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Original Article

Antibacterial Activity of *Astilbe rivularis* Buch. – Ham. Ex D. Don Leaves: Effect of Extraction Solvents

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ABSTRACT

Astilbe rivularis Buch. – Ham. Ex D. Don (*A. rivularis)*, one of the medicinal plants of North East Himalayan region of India, has several pharmacological properties. In the present workantibacterial activity of ethyl acetate, acetone, ethanol, methanol and petroleum ether extracts of *A. rivularis* leaves was evaluated against four Gram - positive bacteriaviz. *Bacillus subtilis, Bacillus megaterium, Streptococcus pyogenes and Staphylococcus aureus*as well as four Gram- negative bacteria like *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Shigella dysenteriae* . Disc diffusion technique was used for *in vitro* antibacterial screening. Result showed that all extracts showed anti bacterial activity but the methanol extract of leaves had larger zone of inhibition in disc diffusion against the said bacteria. Antibacterial activity was more in Gram - positive bacteria than in Gram - negative bacteria. Highest activity was noted against *Bacillus subtilis* and lowest activity was found for *Salmonella typhi.* The MIC (minimum inhibitory concentration) values of extract of leaves of *A. rivularis* against the bacteria ranged from 8 – 64 microgram/mL. Results, therefore, suggest that methanol extract of *A. rivularis* leaves had good antibacterial activity against the tested bacteria, thus, provides a scientific rationale for its use as anti bacterial agent and isolation of anti bacterial compound from it in future.

Keywords : Antibacterial activity, *A. rivularis* leaves, Disc diffusion technique, Zone of inhibition, Minimum inhibitory concentration.

1. INTRODUCTION

Infectious diseases, the world's second leading cause of premature deaths, kill almost 50,000 people every day. Use of antibiotic started to check the death but the use was so rampant and indiscriminate that microorganism started developing resistance. It is reported that approximately 70 per cent of known bacteria have developed resistance to one or more antibiotics. This is specially applicable for the bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*etc. This has created immense problem in treatment of

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infectious diseases.The problem is so severe that antibiotic resistance is now considered a worldwide public health problem[1,2].

To combat the situation continuous effort was going on for synthesis of new chemicals having antimicrobial activity[3].Lot of chemicals were synthesized in laboratory which established their anti bacterial activity[4]. Unfortunately, most of these compounds are potentially toxic and are not free from side effects on the host. This has extended the research even in the field of medicinal plants for search of natural compounds which will be less toxic and will act as a proper substitute of chemical antimicrobial agents [5].Several plants have already been identified for their antimicrobial properties[6] .

Astilberivularis, Buch. – Ham. Ex D. Don (*A. rivularis*), family – Saxifragaceae, is one of the medicinal plants of North Eastern Himalaya. The plant has different names. In Lepcha it is called Pangoand in Nepali the plant is known as Buriokahti[7]. *A. rivularis*has tall herb stem and leaves are covered with hairs [8]. The plant is distributed at a range of 5000 – 9000 feet in Common Temperate of Himalaya. Itis also found on sloppy waste place. In traditional medicine juice of the plant is applied to sprains and muscularswelling. Further, rhizome of this plant is used in curing dysentery, headache, hemorrhages, diarrhoea, prolapse of uterus and to improve fertility [9]. The plant is also known havinganti microbial, anti viral activity[10,11]. Ethnic use of *A.rivularis*, as reported in literature, is in peptic ulcer . Root juice of the plant, two tea spoonful thrice a day, is given to patients suffering from peptic ulcer [12]. We also found anti peptic ulcer activity of *A. rivularis*leaves in experimental animals [13]. Anti oxidant activity of *A.rivularis* is known in literature [14]. We also noted anti oxidant activity of the plant[15].Phytochemical investigation of *A. rivularis*revealed the presence of flavonoids,

terpenoids and bergenin.11-*O*-galloylbergenin, catechin, afzelechin, epiafzelechin and 2-(*β*-D-glucopyranosyloxy)-4-hydroxylbenzenacetonitrilewereisolated from the plant[16].

Adhikari et al. demonstrated [10]highest antibacterial effect of methanol extract of *A.rivularis*grown in Nepal against *E. coli*while antimicrobial susceptibility test conducted by Gyawali et al. showed [17] that methanolic extract of *A. rivularis*of Nepal had highest anti bacterial activity against *Kliebsiellapneumonie.*

Aim of the present study was, therefore,to see effect of extraction solvents on antibacterial activity of *A. rivularis*leaves grown in North Eastern Himalayan region of India against few Gram positive and Gram negative bacteria.

2. METHODOLOGY

2.1 Collection of plant materials

A. rivularis leaves were collected from the medicinal plants garden of the University of North Bengal, Siliguri($26^0 41' 30.9984"$ N, $88^0 27' 4.5756"$ E, elevation, 410 ft), Dist. Darjeeling, West Bengal. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of department of Biochemistry, North Bengal Medical College, Siliguri, West Bengal, India for future references.

