Research Article

Comparative Study on Thrombolytic and anti Helminthes activities of Aqueous Extracts of Each Root, Stem and Leaves of Medicinal Plant *Achyranthes aspera* Linn

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ABSTRACT

Achyranthes aspera is the important medicinal herb found as weed throughout tropical region of Nepal and famous for its folk medicine use, here in this work the different plant parts (root, stem and leaves) were subjected to extractions with different polarity solvent (gradient elution) and based on the presence of phytoconstituents in different extracts, maximum phytoconstituents were present in aqueous extracts of each plant parts, and hence aqueous extracts were subjected to study for antihelmenthics and thrombolytic activity, since these two activities of these specific extracts was aim of our study as because less works were carried out on it. The results imply that phytosterol are found to be active phytoconstituents in all different extract of stem, leaves and root of the same plant. The thrombolytic activity of the aqueous extracts of root showed higher activity as compare to leaves and stem. Among the aqueous extract of root stem and leaves which were subjected to antihelmenthics activity the aqueous extract of stem was found to be better in comparison to root and leaves (in terms of mean death time) but not satisfactory result showed by stem as compare to standard drug. Phytosterol are found to be active phytoconstituents in all different extract of stem, leaves and root of the same plant. The thrombolytic activity of the aqueous extracts of root showed higher activity as compared to leaves and stem. The activity increased with increased concentration of the extracts but less than standard drug (streptokinase).

Keywords: *Achyranthes aspera*, phytoconstituents, antihelmenthics, streptokinase, mebendazole.

INTRODUCTION

Nepal, a Himalayan country represents one of the world's richest pockets in plant diversity[1]. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs.[2] *Achyranthes aspera* is the important medicinal herb found as weed throughout tropical region of Nepal. It belongs to the family Amaranthaceae. It is known as dattiwan in nepali, Apamarg in Sanskrit, prickly chaff flower in English and Naayuruvi in tamil.[3] Phytochemical screening showed the presence of carbohydrates, phenolic compounds, saponins, alkaloids, oil and fats and tannins.[4] The medicinal plants are used for treatment of various diseases because of their safety and effectiveness. Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used medicinally. The plant is used in indigenous system of medicine as emenagogue, antiarthritic, ant fertility, laxative, ecbolic, and anti-helminthes, aphrodisiac, antiviral, anti-plasmodic, and antihypertensive, anticoagulant, diuretic and anti-tumor.

Thrombosis is the formation of a blood clot (thrombus) inside a blood vessel, obstructing the flow of blood through the circulatory system. When a blood vessel is injured, the body uses platelets and fibrin to form a blood clot, because the first step in repairing it (homeostasis) is to prevent loss of blood.

Various works had been carried out regarding thrombolytic and anti-oxidant activities particularly on ethanolic extract of either stems, root of this plant, various authors had reported that due to presence of various phytoconstituents and their synergistic effect are the causes for the above said activities, so here in this work various plant parts were extracted with different polarity solvents and out of which aqueous extracts were chosen for further activity.

MATERIALS AND METHODOLOGY

Plant Collection & Authentication

The fresh plants were collected from the Hasandaha village, Morang, Nepal. The plant was authenticated by Professor Sasinath Jha, Head, Department of Botany, University Post Graduate Campus, Biratnagar. Whole plants were washed with distilled water and sun drying was done.

Pharmacognostical Studies

The Pharmacognostical study of *Achyranthes aspera* was performed by naked eye to detect the specific features of the plant, which helps in easy identification and avoidance of adulteration and contamination due to misidentification of plant species. A macroscopic and microscopic evaluation was done individually of each stem, roots, leaves extract.

Moisture Content (%)

About 1g of the powdered of leaves, stem and roots was accurately weighed in a tarred dish and dried in an oven at 100-105°c for one hour .It was cooled in a desiccators and again weighed. The moisture contents were calculated with reference to the amount of the dried powder taken in dish leaves, stem and roots respectively.

