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Antioxidant and Antimicrobial Activities of *Achyranthes Aspera* from Ghodaghodi Lakeside, Kailali, Nepal

Harish Awasthi
Department of Chemistry, Karnali Secondary School, Kailali, Nepal
Pragati Shah
Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal
Keshari Kunwor
Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal
Surya Kant Kalauni
Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal

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Corresponding Author: Harish Awasthi **Email**: harishawasthi3138@gmail.com

Abstract

Achyranthes aspera L. (Amaranthaceae) has long been used as antimicrobial, larvicidal, antioxidant and antidiabetic activities. The phytochemical screening of the methanolic extract of leaves and stems of *Achyranthes aspera* displayed the presence of almost all important phytochemical parameters. In the quantitative analysis of the plant, total phenolic content of methanolic and ethyl acetate extract of *Achyranthes aspera* plant was observed to be 362.74 ± 10.13 mg/g and 293.96 ± 11.77 mg/g GAE respectively. Total flavonoid in the methanolic and ethyl acetate extract of *Achyranthes aspera* was found to have 96.33 ± 7.67 mg/gm and 60.29 ± 5.98 mg/g QE. The methanolic extract of the plant extract of *Achyranthes aspera* exhibited high antioxidant activity among the plant extract (IC_{50} 61.396 µg/ml). LC_{50} value of methanolic and ethyl acetate extract of the plant was identified to be 56.23 and 1158.77 µg/ml towards brine shrimp larvae respectively. The methanolic extract of *Achyranthes aspera* only active for *Staphylococcus aureus* and *Bacillus subtilis*.

***Keywords*:** Phytochemical screening, methanolic extract, antioxidant activity, phenolic content, flavonoid content, antimicrobial activity

Introduction

Nepal is a landlocked country in South Asia. It is located mainly in the Himalayas but also includes parts of the Indo-Gangetic Plain. It borders China in the north and India in the

south, east and west. Measuring a total area of 147516 square km with average horizontal length of 885 km and vertical length of 193 km.

Nepal has five climatic zones, broadly corresponding to the altitudes. The tropical and subtropical zones lie below 1,200 meters (3,900 ft.), the temperate zone 1,200 to 2,400 meters (3,900 to 7,900 ft.), the cold zone 2,400 to 3,600 meters (7,900 to 11,800 ft.), the subarctic zone 3,600 to 4,400 meters (11,800 to 14,400 ft.), and the Arctic zone above 4,400 meters (14,400 ft.). The altitude arrays from 70 meters (Kechana, Jhapa) to the top of the world, Mt. Everest (8,848). About 70% of Nepal consists of dense forest (Chure and Mahabharat range).

Nepal is a country of diversity from social, cultural, geographical as well as flora and fauna features. The rich geographical variation of Nepal has caused the wider variation in biodiversity. Biodiversity commonly denotes the variety of species and the multiplicity of various forms of life (Bhattarai, 1991).

In Nepal, traditional use of plant resources for medicinal purpose has a long history and is gaining popularity due to a lack of side effects, easy availability at affordable prices; and in many circumstances, it is the only source of health care to the poor communities.

Achyranthes aspera L. (Amaranthaceae) has long been used in different systems of medicine in the treatment of cancer, asthma, fistula, piles, wound, insect and snakebite, malaria, fever, cough, diabetes, toothache etc. The plant has been used as antimicrobial, larvicidal, antioxidant. Due to its important cultural (female use it to brush the teeth in day of haritalika teej) and traditional medicinal value for different types of diseases. The plant *Achyranthes aspera* was taken for this research work. It is commonly known as Apamarg in Hindi, which means that which keeps away the defect. Almost all parts of plant used in traditional system of medicine, seed, roots, stems and leaves. In history, the frequent use of medicinal plants is on record. The reason is that they possess considerable amounts of important biologically active compounds like flavonoids and phenolic acids having strong medicinal benefits (Lattanzio et al., 2009).
Kingdom – Plantae
Subkingdom – Tracheobinota
Super Division - Spermatophyta
Division – Mangoliophyta
Class - Mangoliophsida
Subclass - Caryophyllidae
Order - Caryophyllales
Family - Amaranthaceae
Genus - *Achyranthes*
Species – *Aspera*

It is found on roadsides, field boundaries and waste places as a weed throughout Nepal up to an altitude of 2100 m and in South Andaman Islands (Gupta et al.,1972). The plant is also widespread in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America.

Achyranthes aspera L. Datiwan) is an erect or procumbent, annual or perennial herb of about 1- 2 meter in height, often with a woody base. Stems angular, ribbed, simple or branched from the base, often with tinged purple colour, branches terete or quadrangular, striate, pubescent. Leaves thick, 3.8 - 6.3 × 22.5 - 4.5 cm, ovate – elliptic or obovate – rounded, finely and softly pubescent on both sides, entire, petiolate, petiole 6 – 20 mm long, flowers greenish white, numerous in axillary or terminal spikes up to 75 cm long, seeds subcylindric, truncate at the apex, rounded at the base, reddish brown (Zafar, 2009).

