ORIGINAL ARTICLE

PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL ASSESSMENT OF *Achyranthes aspera L.* STEM EXTRACT AS ANTI-INFLAMMATORY AND ANTI-BACTERIAL POTENTIAL

Deepti Piya Baniya[1] ✉, Sushmita Bohora[1]✉, Usha Giri[1], Mijala Bajracharya[1], Abisa Ghimire[1], Sabita Raut[1], Roshan Paudel[1]

[1]*Department of Pharmacy, Manmohan Memorial Institute of Health Sciences, Soalteemode, Kathmandu, Nepal*

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✉**Deepti Piya Baniya,**
Associate Professor, Department of Pharmacy Manmohan Memorial Institute of Health Sciences, Soalteemode Kathmandu
Email: piyadipti123@gmail.com

✉**Sushmita Bohora,**
Department of Pharmacy Manmohan Memorial Institute of Health Sciences, Soalteemode Kathmandu
Email: sushmibohora135@gmail.com

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ABSTRACT

Introduction: *Achyranthes aspera L.* (Dattiwan in Nepali), belongs to Amaranthaceae family is a medicinal herb of utmost importance which possess anti-bacterial, anti-fungal, anti-inflammatory, anti-spasmodic, astringent, diuretic activity and used for treatment of asthma, cough, diuretics, dental problems, etc.

Method: A descriptive and experimental design was performed. Simple maceration process was carried for extraction. Quantitative phytochemical screening (Total Phenolic Content and Total Flavonoid Content) was conducted by Folin-Ciocalteu method and AlCl3 Colorimetric method respectively. In-vitro anti-inflammatory activity was performed by Human RBC membrane stabilization method and Anti-bacterial activity was carried out by Agar-well dilution method against *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Result: The qualitative phytochemical screening showed the presence of glycoside, flavonoid, saponin and reducing sugar in all three extracts whereas alkaloids and phenolic compounds were present only in ethanolic extract. The TPC of ethanolic extract was found to be 8.058 ±0.168 mg GAE/g. The TFC of aqueous, hydroalcoholic and ethanolic extract were 3.043 ±0.153, 6.23 ±0.042 and 45 ±0.720 mg QE/g respectively. All the three extracts showed significant anti-inflammatory activity, among them hydro-alcoholic extract showed the highest percent of membrane stabilization (i.e. 58.72%, 59.30%, 60.47%, 62.79% and 63.95% at 20, 40, 60, 80, 100 µg/ml concentration) as compared with Standard Diclofenac sodium. Ethanolic extract showed superior anti-bacterial activity against Gram-positive bacteria, showing Zone of Inhibition of 14.6, 16.1, 17.4 and 19.2 mm at 25, 50, 100 and 200 mg/ml respectively against *Staphylococcus aureus* and Zone of Inhibition of 9.1, 16.6, 18.1 mm at 50, 100, 200 mg/ml against *Enterococcus faecalis* respectively.

Conclusion: The stem extracts of *Achyranthes aspera L.* showed presence of different phytoconstituents. The ethanolic extract of *Achyranthes aspera L.* showed higher anti-bacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*). The hydro-alcoholic extract exhibited superior anti-inflammatory activity whereas, other stem extracts of *Achyranthes aspera L.* showed minimal anti-inflammatory activity as compared to Diclofenac sodium.

Key words: ***Achyranthes aspera L.*, Extraction, Phytochemical, anti-inflammatory, anti-bacterial, *Staphylococcus aureus***

INTRODUCTION

Nepal, a Himalayan Country, represents one of the world's richest pockets of plant diversity. According to the World Health Organization, 80% of the population in the world relies on traditional medicine for primary health care[1]. There is a long history of using herbal medicine to treat various diseases. Medicinal herbs are a valuable and affordable source of primary health care, playing a crucial role in treating illnesses, maintaining health, and promoting overall well-being[2].

Achyranthes aspera L. which is the scientific name popularly known as Dattiwan in Nepali, Apamarg in Sanskrit, Prickly chaff flower in English, and Naagyuruvi in Tamil, chirchitaa in Unani and Apaamaarga, Chirchitaa, Shikhari, Shaikharika in Ayurvedic[3] belongs to Amaranthaceae, whose most of the parts (stems, leaves, roots, flowers) containing the main chemical constituent as carbohydrate, protein, glycosides, alkaloids, tannin, saponins, flavonoids, lignin, etc., have been used as medicine in previous days[1]. *Achyranthes aspera* L. is a well-known plant drug in Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic, Home remedies. It is an annual shrub, being distributed and then found throughout the tropical and subtropical regions[4].