Moisture contents = (Weight of empty dish + Sample weight – Weight after drying)/ Sample weight*100%

Extraction

The plant materials were dried in solar drier for 2 days and thereafter fibrous materials were removed along with soils and other debris. It was then cut into pieces and further shade dried at room temperature for one day. Dried samples were crushed into powder by electric blender (electric grinder) and pass through sieve no 30 to get fine powder which was then subjected to extraction by using various polarity solvents using Soxhlet apparatus. The solvent extract was evaporated to dryness using rotator evaporator and the extracts were stored at 4º C in air tight container for further study.

Phytochemical Screening of Extracts

Phytochemical screening of extracts was done to find out the various chemical constituents are present in that extracts of *Achyranthes aspera* by different reagents. Each extracts was treated to alkaloids (Mayer's test, wager's test and Hager test), carbohydrates (Molish's test, Benedicts test, Fehling's test), Glycosides, Saponins, Phytosterols, Phenols, Tannins, Flavonoids, Proteins and Dietpenens tests using standard test procedures.

Thrombolytic activity

Specimen

With all aseptic condition whole blood was drawn for healthy human volunteers without a history of oral contraceptive or anticoagulant therapy. 1 ml of blood was transferred to previously weighed MFT tube to form clots.

Blood Specimen Preparation

(n = no. of plant /herb extract =*A. aspera*)

21 micro centrifuge tubes were taken, sterilized; weighed.1ml blood was drawn from 21 healthy human volunteer without the history of contraceptives and antiplatelet drugs with a given declaration in a bond paper which was submitted to the ethical committee of the organization. The blood was distributed in different pre weighed (W1) micro centrifuge tube, each tube1ml.The blood specimen was centrifuged at 2500 rpm for 5 minutes. Incubated the blood for 45 minutes at 37ºC. After clot formation i.e. incubation, the serum was completely removed by micro pipette, capillary absorption and by removing the serum from the inner surface of the tube carefully by use of cotton bound at top of a glass rod without disrupting the clot and ensure complete removal of serum, or the result will be erroneous. Kept the tubes at lying position on a tray for 6 minutes after first removal of serum and then removed the liquids of the tube surface by the cotton rod. Each tube was weighed (W2) again. Weight of colt was found as, weight of clot = weight of clot containing tube (W2)-weight of tube alone (W1).Finally weighed very carefully, because result varies for inappropriate weighing, checked the balance before weighing. To each micro centrifuge tube containing pre weighed clot, 0.5ml of aqueous extract of 'n' plant/herb (*Achyranthes aspera*) was added separately according to dilution. As a positive control, 0.5ml of streptokinase was added to clot of tube no.6 (Standard). As a negative control, 0.5ml water is added to clot of tube no. 7 (Blank). All the tubes were incubated at 37ºC for 90 minutes and observed if clot lysis has occurred. After 90 minutes of incubation, the released fluid was completely removed by decanted colt containing liquid from the inner surface of the tube carefully by cotton rod without disrupting the clot. The tubes were then weighed again. And ensured complete removal of released fluid or the result will be erroneous. Kept the tubes at lying position on a tray for 6 minutes after first removal of released clot and then removed the liquids of the tube surface by the cotton rod. Weighed the tubes (W3) very carefully because result varies for inappropriate weighing. The difference obtained in weight taken before and after clot lysis is expressed as percentage of clot lysis.[5,6]

