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Study on Phytochemical Profile and Antioxidant Activity of *Achyranthes aspera* Whole Plant

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Authors' contributions

This work was carried out in collaboration between all authors. Author NM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AN and KB managed the analyses of the study. Author KB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to establish qualitative as well as quantitative phytochemical profiles and determine the free radical scavenging activity of phytochemical constituents of the entire plant of *Achyranthes aspera* Linn**.** The extraction of dried plant material was carried out by cold maceration with methanol followed by partitioning with ethyl acetate. Preliminary phytochemical screening of the plant was carried out by performing various chemical tests. Phenolic and flavonoid contents were determined by Folin-Ciocalteu and Aluminum chloride methods, respectively. Antioxidant activity was studied using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The result of study revealed the presence of flavonoids, alkaloids, terpenoids, phytosterols, tannins, saponins, phenolic compounds, and carbohydrates. Total phenolic content, total flavonoids content, and antioxidant activity of the extract were found as 209.007 µg GA/mg, 17.59 µg QE/mg, and 25.12% (100 µg/mL), respectively. The study showed that plant could be a source of antioxidant compounds.

Keywords: Achyranthes aspera; antioxidant activity; ethyl acetate extract; phytochemicals.

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1. INTRODUCTION

Plants have been employed as a wellspring of medicine since the prehistoric period. Various forms of traditional healing practices are cultural assets handed down from generation to generation for many hundreds of years. The enthusiasm has been expanding for the investigation of medicinal plants along with their use in indigenous healing practice in the last few decades [1]. It is evaluated that 80% of the total world population relies on herbal remedies for their essential medicinal services need [2].

The cultural and biological diversity of Nepal offers a massive platform and opportunities for ethnobotanical studies. Ethnic groups live in diverse geographical areas of Nepal depending on various plant species to fulfill their fundamental medicinal requirements and have reserved their indigenous knowledge about herbal medicament [3]. Since the utilization of such traditional information leads to drug discovery, the documentation of traditional knowledge as well as the conservation of plants remains a necessary task. *Achyranthes aspera* is a multi-medicinal plant used as ethnomedicine for the treatment of various diseases in Nepal. It is traditionally used to cure fever, swelling, rheumatism, gastrointestinal problems, respiratory tract problems, common cold, diarrhea, dysentery, toothache, asthma, renal problems, and throat ache as traditional medicine [3-6].

Scientific investigation and evaluation, and pharmaceutical supervision of the plant, are necessary to determine the quality, safety, and efficacy parameters. Various ex vivo and in vivo studies have used traditional knowledge aid to minimize the paucity of evidence-based data to make rational decisions in developing safe and effective herbal therapeutic substances.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The fresh plant of *Achyranthes aspera* was collected from Thakurbaba Municipality, Bardiya, Nepal, and was taxonomically identified by the National Herbarium and Plant Laboratory, Godavari, Lalitpur, Nepal.

2.2 Preparation of Plant Extract

The whole plant materials of *Achyranthes aspera* were cut into pieces and shade dried in a room with active ventilation at ambient temperature for 20 days. The dried sample was pulverized to a powder using a mechanical grinder. 100 g of pulverized powder was extracted by cold maceration using 300 ml of methanol for 48 h. The content was filtered with vacuum filter and concentrated to one-fourth using rotatory evaporator. 50 ml of water was added to methanol extract and then extracted three times in a separating funnel using 100 ml of ethyl acetate each time. The ethyl acetate layer was concentrated to one one-fourth using rotatory evaporator, 10 ml of 10 % Sulfuric acid (H_2SO_4) was added to it and allowed to stand for overnight. The solution was partitioned against ethyl acetate in a separating funnel. The ethyl acetate fraction was taken, evaporated to dryness, leaving dried residue, and stored in a fridge [7,8].

2.3 Phytochemical Screening

Qualitative phytochemical screening was performed for the identification of major groups of chemical constituents present in the crude plant extract of *Achyranthes aspera*. Ethyl acetate extract was subjected to flavonoids, alkaloids, cardiac glycosides, terpenoids, phytosterols, tannins, saponins, phenolic compounds, carbohydrates, and amino acids. The following test procedures were employed for screening with little modification.

2.4 Test for Flavonoids

2.4.1 Shinoda test

The extract was dissolved in alcohol and a few magnesium turnings were added to it, followed by the addition of concentrated hydrochloric acid drop by drop. The development of pink color was indicative of the presence of flavonoids [9].