In customary medicinal system, Achyranthes aspera L. has diuretic, hepatoprotective properties, dysentery, asthma, hypertension, diabetes. Achyranthes aspera L. is one of that is studied for the medicinal properties that it has had most recently and has immunostimulatory properties, wound-healing, anti-oxidant activity, hemolytic activity, anti-inflammatory, anti-bacterial and anti-fungal activity[3]. Active ingredients available on stem of *Achyranthes aspera* L. are Pentatriacontane, 6-penta-Tri-acontanone, Hexa-tritriacontane[2]. Also, some different constituents present in the stems are Dihydroxy ketones-36, 37-dihydroxyhenpentacontan-4-one, aliphatic alcohol, 17-pentatriacontanol, tetracontanol-2, 4-methoxyheptatriacont-1-en-10-ol, E-sitosterol, Spinasterol, 27-cyclohexylheptacosan-7-ol, 16-hydroxy-26-methyleheptacosan-2-one, 20-hydroxyecdysone, quercetin-3-O-β-D galactoside, bisdesmosidic saponins[5].

METHODS

Ethics approval: The study protocol was approved by the Institutional Review Committee of Manmohan Memorial Institute of Health Sciences (MMIHS-IRC) [Reference No:NEHCO-IRC/081/088]

Collection and Identification of plant: The stems of *Achyranthes aspera* were collected from Barlachi, Tanahun and was identified as *Achyranthes aspera L.* by National Herbarium and Plant Laboratories, Godavari, Lalitpur, Nepal.

Processing of plant

The stems were cleaned using distill water, then shade dried for about 3 weeks then cut into small pieces which were then grounded into powder using electric blend.

Preparation of extract from *Achyranthes aspera* L. stem

Distill water, hydro-alcohol (50:50) and ethanol three solvents in a 1:5 ratio were used to macerate the powder. While water

performed a one-day maceration, ethanol and hydro-alcohol solvents underwent a three-day maceration with occasional stirring. Whatman No. 1 filter paper was used for filtration following maceration. The extract was then concentrated using a rotary vacuum evaporator at a lower pressure and temperature below 50°C[6].

Qualitative Phytochemical Screening

Qualitative phytochemical screening of the *Achyranthes aspera* L. stem extract was performed by using the method described in reference[7].

Quantitative Phytochemical Screening:

Determination of Total Phenolic Content

TPC of the extracts of *Achyranthes aspera L.* stem was quantified by Folin-Ciocalteu method where gallic acid and methanol were used as standard and blank respectively[8]. 1 ml of sample (1 mg/ml) was placed to 15 ml test tube. To that 5 ml of Folin-Ciocalteu reagent (10%) and 4 ml of 7.5% Na2CO3 were added to get total of 10 ml. The blue colored mixture was shaken well and incubated for 30 minutes at 40°C in the water bath. Then, the absorbance was measured at 760 nm using UV-visible spectrophotometer. The estimation of the phenolic compounds was carried in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried fraction.

Determination of Total Flavonoid Content

TFC of ethanolic extract of *Achyranthes aspera L.* stem was done by $AlCl_3$ colorimetric method where Quercetin and methanol were used as standard and blank respectively[9]. 1 ml of sample (1mg/ml) was mixed with 0.1 ml of 10% $AlCl_3$ with the help of vortex in different test tube. Then 0.1 ml of 1M Potassium acetate was added and again vortexed, 2.8 ml of distill water was added and incubated at 30° C for 30 minutes. The absorbance of that mixture was taken at 415 nm using UV-Visible spectrophotometer. The TFC was performed in three different runs. The TFC was expressed as milligrams of Quercetin equivalents per g of dried sample.

Anti-inflammatory activity

Anti-inflammatory activity of *Achyranthes aspera L.* stem extracts was evaluated by using in-vitro HRBC membrane stabilization method[10]. Briefly, the assay mixture contains 1 ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36%], 0.5 ml HRBC suspension [10% v/v] with 1 ml of plant extracts, standard drug Diclofenac Sodium and isotonic solution for control sample. The mixture was incubated at 37°C for 30 min and centrifuged at 3000 rpm. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

% Hemolysis= (Absorbance of Test/Absorbance of Control) x100

% Membrane stabilization= 100 - [Absorbance of Test/ Absorbance of Control) x100]

Anti-bacterial activity

Selection of Bacterial strains: The test organism used were *Staphylococcus aureus* (ATCC 25933), Enterococcus faecalis (ATCC 29122), *Pseudomonas aeruginosa* (ATCC 9027) and *Klebsiella pneumoniae* (ATCC 700603).

