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Original Article

In-vitro antibacterial activity of leaves of *Amaranthus spinosus* L.: Seasonal variation

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ABSTRACT

Seasonal variation in antibacterial activity of the leaves of *Amaranthus spinosus* L. was studied against four Gram - positive bacteria viz. *Bacillius subtilis, Bacilus megaterium, Staphylococcus aureus and Streptococcus pyogenes as* well as four Gram- negative bacteria like *Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* and *Salmonella typhi.* Disc diffusion technique was used for in *vitro* antibacterial screening. Results showed that leaves of *Amaranthus spinosus* L. of the period May – June had maximum *in vitro* antibacterial activity.

Keywords: Antibacterial activity, *Amaranthus spinosus* L., seasonal variation, disc diffusion technique, zone of inhibition

INTRODUCTION

Amaranthus spinosus L., a medicinal plant under the family of amaranthaceae, is distributed in lower to middle hills (3000–5000 ft) of entire north eastern Himalayas. The plant grows in cultivated areas as well as in waste places. Leaves of *Amaranthus spinosus* L. are stacked and alternate. The plant is known as "prickly amaranthus" in English and "ban lure" or "dhuti ghans" in Nepali. Medicinal uses of *Amaranthus spinosus* L. as mentioned in Ayurvedic text [1,2] are: Leaf infusion is diuretic and used in anemia. Root paste is used in gonorrhea, eczema, menorrhea etc. Ethnic use of *Amaranthus spinosus* L. is mainly with village-people of Sikkim who use leaf infusion of the plant in stomach disorder specially in case of indigestion and peptic ulcer [3]. In modern research *Amaranthus spinosus* L. has been reported to have anti-inflammatory properties, effect on hematology, immune modulatory activity, anthelmintic properties, antidiabetic, antihyperlipidemic and spermatogenic [4-9]. Recently HarshaVardhana reported [10] anti bacterial activity of *Amaranthus spinosus* L. root. We also noted (unpublished observation*) in vitro* anti bacterial activity of *Amaranthus spinosus* L. leaves. The present work was thus aimed to study seasonal variation, if any, in the anti bacterial activity of *Amaranthus spinosus* L. leaves.

MATERIALS AND METHODS

Collection of plant material: Leaves of *Amaranthus spinosus* L.(Fig. 1) were collected in morning hours (9 – 10 AM) from the Medicinal Plant Garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India during the periods of January – February, March – April, May – June, July – August, September – October and November – December in the year 2012 and authenticated by the expert of the department of Botany of the said University. A voucher specimen was kept in the department of Biochemistry, North Bengal Medical College, Dist. Darjeeling, West Bengal, India for future reference.

Preparation of leaves for Anti bacterial screening: Leaves of *Amaranthus spinosus* L. were shade dried and powdered. 10 grams of this powder was extracted with 50 ml cold ethanol for 30 minutes. The whole extract was filtered and the solvent was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 degrees centigrade. A brownish mass was obtained. 500 micro gram of the mass is extracted with 1 ml water and the solution obtained there from was used to evaluate the anti bacterial activity against the tested bacteria.

Bacteria: Four Gram - positive bacteria *viz. Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus and Streptococcus pyogenes* and four Gram-negative bacteria *viz.*

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Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa and *Salmonella typhi* were employed to determine in *vitro* antibacterial activity. All these bacteria were collected from the department of Microbiology, North Bengal Medical College Hospital, Dist. Darjeeling, West Bengal, India.

Media: Nutrient agar media (Difco laboratories) pH 7.2 was used for antibacterial screening.

Antibacterial screening: In vitro antibacterial screening was carried out by disc diffusion method [11,12]. 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 120 µg per disc concentration of the solution prepared from the leaves of *Amaranthus spinosus* L. were placed on test bacteria-seeded plates. Dose was chosen from our earlier work where it was shown that the dose had maximum anti bacterial activity for one medicinal plant [13]. Blank disc impregnated with water was used as negative control. Zone of inhibition was recorded after 18 hours of incubation. Each sample was used for five times for the determination of anti bacterial activity.

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 $p < 0.05$.

RESULTS AND DISCUSSION

Seasonal variations in *in vitro* anti bacterial activity of the leaves of *Amaranthus spinosus* L. against four Gram- positive bacteria and four Gram-negative bacteria are shown in Tables – 1 and 2 respectively. Results showed that extract of *Amaranthus spinosus* L. leaves exerted anti bacterial activity at 120 µg per disc concentrations for all tested bacteria. Large zone of inhibition in disc diffusion was found out. Antibacterial activity was more in Gram - positive bacteria than Gram - negative bacteria. Highest activity was noted against *Staphylococcus aureus* and lowest activity was found for *Escherichia coli* (Fig.2). Maximum anti bacterial activity against all four Gram-positive and Gram-negative bacteria was noted by the leaves of *Amaranthus spinosus* L. of the period May – June. Seasonal variations in *in vitro* anti bacterial effect of *Amaranthus spinosus* L. leaves against *Staphylococcus aureus* and *Escherichia coli* are shown in figures 3 and 4 respectively. 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 [14]. Under the circumstances, search for antibacterial agent is going on and is extended even in the field of medicinal plants to develop safer antibacterial drugs [15]. We, in our laboratory, screened several medicinal plants for their anti bacterial property and noted that leaves of *Amaranthus spinosus* L. had *in vitro* anti bacterial activity. Since medicinal values of plants vary with season [16-20], we were interested to note the seasonal variation, if any, in the anti bacterial activity of leaves of *Amaranthus spinosus* L. Results of the present study showed that leaves of *Amaranthus spinosus* L. during the period May – June had maximum anti bacterial activity against four Gram-positive and four Gram-negative bacteria. This was probably due to maximum accumulation of active compound in the leaves of *Amaranthus spinosus* L. responsible for anti bacterial activity. Presently we are investigating in this direction.