Antihelmintic activity

The earthworm was collected from local markets of dharan Nepal, it was then washed with normal saline to remove all earthy materials. The entire collected earthworm was kept in laboratory for further study. The activity was evaluated on earthworm, *Phaeritima posthuma*. The different concentration of plant extracts were prepared in distilled water (D/W) in 6% dimethyl sulfaoxide (DMSO). The concentration made were 1000ug/ml, 800ug/ml, 600ug/ml and 400ug/ml for all the extracts. The standard drug (mebendazole) was prepared in 6% DMSO at a dose level of 700ug/ml. The earthworm which served as normal control received 6% DMSO in water only. Then the 6 earthworm of 8-10 cm were placed in each petri dish containing 25 ml of test solutions of extracts received different concentration of 400ug/ml, 600ug/ml, 800ug/ml, 1000ug/ml plant aqueous extracts. And same procedure for standard drug also. Observations were made for the time taken to cause paralysis and death of individual worms. The paralyzing and death times were noted and their mean was calculated. Death was concluded when the worms lost their motility followed with fading away of their body colors.[7,8]

RESULTS

Moisture Content

Moisture contents = (Weight of empty dish + Sample weight – Weight after drying)/ Sample weight*100%

Leaves= (43.82+1)-44.75/1*100% = 7%
Stem=(43.9+.99)-44.78/.99*100% =11%
Roots= (43.83+1)-44.74/1*100% =9%

Table 1: Phytochemical tests of leaves

SN	Tests	Ethyl acetate	Aqueous	Ethanol
1	Alkaloids Mayer's test : Wager's test: Hager test:	 - - -	 + + +	 + - +
2	Carbohydrates: Molish's test: Benedicts test: Fehlings test:	 + - +	 + - +	 + - +
3	Glycosides	+	+	+
4	Saponins	+	+	+
5	Phytosterols	-	+	+
6	Phenols	+	+	+
7	Tannins	-	+	-
8	Flavonoids	+	+	+
9	Proteins	-	+	+
10	Diterpenens	+	+	+

Table 2: Phytochemical tests of stem

SN	Tests	Ethyl acetate	Ethanol	Aqueous
1	Alkaloids Mayer's test : Wager's test: Hager test:	 - - +	- - +	 - + +
2	Carbohydrates: Molish's test: Benedicts test: Fehlings test:	 + + +	+ - +	 + + +
3	Glycosides	+	+	+
4	Saponins	-	-	+
5	Phytosterols	-	+	+
6	Phenols	-	+	+
7	Tannins	-	-	+
8	Flavonoids	+	+	+
9	Proteins	+	+	+
10	**Diterpenens**	+	-	+

Table 3: Phytochemical tests of roots:

SN	Tests	Ethyl acetate	Ethanol	Aqueous
1	Alkaloids Mayer's test : Wager's test: Hager test:	 - - +	 - + -	 + - +
2	Carbohydrates: Molish's test: Benedicts test: Fehlings test:	 + - +	 + _ +	 + - -
3	Glycosides	-	+	-
4	Saponins	+	-	+
5	Phytosterols	-	-	+
6	Phenols	-	-	-
7	Tannins	-	-	+
8	Flavonoids	+	+	+
9	Proteins	-	+	+
10	Diterpenens	+	-	+

Table 4: Percentage clot lysis of aqueous extract

Conc. mg/ml	%clot lysis (Stem extracts)	%clot lysis (Root extracts)	%clot lysis (leaves extracts)
25	15.51	22.95	16.07
12.5	8.19	18.3	10.34
6.25	3.57	15.06	9.61
3.125	1.96	11.26	6.77
1.5625	1.75	9.83	3.5
Streptokinase (30,000 iu)	44	44	44
D/W	1.66	1.66	1.66