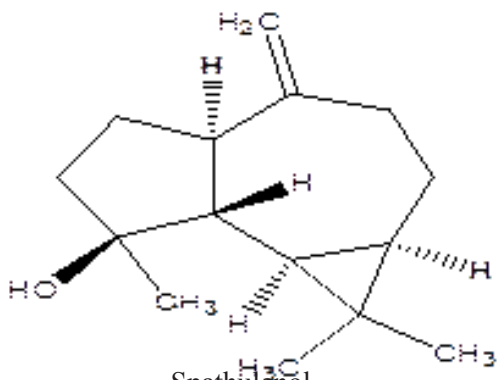
Traditionally, the plant is used in asthma and cough. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in oedema, dropsy and piles, boils and eruptions of skin etc. Crushed plant is boiled in water and is used in pneumonia. Infusion of the root is a mild astringent in bowel complaints. The flowering spikes or seeds, ground and made into a paste with water, are used as external application for bites of poisonous snakes and reptiles, used in night blindness and cutaneous diseases (Nadkarmi, 2009). For snakebites the ground root is given with water until the patient vomits and regains consciousness. Inhaling the fume of *Achyranthes aspera* mixed with *Smilax ovalifolia* roots is suggested to improve appetite and to cure various types of gastric disorders (Bhattari, 2010).

It is useful in haemorrhoids, leaves and seeds are emetic, hydrophobia, carminative, resolve swelling, digestive and expel phlegm. Ash of the plant is applied externally for ulcers and warts. The crushed leaves rubbed on aching back to cure strained back (Sing et al., 1996). A fresh piece of root is used as toothbrush. Paste of the roots in water is used in ophthalmia and opacities of the cornea. Paste of fresh leaves is used for allaying pain from bite of wasps (Gupta, 2010). The plant is useful in liver complaints, rheumatism, scabies and other skin diseases. It also possesses tranquillizing properties (Khare, 2007).

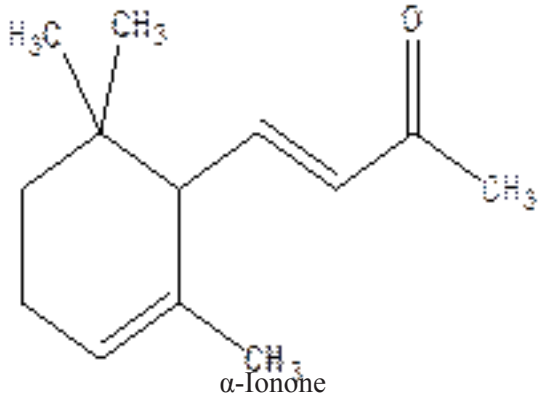
Phytochemicals are the natural chemical substances, which are found in plants. The colour and biological activity of plants are due to presence of phytochemicals. For example, the smell of garlic and the deep purple colour of blueberries are because of phytochemicals. Many of them possess health protective properties and therefore has been used as medicines. Although they are not essential for life (Rouhi, 2003). The plants and foods having anticancer properties include soybean, garlic, ginger, cabbage and carrots. The carotenoids present in carrots and citrus fruits have many health benefits. They neutralize free radicals and modify the working of hormones in the body. Free radicals are short-lived chemicals, which are derived from the oxygen containing compounds having damaging effects on cell structure, particularly cell membrane and DNA (Insel et al., 2004). Phytochemicals are complex chemical compounds, which differ from plant to plant. For example, an Orange plant has more than 170 different phytochemicals (Insel et al., 2004). Many of the phytochemicals classified as secondary metabolites. Secondary metabolites are organic compounds, which are although not directly involved in the normal growth and development, or reproduction of an organism but help in growth and development. These compounds further divided into Alkaloids, Carbohydrates, Flavonoids, Phenolic compounds, Saponin, caumarins, Terpenoids and Quinones.

Michl et al. (2000) reported two new bisdesmosidic triterpenoid saponins were isolated, besides the three known saponins from the Methanolic extract of the aerial parts of *Achyranthesaspera*. Rameshwar (2007) isolated chemical compounds of the volatile oil from *Achyranthes aspera* leaves, growing in Dehra Dun were analyzed by G.C. M.S. Seven compounds viz., pbenzoquinone, hydroquinone, spathulenol, nerol, *α*-ionone, etc.

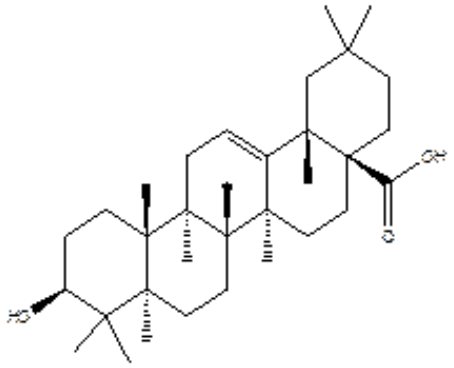
Ecdysterone isolated from the whole plant (Banerji et al., 1971). Asarone and eugenol constituting 63.05% of the oil were identified. Hydroquinone (57.7%) was found to be the chief constituent (Srivastav et al., 2011). Some chemical compounds, which are isolated from *Achyranthes aspera*, are as follows according to above literature.



