

In Vitro Evaluation of Antimicrobial and Cytotoxic Potential of Dry Rhizome Extract of *Astilbe Rivulari*

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ABSTRACT

Antimicrobial and cytotoxic activity of methanol crude extract of Nepalese species *Astilbe rivularis* has been evaluated. Methanol crude extract of *Astilbe rivularis* showed marked antimicrobial properties against *E. coli*. In comparison with standard antibiotic, it had moderate activity. Phytochemical screening revealed the presence of alkaloid, tannin, flavonoid, coumarin, and glycoside as the main phytochemical group. LC₅₀ values were calculated by using brine shrimp lethality test. The LC₅₀ value for *Astilbe rivularis* was found to be 92.01 ppm. The MIC of methanol crude extract of *Astilbe rivularis* was found to be 0.0011% w/v or 0.011mg/ml. Thus we can say methanol crude extract of *Astilbe rivularis* showed strong antibacterial activity against *E.coli*. Use of *Astilbe rivularis* locally for the treatment of diarrhea and dysentery is justifiable.

INTRODUCTION

Traditional medicine is undoubtedly the oldest form of medicine and probably evolved simultaneously with the evolution of human beings or even much earlier (Wanzala et al. 2005). In fact the primary health care of about 80% of the world's population is dependent on the use of medicinal plants derived from traditional medicine (Bajaj et al. 1995).

In Nepal, more people seek medical attention from traditional medical practitioners than from medical doctors. Medicinal plants especially serve as the main source of medicine to rural poor communities that do not have access to modern medical services. About 25% of conventional drugs are derived from plants that are been used traditionally. Publications showed that almost all parts of the plant are used locally as medicine (Evans, W. 2002). The root and the stem-bark are used in disease conditions such as diarrhoea, dysentery, stomach ache, ascites, headache, cough, rheumatism, back pain, wound healing, weakness, avian plague, yellow fever, and malaria. The fruit pulp is used as laxative while the leaves are used as diuretic, antipyretic, analgesic and in the treatment of pleurisy and burns. The seed is used for treatment of pneumonia. However little scientific information is available on traditional plants.

Different kind of studies on the mechanisms of action, interaction with antibiotics or other medicinal plants or compounds and the pharmacokinetic profile of the extracts has been studied extensively and stimulated the use of natural products throughout the world. Because of the innumerable biologically active compounds that are found in plants possess antibacterial properties; now days numerous investigations are going on in isolation of potent compounds for antimicrobial therapy (Clark AM et al.). Thus all these conditions were taken in account in

order to conduct this research aimed to assess the phytochemical and biological properties of Nepalese plant *Astilbe rivularis*.

MATERIAL AND METHODS

Plant Material: The whole aerial plant (Table 1) was collected from Dhungharka Community of Kavrepalanchok District in Bagmati zone, Nepal (Latitude 27°31'52.22"N to 27°31'42.15"N and Longitude 85°29'44.57"E to 85°29'35.79"E) during March, 2011. The elevation of the area is about 1820-1921 m above Sea level. Voucher specimens identified by Tirtha Maiya Shrestha, (Department of Pharmacy, Kathmandu University, Dhulikhel, Nepal) have been deposited in Department of Pharmacy, Kathmandu University.

The collected plant rhizome was dried in shade and stored at room temperature before the experiments.

Preparation of the plant extract: Extraction was carried out using methanol. The rhizome was blended in home blender and powdered sample was initially soaked in methanol in a conical flask. The mouth of the flask was closed with aluminum foil to reduce the volatilization of the solvent. The flask was allowed to stand for 7 days with occasional shaking. After 7 days, the solvent along with solubilised components were collected. Traces of the methanol from the extract were removed by keeping the extract on a water bath at low temperature. The extract obtained was then weighed and percentage of yield evaluated.

Phytochemical screening: The crude methanol extract of rhizome of *Astilbe rivularis* was screened for the presence of Alkaloids, Flavanoids, Tannin, Coumarins and Glycoside according to standard screening procedure (Evans, W. 2002 and Harborne 1993)

