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Research Article

Comparative Study on Anticoagulant Activity of Different Parts of Achyranthes Aspera

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Abstract

Achyranthes aspera is a perennial herb belonging to the family of Amaranthaceae (Hossain et al 2013). It is known as "Prickly chaff flower".The plant was found to have antibacterial property against hospital origin gram positive bacteria (Singh et al 2019). Achyranthes aspera contain coumarin which having protease activity. Coumarin is a potent enzyme that naturally supports the body's ability to break down blood clots caused by the blood-clotting protein fibrin. The aim of this work was to potent clot lysis activity (anticoagulant activity) of different parts of Achyranthes aspera with different solvent to get a fruitful comparative output. Achyranthes aspera contain coumarin which having protease activity. Extracts prepared using acetone as solvent gave maximum clot lysis activity (48.3%) as compare to other. However extracts prepared by taking ethanol and methanol were gave approximately same clot lysis. Extracts prepared by taking water as solvent gave minimum clot lysis <10% in all sample of Achyranthes aspera. Heparin as a positive control for anti coagulant activity shows the maximum percentage of clot lysis that is 76.1% at 0.5ml and 46% at 0.1 ml which is near to clot lysis percent of leaf extract prepared by using acetone as a solvent.

INTRODUCTION

Achyranthes aspera is a perennial herb belonging to the family of Amaranthaceae (Hossain *et al* 2013). It is known as "Prickly chaff flower" in English and "Chirchita", "Onga", "Latjeera" or "Apamarga" in local language (Hasan 2014). It is an erect, annual herb, distributed in the hilly districts of India (Londonkar *et al.,* 2011). The phytochemical studies with solvent i.e. methanol,ethanol,acetone,diethylether and water extracts of various parts of the plant *Achyranthes aspera* by shaking and boiling method showed to possess secondary metabolites (Tiwari *et al.,* 2018*).* Phytochemical evaluation of medicinal plants is very imperative in recognizing new sources of therapeutically and industrially important chemical compounds (kamboj, 2000).Medicinal plants have historically been the first source of anticoagulant and antithrombotic molecules (Chaves et al 2010). *In vitro* haemolytic activities are becoming a new area of drug research to discover new interventions for diseases such as thromboembolic disorders. Efforts have been shifted towards discovery and development of natural products from various plant and sources which have anticoagulant, antithrombotic and thrombolytic activity. Studies have proven that this plant has a great potential in inhibiting plate aggregation and enhancing fibrinolytic activity. *Achyranthes aspera* contain coumarin which having protease activity. Coumarin is a potent enzyme that naturally supports the body's ability to break down blood clots caused by the blood-clotting protein fibrin. Coumarin used to prevention of platelet aggregation and also prevention and treatment of thrombosis and thrombophebitis. (Evangelista *et al* 2012).

MATERIALS AND METHODES

Collection of plant material

The fresh, healthy, mature plants were collected from roadside area of Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.).The plant materials were identified, on the basis of flower and

inflorescence part of *Achyranthes Aspera* .The leaves, stem and roots were washed and used for the present study.

Preparation of plant extract

The fresh plant parts (leaf, inflorescence, stem and root) were collected and washed with tap water. The sample were dried under sunlight for two days after that partially dried root, shoot, and inflorescence were dried in hot air oven at 50°C for 24, 24 and 6 hour respectively. The dried plant material was powdered with mixer grinder and stored in air tight poly bags for further use.

(1)Shaking method-The powdered sample was soaked or immerged with solvent (methanol, ethanol, acetone and water) in (1:10) shaking incubator for 48 hour and filtered through whatmann No.1 filter paper (Ekpo *et al*., 2009)

(2)Boiling method-The powdered sample were mixed with solvent. The mixture was ground using morter and pestle, boiling in water bath for 10mins at 60°C and filtered through whatmann No.1 filter paper (Usha C. *et al* 2014)

Study of anticoagulant activity at different concentration

Material- Fresh blood, microtube, micropipette, micropipette stand.