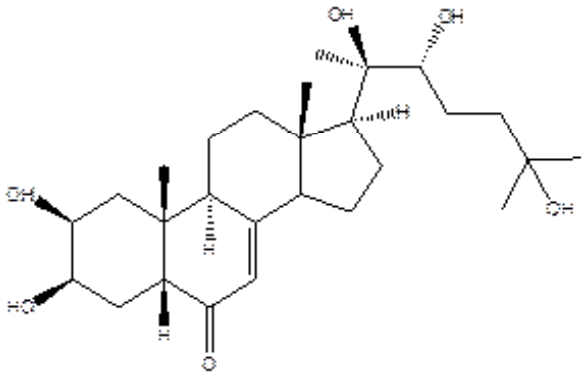
Spathulenol



α-Ionone



Oleanolic Acid



Ecdysterone

Extract of *Achyranthes aspera* were also useful for different kind of disease such as spermicidal activity (Paul et al., 2010) antiparasitic activity (Bagavanet al., 2008) hypoglyceamic activity (Akhtar and Iqbal, 1991) cancer chemo preventive activity (Chakraborty et.al, 2002) hepatoprotective activity (Bafna and Mishra, 2006) Analgesic and antipyretic activity, anti-inflammatory, anti-arthritic activity, antimicrobial activity and antioxidant activity (Srivastav et al., 2011). Nephroprotective activity (Jayakumar et al., 2009; Barua et al., 2009) diuretic activitybronchoprotective activity (Goyal et al., 2007) cardiovascular activity (Neogi et al., 1970; Gupta et al., 1972) antiallergic activity (Datir et al., 2009) wound healing activity (Edwin et al., 2008) immuno

modulatory activity (Chakrabarti et al., 2006) hypolipidemic activity (Khanna et al., 1992) antioxidantsantimicrobial activity antidiabetic study of plants.

Research Hypotheses

If this research work, give us to good result for phytochemical analysis, antimicrobial and antioxidant activities test or any extra Nobel activities during research period then we can elaborated much more for further research in this plant world widely.

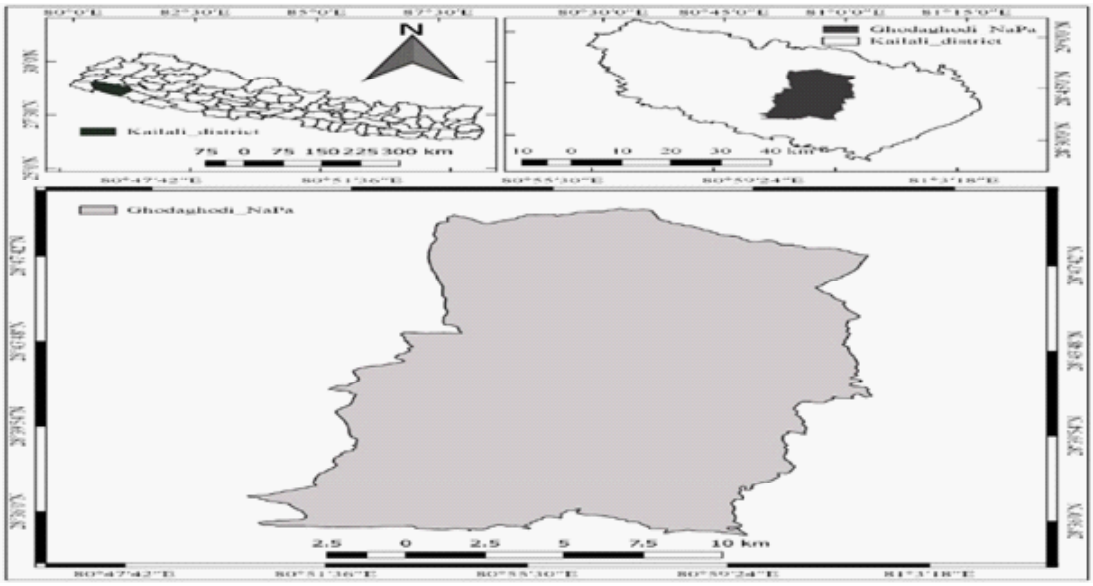
Methods and Procedures

Study Area

The study area is Ghodaghodi Municipality, Kailali, Nepal.

Figure 1

Ghodaghodi Municipality, Kailali, Nepal



Collection Identification and Processing of Plants

For this research study *A. aspera* were selected on the basis of their medicinal importance in literature and traditional use especially in the Kailali district of Nepal. The plant was identified from Central Department of Botany Tribhuvan University Kathmandu. The stems and leaves of plant were collected and washed thoroughly in distilled water. They were cut in to small pieces and dried shade place at refrigerator to get powder. After that, the powdered sample was stored in clean plastic bag until the further use. Then, the powered sample used for extraction.

Extraction

Extraction of plant sample was carried out by cold percolation and soxhlet apparatus method. The cold percolation extracts was filtered first by Whatmann filter paper and it was concentrated. The concentrated filtrate were kept in a beaker wrapping with aluminium foil containing small pores to facilitate the evaporation of the solvent. After complete evaporation of the solvent, (semi) solid metabolic extracts were obtained, and were taken in a freeze for further use (Srivastav et al., 2011).

Phytochemical screening and its analysis

The method employed for phytochemical screening and analysis of the natural compounds present in the *Achyranthes aspera* extract were detected by using specific reagents for each as well as total phenolic, total flavonoids, carbohydrates, tannin, alkaloid, coumarin and saponin was investigated by using standard procedures by short modification (Srivastav et al., 2011).

Antioxidant activity

DPPH free radical scavenging activity assay was performed.

Preparation of ascorbic acid solution (standard)

15mg of ascorbic acid was weighted out and it was dissolved in 15 ml methanol to make the stock solution of 1000µg/ml (ppm). Then by serial dilution, ascorbic acid solutions having concentration 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm were prepared.

Preparations of sample solutions

17mg of (methanolic and ethyl acetate) extracts were weighted out and it was dissolved in 17 ml methanol to make the stock solution of 1000µg/ml (ppm). Then by serial dilution, ascorbic extracts solutions having concentration 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm were prepared.

