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Research Article

Phytochemical and Pharmacological Investigation of *Achyranthes Aspera* Linn.

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Abstract: The methanol extract of *Achyranthes aspera* Linn. (Family- *Amaranthaceae*) the herb was studied for its Phytochemical and some Pharmacological activities. The study of the herb extracts indicated the presence of flavonoides, tannins, saponins, and alkaloids. The pharmacological study includes thrombolytic and cytotoxic activity. They showed significant cytotoxic effect in Brine shrimp lethality bioassay where the plant extracts exhibited LC_{50} =3.80µg/ml and LC_{90} =7.04µg/ml (after 18 hour); LC_{50} =3.31µg/ml and LC_{90} =6.36µg/ml (after 21 hour); LC_{50} =2.18µg/ml and LC_{90} =5.29µg/ml (after 24 hour). This study gave a significant indication to the use of the plant extract as a potential source for cytotoxic compounds. In the thrombolytic activity of standard was found 76.08% and for *A. aspera* was 36.49%, which indicates moderate thrombolytic activity. Based on the findings of thrombolytic and toxicological activity, we can say that *Achyranthes aspera* may contain some novel compounds that possess potent anti-mutagenic activity and clot lysis property.
Keywords: *Achyranthes Aspera,* Phytochemical, Pharmacological.

INTRODUCTION:

Phytotherapy, The treatment of disease by the use of plants, was the beginning of pharmacotherapy or treatment of disease by means of drug [1]. Therapeutic uses of plants had in effect stored at the very beginning of human life on earth when the primitive man, out of necessity and by intuition, resorted to using plants to alleviate his sufferings from injuries and diseases. The medicinal plants have been used in traditional medicine for hundreds of years with reputation as efficacious remedies although there may not sufficient scientific data to substantiate their efficacy of these, surprisingly large number is still of importance in modern medicine [2]. In this way, phytotherpy laid the foundation stone of all forms of medical treatment that are practiced today. With the development of human civilization, the implementation of phytotherpy exhibits Stepwise development, which can be enumerated as –

1st stage: Crude drugs were employed, prepared in the roughest manner, such as powdered willow bark in the management of pain.

2nd stage: This was converted into more active and manageable forms, such as: extracts or solutions, watery or alcoholic.

3rd stage: The pure active principles separated from the crude drug were employed, e.g. salicylic acid.

4th stage: Attempt to synthesize the active drug in the laboratory and indeed structural modification, e.g. aspirin, the wonder drug.

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal plants". According to WHO consultative group on medicinal plants, "A medicinal plant is any plant which, in one or more of its organs contains substances that can be used for therapeutic purposes or which, is a precursor for synthesis of useful drugs. The contributions of the plants are numerous in every sector of human life. It helps to growing up of the human body and also protects human being from sickness by being used as medicine. The current list of medicinal plants growing around the world includes more than a thousand items Specifically in Bangladesh about two hundred fifty species are used as medicinal plants [3].It has now been established that the plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils and contain, minerals and vitamins possesses medicinal properties [1].

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant products to produce drugs and medicines. This huge foreign exchange can be saved if the indigenous medicinal plants or their semi-processed products are utilized by the manufacturers to satisfy their needs. There is a resource of this genus (*Amaranthaceae*) in Bangladesh. Moreover, *Achyranthes aspera* Linn. is used traditionally and pharmacologically. The present study also will provide some valuable information

about the pharmacological properties of this plant. However, the objective of the present study is to find out the reason of traditional use of this plant. This study includes the following pharmacological action: phytochemical investigation, cytotoxic activities, thrombolytic activity.

Phytochemicals

Phytochemicals are chemical compounds such as reducing sugar (carbohydrate), tannins, saponins, alkaloids etc. that occurs naturally in plants. The term is generally used to refer to those chemicals that may affect health , hence there is growing evidence to support the health benefits of diets rich in fruits and vegetables, there is only limited evidence to suggest these effects are due to specific phytochemicals [4].

Phytochemistry

These are chemicals derived from plants. in a narrower sense the terms are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers. a knowledge of the phytochemicals constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies. Techniques commonly used in the field of phytochemistry are extraction, isolation and structural elucidation (MS,1D and 2D NMR) of natural products, as well as various chromatography techniques (MPLC, HPLC, LC-MS) and chemical group test.