For determination of anticoagulant activity Evangelista et al 2012 was followed. In this method venous blood was withdrawn and transferred in different pre-weighed sterile microtubes (0.5ml/tube) and was incubated at 37°C for 45 minutes. After clot formation, the incubated microtubes were centrifuged for 2 minutes and serum was removed completely using a micropipette (aspirated out without disturbing the clot formed). Each microtubes with clotted blood were weighed again to determine the initial clot weight (1), wherein Cwi is the initial clot weight; Cwt is the weight of tube with clot; and wt is the weight of tube alone

$$Cwi = Cwt - wt \qquad (1)$$

After the determination of the initial clot weight, each micro tube containing the clot was labelled properly and 0.1 ml of each of the prepared plant extract were added. For the controls, 0.1ml of Heparin and 0.1ml of distilled water were separately added to the control tubes and were labelled accordingly. The micro tubes containing the treated clots were incubated again at 37°C for 90 minutes and clot lysis was observed. The incubated tubes were centrifuged for 2 minutes and the fluid obtained was removed. The micro tubes were weighed again to determine the weight after clot disruption (2), where Cwf is the final weight of clot; CwT is weight of tube with treated clot; and wt is the weight of tube alone. The difference obtained taken before and after clot lysis were expresses as percentage of clot lysis (3). The procedure was repeated 5 times with different concentration of plant extract.

$$Cwf = CwT - wt \qquad (2)$$

$$\% \text{ Clot lysis} = (Cwi-Cwf)/Cwi \times 100 \qquad (3)$$

RESULTS AND DISCUSSION

The positive control, 0.1ml Heparin (10,000 I.U.) to the clots after incubation for 90 minutes at 37°C showed a 46.03%, 47.03%, 48.5%, 52.2%, 76.10% of clot lysis with different concentrated volume (0.1, 0.2, 0.3, 0.4, 0.5ml). The clot treated with solvent (methanol, ethanol, acetone, water) as a negative control shows in figure 3,4,5,6,7,8,9,10,11,12. The leaf part of plant shows the maximum activity as compare to other part of plant in shaking method. Blood clots due to some blood clotting chemicals like thrombin, prothrombins, fibrinogens, fibrins etc. some drug like heparin are used for this clot lysis. Clot lysis is require to cure diseases like sickle cell anaemia, thromboembolic disorders. However several plants such as *Allium sativum* (garlic), *Cucurma longa* (luyang dilaw), *Ananas comosus* (pineapple) and *Lycopersicum esculentum* (tomato) has been used as anti clot lysis activity reported by Evangelista (2012). Coumarin as bioactive component for anti clot lysis which inhibit the function of vitamin K which is required for the biosynthesis of prothrombin. In present study it was found that extracts prepared by shaking method is giving more clot lysis as compare to extracts prepared by boiling method. It may be due to different extracts may contain different amount and structurally altered bioactive component responsible for clot lysis. Extracts prepared using acetone as

solvent gave maximum clot lysis activity (48.3%). However extracts prepared by taking ethanol and methanol were gave approximately same clot lysis. Extracts prepared by taking water as solvent gave minimum clot lysis <10% in all sample of *Achyranthes aspera.* As volume of extracts was increased, then percentage of clot lysis was observed as increasing. But in some cases percentage of clot lysis was decreasing by increase in percentage, the reason behind this is due to some contamination during the incubation of clot with plant extracts. It may also be due to instrumental error of weighing balance (used to weigh the clot samples before and after incubation). Negative control that is solvents without plant extracts were showing negligible clot lysis, so it can be ensure that chemicals present in plant extracts shows the percentage clot lysis activity. Heparin as a positive control for anti coagulant activity shows the maximum percentage of clot lysis that is 76.1% at 0.5ml and 46% at 0.1 ml which is near to clot lysis percent of leaf extract prepared by using acetone as a solvent.

Table No.2 (a) Percentage of clot lysis in presence of different solvent with different parts of *Achyranthes aspera* extract prepared by shaking method.