Preparations of 0.2 mM DPPH solution

0.0079 g of DPPH was weighed out and it was dissolved by methanol in 100 ml Volumetric flask and the final volume was made up to the mark to make 0.2 mm DPPH solution. Then it was stored in dark until further use.

Measurement of DPPH radical scavenging activity

2 ml of ascorbic acid solution from each concentration were pipetted out and mixed with 2 ml 0.2 mm DPPH solution in triplicate then the mixed solution were kept in dark for 30 minutes. 2 ml of methanol was mixed with 2 ml 0.2 mm DPPH and it was also kept in dark. Then their absorbance value was measured at 517 nm by using UV–Vis Spectrophotometer against methanol as a blank.

Similarly, absorbance value for extract and DPPH solution was measured following the procedure as ascorbic acid.

DPPH free radical scavenging activity assay was performed by using standard procedures by short modification.

The capability toscavenge the DPPH radical was calculated by:

DPPH scavenged (%) = $\frac{(Ac - At)}{Ac}$ x 100

Where, Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extract.

Total phenolic content

The total phenolic content in the plant extract was analyzed by Folin- Ciocalteu colorimetric method based on oxidation-reduction reaction as described by standard protocol (Pallab et al., 2013). Gallic acid was used as a standard**.**

Preparation of the standard Gallic acid solution

11mg of gallic acid was weighted out and it was dissolved in 11 ml methanol to make the stock solution of 1000µg/ml (ppm). Then by serial dilution, gallic acid solutions having concentration 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm were prepared.

Preparation sample solution

20mg of extract (methanolic and ethyl acetate) were weighted out and it was dissolved in 20 mL methanol to make the stock solution of 1000µg/ml (ppm). Then by serial dilution, extracts solutions having concentration 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm were prepared.

Construction of the calibration curve

Gallic acid solution (1ml) from each concentration was poured into test tubes. Then, 5 ml of 10 % Folin- Ciocalteu reagent (FCR) and 4 ml of 7 % sodium carbonate (Na_2CO_3) were added to these test tubes to get a total volume of 10 ml. The blue coloured mixture was shaken well and incubated for 30 minutes at 40 ºC in a water bath. Finally, the absorbance of the solution was measured at 760 nm wavelength using UV-spectrophotometer against blank solution containing all reagent except Gallic acid.

All the experiment were carried out in triplicate the average absorbance values obtained at different concentration of Gallic acid were used to plot calibration curve.

Total flavonoid content (TFC)

The total flavonoid content of the plant extract was determined by aluminium chloride colorimetric Assay (Bag et al., 2015). Quercetin was used as a standard.

Preparation of reagent

1 M $NaCO_3$ solution was prepared by dissolving 5.29 gram of $NaCO_3$ in 50 ml distilled

water. 1:10 V/V FCR reagent was prepared by diluting 10 ml of commercially available F-C reagent in 100 ml of distilled water in 100 ml volumetric flask. 5 gram of $NaNO_2$ was weighted in watch glass for making 5 % $NaNO_2$ and it is poured in 100 ml volumetric flask then distilled water was added up to mark. Similarly, 10 % of $AlCl_3$ was also prepared in 100 mL volumetric flask.

Preparation of standard stock solution (Quercetin)

Quercetin stock solution of concentration 1000 µg/ml (ppm) was prepared by dissolving 18 mg of quercetin in 18 ml of methanol as a solvent. The various concentrations of quercetin such as 10 ppm, 20 ppm, 40 ppm, 60 ppm 80 ppm, and ppm were prepared by serial dilution of the stock solution.

Construction of the calibration curve

For this experiment, first of all 1 ml of quercetin in methanol was taken in test tube containing 4 ml of distilled water. Then, at the initial time (at zero time), 0.3 ml of Na_2NO_3 was added to the test tube. After 5 minutes, 0.3 ml of 10% of $AlCl_3$ and after 6 minutes, 2 ml of 1 M NaOH were added to the mixture. Immediately the total volume of the mixture was made up to 10 ml by addition of 2.4 ml distilled water and mixed thoroughly. Finally, the absorbance of the pink colour mixture was measured at 510 nm wavelength using UV-spectrophotometer against blank solution containing all reagent except quercetin.Experiment was done triplicate. The average absorbance values obtained for different concentration of quercetin were used to plot the calibration curve.

Preparation of samples solution

Extracts (methanolic and ethyl acetate) stock solution of concentration 1000 µg/ml (ppm) were prepared by dissolving 18 mg of quercetin in 18 mL of methanol and ethyl acetate respectively as a solvent. The various concentrations of extract such as 10 ppm, 20 ppm, 40ppm, 60 ppm 80 ppm, and 100 ppm were prepared by serial dilution of the stock solution.

Measurement of total flavonoid content in extract

For measurement of TFC First of all 1 ml of extract in methanol and ethyl acetate were taken in test tube containing 4 ml of distilled water. Then, at the initial time (at zero time), 0.3 ml of Na_2NO_3 was added to the each test tube. After 5 minutes, 0.3 ml of 10% of $AlCl_3$ and after 6 minutes, 2 ml of 1 M NaOH was added to the mixture of the solution in each test tube. Immediately the total volume of the mixture was made up to 10 ml by addition of 2.4 ml distilled water and mixed thoroughly. Finally, the absorbance of the pink colour mixture were measured at 510 nm wavelength using UV- spectrophotometer against blank solution containing all reagent except extract.