COLLECTION AND IDENTIFICATION

Leaves of *Achyranthes aspera* Linn. were collected from Gaibandha District of Bangladesh in November 2009 and were identified by the Bangladesh National Herbarium, Mirpur, and Dhaka. One voucher specimen was deposited in Bangladesh National Herbarium, Accession number is 34420.

PREPARATION OF EXTRACT

The collected plant parts (herb) were separated from undesirable materials or plants or plant parts. They were dried in open air under shade for two weeks. The shade dried plants part ground into a coarse powder with the help of a suitable grinder from. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 225gm of powder material of leaf were taken in a clean glass container and soaked in 1500ml of distilled methanol. The container with it contain was sealed and kept for a period of 5 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white, cotton material. Then it was filtrate through Whatman filter paper. The filtrate (Methanol extract) thus obtained was evaporated under ceiling fan and in a water bath until dried. It rendered a greenish black color. The greenish black color extract was designated as crude extract of methanol.

PERCENTAGE YIELD

225gm of powdered *A. aspera* was taken and after evaporation it yields 8gm of *A. aspera* extract. So, percent yield is $\{(8/225) \times 100\} = 3.56\%$.

CHEMICAL GROUP TEST

0.5gm extract was dissolved in 10 ml alcohol and following test were performed for identifying different chemical groups present in the extracts [1,5,6].

REAGENTS USED FOR THE DIFFERENT CHEMICAL GROUP TEST

The following reagents were used for the different chemical group test [5]-

a) **Mayer's reagent**
   1.36gm mercuric iodide in 60ml of water was mixed with a solution contains 5gm of potassium iodide in 20ml of water.
b) **Dragendroff's reagent**
   1.7gm basic bismuth nitrate and 20gm tartaric acid were dissolved in 80ml water. This solution was mixed with a solution contain 16gm potassium iodide and 40ml water.
c) **Fehling's solution A**
   34.64gm copper sulphate was dissolved in a mixture of 0.50ml of sulfuric acid and sufficient water to make 500ml.
d) **Fehling's solution B**
   176gm of sodium potassium tartarate and 77gm of sodium hydroxide were dissolved in sufficient amount of water to produce 500ml. Equal volume of above solution were mixed at the time of use.
e) **Benedicts reagents**
   1.73gm cupric sulphate, 1.73gm sodium citrate and 10ml anhydrous sodium carbonate were dissolved in water and volume was made up to 100ml with water.
f) **Molish reagent**
   2.5gm of pure α-naphthol was dissolved in 25ml of methanol.
g) **Wagner's reagent**
   Dissolved 12.7gm iodine in a solution of 20gm of pure potassium iodide in 50ml water and dilute to 1L with water.
h) **Hager's reagent**
   A 1% solution of picric acid in water

1. Tests for reducing sugar

a) **Benedict's test:** 0.5ml of aqueous extract of the plant material was taken in a test tube. 5ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool

spontaneously. A red color precipitate of cuprous oxide was formed in the presence of a reducing sugar. Observation- Brick red colored precipitate was not found. Inference- Absence of reducing sugars.

b) Fehling's test: 2ml of aqueous extract of the plant was added 1ml of a mixture of equal volumes of fehling's solutions A and B. Boiled for few minutes. A red color or brick color precipitate was formed in the presence of a reducing sugar. Observation, Brick red colored precipitate was not found. Inference- Absence of reducing sugars.

2. Test for tannins

a) Ferric Chloride test: 5ml solution of the extract was taken in a test tube. Then 1ml of 5% Ferric Chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins. Observation- Greenish black precipitate was formed. Inference- Presence of tannin.

b) **Potassium dichromate test:** 5ml solution of the extract was taken in a test tube. Then 1ml of 10% Potassium dichromate solution was added. A yellow precipitate was formed in the presence of tannins. Observation-Yellowish brown precipitate was formed, Inference- Presence of tannin.

