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Original Article

Effect of Season on Antibacterial Activity of *Astilbe rivularis* Buch.–Ham.Ex D. Don Leaves

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ABSTRACT

Seasonal variation in antibacterial activity of the leaves of *Astilbe rivularis*, Buch. – Ham. Ex D. Don (*A. rivularis)*, a medicinal plants of North Eastern Himalaya, was studied against four Gram - positive and four Gram negative bacteria. Gram positive bacteria were *Bacillius subtilis, Bacilus megaterium, Staphylococcus aureus* and *Streptococcus pyogenes* while Gram negative bacteria included *Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* and *Salmonella typhi.* Disc diffusion technique was used for ascertaining *in vitro* antibacterial activity. Results showed that leaves of *A. rivularis*of the months of July and August had maximum *in vitro* antibacterial activity.

Keywords: Antibacterial activity, *A. rivularis* leaves, seasonal variation, disc diffusion technique, zone of inhibition

1. INTRODUCTION

Astilbe rivularis, Buch. – Ham. Ex D. Don (*A. rivularis*), family – Saxifragaceae, is one of the medicinal plants of Sikkim Himalaya. The plant has different names. In Lepcha it is called Pango and in Nepali the plant is known as Buriokahti[1]. *A. rivularis*h as tall herb stem and leaves are covered with hairs [2]. 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 muscular swelling. Further, rhizome of this plant is used in curing dysentery, headache, hemorrhages, diarrhoea, prolapse of uterus and to improve fertility [3]. The plant is also known havinganti

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microbial, anti viral activity[4,5]. Ethnic use of *A. rivularis*, as reported in literature, is in peptic ulcer [1]. Root juice of the plant, two tea spoonful thrice a day, is given to patients suffering from peptic ulcer [6]. We also found anti peptic ulcer activity of *A. rivularis* leaves in experimental animals [7]. Anti oxidant activity of *A. rivularis* is known in literature [8]. We also noted anti oxidant activity of the plant [9]. 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-hydroxylbenzenacetonitrile were isolated from the plant[10].

Adhikari et al. demonstrated [4]highest antibacterial effect of methanolic extract of *A.rivularis* grown in Nepal against *E. coli* while antimicrobial susceptibility test conducted by Gyawali et al. showed [11] that methanolic extract of *A. rivularis* of Nepal had highest anti bacterial activity against *Kliebsiella pneumonie*. Recently we confirmed (paper is under communication) in vitro anti bacterial activity of methanol extract of *A. rivularis* grown in North East Himalayan region of India.

It is known that season has influence on production of secondary metabolites / active compounds in medicinal plants responsible for medicinal values[12-17]. The present work was, therefore, aimed to see the influence of season, if any, on in vitro anti bacterial activity of *A. rivularis*.

2. METHODOLOGY

2.1 Collection of plant materials

A. rivularis leaves were collected from the medicinal plants garden of the University of North

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

Bengal, Siliguri ($26^0 41' 30.9984"$ N, $88^0 27' 4.5756"$ E, elevation, 410 ft), Dist. Darjeeling, West Bengal randomly during the periods of January – February, March – April, May – June, July –August, September - October and November – December. Leaves were authenticated by the experts of the department of Botany of the said University. Voucher specimens of the leaves were deposited in the department of Biochemistry, North Bengal Medical College, Siliguri, West Bengal, India for future references.

2.2 Preparation of leaves for Anti bacterial screening

Leaves of *A. rivularis* were washed thoroughly, shed dried and powered. 50 grams of this powder was extracted separately with 500 ml of methanol in soxhlet at 37^0C for 15 minutes. Methanol extract was chosen as we have noted maximum anti bacterial effect of methanol extract of *A. rivularis.* 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* 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* (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 37^0C. 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) 20^{th} versions. Differences between means were tested employing Duncan's multiple comparison tests and significance was set at $p < 0.05$.

3. RESULTS

Table – 1 shows effect of season on *in vitro* anti bacterial activity of *A. rivularis* leaves against four Gram- positive bacteria. Results showed that acetone extract of *A. rivularis* leaves exerted anti bacterial activity at 40 µg per disc concentrations for all tested bacteria. In disc diffusion method large zone of inhibition was found for *Bacillus* subtilis (30 ± 0.4 mm) and small zone of inhibition was noted against *Streptococcus pyogenes* (20 ± 0.4 mm).