Bioassay

Biological activity is the study of effect of the crude plant of fraction or isolated compounds at arbitrarily fixed dose level in a species of an organism and predicting its effect over the entire dose range. In this dissertation work, *Achyranthes aspera* is used as general bioassay tool to perform brine shrimp bioassay as given by Meyer et al. (1982).

Brine shrimp bioassay

Michael et al. (1956) introduced the Brime shrimp lethality testing of plants. Artemia also called Brine shrimp is used for phtotoxicity analysis of plants. Artemia is also named as "sea monkeys" or fairy shrimp. For preliminary evaluation of toxicity, this method is the very useful tool, and it has also been successfully employed to examine fungal toxicity, toxin analysis of plant extracts, pesticides, heavy metals and the compounds which are newly synthesized (Gul farzana, 2015).

High concentrations of bioactive compounds are mostly toxic. A simple organism may be used *in vivo* lethality test for exploration, isolation and identification of biologically active natural products. The brine shrimp (*Artemia salina*) eggs are cheaper and simply accessible from pet shops. In the dry form, they are effective for years. If they are kept in seawater, the hatching of eggs takes only 48 hours and produces a large number of larvae (nauplii) for experimental use. Many other research reports have showed the successful application of the brine shrimp to test plants commonly used as pesticides, plants used as anticancer agents, and tropical plants used medicinally (thesis). This method is rapid inexpensive, simple and in- house approach for screening and monitoring physiologically active plant extracts. It determines the LC_{50} values (ppm) for the crude extracts. Compounds having LC_{50} values less than 1000 ppm (µg/ml) are considered as potentially pharmalogically active.

General Procedure of Brim Shrimp Bioassay

Hatching of the Brine Shrimp

About 50 gm of brine shrimp were sprinkled on the artificial seawater taken in the beaker and the beaker was covered with aluminium foil. Several small pores were made to facilitate the passage of heat and light then the beaker was kept for 96 hours illuminating with the bulb (200 watt) at room temperature. The time taken for hatching of brine shrimp is much more than the normal due to the winter season the room temperature about 8-15 ºC.

Preparations of Sample

20 mg extract was weighted out and dissolved in 2 ml methanol to make stock solution of concentration 10,000 ppm (µg/ml). From that stock solution, solutions of concentration 1000 µg/ml, 100 µg/ml and 10 µg/ml were prepared by serial dilution method. 2 ml of solution from each solution (1000 ppm, 100 ppm and 10 ppm) were transferred to nine different test tubes three for each concentration. Similarly, 2 ml methanol was taken in three test tubes (as a blank). After labelling these test tubes, they were kept for 24 hours to evaporate the solvent (methanol).

Procedure for Bioassay

After complete evaporation of the solvents, 5 ml of artificial seawater was added and the solution was mixed thoroughly to suspend the residue. Then ten mature brine shrimp nauplii were transferred into all twelve test tubes. After 24 hours, the numbers of the survivors were counted with the help of the disposable pipettes.

Antimicrobial assay

Inhibition of bacterial growth was tested by using agar well diffusion plate method and measured in the form (ZOI) as given by Dingel et al., with some modification. Antibacterial assay was performed at central department of microbiology Tribhuvan University.

Results

Percentage yield

The percentage yield of hexane, ethyl acetate and methanol of *Achyranthes aspera* were found to be 1.65 %, 5.04 % and 9.17 % respectively.

Table 1
Percentage Yield of Plant Extract

Plant part extract	Amount macerated (g)	Yield (g)	Yield (%)	Solvent
1	80	1.321	1.65	Hexane
2	80	4.032	5.04	Ethyl acetate
3	80	7.335	9.17	Methanol

Phytochemical screening

Table 2
Phytochemical Analysis of Achyranthes Aspera

S.N.	Phytochemicals	Hexane	Ethyl acetate	Methanol
1	Alkaloids	+	++	+++
2	Carbohydrates	+	+	++
3	Flavonoids	-	+	++
4	Phenolic compounds	-	+	+++
5	Coumarin	-	-	-
6	Saponin	-	-	+++
7	Terpenoids	-	-	++
8	Quinones	-	-	++
9	Tannin	+	+	++

(+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent

In phytochemical screening of *Achyranthes aspera* alkaloids, carbohydrates, tannins, oil, and fats were found to be present in trace amount but flavonoids, phenolic compounds, saponin coumarin, terpenoids and quinones were found to be absent in the hexane extract.

In the ethyl acetate extract alkaloids carbohydrates, phenolic compounds and tannins were found to be present but coumarin saponins, terpenoids and quinones were found to be absent.

Similarly, in the methanolic extract alkaloids, flavonoids, phenolic compounds and saponins were found to be present in appreciable amount whereas carbohydrates, terpenoids, tannins and quinones were found to be present in moderate amount but coumarin were found to be absent.

Antioxidant activity

The antioxidant activity of ethyl acetate extract and methanolic extract of *Achyranthes aspera* were obtained by plotting % of free radical scavenging verses concentration which are shown as following figure (fig. 2 and fig. 3) and IC_{50} value of respective extract were calculated.