CONCLUSION

Seasonal variation in antibacterial activity of the leaves of *Amaranthus spinosus* L. was studied against four Gram - positive and four Gram-negative bacteria. Results showed that leaves of *Amaranthus spinosus*L. of the period May - June had maximum antibacterial activity for all tested bacteria.

Fig. 1 :*Amaranthus spinosus* L.

Table – 1: Showing seasonal variation in the *in vitro* anti bacterial activity of leaves of *Amaranthus spinosus* L. against four Gram- positive bacteria.

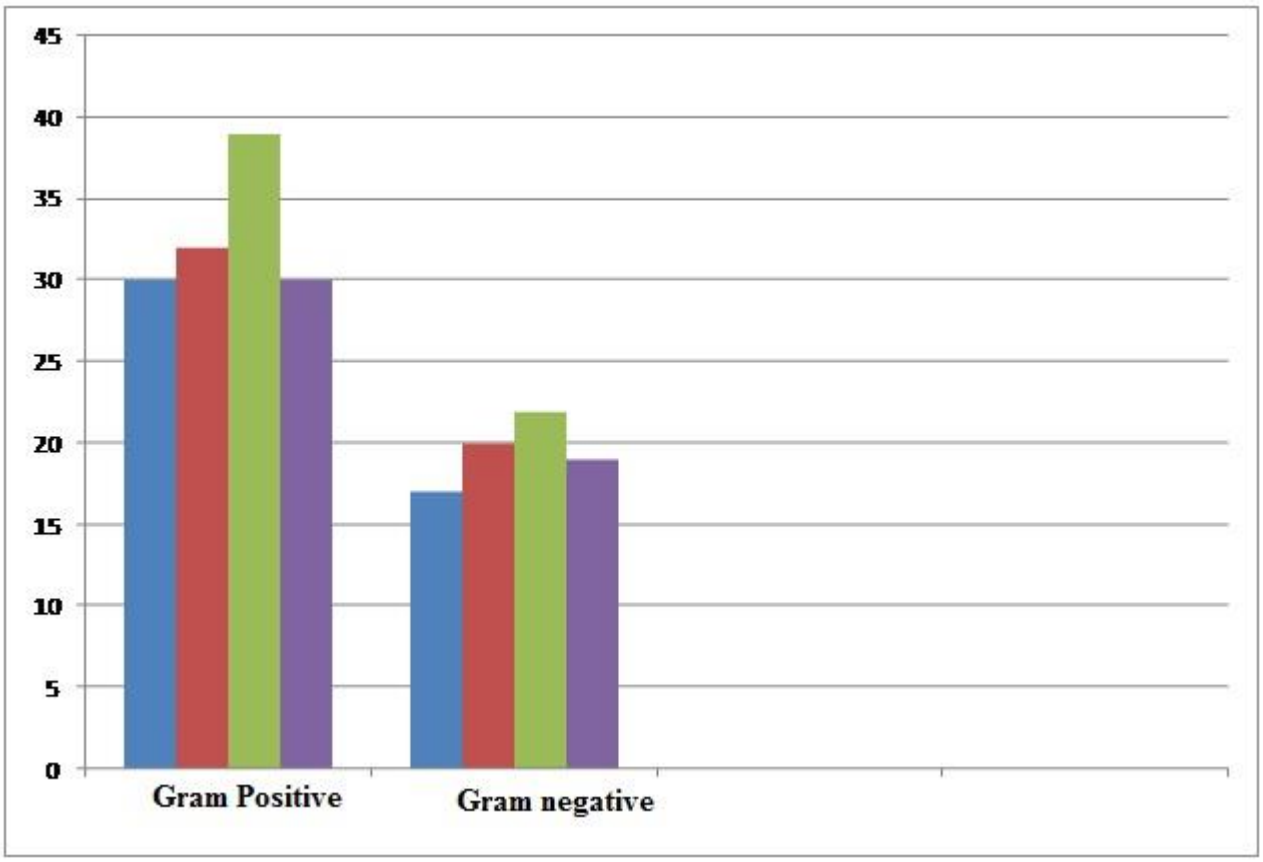
Gram-positive Bacteria (Strain)	*Amaranths spinosus* L.(January – February) 120 µg per disc	*Amaranthus spinosus* L.(March – April) 120 µg per disc	*Amaranthus spinosus* L.(May – June) 120 µg per disc	*Amaranthus spinosus* L.(July – August) 120 µg per disc	*Amaranthus spinosus* L.(September – October) 120 µg per disc	*Amaranthus spinosus* L. (November-December) 120 µg per disc
Bacillus subtilis (ATCC 19659)	10 ± 0.3	19 ± 0.6	30 ± 0.7	25 ± 1.7	20 ± 0.5	18 ± 0.2
Bacillus megaterium (NBMC 1122)	15 ± 0.1	21 ± 0.3	32 ± 0.4	26 ± 1.3	22 ± 0.4	19 ± 0.5
Staphylococcus aureus (ATCC 25923)	20 ± 1.1	29 ± 1.4	39 ± 1.9	30 ± 1.4	26 ± 1.3	22 ± 1.1
Streptococcus pyogenes (NBMC 1321)	12± 0.2	15± 0.7	30± 0.7	27± 1.1	18± 0.1	15± 0.3