But for all bacteria anti bacterial activity of acetone extract of *A. rivularis*leaves was maximum during the months of July and August. Results were statistically significant when compared to that of other seasons of the year.

Table 1: Showing seasonal variation in the *in vitro*anti bacterial activity of leaves of *A. rivularis* against four Gram- positive bacteria.

Gram-positive Bacteria (Strain)	*A. rivularis*(January – February (40 µg per disc)	*A. rivularis*(March – April) (40 µg per disc)	*A. rivularis*(May – June) (40 µg per disc)	*A. rivularis*(July – August) (40 µg per disc)	*A. rivularis*(September – October) (40 µg per disc)	A. *rivularis* (November-December) (40 µg per disc)
Bacillus subtilis (ATCC 19659)	15 ± 0.2	20 ± 0.3	25 ± 0.4	30 ± 0.4*	24 ± 0.3	20 ± 0.3
Bacillus megaterium (NBMC 1122)	12 ± 0.1	20 ± 0.3	24 ± 0.4	28 ± 0.5*	22 ± 0.4	18 ± 0.2
Staphylococcus aureus (ATCC 25923)	10 ± 0.1	15 ± 0.2	20 ± 0.4	25 ± 0.4*	18 ± 0.2	16 ± 0.1
Streptococcus pyogenes (NBMC 1321)	8 ± 0.1	14 ± 0.2	16 ± 0.3	20 ± 0.4*	15 ± 0.2	12 ± 0.1

Data was for Zone of inhibition (diameter in mm). It 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

Effect of season on *in vitro* anti bacterial activity of *A. rivularis* leaves against four Gram- negative bacteria is shown in Table – 2. Acetone extract of *A. rivularis* leaves at 40 µg per disc concentrations exerted anti bacterial activity against all tested Gram negative bacteria. In disc diffusion method large zone of inhibition was found against *Escherichia coli* (24 ±.4 mm) while smallzone of inhibition was noted against *Salmonella typhi*(18 ± 0.3 mm). But

for all bacteria anti bacterial activity of acetone extract of *A. rivularis* leaves was maximum during the months of July and August. Results were statistically significant when compared to that of other seasons of the year.

Table 2: Showing seasonal variation in the *in vitro*anti bacterial activity of leaves of *A. rivularis* against four Gram- negative bacteria.

Gram-negative Bacteria (Strain)	*A. rivularis*(January-February) (40 µg per disc)	*A. rivularis*(March – April) (40 µg per disc)	*A. rivularis* (May – June) (40 µg per disc)	*A. rivularis* (July – August) (40 µg per disc)	*A. rivularis* (September – October) (40 µg per disc)	*A. rivularis* (November-December) (40 µg per disc)
Escherichia coli (ATCC 25922)	15 ± 0.1	18 ± 0.2	20 ± 0.3	24 ± 0.4*	15 ± 0.2	12 ± 0.2
Shigelladysenteriae (NBMC 1127)	14 ± 0.3	16 ± 0.2	20 ± 0.3	22 ± 0.3*	16 ± 0.2	12 ± 0.1
Pseudomonas aeruginosa (NBMC 1243)	12 ± 0.2	14 ± 0.3	16 ± 0.4	20 ± 0.4*	14 ± 0.2	10 ± 0.2
Salmonella typhi (MTCC 733)	10 ± 0.1	12 ± 0.2	14 ± 0.3	18 ± 0.3*	14 ± 0.1	12 ± 0.2

Data was for Zone of inhibition (diameter in mm). It 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

4. DISCUSSION

Anti microbial activity of medicinal plants varies with season. The best antimicrobial activities of *Hypoxishemerocallidea*, *Drimiarobusta,Tulbaghiaviolacea* and *Merwillaplumbea* against *K. pneumoniae* and *S. aureus*were recorded in winter and autumn seasons byNcubeetal.[20].Ranwan and Yadav, however, showed maximum anti bacterial activity of *Achyranthes aspera* (L). against two Gram positive (Staphylococcus aureus MTCC96, Bacillus cereus MTCC 430,) and three Gram negative (Pseudomonas aeruginosa MTCC 424, E.coli MTCC 433 and Proteus mirabilis MTCC425) bacteria during the month of January[21]. Chaves et al observed that *Guapira graciliflora* and *Pseudobombax marginatum*, two species used in the treatment of various diseases in

traditional medicine of the Brazilian, have maximum anti bacterial activity in summer and winter respectively [22].