Figure 2

% of free radical scavenging vs concentration for ascorbic acid and methanolic extract of *Achyranthes aspera*

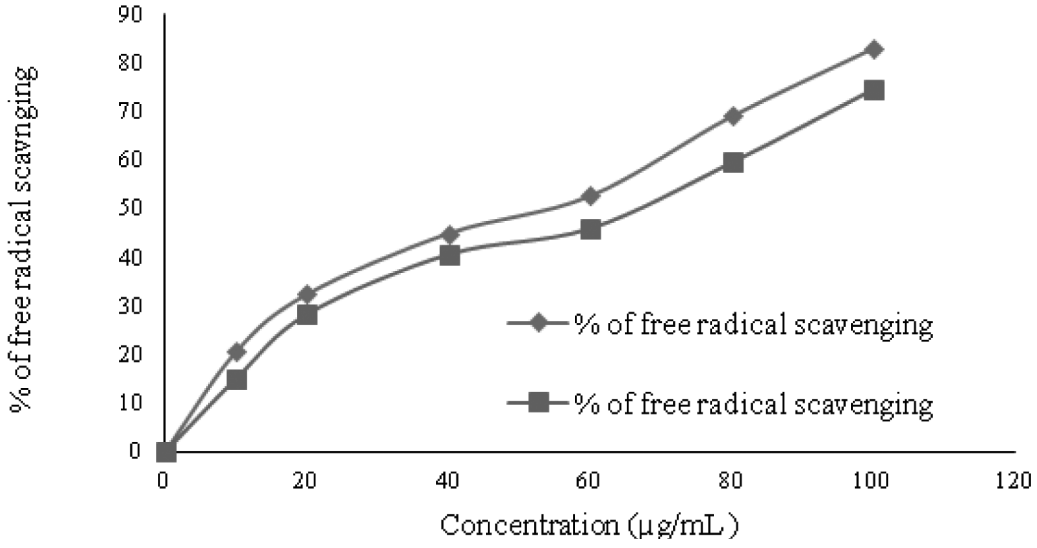
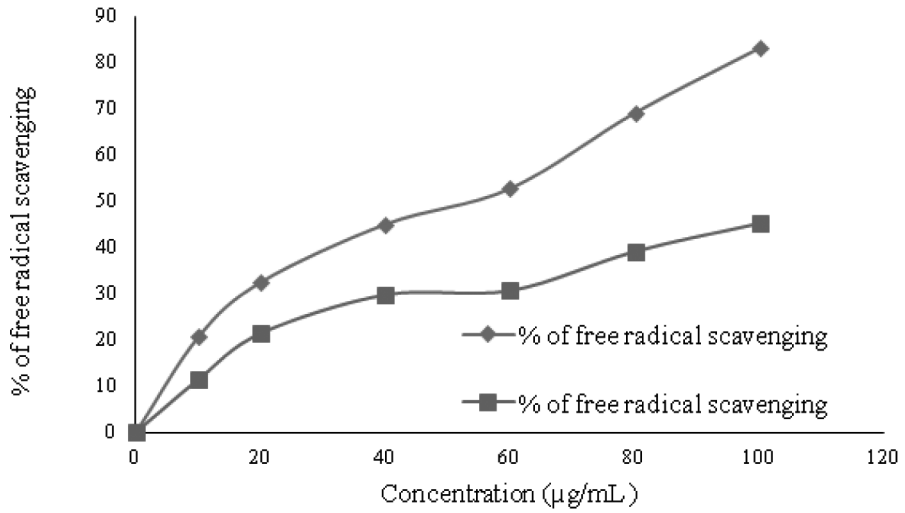


Figure 3

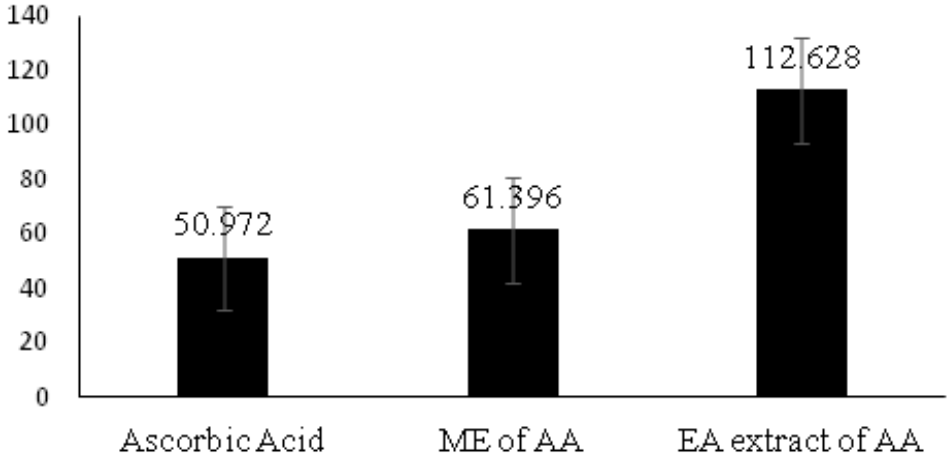
% of free radical scavenging vs concentration for ascorbic acid and ethyl acetate extract of Achyranthes aspera



IC_{50} value of ethyl acetate and methanolic extracts were calculated and compared with IC_{50} value of ascorbic acid to determine their potential antioxidant activity. (Fig. 4).

Figure 4

IC_{50} value for ascorbic acid and different extract of *Achyranthes aspera* for DPPH free radical scavenging



The IC_{50} value of methanolic extract and ethyl acetate extract of leaves and stems of *Achyranthes aspera* were found to be 61.396 µg/ml and 112.628 µg/ml respectively. IC_{50} value of both extract are higher than that of ascorbic acid (50.972 µg/ml)among these two extract methanolic extract of *Achyranthes aspera* showed higher antioxidant activity.

Total Phenolic content and total flavonoid content

The total phenolic content and total flavonoid content of ethyl acetate and methanolic extract of *Achyranthes aspera* were calculated by plotting concentration verses absorbance and using calibration curve (fig. 5 and fig. 6).