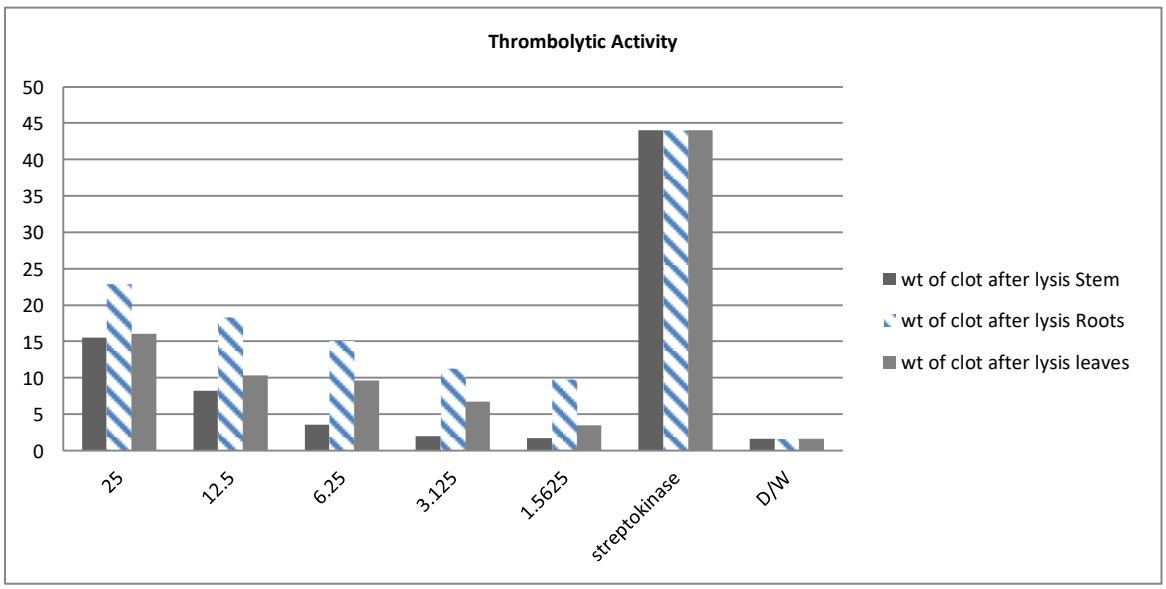

Figure 4: Comparisons of thrombolytic activity of aqueous extra different extracts

Table 5: Antihelmintic activity of plant aqueous extracts:

Plant extracts	Antihelmintic activity					
	Concentration (ug/ml)	Mean concentration (µg/ml)	Paralyzing time (min)	Death time (min)	Mean paralyzing time (min)	Mean death time (min)
Leaves	1000ug/ml	700µg/ml	55	74	71	87.5
	800ug/ml		64	82		
	600ug/ml		70	89		
	400ug/ml		95	105		
Stem	1000ug/ml	700µg/ml	30	63	35.75	77.5
	800ug/ml		33	75		
	600ug/ml		35	79		
	400ug/ml		45	93		
Roots	1000ug/ml	700µg/ml	35	78	48.75	92
	800ug/ml		45	83		
	600ug/ml		52	98		
	400ug/ml		63	109		
*Std.	700ug/ml		28	40	28	40
Blank (6% DMSO)	-		-	-	-	-

*standard = Mebendazol for antihelmintics activity, the antihelmintics activity were reported as mean paralyzing time and mean death time.