Fig. 1: *Astilberivularis*Buch. – Ham. Ex D. Don

2.2 Preparation of leaves for Anti bacterial screening

Leaves of*A. rivularis*L. were washed thoroughly, shed dried and powered. 50 grams of this powder was extracted separately with 500 ml of ethyl acetate, ethanol, petroleum ether, methanol and acetone in soxhlet at 37^0C for 15 minutes. The whole extract was filtered and the solvent was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50^0C. A brownish mass was obtained. 500 micro gram of the mass was extracted with 1 ml water and the solution obtained was used to evaluate the anti bacterial activity against the tested bacteria.

2.3 Bacteria

Four Gram - positive bacteria *viz. Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus* and *Streptococcus pyogenes* and four Gram-negative bacteria *viz. Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* and *Salmonella typhi* were employed to determine antibacterial activity and minimum inhibitory concentration. All these bacteria were collected from the department of Microbiology, North Bengal Medical College Hospital.

2.4 Media

Nutrient agar media (Difco laboratories) pH 7.2 and nutrient broth media (Difco laboratories) pH 6.8 were used for antibacterial screening and minimum inhibitory concentration determination.

2.5 Antibacterial screening

In vitro antibacterial screening was carried out by disc diffusion method [18]. According to this method, 20 ml quantities of nutrient agar were placed in a petri dish with 0.1 ml of 10^{-2} dilution of bacterial culture of 20 hours old. Filter paper discs (6 mm diameter) impregnated with 60 µg per disc and 120 µg per disc concentration of the solution prepared from *A. rivularis* L. were placed on test bacteria-seeded plates. Blank disc impregnated with water was used as negative control. Zone of inhibition was recorded after 18

hours of incubation. Diameters of zone of inhibition produced by the solution prepared from *A. rivularis* L. were compared with that of standard antibiotic kanamycin 40 µg per disc, Each sample was used for five times for the determination of anti bacterial activity.

2.6 Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration is defined as the lowest concentration of antibiotic completely inhibiting visible growth of bacteria after 18 – 24 hours of incubation at 37 ^{0}C. This was done by the method of Mosaddik and Haque[19]. According to this method, extract of *A. rivularis* L. (1.0 mg) was dissolved in 2 ml nutrient broth media to obtain a stock solution of concentration 500 µg/ml. 3 drops of Tween 80 was added in nutrient broth to facilitate dissolution. Serial dilution technique was followed to obtain 250 µg/ml concentration of the compound. One drop (0.02ml) of prepared suspensions of organism (10^6organism/ml) was added to each broth dilution. These dilutions were then incubated for 20 hours at 37^{0}C. Growth of bacteria was examined by noting turbidity of the solution. The nutrient broth media with 3 drops of Tween 80 was used as negative control while kanamycin was used as positive control.

2.7 Statistical analysis

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th versions. Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

3. RESULTS

Table – 1 shows antibacterial activity of different solvent extracts of *A. rivularis* L. and kanamycin against four Gram positive bacteria. All solvent extracts of *A. rivularis* L. had antibacterial activityagainst the tested bacteria but methanol extract exhibited maximum antibacterial effect. Results were statistically significant ($p<0.001$) when compared with anti bacterial activity of other extracts. Minimum anti bacterial activity, however, was noted for petroleum ether extract of *A. rivularis* L. Highest antibacterial activity of methanol extract of *A. rivularis* L. was found against *Bacillus subtilis.* In the dose of 40 µg per disc concentrations zone of inhibition in disc diffusion method was 33 ± 0.6 for *Bacillus subtilis*. Result was comparable to that of standard antibiotic kanamycin where in the same dose zone of inhibition for *Bacillus subtilis* came 36 ±0.7. Lowest anti bacterial activity of methanol extract of *A. rivularis* L. was noted for *Streptococcus pyogenes.* In this case with 40 µg per disc concentrations zone of inhibition in disc diffusion method was 22± 0.5. Other bacterial strains like *Bacillus megaterium*, *Staphylococcus aureus*, however, vary in their sensitivity.

Table 1 :Antibacterial activity of different solvent extracts of *A. rivularis*L. and kanamycin [Zone of inhibition (diameter in mm)] against four Gram positive bacteria.