2.4.2 Alkaline reagent test

A small portion of the extract was dissolved in alcohol and treated with a few drops of sodium hydroxide solution. Observation of an intense yellow color, which vanished upon the addition of dilute hydrochloric acid, was an indication of the presence of flavonoids [9,10].

2.4.3 Tests for alkaloids

The extract was dissolved in dilute hydrochloric acid, filtered, and the filtrate was treated with Mayers's reagent (potassium mercuric

iodide solution), Wagner's reagent (iodine and potassium iodide solution), and Hager's reagent (saturated solution of picric acid). The cream, yellow and reddish-brown precipitate obtained with Mayer's reagent, Hager's reagent, and Wagner's reagent, respectively, indicated the presence of alkaloids [9,11].

2.5 Tests for Cardiac Glycosides

2.5.1 Legal's test

The extract solution was dissolved in 1 mL pyridine and 1 mL alkaline sodium nitroprusside. Blood red color was taken as an indication of the presence of cardiac glycosides [9].

2.6 Test for Terpenoids

2.6.1 Salkowski test

The extract was dissolved in chloroform, and a few drops of concentrated sulfuric acid were added gradually to produce a layer. The formation of a reddish-brown color at the interface indicated the presence of terpenoids [12].

2.7 Test for Phytosterols

2.7.1 Libermann-Burchard test:

The extract was dissolved in chloroform, and a few drops of acetic acid and concentrated sulfuric acid were added to it. The formation of a deep green color indicated the presence of steroids [10,13].

2.8 Test for Tannins

2.8.1 Gelatin test

The extract was treated with 1% gelatin solution containing 10% sodium chloride. The formation of a white precipitate indicated the presence of tannins [9,14].

2.9 Test for Saponins

2.9.1 Foam tests

A small amount of the extracted sample was shaken with water. The persistence of form above the liquid surface for a few minutes indicated the presence of saponins [10].

2.10 Test for Phenolic Compounds

2.10.1 Ferric chloride test

The extract was dissolved in a ferric chloride solution. The green color was indicative of the presence of phenolic compounds in the extract [9, 10].

2.11 Test for Carbohydrates

2.11.1 Molisch's test

200 mg of the extract was dissolved in 5 mL of distilled water and filtered. To the filtrate solution, A few drops of alcoholic alpha-naphthol were added and subsequently followed by concentrated sulfuric acid from the sides of the test tube. The Violet ring visible at the junction indicated the presence of carbohydrates [9,15].

2.12 Test for Amino Acids

2.12.1 Ninhydrin test

A portion of the extract was dissolved in water and heated with a few drops of Ninhydrin solution in a water bath. The appearance of blue-violet color was the indication of the presence of amino acids [16].

2.12.2 Total phenolic contents

Total phenolic content was determined by the Folin-Ciocalteu method with slight modification [17,18]. The same volume (0.5 mL) of the plant extract (1 mg/mL) and different concentrations of gallic acid as the standard (10-120 µg/mL) were taken and 0.1 mL 1:2 Folin-Ciocalteu reagent was added along with 0.8 mL deionized water. After 3 min of incubation, 0.3 mL of sodium carbonate was added and kept at room temperature for 30 min. After 30 min, absorbance was taken at 765 nm against the methanol as blank in 96 wells plate enzyme-linked immune sorbent assay (ELISA) reader. The total phenolic content of the sample was calculated as mg of gallic acid equivalents (GAE) of each gram of dried extract with the help of the equation obtained from the standard curve. All tests were done in triplicate.

2.12.3 Total Flavonoid Content

The total flavonoid content was determined according to the procedure followed by Zhishen et al. (1999), with slight modifications [19]. An equal volume (1.5 mL) of the plant extract (1 mg/mL) and standard of different concentrations (10-60 mg/mL) were mixed with 75 µL 5% sodium nitrite and incubated for 6 min. Subsequently, 150 µL of 10 % aluminium

chloride was added to the content and again incubated for 6 min. After 6 min, 0.5 mL of 1 M sodium hydroxide was added and the final volume was made up to 3 mL with deionized water. The absorbance was taken at 510 nm in 96 wells plate ELISA reader after shaking the reaction mixture. The total flavonoid content was expressed as mg of quercetin equivalents (QE) per gram of dried extract with the help of the equation obtained with the standard, and the test was conducted in triplicate.