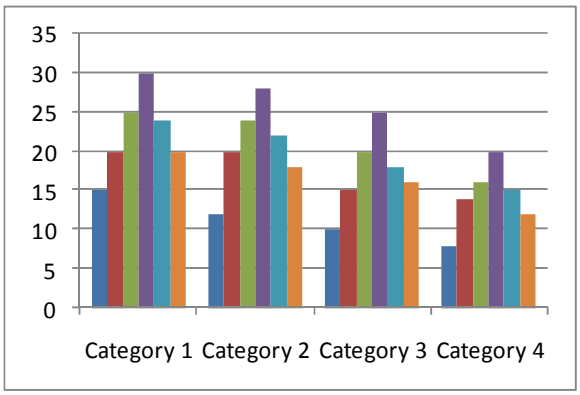

Fig 1: Showing seasonal variations in anti bacterial effect [Zone of inhibition (diameter in mm)] of acetone extract of*A. rivularis*leaves on Gram positive bacteria.

Category 1: *Bacillus subtilis* Category 2: *Bacillus megaterium* Category 3: *Staphylococcusaureus* Category 4: *Streptococcus pyogenes*
■January - February■March - April■May - June■July - August■September - October ■November - December

Osadebe and coworkers, however, found maximum anti bacterial activity of *Loranthusmicranthus* leaves in the month of January [23].Chokoe et al noted maximum anti bacterial activity of *Carpobrotus edulis* L.against *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* during autumn[24] .

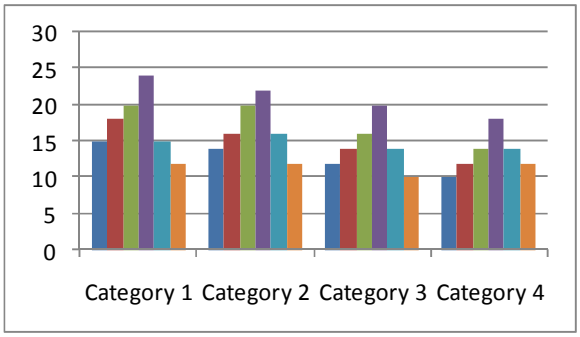

Fig 2: Showing seasonal variations in anti bacterial effect [Zone of inhibition (diameter in mm)] of acetone extract of *A. rivularis* leaves on Gram negative bacteria.
Category 1: *Excherichia coli* Category 2: *Shigelladysenteriae* Category 3: *Pseudomonas aeruginosa* Category 4: *Salmonella typhi*
■January - February■March - April■May - June■July - August■September - October ■November - December

In the present study we,perhaps for the first time, showed that *A rivularis* leaves exerted maximum anti bacterialactivity against four Gram positive (*Bacillius subtilis*, *Bacilusmegaterium*, *Staphylococcus aureus* and *Streptococcus pyogenes*) and four Gram negative (*Escherichia coli*, *Shigelladysenteriae*, *Pseudomonas aeruginosa* and *Salmonella typhi*)bacteria during the months of July and August (Figures – 1, 2). This is due to large accumulation of the active compound in the plant leaves during this period responsible for anti bacterial activity.

Anti bacterial compound(s) present in *A rivularis* leaves needs isolation and characterization especially in view of the fact that a large number of antibacterial agents have been discovered but pathogenic bacteria are constantly developing resistance to these agents. Due to this, life threatening bacterial infection has been increased worldwide and is becoming an important cause of morbidity and mortality[25].

Work on isolation and characterization of anti bacterial compound(s) from the leaves of *Arivularis*is now in progress in our laboratory.

5. CONCLUSION

Seasonal variation in antibacterial activity of the leaves of *A. rivularis* was studied against four Gram - positive and four Gram-negative bacteria. Results showed that *A. rivularis* leaves during the period July - August had maximum antibacterial activity against all the tested bacteria. *A. rivularis* leaves of the months of July and August may, therefore, be used for isolation of the anti bacterial compound.

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