		PERCENTAGE OF CLOT LYSIS				
SOLVENT	**PLANT PARTS**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Methanol	Leaf	25.5	21.7	29.3	29.2	29.3
	inflorescence	23.1	27.2	22.2	24.0	25.3
	Stem	21.3	25.4	22.1	24.3	26.2
	Root	17.1	21.2	12.2	19.3	23.2
Ethanol	Leaf	23.2	29.4	28.1	29.8	31.1
	inflorescence	20.1	25.0	28.2	26.9	32.2
	Stem	22.1	21.0	26.2	28.3	29.2
	Root	18.3	13.2	13.2	19.5	21.0
Acetone	Leaf	42.8	47.2	48.3	43.2	44.2
	Inflorescence	3.4	12.0	14.2	14.1	14.2
	Stem	1.52	7.7	7.2	7.8	7.9
	Root	6.6	4.2	4.3	6.8	9.2
water	Leaf	1.8	2.8	9.2	9.8	11.1
	inflorescence	2.7	4.4	6.8	7.2	8.0
	Stem	4.4	7.3	3.1	4.2	5.3
	Root	13.3	12.2	6.4	19.2	20.0
Negative control	Methanol	2.2	2.5	7.1	8.1	9.2
	Ethanol	1.7	2.0	3.2	2.8	3.4
	Acetone	2.4	3.0	3.2	4.2	5.8
	water	1.1	2.7	2.9	4.6	6.2
Positive control	heparin	46.03	47.03	48.5	52.5	76.1

Table No.1 (b) Percentage of clot lysis in presence of different solvent with different parts of *Achyranthes aspera* extract prepared by boiling method.

PERCENTAGE OF CLOT LYSIS

SOLVENT	PLANT PARTS	0.1	0.2	0.3	0.4	0.5
Methanol	Leaf	13.8	10.2	14.3	19.3	19.4
	Inflorescence	14.2	14.2	17.2	18.2	17.2
	Stem	11.0	13.2	18.3	18.4	17.1
	Root	15.6	14.6	14.8	15.9	16.2
Ethanol	Leaf	15.8	12.4	11.1	16.2	19.2
	Inflorescence	20.5	13.1	14.2	19.8	20.1
	Stem	6.6	4.5	7.2	8.9	9.2
	Root	22.1	16.2	18.7	23.2	24.2
Acetone	Leaf	3.2	2.1	2.3	4.2	5.2
	Inflorescence	1.8	3.0	3.8	4.2	5.0
	Stem	2.3	1.3	2.4	2.8	3.0
	Root	2.2	10.6	5.5	9.2	9.8
Water	Leaf	2.6	4.6	7.2	8.1	9.2
	Inflorescence	14.2	3.0	12.2	13.2	12.9
	Stem	3.8	4.8	5.9	6.2	7.3
	Root	6.4	6.8	6.9	8.3	9.2
Negative control	Methanol	2.2	2.5	7.1	8.1	9.2
	Ethanol	1.7	2.0	3.2	2.8	3.4
	Acetone	2.4	3.0	3.2	4.2	5.8
	Water	1.1	2.7	2.9	4.6	6.2
Positive control	Heparin	46.0	47.0	48.5	52.5	76.1

CONCLUSION

Medicinal plants form a large group of economically important plants that provide the basic raw materials for pharmaceuticals purpose. *Achyranthes Aspera* is present a large source of novel active biological compounds showes different activities, including anti-inflammatory, anti-cancer, antiviral, and antibacterial and cardio protective activities and anticoagulant activity. Now a day, plant materials continue to play a major role in primary health care as therapeutic remedies in developing countries.According to rural area people, it was used for various diseases but still not used for commercial production of drugs. The study showed that the leaf with acetone extract have exhibited the greatest clot lysis activity as compare to other part in shaking method as compare to boiling method. It also shows the better alternative source of pharmacologic agent. Further study is required to isolate the bioactive compound from *Achyranthes aspera* that is act as anticoagulant agent, so that this wide spread plant will be used as pharmacological purpose. Purity assessment and detail study about active anticoagulant compound may reveal a data, so that new drug can be formulated using *Achyranthus aspera.*

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