The anti-bacterial activity of *Achyranthes aspera L.* stem extracts were determined by Agar well-dilution method. In MHA plates, diameter of 6 mm wells was made and 100 µl solution from the concentration of 25, 50, 100, 200 mg/ml of extracts which was dissolved using 1% DMSO were placed. Ciprofloxacin- 10 µg/ml, 20 µg/ml as positive control and 1% DMSO as Negative control were used. Then, plates were placed in a 37°C air incubator for 16-18 hours[11]. The anti-bacterial activity was evaluated by measuring Zone of Inhibition (in mm) using Digital Zone Reader.

RESULTS AND DISCUSSIONS

Extractive value

The extractive value of plant material with Water, hydro-alcohol and ethanol as a solvent through maceration were calculated as follow

Table 1: Total percentage yield of extracts of Achyranthes aspera

S.N.	Extracts	Yield %
1	Aqueous	11.46 ± 0.05
2	Hydroalcoholic	10.11 ± 0.10
3	Ethanolic	1.68 ± 0.02

Among the three extracts, highest extractive value had been shown by the Aqueous extract and least by ethanolic extract.

Table 2: Result of Phytochemical screening of different extracts of Achyranthes aspera

Phytoconstituents	Aqueous extract	Hydroalcoholic extract	Ethanolic extract
1.Alkaloids			
a. Dragendroff's test	-	-	+
b. Wagner's test	-	-	+
c. Hager's test	-	-	-
d. Mayer's test	-	-	+
2. Phenolic compounds			
a. Ferric chloride test	-	-	-
b. Gelatin test	-	-	+
c. Lead acetate test	-	-	-
3. Glycoside (General test)	+	+	+
4. Flavonoids			
a. Lead acetate test	+	+	+
b. Alkaline reagent test	-	+	-
c. Shinoda test	-	+	+
d. Conc. H2So4	+	+	+
5. Saponin	+	+	+
6. Reducing sugars			
a. Fehling's test	+	+	+
b. Benedict's test	-	+	+

(Note: + = Presence, - = Absence)

Phytochemical analysis indicated the presence of Glycosides, Saponin, Flavonoid in all of the three extracts but the presence of Alkaloid and phenolic compounds were indicated only by the Ethanolic extract.

Research conducted by Dhital KS. (2018) showed Presence of Alkaloids, Saponin, Phenolic compounds in all 3 extracts (i.e. Aqueous, Hydroalcoholic and Alcoholic extract), Presence of Flavonoid, reducing sugar and Glycoside in only Alcoholic

and Hydroalcoholic extract[12]. Whereas in comparison with the study, similarity is seen only in context of saponin only and the differences in other phytochemical screening may be due to collection time as secondary metabolites vary during plant development, Extraction method, Extraction time and temperature.

Quantitative Phytochemical Screening

Total Phenolic Content (TPC)

The total phenolic content of the extract was determined by Folin-Ciocalteu method which was reported as Gallic acid equivalents. The results were derived from a calibration curve (y=0.012x+0.1613, R^2 = 0.996) of Gallic acid (20-100µg/ml).

Table 3: Total Phenolic Content of Extract

Sample	Extract	TPC (mg GAE/gm)
Achyranthes aspera L. (Stem)	Ethanolic	8.058 ±0.168

The total phenolic content was found to be 8.058 ±0.168 mg GAE/gm.

Total Flavonoid Content (TFC)

The concentration of flavonoid in extract was calculated from the calibration curve of Quercetin (20-100 µg/ml) using the regression equation (y = 0.0138x + 0.061, R^2 = 0.9985).

Table 4: Total Flavonoid Content of Extract

Sample	Extract	TFC (mg QE/gm)
Achyranthes aspera L. stem	Aqueous	3.043 ±0.153
	Hydro-alcoholic	6.232 ±0.042
	Ethanolic	45 ±0.072

The total flavonoid contents of aqueous, hydro-alcoholic and ethanolic extract were 3.043, 6.23 and 45 mg QE/gm respectively where highest flavonoid content was observed in Ethanolic extract.

TPC and TFC of alcoholic extract of *Achyranthes aspera L.* plant was observed to be 362.74 mg GAE/gm and 96.33 mg QE/gm respectively in research conducted by Awasthi H et al., (2021)[13], which is considerably higher for TPC and two times higher for TFC in comparison with this study's ethanolic extract. This substantial difference in Quantitative phytochemical screening may be due to the variations in the type of solvent used, geographical and environmental factors or differences in extraction procedure.

Anti-inflammatory activity of extract

The invitro anti-inflammatory activity of extract were concentration dependent and the results of the anti-inflammatory activity are shown in Table 5.

