

## ANTIOXIDANT AND THROMBOLYTIC ACTIVITIES OF METHANOLIC EXTRACTS OF *ACHYRANTHES ASPERA* LINN.

Raut B<sup>1</sup>, Khanal DP, Bhandari K

<sup>1</sup>Department of Pharmacy, Manmohan Memorial Institute of Health Sciences, Solteemode, Kathmandu, NEPAL

†**Corresponding author:** Bechan Raut, Associate Professor, Department of Pharmacy, Manmohan Memorial Institute of Health Sciences, Solteemode, Kathmandu, Email: [rautbechan@yahoo.com](mailto:rautbechan@yahoo.com)

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### ABSTRACT

#### Background

Plants have been used since a long time for the therapeutic use and precursors of therapeutic agents. People depend upon the herbal and traditional medicine to satisfy their health care needs. *Achyranthes aspera* L. is a multifunctional plant used to treat various kinds of diseases. It possesses various type of activities such as antioxidant, anti-inflammatory, antimicrobial and thrombolytic activity. Study of various biological activities helps to provide evidence to the traditional knowledge. Therefore, antioxidant and thrombolytic activities of its stem-leaves were aimed to explore in the present study.

#### Methods

Quantitative experimental method was applied to its antioxidant and thrombolytic activities. Stem-leaves of *Achyranthes aspera* collected from Thankot, Kathmandu was subjected for methanolic extraction by maceration method. The extract was screened for the antioxidant activity by using DPPH radical scavenging assay as well as thrombolytic activity through the clot lysis assay in human blood.

#### Results

The methanolic extractive value by using maceration was found to be 6.12%. Extract showed their antioxidant and thrombolytic potentialities. The concentration of antioxidants needed to

decrease the initial DPPH concentration by 50% (IC<sub>50</sub>) was found to be 30.5µg/ml for its extract and it was almost 5-fold lesser than ascorbic acid, 6.62 µg/ml. Similarly, the% clot lysis was found to be 7.29 % and 15.35 % in 10 mg/ml and 25 mg/ml concentration respectively.

## Conclusion

The study suggests that stem-leaves of *Achyranthes aspera* are the possible sources of natural radical scavenger as well as thrombolytic agents. Thus, they could be used as natural antioxidants as well antithrombosis in the beverage, functional food and pharmaceutical industries that need further wide range in vivo studies.

## Keywords

*Achyranthes aspera*, Antioxidant activity, Thrombolytic agent, DPPH assay

## INTRODUCTION

Medicinal plants are the oldest form of health-care known to mankind and constituted an integral part of development of modern civilization. According to World Health Organization (WHO) about 80 percent of world population still rely on traditional or herbal medicines for their primary health-care needs. Approximately 25% of drugs in modern pharmacopoeia were derived from plants.<sup>1</sup> World Health Organization also notes that about three fourth of plant-derived drugs correlate directly with their traditional uses in native cultures. Herbal medicinal products have become a subject of increasing global importance for their health benefits and economic considerations.<sup>2</sup>

The cultural and biological diversity of Nepal offers massive platform and opportunities for ethno-botanical studies. In Nepalese traditional medicine, more than 2300 plant species are used by 125 cast/ethnic communities speaking approximately 123 different languages.<sup>3</sup>

*Achyranthes aspera* Linn belongs to the family Amaranthaceae, is an annual, stiff erect herb. The plant is widespread as a weed globally as in Temperate Asia, Tropical Asia, Africa, Europe, America and Australia. It is found as weed throughout tropical region of Nepal.<sup>4,5</sup> It is a well-known plant drug in Ayurvedic, Unani, Siddha, Allopathic, Homeopathic systems. It is also called devil's horsehip, prickly chaff flower. Plant is known by different names such as Chirchita in Hindi, Apamarga in Sanskrit, Apang in Bengali, Nayurivi in Tamil and Agadhain

Marathi.<sup>6</sup> The plant is known as Datiwan in Nepalese society possessing various medicinal properties.<sup>7</sup> According to Yajurveda, Apamarga can enhance the will power and controls the minds of the persons whoever uses it. Atharvaveda considers this plant as the Lord of all plants on earth because it can increase life span by wiping away various diseases.<sup>8</sup> Ayurvedic practitioners and Kabirajs uses different parts of the plant to treat leprosy, asthma, fistula, piles, arthritis, wound, insect and snake bite, kidney stone, diabetes, dermatological disorders, gonorrhoea, malaria, pneumonia, fever, cough, toothache, dysentery, rabies, hysteria, toothache etc.<sup>9</sup>