3. Test for flavonoids: Added a few drops of concentrated hydrochloric acid to a small amount of an alcoholic extract of the plant materials. Immediate development of a red color indicates the presence of Flavonoids. Observation- A red color was not developed, Inference- Absence of Flavonoids.

4. Test for saponins: 1ml solution of the extract was diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of indicated the presence of saponins. Observation- Layer was formed, Inference- Presence of saponin.

5. Test for gums: 5ml solution of the extract was taken and then molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicated the presence of gums and carbohydrate. Observation-A red violet ring produced at the junction was not formed, Inference-Absence of gums.

6. Test for Alkaloids

a) **Mayer's test:** 2ml solution of the extract and 0.2ml of dilute hydrochloric acid were taken in a test tube. Then 1ml of Mayer's reagent was added. Yellow color precipitate was formed and that was indicated as the presence of alkaloids. Observation-Yellow color precipitate was formed, Inference-Presence of Alkaloids.

b) **Dragendroff's test:** 2ml solution of the extract and 0.2ml dilute hydrochloric acid were taken in a test tube. Then 1ml of Dragendroff's reagent was added. Orange brown precipitate was formed and that was indicated as the presence of alkaloids.Observation-Orange brown precipitate was formed, Inference- Presence of Alkaloids.

c) **Wagner's test:** 2ml solution of the extract and 0.2ml dilute hydrochloric acid were taken in a test tube. Then 1ml of iodine solution (Wagner's reagent) was added. Reddish brown precipitate was formed and that was indicated as the presence of alkaloids. Observation- Reddish brown precipitate was not formed, Inference- Absence of Alkaloids.

d) **Hager's test:** 2ml solution of the extract and 0.2ml dilute hydrochloric acid were taken in a test tube. Then 1ml of picric acid solution (Hager's reagents) was added. Yellowish precipitate was formed and that was indicated as the presence of alkaloids. Observation-Yellowish precipitate was not formed, Inference- Absence of Alkaloids.

BRINE SHRIMP LETHALITY BIOASSAY OF *ACHYRANTHES ASPERA* LINN.

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc. [7]. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (in-vivo) lethality, a simple zoological organism (brine shrimp napulii- *Artemia salina*) can be used as a convenient monitoring for screening and fractionation in the discover of new bioactive natural products [8]. Natural product extracts, fractions or pure compounds can be tested for there bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus *Artemia* of aquatic crustaceans. *Artemia*, the only genus in the family Artemiidae, have evolved little since the Triassicperiod. The historical record of existence of *Artemia* dates back to 982, more than one thousand years ago, from Lake Urmia, Iran, while Schlösser was the first person to give drawings of *Artemia* in 1756. *Artemia* are found worldwide in inland saltwater lakes, but not in oceans [9].

Life Cycle

Brine shrimp eggs are metabolically inactive and can remain in total stasis for two years while in dry oxygen-free conditions, even at temperatures below freezing. This characteristic is called cryptobiosis

meaning "hidden life" (also called diapause). While in cryptobiosis, brine shrimp eggs can survive temperatures of liquid air (−190 °C, −310 °F) and a small percentage can survive above boiling temperature (105 °C, 221 °F) for up to two hours. Once placed in brine (salt) water, the cyst-like eggs hatch within a few hours. The nauplii, or larvae, are less than 0.5 mm in length when they first hatch. Brine shrimp have a biological life cycle of one year, during which they grow to a mature length of around one centimeter on average. This short life span, along with other characteristics such as their ability to remain dormant for long periods, has made them invaluable in scientific research, including space experiments [10].

Tolerance to salinity

Brine shrimp can tolerate varying levels of salinity. A common biology experiment in school is to investigate the effect of salinity levels on the growth of these creatures. The preferred level of salinity is about 120ppt.

Nutritional Benefits

The nutritional properties of newly hatched brine shrimp make them particularly suitable to be sold as aquarium food as they are high in lipids and unsaturated fatty acids (but low in calcium).