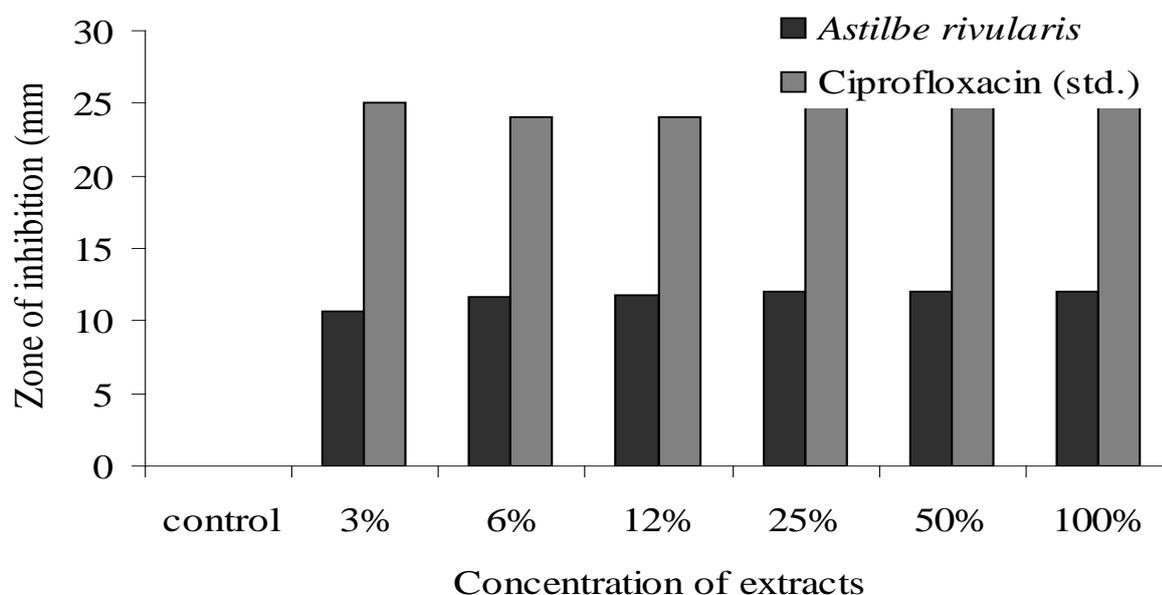
Table 1: showing phytochemical screening results of methanolic extract of dry rhizome of *Astilbe rivularis*

Methanolic extract of <i>Astilbe rivularis</i>	Parts Used	Alkaloids	Tannins	Flavonoids	Coumarins	Glycosides
	Rhizome	++	++	++	++	++

++ : Presence --: Absence

Table 2 Antimicrobial activity of methanolic extract of *Astilbe rivularis* in varying concentration against *E. coli* using Ciprofloxacin (30mcg) as standard. Concentration of extract: 100 % (w/v in 1 % DMSO) and Ciprofloxacin (30mcg) as standard.

Extract (g/100ml)	Zone Of Inhibition (mm)				
	Plate I	Plate II	Plate III	Average	Ciprofloxacin in
0	0	0	0	0	24
3	10	11	11	10.67	25
6	10	14	13	12.33	23
12	14	10	11	11.67	24
100	13	12.5	13.5	13	25

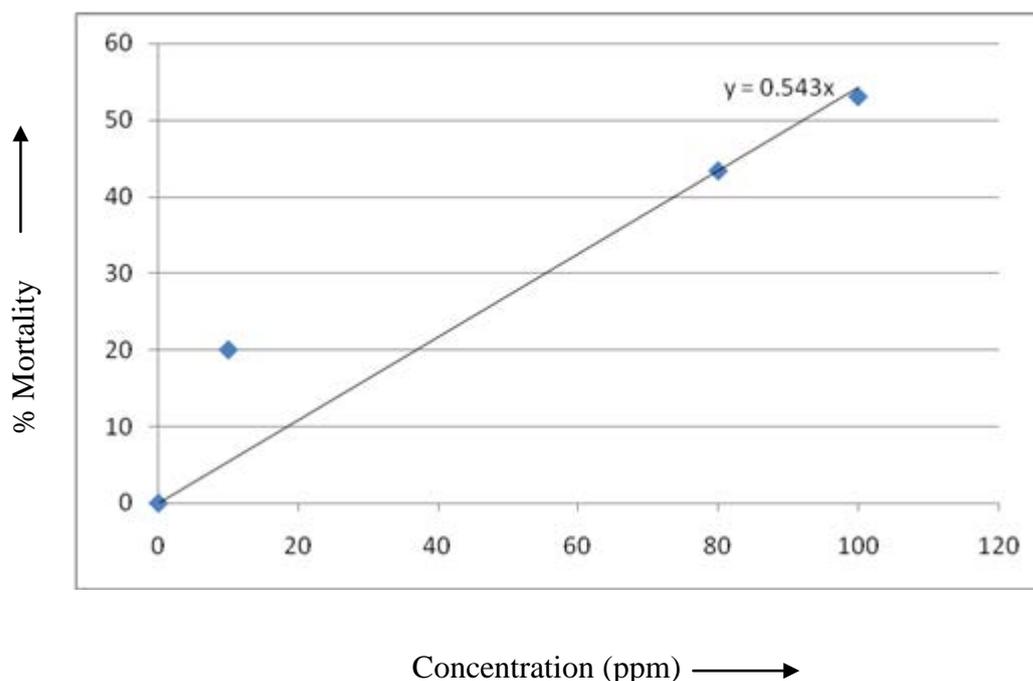
Figure 1: Graph showing antimicrobial activity of methanolic extract of *Astilbe rivularis* varying concentration against *E. coli* using Ciprofloxacin (30mcg) as standard.