The ash from the burnt plant, often mixed with mustard oil and a pinch of salt, and is used as a tooth powder for cleaning teeth. It is believed to relieve pyorrhea and toothache.<sup>10</sup>

Oxygen based free radical obtained from oxidation can attack cell and cause initiation of various diseases like diabetes, liver cirrhosis, premature ageing etc. Thrombosis obstructs the blood flow in the circulatory system and causes various problems such as heart attack, and massive pulmonary embolism. So, development of newer antioxidant and thrombolytic drugs is today's need. The medicines used for the treatment of these problems may have various side effect and costly. So, there is a need to validate the traditional use of this plant and develop it as source of new medicine.<sup>11</sup>

*A. asperad* despite of having various uses, in Nepalese community it is found that the plant is just used for one day at Nepalese festival, Rishi Panchami, while during other time proper utilization of plant is not done and they are wasted. Moreover their therapeutic properties are still largely unexplored.

## MATERIAL AND METHODS

### Chemicals and Instruments

All chemicals and solvents used during the experiment were of analytical grade. Methanol was used as principle solvent for maceration and it was supplied by Changshu Homgsheng Fine Chemical Co. Ltd. Chemicals like ascorbic acid and DPPH were purchased from Merck Industrial and Lab Chemicals. Instruments like UV-VIS spectrophotometer and Rotary Evaporator were acquired from Electronics India and Accumax India respectively.

### **Collection of Plant Material**

Plant leaves-stems were collected from Chandragiri-6, Thankot, Kathmandu having altitude of about 1480 m on the month of April 2019.

### **Identification of Plant Material**

Herbarium sample was submitted to National Herbarium and Plant Laboratory, Godavari, Lalitpur and was identified as *Achyranthes aspera* Linn.

### **Preparation of Extract**

Plant sample, leaves and stems, were washed with distilled water, cut into small pieces and shade dried. The dried sample was grounded and 50 g powder was allowed for cold maceration with 500 ml methanol (in the ratio of 1:10) at room temperature for 24 h. It was filtered using Whatman No.1 filter paper to obtain methanolic extract. The residue left was again subjected for second maceration with 500 ml methanol for another 24 h as per previous conditions. Both the filtrates were mixed. Finally, it was transferred to a stainless-steel plate for the evaporation of solvents and concentrated at room temperature. Thus, the extract was obtained and stored at 4°C in refrigerator. The extracts were subjected for screening of their antioxidant and thrombolytic potentialities.

### **Antioxidant Activity**

Antioxidant activity was tested by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay<sup>12</sup>. This assay is based on the fact that when DPPH (1,1-diphenyl-2-picrylhydrazyl) react with an antioxidant compound or extract, donate hydrogen, it is then reduced. The change in color (from deep-violet to light yellow) can be measured at 517nm on a UV visible light spectrophotometer. Ascorbic acid was used as positive control which is standard antioxidant. DPPH solution without sample extract served as negative control.

### **Preparation of Stock Solution**

The stock solution of 1 mg/ml of plant extract and ascorbic acid were prepared using methanol.

### **Preparation of Plant Samples**

Different concentrations (5, 10, 15, 20 and 25 µg/ml) were prepared by serial dilution using methanol.

### **Preparation of Ascorbic Acid Solution**

Ascorbic acid was taken as standard. Different concentrations (5, 10, 15, 20 and 25µg/ml) of ascorbic acid were prepared by serial dilution using methanol.

### **Preparation of DPPH Solution**

0.1 mM solution of DPPH in methanol was prepared by dissolving 3.94 mg DPPH in 100 ml of methanol

### **DPPH Radical Scavenging Activity**

4 ml of DPPH solution in methanol (0.1 mM) was added to 1 ml of methanolic extract and ascorbic acid solutions separately. The mixtures were kept in dark for 30 minutes. Similarly, as control, 4 ml of 0.1 mM DPPH was mixed with 1 ml of methanol (solvent) and kept in dark for 30 minutes.