Materials

Equipments- Small tank (glass jar) to grow shrimp, cover and Lamp to attract shrimp, Pipettes (5ml, 1ml), Micro-pipettes (10 µl, 200µl adjustable), Test tube (15ml), Volumetric flask (10ml), Spoon, Electric water blower to produce current, Electric bulb to produce heat, Stand to hold the bulb, Petri dish, Test tube stand, Beaker (1L), Chemical and Reagents-Table salt, Pure NaCl, Test Compounds-Methanol extract of Achyranthes aspera Linn. Test Organism- Artemia salina Leach (brine shrimp) .The egg of the shrimp was collected from Katabon University Market.

Method

The study was performed according to the Brine shrimp lethality bioassay method [11].

Preparation of Stock Solution

100 mg of dried methanol extract was taken in 10 ml volumetric flask and volume was adjusted by water. The concentration of this solution was 10µg/µl.

Preparation of Simulated Sea Water

20g of NaCl and 18g of table salt were weighed accurately, dissolved in distilled water to make one liter and then filtered off to get a clear solution.

Hatching of Brine Shrimp

Sea water was taken in the small tank and shrimp eggs were added to the one side to the divided tank and the side was covered. The shrimps were allowed for one day to hatch and mature as nauplii (larvae). The hatched shrimps were attracted to the lamp through the perforations in the dam and they were taken for bioassay. In hatching time was 22 hours.

Application of Test Solution and Brine Shrimp Nauplii to the Test Tubes

Seven (07) clean test tubes were taken, six (06) of which were for the samples in six concentrations and one (1) for negative control test. Then 5ml of seawater was given to each of the test tubes. Then with the help of the micropipette specific volumes (1, 3, 5, 7, 9, 11µl) of samples were transferred from the stock solutions to the test tubes to get final sample concentrations of 1, 3, 5, 7, 9, 11µl/ml respectively. The concentration of distilled water in these test tubes did not exceed 40µg/ml. For the control, same volumes of distilled water (as in the sample test tubes) were taken in the rest of the one (1) test tube. Finally with the help of a Pasteur pipette 15 living shrimps were kept to each of the test tubes [11].

Counting of Nauplii

After 18hrs the test tubes were observed and the number of survived nauplii in each test tube was counted and the results were noted. Form this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample. Like above procedure after 21hrs and 24 hour's the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

THROMBOLYTIC EFFECT OF *ACHYRANTHES ASPERA* LINN.

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. If that mechanism causes too much clotting and the clot breaks free, an embolus is formed [12.13]. Thrombolysis is the breakdown (lysis) of blood clots by pharmacological means. The name "thrombolysis" comes from two Greek words that mean "clot" and "loosening."It is colloquially referred to as clot busting for this reason. It works by stimulating fibrinolysis by plasmin.

Thrombolytic therapy

It is the use of drugs that dissolve blood clots. The name "thrombolytic" comes from two Greek words that mean "clot" and "loosening." All thrombolytic agents work by activating the enzyme plasminogen, which clears the cross linked fibrin mesh (the backbone of a clot). This makes the clot soluble and subject to further proteolysis by other enzymes, and restores blood flow over occluded blood vessels [14].

Streptokinase (SK)

A protein secreted by several species of streptococci can bind and activate human plasminogen.

SK is used as an effective and inexpensive clot-dissolving medication in some cases of myocardial infarction (heart attack) and pulmonary embolism. Streptokinase belongs to a group of medications known asfibrinolytics, and complexes of streptokinase with human plasminogen can hydrolytically activate other unbound plasminogen by activating through bond cleavage to produce plasmin. There are three domains to Streptokinase, denoted α (residues 1–150), β (residues 151–287), and γ (residues 288–414). Each domain binds plasminogen, although none can activate plasminogen, independently [15,16].

Blood Specimen Preparation

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

- 5 micro centrifuge tubes were taken, sterilized, weighed. (Let n= 1), 5 ml blood was drawn from volunteer. The blood was distributed in 5 different pre weighed (W_1) micro centrifuge tube, each Tube 1ml.
- The blood specimen was centrifuged at 2500 rpm for 5 minutes. Incubated the blood for 45 minutes a 37ºC.
- After clot formation i.e. incubation, the serum was completely removed by decantation, capillary absorption and by removing the serum from the inner surface of the tube carefully by cotton bar or 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 (W_2) again. Weight of colt was found as, weight of clot = weight of clot containing tube (W_2)-weight of tube alone (W_1).Finally weighed very carefully, because result varies for inappropriate weighing, checked the balance before weighing.
- To each micro centrifuge tube containing pre-weighed clot, 100µl of aqueous extract of 'n' plant/herb (*Achyranthes aspera*) was added separately. As a positive control, 100µl of streptokinase was added to clot of tube no.5 (Standard). As a negative control, 100µl water is added to clot of tube no.4 (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 bar or by use of cotton tightly bound at top of a glass 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 (W_3) 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.

