavonoids	-	-	+	-	++	++
7.	Gums and mucilage	-	-	-	-	-	-
8.	Volatile oils	-	-	-	-	-	+

+ = present, - = absent

Table 7: It shows anthelmintic potential of *Bauhinia variegata* Extracts

Treatment	Conc. (mg/ml)	B. Variegata stem		B. Variegata leaf	
		Time(min.)		Time(min.)	
		Paralysis	Death	Paralysis	Death
Vehicle	-	-	-	-	-
Chloroform	10	10.5±0.57	258±0.57	23±1.02*	60±1.02
	20	56±0.57	250±0.54	19±0.86*	51±0.86
	30	50±0.57	202.8±0.57	15±1.58**	50±1.58
	40	52±1.02	183±1.02	18±0.57*	43±0.57*
Alcohol	10	30±0.25	69±0.28	12±0.96**	22±0.96*
	20	35±0.74	78±0.71	10±0.12**	30±0.12*
	30	30±1.34	55±1.34	12±0.85**	18±0.85**
	40	09±0.23**	72±0.27	04±0.56***	10±0.48***
Aqueous	10	45±0.25	69±0.25	48±0.14	93.6±0.14
	20	25±0.89	136.9±0.84	28±0.26	34.8±0.35
	30	15±0.45	114±0.48	23±0.21	76.3±0.11
	40	08±0.55***	58±0.55	18±0.65*	86.9±0.65
Standard	10	10±0.22***	15±0.22***	10±0.22***	15±0.22***
	20	06±0.57***	08±0.57***	06±0.57***	08±0.57***
	30	05±0.78***	07±0.80***	05±0.78***	07±0.80***
	40	05±1.02***	05±1.02***	05±1.02***	05±1.02***

The values are expressed as mean ± SEM; n=6 animals in each group.
Results are expressed as (mean ± SEM) *: $p<0.05$, **: $p<0.001$ & ***: $p<0.0001$ compared to control.
Data were analyzed by using one way analysis of variance (ANOVA) followed by Tukey-kramer multiple comparison test.
p-values <0.05 considered to be statistically significant.

3.3 Anthelmintic activity

The results of anthelmintic activity are shown in Table 7. In the present study it was observed that all the extracts have shown positive response to a certain degree of anthelmintic activity. Chloroform, ethanol and aqueous extracts of plant leaf as well as stem of plant showed significant activity as

comparable to the standard drug. Whereas petroleum ether and benzene extract of leaf and stem of plant failed to show anthelmintic effect. When compared among the extracts alcohol and chloroform extracts of leaf were found to possess maximum anthelmintic activity was comparable to the standard when compared with that of control and standard.
When compared among the parts leaf chloroform, ethanol and aqueous extracts were found to possess more significant effect than the stem extracts.

4. Discussion

Chloroform and alcohol extract of leaf as well as stem were found to possess a statically significant ($p<0.5$) anthelmintic potential as given in the Table 7.Among the extracts chloroform and alcohol extract of leaf demonstrated paralysis as well as death of worms at a time comparable to albendazole at (10, 20, 30, 40 mg/ml) concentration.
Albendazole is found to inhibit the polymerization of the parasite tubulin into microtubules. Mechanism of action of albendazole involves higher affinity of the drug to the parasite's tubulin and so the activity is mediated mainly against the parasite rather than on the host. The loss of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the parasites. The worm is then unable to maintain energy production, which leads to immobilization and eventual death [18].
Phyto-chemical screening of the crude extracts revealed the presence of flavonoids, alkaloids and phenolic compounds. Some synthetic phenolic anthelmintics e.g., niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [19]. It is possible that phenolic compounds present in the extract produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [20]. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [21].

5. Conclusions

From the present findings it can be concluded that *B. variegata* chloroform, alcohol and aqueous extracts were found to possess a statistically significant anthelmintic activity whereas pet. Ether, benzene and acetone extracts were found to possess insignificant anthelmintic activity. When compared among the extracts alcohol extracts of both stem and leaf were found to possess statistically significant anthelmintic activity.

6. Acknowledgement

Authors are thankful to the management of Devsthali Vidyapeeth College of Pharmacy, Rudrapur for their kind support.

7. Conflict of Interest Statement

The author(s) confirm that this article content has no conflict of interest.

8. References

1. Jackson F, Coop RL. The development of Anthelmintic resistance in sheep nematodes. Parasitology 2000; 120:95-107.
2. Niezen JH *et al.* Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or Sulla (*Hedysarum coronarium*) which contain condensed tannins. J Agric Sci 1995; 125:281-289.
3. Paolini V *et al.* Effects of Sainfoin hay on gastrointestinal nematode infections in goats. Vet Rec 2003; 152:600-601.
4. Marley CL *et al.* The effect of dietary forage on the development and survival of helminth parasites in ovine faeces. Vet Parasitol 2003; 118:93-107.
5. Anonymous. The Wealth of India. New Delhi: Publication and information Directorate, CSIR, 1988.
6. Kapoor SL, Kapoor LD. Medicinal plant wealth of the Karimnagar district of Andhra Pradesh. Bull Med Ethnobot Res 1980; 1:120–44.
7. Sahu TR. An ethnobotanical study of Madhya Pradesh I: Plants used against various disorders among tribal women. Ancient Sci Life 1981; 7:178–81.
8. Thakur MJ, Misra IN, Chaudhary BK. Ethnobotanical studies of some plants of Madhubani district, Bihar. J Econ Tax Bot 1992; 16:383–90.
9. Singh PB, Aswal BS. Medicinal plants of Himachal Pradesh used in Indian pharmaceutical industry. Bull Med Ethnobot Res 1992; 13:172–208.
10. Kirtikar KR, Basu BD. Indian Medicinal Plants. Dehra Dun: International Book Distributor, 1990.
11. Bodakhe SH, Alpana R. Hepatoprotective properties of *Bauhinia variegata* extract. Pharma J Jpn 2007; 127:1503–7.
12. Mali RG, Mahajan S, Mehta AA. Evaluation of *Bauhinia variegata* Linn stem bark for anthelmintic and antimicrobial properties. J Nat Rem 2008; 8:39–43.
13. Anonymous. Indian Pharmacopoeia. New Delhi:Controller of Publications, 1996, 2.
14. Koti BC, Biradar SM, Karadi RV, Taranalli AD, Benade VS. Effect of *Bauhinia variegata* bark extract on blood glucose level in normal and alloxanised diabetic rats. J Nat Rem 2009; 9:27–34.
15. Harborne JB. Phytochemical Methods- A guide to modern techniques of plant analysis. London: Chapman and Hall, 1998.
16. Mali RG, Shailaja GM, Mehta AA. *In vitro* screening of *Cleome viscosa* extracts for anthelmintic activity. Pharm Biol 2007; 45:766-8.
17. Lakshmi SP, Bindu RN. A comparative study of the anthelmintic potential of *Cleome viscosa* L. and *Cleome burmanni* W. IJPS 2011; 73 (1):98-100.
18. Dayan AD. Review of non-clinical toxicity and pharmacokinetics of Albendazole, mebendazole and praziquantel. Acta Trop 2003; 86(2-3):141-59.
19. Smith EC. The phenolic constituents of plants and their taxonomic significance. J Linn Soc (Bot.) 1962; 58:95-173.
20. Martin RJ. Modes of action of Anti-helmentic Drugs. Vet J 1997; 154:11-34.
21. Mali RG, Wadekar RR. Anthelmintic activity of *Platycladus orientalis* leaves extract. IJPS 2008; 70:131-133.