Table 5: Percent of Membrane stabilization of plant extracts and Diclofenac sodium standard

Concentration (µg/ml)	% Membrane Stabilization			
	Aqueous extract	Hydro-alcoholic extract	Ethanolic extract	Diclofenac sodium (Standard)
20	34.88	58.72	8.82	31.37
40	54.07	59.30	18.63	38.24
60	56.40	60.47	23.53	39.22
80	59.88	62.79	30.39	40.20
100	62.21	63.95	34.31	43.14

The maximum membrane stabilization was shown by Hydro-alcoholic extract at concentration of 100 µg/ml followed by aqueous extract. In comparison with the standard, hydro-alcoholic extract showed superior anti-inflammatory activity, moderate activity by aqueous extract and minimalist activity by ethanolic extract. The anti-inflammatory activity in aqueous and hydro-alcoholic extract may be due to presence of different phytoconstituent as mentioned in the Nagaharika Y et al. study[14].

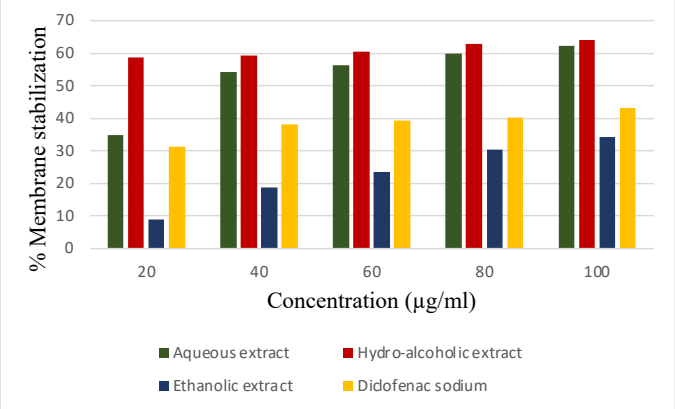

Figure 1: % Membrane stabilization comparison of plant extracts and Diclofenac sodium standard

Table 7: Anti-bacterial activities of *Achyranthes aspera L.* stem in different Extract

Micro-organisms	Concentration(mg/ml)	Zone of Inhibition (mm)		
		Aqueous	Hydro-alcoholic	Ethanolic
Staphylococcus aureus ATCC 25933	25	-	-	14.6
	50	-	8.7	16.1
	100	-	10.3	17.4
	200	-	11.9	19.2
Enterococcus faecalis ATCC 29122	25	-	-	-
	50	-	-	9.1
	100	-	-	16.6
	200	-	-	18.1
Pseudomonas aeruginosa ATCC 9027	25	-	-	-
	50	-	-	-
	100	-	-	-
	200	9.3	-	-
Klebsiella pneumoniae ATCC 700603	25	-	-	-
	50	-	-	-
	100	-	-	-
	200	-	7.5	-

Table 8: Anti-bacterial activity of Standard (Ciprofloxacin) and Blank

Micro-organisms	Zone of Inhibition (mm)		
	Ciprofloxacin		Blank (1% DMSO)
	10 mcg/ml	20 mcg/ml	
Staphylococcus aureus	17	20	-
Enterococcus faecalis	9	11	-
Pseudomonas aeruginosa	23	28	-
Klebsiella pneumoniae	16	19	-

Ethanolic extract showed the superior anti-bacterial activity among 3 extracts against Gram positive bacteria (i.e. *Staphylococcus aureus* followed by *Enterococcus faecalis)* and moderate activity in comparison with the standard (Ciprofloxacin). However, Hydro-alcoholic extract less anti-bacterial activity against Gram positive bacteria (*Staphylococcus aureus*) in comparison with the ethanolic extract. Where, aqueous extract doesn't show any anti-bacterial activity. Therefore, it is able to extract non-polar compounds as well[15]. The differences in sensitivity among the bacterial strains may be from their intrinsic resistance mechanisms along with the nature and composition of the phytocompounds in the extract[16]. Whereas, Standard (Ciprofloxacin) showed Zone of Inhibition against both Gram-positive and Gram-negative bacteria.

CONCLUSION

This study reveals *Achyranthes aspera L.* stem extract showed phytoconstituents like alkaloid, flavonoid, saponin, glycoside, phenolic compounds and reducing sugar. Among three extracts, ethanolic extract showed superior anti-bacterial activity against gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), moderate activity by hydro-alcoholic extract whereas aqueous extract doesn't show anti-bacterial activity. Hydro-alcoholic extract showed the superior anti-inflammatory, moderate activity by aqueous extract and minimal activity by ethanolic extract in comparison with the standard Diclofenac sodium.

LIMITATIONS

Plants were collected from a single geographical area. Extract was used for the pharmacological assessment. Active chemical constituent wasn't isolated and purified for assessment and limited phytochemical profiling.

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COMPETING INTERESTS

All the authors declare no competing interest