Bacteria (Strain)	Ethyl acetate extract of *A. rivularis* L. (40 µg per disc)	Acetone extract of *A. rivularis* L. (40 µg per disc)	Ethanol extract of *A. rivularis* L. (40 µg per disc)	Methanol extract of *A. rivularis* L. (40 µg per disc)	Petroleum ether extract of *A. rivularis* L. (40 µg per disc)	Kanamycin (40 µg per disc)
Gram – positive						
Bacillus subtilis (ATCC 19659)	22 ± 0.5	21 ± 0.5	20 ± 0.6	33 ± 0.6*	16 ± 0.6	36 ± 0.7
Bacillus megaterium (NBMC 1122)	19 ± 0.7	18 ± 0.6	18 ± 0.7	30 ± 0.5*	14 ± 0.5	33 ± 0.8
Staphylococcus aureus (ATCC 25923)	17 ± 0.5	16 ± 0.7	16 ± 0.5	29 ± 0.3*	12 ± 0.3	34 ± 0.7
Streptococcus pyogenes (NBMC 1321)	15 ± 0.4	14± 0.3	14 ± 0.4	22 ± 0.5*	10 ± 0.7	30 ± 0.5

Data was in mean SEM (n = 5). Control was made with water. It had no zone of inhibition. So data has not been shown. * $p<0.001$

Antibacterial activity of different solvent extracts of *A. rivularis*L. and kanamycin against four Gram negative bacteria was shown in Table - 2. All solvent extracts of *A. rivularis*L. had antibacterial activity against the tested bacteria. Methanol extract, however,exhibited maximum antibacterial effect. Results were statistically significant ($p<0.001$) when compared with anti bacterial activity of other extracts. Minimum anti bacterial activity was noted for petroleum ether.

extract of *A. rivularis*L. Highest antibacterial activity of methanol extract of *A. rivularis*L. was found against *Escherichia coli.* In the dose of 40 µg per disc concentrations zone of inhibition in disc diffusion method was 25 ± 0.7 for *Escherichia coli*. Result was comparable to that of standard antibiotic kanamycin where in the same dose zone of inhibition for *Escherichia coli* came 27 ± 1.0. Lowest anti bacterial activity of methanol extract of *A. rivularis*L. was noted for *Salmonella typhi.* In this case with 40 µg per disc concentrations zone of inhibition in disc diffusion method was 18± 0.2. Other bacterial strains like *Shigelladysenteriae*, *Pseudomonas aeruginosa*, however, vary in their sensitivity.

Table 2 :Antibacterial activity of different solvent extracts of *A. rivularis*L. and kanamycin [Zone of inhibition (diameter in mm)] against four Gram negative bacteria.

Bacteria (Strain)	Ethyl acetate extract of *A. rivularis* L. (40 µg per disc)	Acetone extract of *A. rivularis* L. (40 µg per disc)	Ethanol extract of *A. rivularis* L. (40 µg per disc)	Methanol extract of *A. rivularis* L. (40 µg per disc)	Petroleum ether extract of *A. rivularis* L. (40 µg per disc)	Kanamycin (40 µg per disc)
Gram – negative						
Escherichia coli (ATCC 25922)	17 ± 0.3	18 ± 0.3	20 ± 0.4	25 ± 0.5*	16 ± 0.5	27 ± 0.8
Shigelladysenteriae (NBMC 1127)	15 ± 0.2	16 ± 0.2	18 ± 0.2	22 ± 0.4*	14 ± 0.4	25 ± 0.7
Pseudomonas aeruginosa (NBMC 1243)	13 ± 0.3	14 ± 0.2	16 ± 0.2	20 ± 0.3*	12± 0.3	22 ± 0.6
Salmonella typhi (MTCC 733)	11 ± 0.1	13 ± 0.1	14 ± 0.1	18 ± 0.2*	10 ± 0.2	20 ± 0.4

Data was in mean SEM (n = 5). Control was made with water. It had no zone of inhibition. So data has not been shown. * p <0.001

Table – 3 indicates results of minimum inhibitory concentration of acetone extract of *A. rivularis*L. leaves and kanamycin. The MIC (Minimum Inhibitory Concentration) of methanol extract of *A. rivularis*L.leaves ranged from 4 to 32 for Gram positive bacteria and 8 to 64 microgram/mL for Gram negative bacteria respectively.For kanamycin MIC value for Gram positive bacteria was found to vary between 2 and 16 and for Gram negative bacteria it came between 4 and 16.

Table 3: Minimum inhibitory concentration of *A. rivularis*L. and kanamycin

Bacteria	MIC values of *A. rivularis*L. (microgram/mL)	MIC values of kanamycin (microgram/mL)
Gram – positive		
Bacillus subtilis	4	2
Bacillus megaterium	8	4
Staphylococcus aureus	16	8
Streptococcus pyogenes	32	16
Gram – negative		
Escherichia coli	8	4
Shigelladysenteriae	16	8
Pseudomonas aeruginosa	32	8
Salmonella typhi	64	16

Negative control containing water had no MIC value. Thus, it has not been shown

4. DISCUSSION

Several medicinal plants showed antimicrobial property[20-22]. We have reported antibacterial activity of Titeypati (*Artemisia vulgaris* Linn.) [23], *Amaranthusspinous*[24], *Cassia alata*Linnaeus[25] against various Gram positive and Gram negative bacteria.