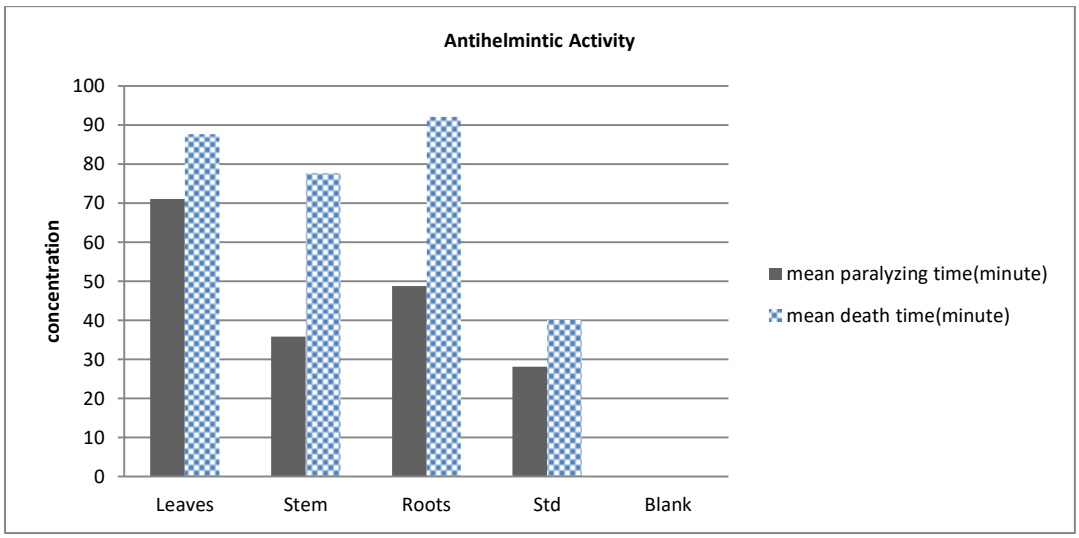

Figure 5: Antihelmintic activity of aqueous extracts of *A. aspera*

Root stem leaves of *A. aspera* extracted with ethylacetate, ethanol and water in isocratic manner, phytochemical screening showed positive test for various phytochemicals. Aqueous extracts were selected for entire activity. The aqueous extracts of stem, roots and leaves showed 15.51%, 22.95% and 16.07% at the concentration 25 mg/ml whereas 8.19%, 18.3% and 10.07% at the concentration 12.5mg/ml.

Accordingly 3.57%, 15.06% and 9.61% at the concentration 6.25 mg/ml. Then 1.96%, 11.26% and 6.77% at the concentration 3.125 mg/ml and 1.75%, 9.83% and 3.5% at concentration 1.5625mg/ml. Addition of 500 µl Streptokinase has showed 44% clot lysis However, distilled water (negative control) shown only negligible clot lysis 1.66% .The mean difference in clot lysis percentage between positive and negative control was significant. The mean percentage of clot lysis by different extracts of *Achyranthes aspera* was statistically more significant when compared to those of both positive control streptokinase and negative control water.

By comparing with this positive & negative control, a significant thrombolytic activity was observed after treating the clots with aqueous extracts of leaves, stem and roots whereas aqueous extract of stem of *Achyranthes aspera* result indicates less potential to lysis the clot. It has been reported that Tannins are remarkable also for their astringent actions. This astringent property affords them the therapeutic value in arresting hemorrhage by constricting blood vessels and protecting wounds.[9]

The preliminary studies carried out for screening of phytochemicals from aqueous extract of leaf has shown the presence of phenolics, tannins, flavonoids, saponins, glycosides and terpenes) Antihelmintic activity three extracts were taken each from root, stem and leaves (aqueous) 6% DMSO solution in distilled water was taken as negative control towards worm, the individual time for paralysis and death of worms were recorded and compared with standard Mebendazol. The Antihelmintic activity of *aspera* extracts was found poor then the standard drug previous research revealed that Tannins might have Antihelmintic activity by binding with free protein in gastrointestinal tract of the earthworm and cause death. [10].

CONCLUSION

Using different solvents different yield value was obtained, aqueous extract of all stem, root and leaves produced highest yield value by extraction as compared to other solvents. Alkaloids, Carbohydrates and phytosterol are found to be active phytoconstituents in all different extract of stem, leaves and root of the same plant. The thrombolytic activity of the aqueous extracts of root showed higher activity as compare to leaves and stem. The activity was increased with increased concentration of the extracts but less than standard. Among the aqueous extract of root stem and leaves which were subjected to antihelmenthics activity the aqueous extract of stem was found to be better in comparison to root and leaves (in terms of mean death time) but not satisfactory result showed by stem as compare to standard drug. All the above activities of *Achyranthes aspera* extract may be the result of the combinatorial effect of the active compounds present or by the individual compounds. Further isolation may lead to the findings of individual components responsible for individual activity. The study hopefully provides some important identifying characters for considered plants & guidelines for further related works.

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