After 30 min, the absorbance was measured at 517nm. Each assay was performed in triplicates the capability to scavenge the DPPH radical was calculated by using the following formula

$$\text{Percentage scavenging} = [(A_0 - A_t) / A_0] \times 100$$

Where,  $A_0$ = absorbance of control (DPPH solution and methanol),  $A_t$ = absorbance of test or reference sample.

### **STATISTICAL ANALYSIS**

The % scavenging was then plotted against concentration and regression equation was obtained. IC50 (microgram concentration required to inhibit DPPH radical formation by 50%) values were calculated for each plant extracts by using this inhibition curve. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

## Thrombolytic Activity

### Test Specimen

Whole blood (5ml) was drawn from healthy human volunteer without the history of contraceptives and antiplatelet drugs. The blood was distributed in different pre weighed ( $W_1$ ) micro centrifuge tube, each tube with 1ml. The blood specimen was centrifuged at 2500 rpm for 5 minutes and incubated it for 45 minutes at 37°C to form clot.

### Clot Lysis Study

After clot formation i.e. incubation, the serum was completely removed by micro pipette and finally ensured 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. Each tube was weighed ( $W_2$ ) again.

To the micro centrifuge tube containing pre weighed clot, 0.5ml of plant (*A. aspera*) extract of concentration 10 mg/ml and 25mg/ml was added separately. All the tubes were incubated at 37°C 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 ( $W_3$ ) again. The difference obtained in weight taken before and after clot lysis is expressed as percentage of clot lysis.

$$\% \text{ Clot lysis} = \frac{W_3 - W_2}{W_2 - W_1} \times 100$$

As a positive control, 0.5ml of streptokinase was added to next tube. As a negative control, 0.5ml water is added to clot of tube.

## RESULTS

Percentage yields of extract from stem-leaves powder of *Achyranthes aspera* Linn by methanol as 6.12%.

### Antioxidant activity by DPPH method

The antioxidant potential was assessed in term of  $IC_{50}$  by DPPH free radical scavenging capacity. It is the concentration of antioxidants required to decrease the initial DPPH

concentration by 50%. IC<sub>50</sub> value of methanolic extract was calculated through regression equation. and was found to be (IC<sub>50</sub>= 30.12 µg/ml). Ascorbic Acid was used as standard and its IC<sub>50</sub> value was found to be 6.12µg/ml.

Table 1: % scavenging of ascorbic acid and extract

Concentration (µg/ml)	% Scavenging of ascorbic acid	% Scavenging of plant Extract
5	41.19	11.39
10	61.69	19.77
15	75.58	26.32
20	92.56	35.36
25	97.35	40.92

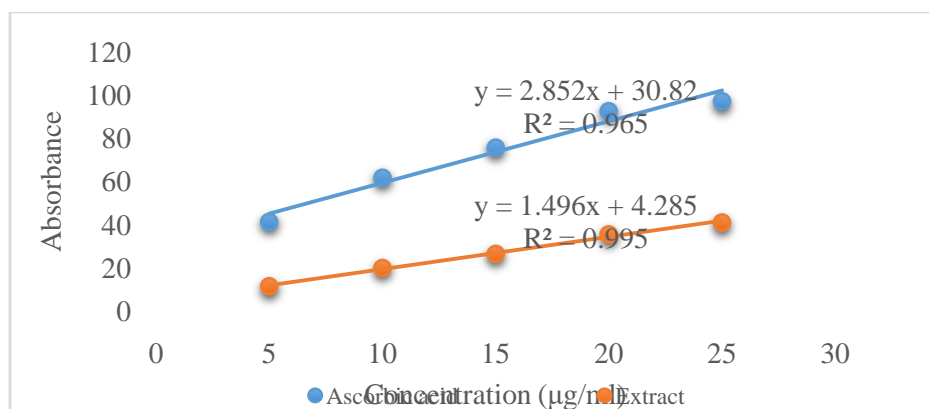


Figure 1: Antioxidant activity of the extract

### Thrombolytic activity by clot lysis in Human blood

The % clot lysis at the concentration of 25 mg/ml was found to be 15.35 % and at 10 mg/ml it was 7.29 %. Similarly, for the standard streptokinase the clot lysis was found to be 40.77 %.