Figure 5

Absorbance Vs. concentration curve for Quercetin

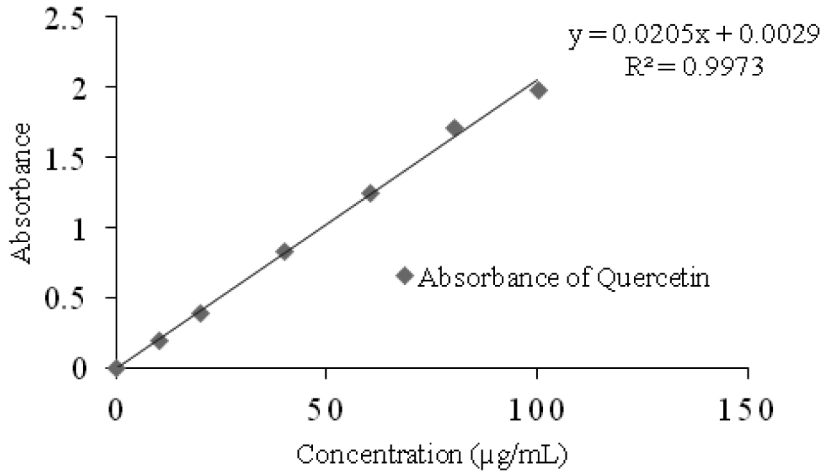
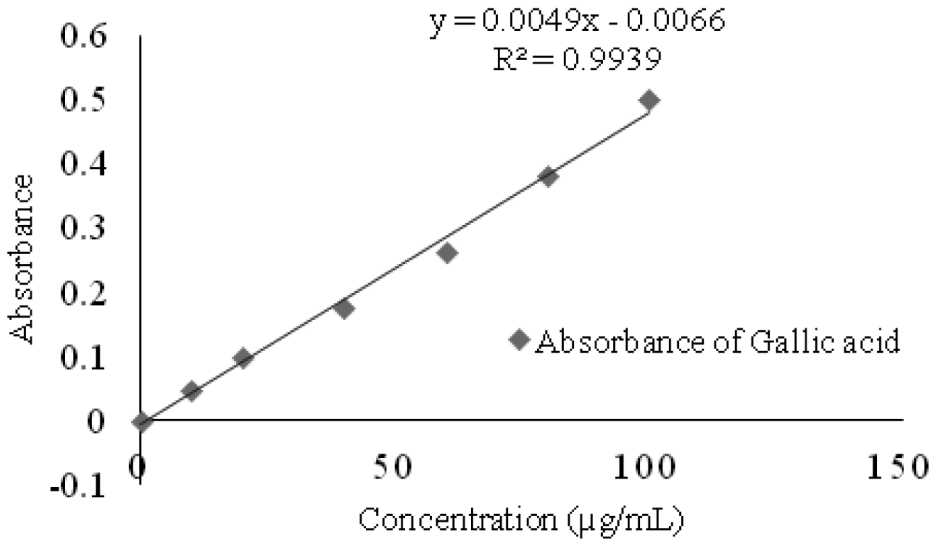


Figure 6

Absorbance Verses concentration curve for Gallic acid



Using calibration curve and absorbance value of methanolic and ethyl acetate extract of leaves and stems of the *Achyranthes aspera* (10 µg/mL, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml), total phenolic content was calculated as 362.75 ± 10.13 and 293.96 ± 11.77 mg per Gallic acid equivalent (mg/g GAE**)** respectively.

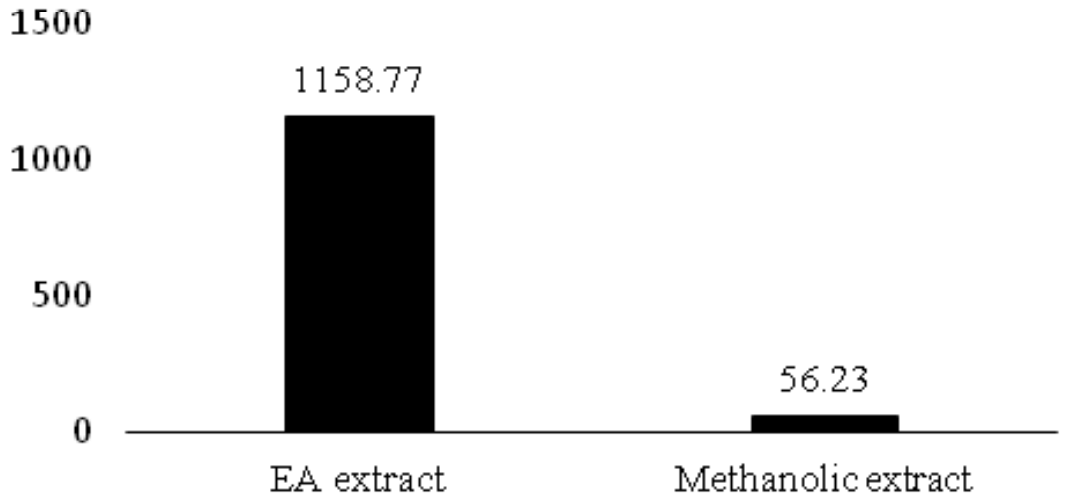
Using calibration curve and absorbance value of methanolic and ethyl acetate extract of leaves and stems of the *Achyranthes aspera* (10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) total flavonoid content was calculated as 96.33±7.67and 69.290 ±5.98 mg per quercetin equivalent (mg/g QE) respectively.