Microbial cultures and growth conditions: *Escherichia coli* (0157: H) were used as test microorganism. Cultures of bacteria were grown for 24 h in 50 ml of nutrient broth (Himedia, India) at 37°C and were maintained at 4°C. Subcultures of the organisms were grown in nutrient broth (Himedia, India) at 37°C, 24 h before each experiment.

Antimicrobial activity assay: Required amount of extract of the rhizome of *Astilbe rivularis* was dissolved in 1% (v/v) DMSO to give concentrations of (0, 3, 6, 12, 100) gm/100ml; membrane filter (Pore size 0.47 micrometer) sterilized and tested for antimicrobial activity using the agar disk diffusion method. Sterile, 6 mm diameter Grade 1 Whatman filter paper discs were impregnated with 100% (w/v) methanol crude extract. And placed in duplicates onto MacConkey agar (Himedia, India) plates, surface spread with 1.5×10^6 cells/ml (adjusted to

the 0.5 McFarland turbidity standards) bacteria cultures. The plates were then incubated for 24 h at 37°C. The experiments were carried out in duplicate three times. The results (mean value, n=3) was recorded by measuring the zones of growth inhibition surrounding the discs. Inhibition zone values were corrected i.e. disk diameter was subtracted from the value of the inhibition zone. Control discs contained DMSO only. For comparative purposes standard ciprofloxacin (30 mcg: disc), was included in the assay.

Cytotoxic assay: Methanolic extract of dried rhizome of *Astilbe rivularis* sample was evaluated for lethality to brine shrimp larvae (*A. salina* Leach) according to the procedures described by Meyer et al. 1982. Brine shrimp eggs were hatched for 48 hours in a conical flask containing 300 ml of artificial seawater. The flasks were

Figure 2: Graph showing the relationship between concentration and % mortality of *Astilbe rivularis*

well aerated with the aid of an air pump and kept in a water bath at 29–30 °C. The extracts were dissolved in 1 % (v/v) DMSO and then in sea water to obtain a concentration of 1,000 ppm, 750 ppm, 500 ppm, 100 ppm, 80 ppm and 10 ppm. An aliquot of each concentration (1 ml) was transferred, in triplicate, into clean sterile vials with pipette, and aerated seawater (9 ml) was added. Ten shrimp nauplii were transferred to each vial and incubated at 37°C for 24 hours. After 24 h the numbers of survivors were counted and percentage of death calculated. Graph was then plotted with percentage mortality of brine shrimp in Y-axis and concentration of the extract (ppm) in the X-axis. From the curve obtained, concentration required to kill 50% mature shrimps (LC₅₀) was obtained.

Assessment of minimum inhibitory concentration: According to Performance Standards for Antimicrobial Susceptibility Testing; 2009, Serial dilution technique was used for the determination of MIC. Agar plates were prepared to contain 500,000 cells/mL microorganism and two fold serial dilution technique was used to prepare different concentration (100, 50, 25, 12.5 and so on) % (w/v) of extracts in DMSO and nutrient broth. The negative and positive controls were also performed using 10% DMSO and standard antibiotics. Duplicate wells were run for each concentration of dry rhizome extract of *Astilbe rivularis*. The plates were bored with 6 mm diameter borer which was loaded with dry rhizome extract of *Astilbe rivularis*. The plates were allowed to set for 2 hrs at room temperature and then were incubated at 37 ° C for 24 hrs. Preliminary result and inferences was noted. There was no turbidity observed until concentration of 0.00152% but turbidity was seen at 0.0007%. MIC value may lie between 0.0007% and 0.00152%. Dry rhizome methanolic extracts of concentration between (0.0008% to 0.0014) w/v in

DMSO (1%v/v) was prepared. MacConkey media (Himedia, India) was prepared consisting two distinct layers with different concentration of MacConkey Agar. Dry rhizome methanolic extracts of various concentration was inoculated into the formed bore into each petridish. Similarly, Standard Ciprofloxacin discs (6mm diameter) were also placed on the surface of each media. The petridishes were then incubated at 37°C for 24 hours and observations were made for zone of inhibition.

RESULT AND DISCUSSION

In this report, first the methanolic extract of dried rhizome of *Astilbe rivularis* was subjected to phytochemical screening. Methanolic extract of dried rhizome of *Astilbe rivularis* was tested positive for Alkaloids, Flavanoids, Tannin, Coumarins and Glycoside (Table 1). It was in agreement with Kunwar et al. (2008) and Bhattarai N.K (1998) these plants had been used in local folk medicinal remedies in different forms for various afflictions.