In the present study antibacterial activity of *A. Rivularis* L. leaves was observed against four Gram - positive bacteriaviz. *Bacilliu subtilis, Bacilusmegaterium, Staphylococcus aureus,* and *Streptococcus pyogenes* as well asfour Gram- negative bacteria like *Escherichia coli, Pseudomonas aeruginosa, Shigelladysenteriae*and *Salmonella typhi.* Maximum antibacterial activity was found by the methanol extract of *A. rivularis*L. leaves when compared with ethyl acetate, ethanol, acetone and petroleum ether extract of the plant leaves for both Gram positive (Figure – 1) and Gram negative (Figure – 2) bacteria. Adhikari et al.[10]and Gyawali et al. [17] also found highest antibacterial activityof methanol extract of *A.rivularis*grown in Nepal against *E. coli* and *Kliebsiella pneumonie*. Jayati et al.[26]also found antimicrobial activityof methanol extract of *A. rivularis* grown in Darjeeling district of West Bengal against various Gram positive and Gram negative bacteria, yeast and mold. Galvez [27], however, did not find anti bacterial activity of *A. rivularis* L. grown in Philippines. Of course, Galvez used ethanol extract and not methanol extract of A. rivularis L.

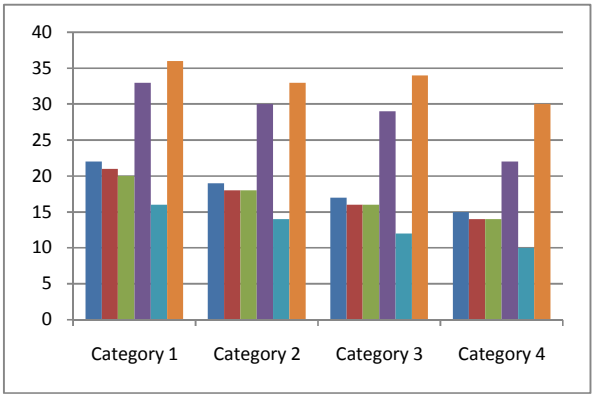

Fig 1: Showing anti bacterial effect [Zone of inhibition (diameter in mm)] of different solvent extracts of *A. rivularis*L. leaves on Gram positive bacteria.

Category 1: *Bacillus subtilis* Category 2: *Bacillus megaterium* Category 3: *Staphylococcusaureus* Category 4: *Streptococcus pyogenes*

■Ethylacetate■Acetone■Ethanol■Methanol■Petroleum ether■Kanamycin

Present study shows that methanol extract of *A. rivularis* L. has maximum anti bacterial activity which is comparable to that of standard antibiotic kanamycin. This indicates that the anti bacterial factor, may be the secondary metabolite, present in *A. rivularis* L. leaves may act as antibiotic in future. Therefore, isolation of this anti bacterial factor from *A. rivularis* L. leaves seems to be important specially in view of bacterial resistant to antibiotics globally.

It is known that season can change biological activity of plants [28-30] . Studies in relation to effect of season on anti bacterial activity of *A. rivularis* L. leaves are now in progress in our laboratory.

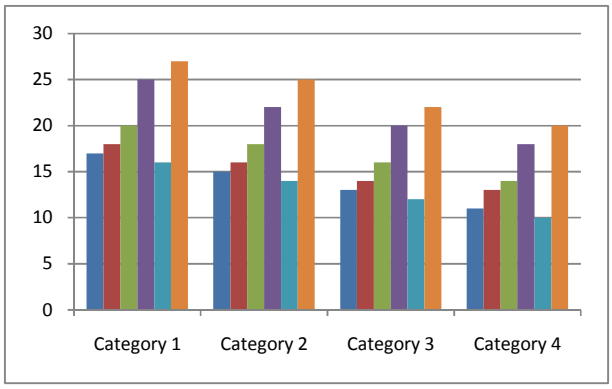

Fig 2: Showing anti bacterial effect [Zone of inhibition (diameter in mm)] of different solvent extracts of *A. rivularis*L. leaves on Gram negative bacteria.

Category 1: *Excherichiacoli* Category 2: *Shigelladysenteriae* Category 3: *Pseudomonas aeruginosa* Category 4: *Salmonella typhi*

■Ethyl acetate■Acetone■Ethanol■Methanol■Petroleum ether■Kanamycin

5. CONCLUSION

Present study showed that methanol extract of *A. rivularis* L. leaves possess anti bacterial effect against number of gram positive and gram negative bacteria and the effect is comparable to that of standard antibiotic kanamycin. The plant, therefore, can be developed further as plant-based antibacterial drug which needs isolation and characterization.

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