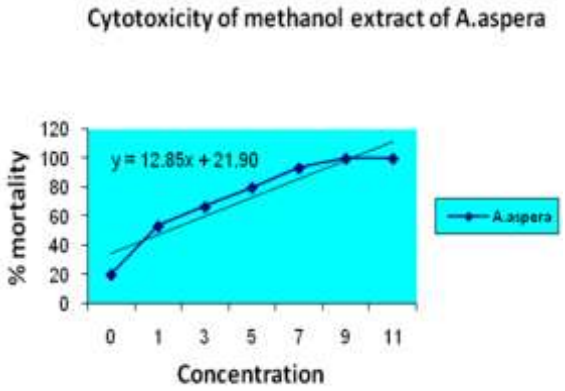
RESULTS AND DISCUSSION

As the chemical constituents present in a plant are directly responsible for its therapeutic and other pharmacological properties, the constituents of the plant which were reported and detected during this investigation should have some direct relationship with local medicinal uses The result showed that the extract of leaves contain, tannins, saponins, flavonoids, and alkaloids. So the plant is rich of polar compound. Approximately all the samples of *Achyranthes aspera* Linn. had been found to contain flavonoids, alkaloids, saponins and tannins. Alkaloids being bitter substances exert notable antimicrobial actions. So it is quite reasonable that the plant containing alkaloids exert beneficial therapeutic effects against infectious diseases for which it is used. Tannins are not only remarkable for their antiseptic property but also for their astringent actions. This astringent property affords them the therapeutic value in arresting hemorrhage by constricting blood vessels and protecting wounds, Inflammation and ulcer form external irritations by precipitating surface protein which forms impervious coating on them. Thus it is evident that the constituents (alkaloids, flavonoids, saponins) are sufficient to cure infections and tannins are also responsible to cure inflammatory diseases. Toxicity means adverse or poisonous effects of drugs, toxins or their metabolites. Though the extracts of the plant *A. aspera* possess potent cytotoxic activity, hence it may say that they may show some anti microbial activity. Brine Shrimp Lethality Bioassay, a bench top bioassay method for evaluating anticancer, antimicrobial and other pharmacological activities of natural products are a recent development in the bioassay for the plant extracts of the plant, *A. aspera* were examined on the larvae of brine shrimp, *Artemia salina* Leanh. This bioassay method is indicative of cytotoxicity and a wide range of pharmacological activities of the compound. Test sample showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample and plot of percent mortality versus concentration on the graph paper produced an approximate linear correlation between them. From the Table-1, the concentration at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred were obtained by extrapolation, the values were found 3.81µg/ml, 3.31µg/ml, 2.18µg/ml, after 18hour, 21 hour, and 24 hour respectively for the crude extract. The 90% mortality (LC_{90}) values were found 7.04µg/ml, 6.36µg/ml,5.29µg/ml, after 18hour, 21 hour, and 24 hour respectively from the same table. The crude extracts were found to show potent lethality against the brine shrimp nauplii. These results tend to suggest its possible antitumer, antibacterial or

pesticidal activities.However, further researches are necessary particularly with its purified fraction. The purpose of brine shrimp lethality bioassay is to discover a new cytotoxic(Anticancer) drug.