Brine shrimp bioassay of ethyl acetate and methanolic extract of *Achyranthes aspera*

The newly hatched brine shrimp nauplii were exposed to different concentrations of extract (10 ppm, 100 µg/ml and 1000 µg/ml) and their toxicity toward nauplii were evaluated to calculate LC_{50} (µg/ml) value. Extracts having LC_{50} values less than 1000 µg/ml are supposed to be pharmalogically active. LC_{50} value of methanolic and ethyl acetate extracts of *Achyranthes aspera* were calculated as 1158.77 (µg/ml). Thus, it concluded that ethyl acetate extract was not significantly toxic toward *Artemia salina* (brine shrimp) larvae. The extract can be regarded as
Pharmalogically not active since the LC_{50} value was found higher than 1000.

Figure 7

LC_{50} value of Ethyl acetate (EA) extract and methanolic extract of *Achyranthes aspera* for brine shrimp bioassay



Similarly, the LC_{50} value of methanolic extract of *Achyranthes aspera* was calculated as 56.23 µg/mL. Thus, it concluded that methanol extract was toxic toward *Artemia salina (*brine shrimp) larvae. The extract can be regarded as pharmalogically active since the LC_{50} value was found less than 1000 µg/mL.

Antibacterial activity

The antibacterial activity of *Achyranthes aspera* was performed toward two-Gram positive and two-Gram negative bacteria and the ZOI value were measured as follows (Table 3) :

Table 3

Antibacterial activity of methanolic extract of Achyranthes aspera

% of Concentration	**Mean, Zone of inhibition**			
	SA	BS	EC	KP
1 %	12.00	10.00	0.00	0.00
5 %	14.00	14.00	0.00	0.00
10 %	16.00	18.00	0.00	0.00
DMSO	0.00	0.00	0.00	0.00
Chloramphenicol	24	21	22	18
Reference Culture	ATCC 25923		ATCC 25922	ATCC 700603

SA = *Staphylococcus aureus,* BS = *Bacillus subtilis* EC = *Escherichia coli,* KP = *Klebsiella pneumonia*

The antimicrobial activity for methanolic extract of leaves and stems of *Achyranthes aspera* resulted 12, 14, 16 mm and 10, 14, 18 mm for *Bacillus subtilis* and *Staphylococcus aureus* with 1 %, 5 % and 10 % respectively while the rest of the microorganism were found to be resistant.

Discussion

From the result of phytochemical screening of *Achyranthes aspera* alkaloids, carbohydrates, tannins, oil, and fats were found to be present in trace amount but flavonoids, phenolic compounds, saponin coumarin, terpenoids and quinones were found to be totally absent in the hexane extract. In the ethyl acetate extract alkaloids carbohydrates, phenolic compounds and tannins were found to be present but coumarin saponins, terpenoids and quinones were found to be absent. Similarly, in the methanolic extract alkaloids, flavonoids, phenolic compounds and saponins were found to be present in appreciable amount whereas carbohydrates, terpenoids, tannins and quinones were found to be present in moderate amount but coumarin were found to be absent. It has been discussed that polar solvent is best for the plant extract.

From the result of the antioxidant activity, it is found to be higher than that of the literature data Pandey et al. (2014). It is due the presence of larger amount of total phenolic content in a plant extract. Methanolic extract solution shows good antioxidant activity among three plant extract.

Viswanathan et al. (2019) total phenolic content of alcoholic extract of *Achyranthes aspera* was found to be 72.48 mg/g GAE it is much more less than that of this calculated value 362.75 ± 10.13 mg/g. it is due to the geographical condition of plant growth area and other instrumental error.

From this result, it has been discussed that gram positive *Bacillus subtilis* bacteria is sometimes toxic to liver disease and *Staphylococcus aureus* can cause food poisoning, bone and joint infection ,mastitis in cow. As the methanolic extract of *Achyranthes aspera* leaves and stems has been found to be effective in inhibition of these bacteria, it can be administered to control that disease.

Conclusion

The phytochemical screening of the methanolic extract of leaves and stem of Achyranthes aspera revealed the presence ofalkaloids, carbohydrates, tannins, flavonoids, phenolic compounds, saponin coumarin and terpenoids.

The total phenolic content of methanolic extract was found to be higher than ethyl acetate extract of Achyranthes aspera. Similarly total flavonoid content methanolic extract was found to be higher than ethyl acetate extract of *Achyranthes aspera.*

The methanolic extract of *Achyranthes aspera* showed 61.396 µg/ml (IC_{50}) that it has highly antioxidant property among the plant extract.

LC_{50} value towards the brine shrimp larvae by methanolic extract and ethyl acetateextract was found to be 56.23 and 1158.77 µg/ml respectively. Thus, methanolic extract of *Achyranthes aspera* found to be toxic toward *Artemia salina* larva.

The antimicrobial activity for methanolic extract of leaves and stems of *Achyranthes aspera* resulted 12, 14, 16 mm and 10, 14, 18 mm for *Bacillus subtilis* and *Staphylococcus aureus* with 1 %, 5 % and 10 % respectively while the rest of the microorganism were found to be resistant.

Thus, *Achyranthes aspera* plant selected showed major biological activities, they cannot directly be referred for pharmaceutical usage. Further extensive phytochemical and pharmacological studies along with mechanisms of action are crucial not only to eliminate this preliminary experiment but also to characterize and isolate the unknown compounds to inaugurate their pharmacological properties.

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