Table 2: % clot lysis of extract

Sample	Concentration (mg/ml)	% clot lysis
Sample 1(extract)	10	7.29±0.000816
Sample 2(extract)	25	15.35±0.012257
Streptokinase	30,000 I.U	40.77±0.0017
Negative control	Distilled water	2.03

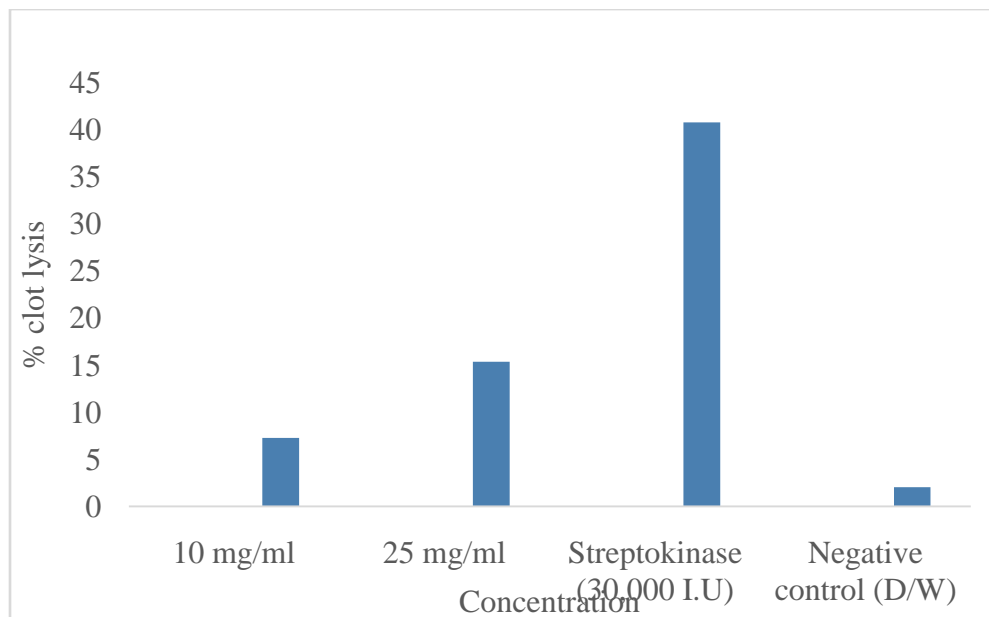


Figure2: *Thrombolytic activity of the extract*

## DISCUSSION

Stem-Leaves of *Achyranthes aspera*, being the multifunctional plant, it was selected to find its antioxidant and thrombolytic potentialities. Methanolic extraction was carried out by maceration

The percentage yield was found to be 6.12 % asmethanolic extract. This extract was used for further study of biological activity. In the previous study conducted by Veena Sharma et al the extractive value on methanol was found to be 7%.<sup>12</sup> This value is slightly higher than the present study. It may be due to varying in collection time, season and extraction method of test sample.

It has been recognized that the compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants through scavenging or chelating process and phenolic compounds are a class of antioxidant compounds which act as free radical terminators. Such phenolic compound and flavonoids are used for the prevention and cure of disease which are associated with free radicals. In DPPH method the IC<sub>50</sub> value of the ascorbic acid was found to be 6.62(μg/ml) and for plant extract it was 30.2 (μg/ml). Previous study showed that methanol extract had scavenging activity of 91 % at 200 μg/ ml and the IC<sub>50</sub> value was 32.84 μg/ ml. This value is almost similar to the present study. Plants contain many



phenolic compounds Flavonols such as (quercetin, myricetin, kaempheroletc) which are contain hydroxyl groups on an aromatic ring. These phenolic compounds interrupt chain of oxidation reactions by donation of hydrogen atoms.

The thrombolytic activity at the concentration of 10 mg/ml was found to be 7.29% and at 25 mg/ml it was 15.35 %.For standard streptokinase clot lysis was found to be 40.77%and for negative control distilled water it was 2.03%. In the previous study conducted by Kamana Ghimire et al. the plant showed clot lysis of 15.51% and 8.19% for 25mg/ml and 12.5 mg/ml respectively and for standard and negative control it was 44% and 1.66% respectively.<sup>13</sup>

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### CONTRIBUTIONS

BR and DPK supervised the research works, contributed in data analysis and manuscript submission. KB has made significant contribution to conception the project as well as to conduct the research work. All authors read and approved the final manuscript.

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