Yield percentage of methanolic extract of *Astilbe rivularis* was also evaluated. Yield percentage of methanolic extract of *Astilbe rivularis* was found to be 14.77%. Yield value quantifies the amount of active constituents relative to amount of the crude drug material. Due to unavailability of standard yield values data, we can only assume that lower yield value may be due to either the plant contains lower amount of active constituents or relatively more number of active constituents but are not solubilized in methanol.

Methanolic extract of dried rhizome of *Astilbe rivularis* was tested for antimicrobial activity against *E. coli*. Out of the various concentrations of methanolic extract of dried rhizome, it was found most effective at concentration of 100% (Table 2). Antimicrobial activity of methanolic extract of *Astilbe rivularis* in varying

Table 3: Effects of the *Astilbe rivularis* extracts on the viability of brine shrimp (% Mortality)

Concentration(ppm)	Viable Brine Shrimps Count			% Mortality
	Replicate 1	Replicate 2	Replicate 3	
1000	0	0	0	100
750	0	0	0	100
500	1	2	1	56.67
100	6	4	4	53.33
80	7	6	4	43.33
10	9	7	8	20
0	10	10	10	0

Table 4: Results for MIC value between 0.0007% and 0.00152% on the basis of Zone of Inhibition (mm)

Extract (%)	ZOI (mm)	ZOI of C _{AD} (mm)
0.0014	8	20
0.0013	7	22
0.0012	7	21
0.0011	7	22
0.001	-	21
0.0009	-	20
0.0008	-	21

concentration against *E. coli* using Ciprofloxacin (30mcg) as standard is shown in Figure 1. *Astilbe rivularis* chiefly contains compounds: arbutin and bergenin (Ratnayake et al.(2009). According to the study conducted on antimicrobial activity of berginin (Yoshida et al. 1982), isolated bergenin showed moderate antimicrobial activity against *E. coli* (SG 485) with zone of inhibition of 15mm. Since, arbutin also possesses antimicrobial activity (Budaveri S 1996). The antimicrobial property of *Astilbe rivularis* in our study may be due to the synergistic effect of bergenin, arbutin and other methanol soluble constituents.

Cytotoxic assay was carried out to determine the LC₅₀ (Table 3). Graph was plotted showing the relationship between concentration and % mortality of *Astilbe rivularis* (Figure 3). LC₅₀ of methanolic extract of *Astilbe rivularis* was found to be 92.01 ppm. Methanolic extract of *Astilbe rivularis* (LC₅₀ 92.01 µg/ml) was found to be mildly toxic [Moshi et al. 2010] and probably have no danger of outright toxicity during acute exposure. Thus extract from this plant used as traditional medicines is unlikely to have any acute ill effects on patients as they are not on the highly toxic category. In the study using brine shrimps, *Phyllanthus engleri* gave an LC₅₀ of 0.47 µg/ml[Moshi et al 2004] and recently the plant yielded englerin, a selective anti-cancer compound against kidney cancer cells(Ratnayake et al.2009), which provides further corroborative evidence on the potential of brine shrimp to predict the presence of anti-cancer compounds in plant extracts. As compared to cyclophosphamide (Moshi et al. 2010), obtained LC50 value for *Astilbe rivularis* is 5.64 times higher and probably not too farfetched to speculate its possibility to yield cancer cell line active compounds. In conclusion the methanolic extract of dry

rhizome of *Astilbe rivularis* seems to be innocuous on short term use and tested extract suggests a remote possibility that it will act as an anti- cancer agent.

Determination of MIC was done by serial dilution technique. There was no turbidity observed until concentration of 0.00152% but turbidity was seen at 0.0007%. MIC value may lie between 0.0007% and 0.00152%. Exact value of MIC was determined by using zone of inhibition method. The Minimum Inhibitory Concentration of methanolic extract of *Astilbe rivularis* was found to be 0.0011% w/v or 0.011mg/ml. There are no validated criteria for the Minimum Inhibitory Concentration end points for in vitro testing of plant extracts, but a criterion based on MIC results as proposed by (Aligiannis et al. 2001) showed that *Astilbe rivularis* has strong antibacterial activity against *E. coli*.

This plant *Astilbe rivularis* used in this study was chosen on the basis that it is used traditionally for treatment of conditions like peptic ulcer, diarrhoea and dysentery. The study is premised on establishing proof of traditional claims for antimicrobial activity, but also to roughly ascertain their safety. Antimicrobial tests indicate *Astilbe rivularis* plants have antibacterial activity against *E. coli*. Its potential use as an anticancer agent can also be further established.

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