2.12.4 Antioxidant assay (DPPH free radical-scavenging assay)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a widely known method used for the determination of free radical scavenging capacity of a molecule with antioxidant potential and is recognized as a simple and standard colorimetric method for the estimation of antioxidant properties of compounds [20]. For the DPPH assay, the procedure carried out by Mensor et al. (2001) was followed [21]. 2.5 mL of 100 µg/mL of plant extract as well as the different concentrations of the standard (ascorbic acid), was made in test tubes. Then, 1 mL of 0.3 mM freshly prepared DPPH was added to the sample and standard with proper mixing and incubated in the dark for 30 min. After 30 min, absorbance was measured at a wavelength of 518 nm in 96 wells plate ELISA reader and all the tests were performed in triplicate. Absorbance for control was taken without using the plant extract. The percentage scavenging of DPPH by the extracts was estimated using the following formula [22]:

% inhibition activity = (absorbance of control - absorbance of sample)/ (absorbance of control) ×100

2.13 Data Analysis

Linear correlation coefficient (R^2) and linear regression equation for a straight line (y = mx + c) were calculated using Excel Microsoft Excel 2010. Total phenolic content, total flavonoid content, and antioxidant activity were determined with the help of linear regression equations.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of Extract

The percentage yield of ethyl acetate extract of *Achyranthes aspera* was found to be 1.103 %.

3.2 Phytochemical Analysis

3.2.1 Preliminary phytochemical screening

Phytochemical screening of ethyl acetate extract of the plant showed the presence of different groups of active constituents. The results obtained are tabulated below in Table 1.

3.2.2 Total phenolic content

The regression equation of the calibration curve (y = 0.0038x + 0.0976, R2 = 0.988) was used to calculate the total phenolic content of the plant extract, where x is the absorbance and y is the concentration of gallic acid solution (µg/mL) expressed as µg GAE/mg. The total phenolic content in ethyl acetate extracts was found to be 209.007 µg GAE/mg.

3.2.3 Total flavonoid content

The total flavonoid content of plant extracts was calculated from the regression equation of the calibration curve (y = 0.0129x + 0.1962, R2 = 0.9705), where x is the absorbance and y is the concentration of quercetin solution (µg/mL) expressed as µg QE/mg. The total flavonoid content ethyl acetate extract was found to be 17.59 µg QE/mg.

3.3 Antioxidant Activity

In the current investigation, the hydroxyl radical scavenging activity of the plant extract was found to be 25.12% (100µg/mL). Fig. 3 shows the hydroxyl radical scavenging activity of different concentrations of ascorbic acid used as the standard antioxidant compound.

Various secondary metabolites present in plants are responsible for producing biological effects; therefore, they portray a pivotal role in alleviating many ailments. This study shows that metabolites namely flavonoids, alkaloids, terpenoids, phytosterols, tannins, saponins, phenolic compounds, and carbohydrates are present in the crude extract of *Achyranthes aspera*. Alkaloids represent a substantial group of compounds present in plants possessing diverse medicinal properties [23]. Phytosterols naturally show lipid-lowering effects by diminishing cholesterol absorption and also produce protective outcomes against cardiovascular illness, diabetes, possible cancer, and hepatic disease [24].

Table 1. Phytochemical screening of ethyl acetate extract of *Achyranthes aspera*

Presence of components	Name performed of the test	Results
Flavonoids	Shinoda test	+
	Alkaline reagent test	+
Alkaloids	Mayer's reaction	+
	Wagner's Test	+
	Hager's Test	+
Cardiac Glycosides	Legal's Test	-
Terpenoids	Salkowski Test	+
Phytosterols	Libermann-Burchard Test	+
Tannins	Gelatin Test	+
Saponins	Foam Test	+
Phenolic compounds	Ferric Chloride Test	+
Carbohydrates	Molisch's Test	+
Amino acids	Ninhydrin Test	-