Table 1: After 18, 21 and 24 hours later result of Brine shrimp lethality bioassay of distilled methanol extract of the herb (A. aspera)

Conc. of Extract µg/ml	After 18 hour % Mortality	LC_{50} µg/ml	LC_{90} µg/ml	After 21 hour% Mortality	LC_{50} µg/ml	LC_{90} µg/ml	After 24 hour % Mortality	LC_{50} µg/ml	LC_{90} µg/ml
0(blank)	13.34	3.81	7.04	13.34	3.31	6.36	20	2.18	5.29
1	33.34			40			53.34		
3	40			46.67			66.67		
5	53.34			60			80		
7	60			73.34			93.34		
9	66.67			80			100		
11	100			100			100		



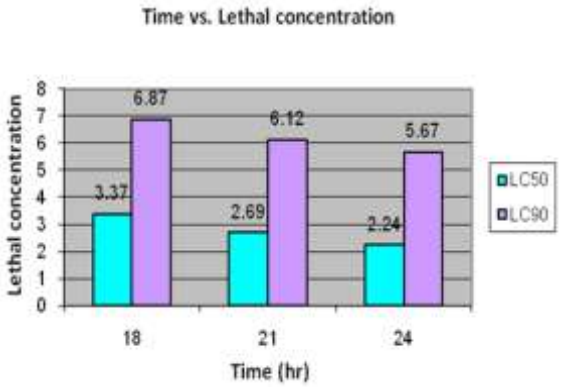


Figure-1 : After 24 hour later LC_{50} and LC_{90} **Figure - 2: Comparison among time vs. lethal concentration**

Thrombolytic effect of *achyranthes aspera* linn.:

From the following equation, the thrombolytic activity of *A. aspera* was calculated. Since two samples of *A. aspera* extract were taken for the experiment, the average value was counted. By regretting the value of Blank we found the % of clot lyses of Standard and sample 76.08 % and 36.49% respectively. The thrombolytic activity of standard was found 76.08% and for *A.aspera* was 36.49% which indicates moderate thrombolytic activity. However, further researches are necessary to find out the thrombolytic activity of the active principles.The purpose of thrombolytic activity study is to discover a new thrombolytic drug.

% of clot lysis = (wt. of Released clot/clot wt) × 100
$= (W_2 – W_3/W_2 – W_1) \times 100$

Where, W_1= the weight of empty centrifuge-tubes, W_2= weight of tube and clot, W_3= weight of centrifuge-tubes and the clot that remained after the loss in weight of clot dissolved by the action of extract solution.

Table- 2: Thrombolytic activity of *A. aspera*

Observation	Wt. of empty test tubes (W_1) gm	Wt. of clot containing tube (W_2) gm	Wt. of test tube after incubation (W_3) gm	Avg. of clot lyses, gm	% of clot lyses
Sample 1	4.1863	4.7035	4.5272	0.415	36.49
Sample 2	4.1455	4.6237	4.4149		
Sample 3	4.1680	4.6137	4.4049		
Sample 4(Blank)	4.1680	5.0173	4.9745	-	0
Standard	4.1277	4.9432	4.2816	-	76.08

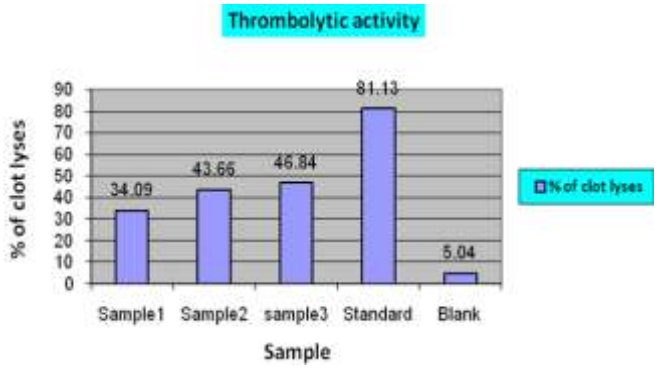


Figure - 3: **Thrombolytic activity of *A. aspera.***

CONCLUSION:

The methanol extract of *Achyranthes aspera* Linn. (Family- *Amaranthaceae*) the herb was studied for its Phytochemical and some Biological (Pharmacological) activities. To get preliminary idea about the active constituents present in the plant extracts different chemical test were performed and showed the presence of reducing flavonoids, tannins, saponins, and alkaloids. Further studies needed to determine the active compounds responsible for these activities and this development effort will help to identify the active constituents, structures and their action mechanism responsible for the activity and this project will also help a new investigator to proceed his research study.

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