'+' = Presence and '-'=Absence

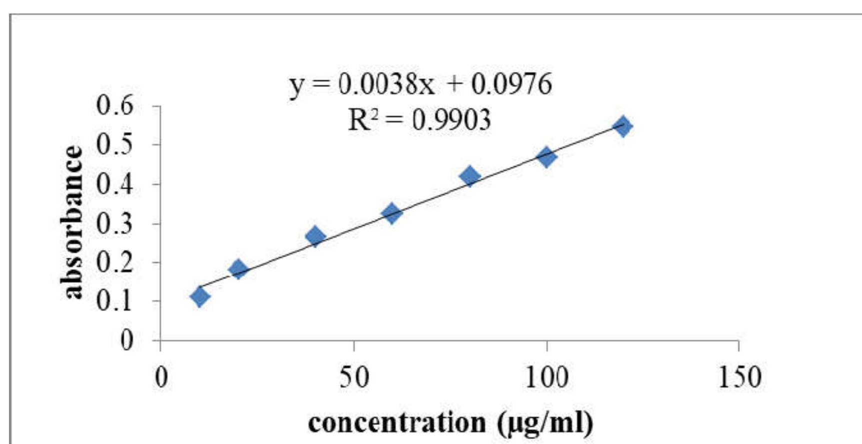

Fig. 1. Standard calibration curve of gallic acid at 765 nm

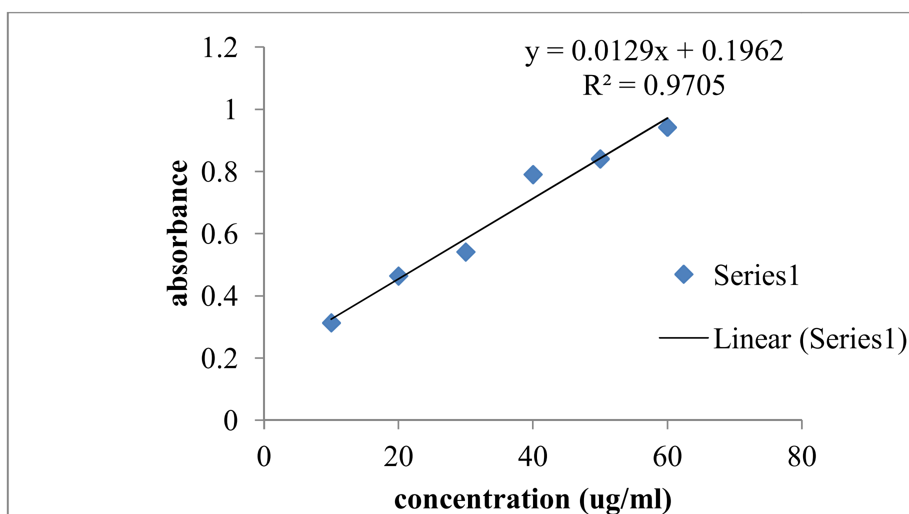

Fig. 2. Standard calibration curve of quercetin at 510 nm

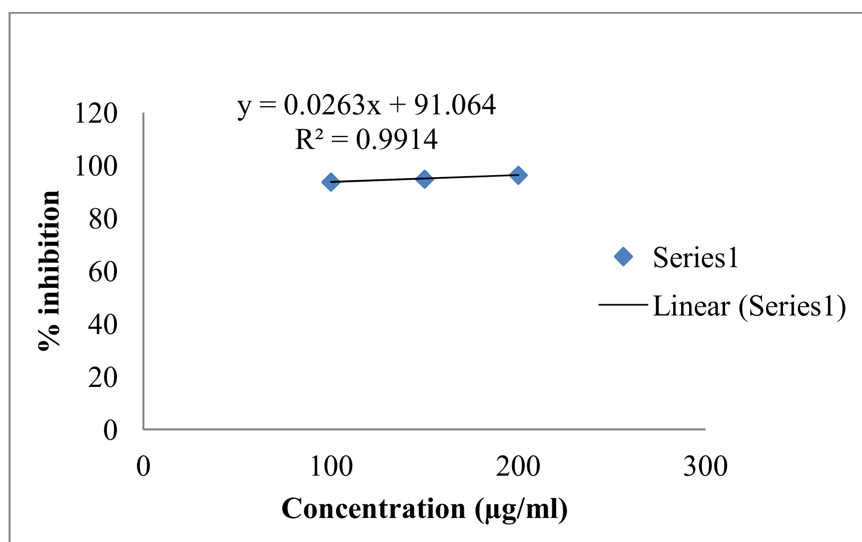

Fig. 3. DPPH free radical scavenging activity of standard ascorbic acid

Antioxidant activity of a substance is responsible for countering diseases, which are accompanied by oxidative stress [25]. Phytochemicals present in the plant *Achyranthes aspera* as flavonoids, terpenoids, tannins, saponins, and phenols may be accountable for their antioxidant property [26]. Quantitative phytochemical screening showed a significant amount of flavonoids and phenols in the plant extract. Antioxidant actions of flavonoids are attributed to their scavenging and chelating power of the hydroxyl group, while the free-radical terminating property of phenols liable for its antioxidant activities [27].

4. CONCLUSION

Phytochemical screening and study of the antioxidant potential of ethyl acetate extract of *Achyranthes aspera* whole plant exhibited the presence of bioactive constituents. These bio-constituents may provide essential ingredients for the development of new therapeutic products. The antioxidant activity shown by the plant may be taken into consideration for the treatment of various disease-related to oxidative stresses. It is recommended that further study aimed at isolation and characterization of the molecules responsible for the antioxidant activity should be carried out.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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