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.

Table – 2: Showing seasonal variation in the *in vitro* anti bacterial activity of leaves of *Amaranthus spinosus* L. against four Gram- negative bacteria.

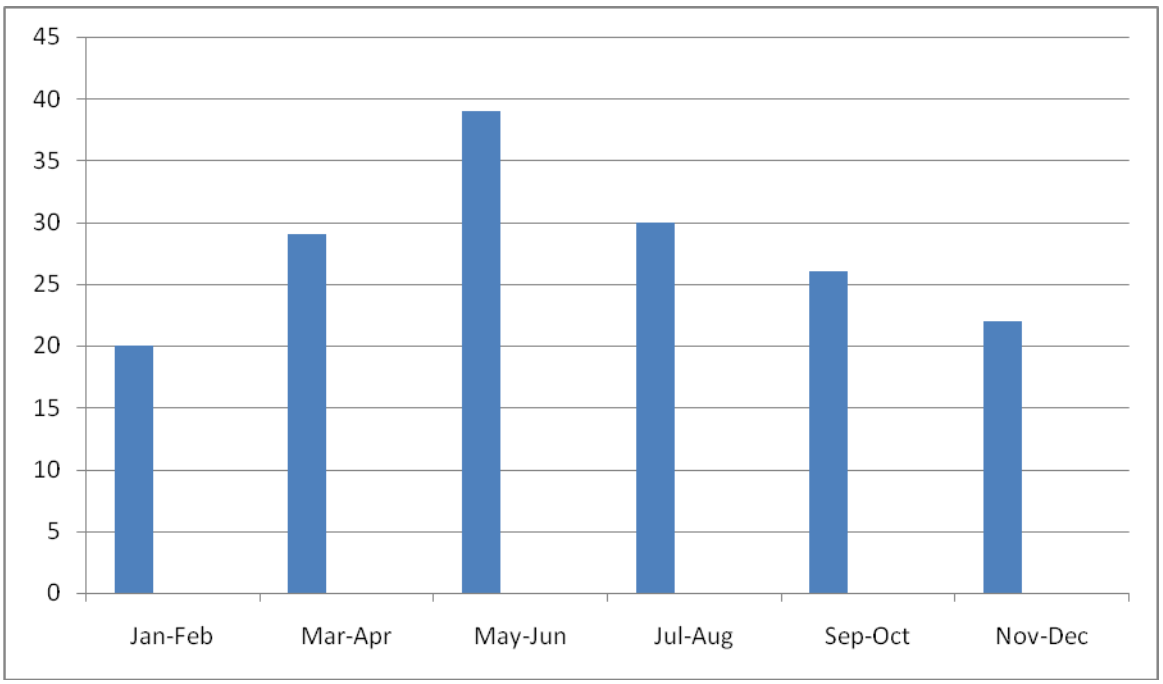
Gram-negative Bacteria (Strain)	*Amaranths spinosus* L.(January – February) 120 µg per disc	*Amaranthus spinosus* L.(March – April) 120 µg per disc	*Amaranthus spinosus* L.(May – June) 120 µg per disc	*Amaranthus spinosus* L.(July – August) 120 µg per disc	*Amaranthus spinosus* L.(September – October) 120 µg per disc	*Amaranthus spinosus* L.(November-December) 120 µg per disc
Escherichia coli (ATCC 25922)	9 ± 0.2	12 ± 0.5	17 ± 0.5	15 ± 0.5	14 ± 0.1	12 ± 0.2
Shigella dysenteriae (NBMC 1127)	11 ± 0.1	15 ± 0.3	20 ± 1.1	17 ± 1.1	16 ± 0.5	14 ± 0.3
Pseudomonas aeruginosa (NBMC 1243)	12 ± 0.2	17 ± 0.6	22 ± 0.8	20 ± 1.2	19 ± 0.5	16 ± 0.2
Salmonella typhi (MTCC 733)	11 ± 0.2	14 ± 0.3	19 ± 0.7	19 ± 0.5	15 ± 0.4	14 ± 0.3

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.



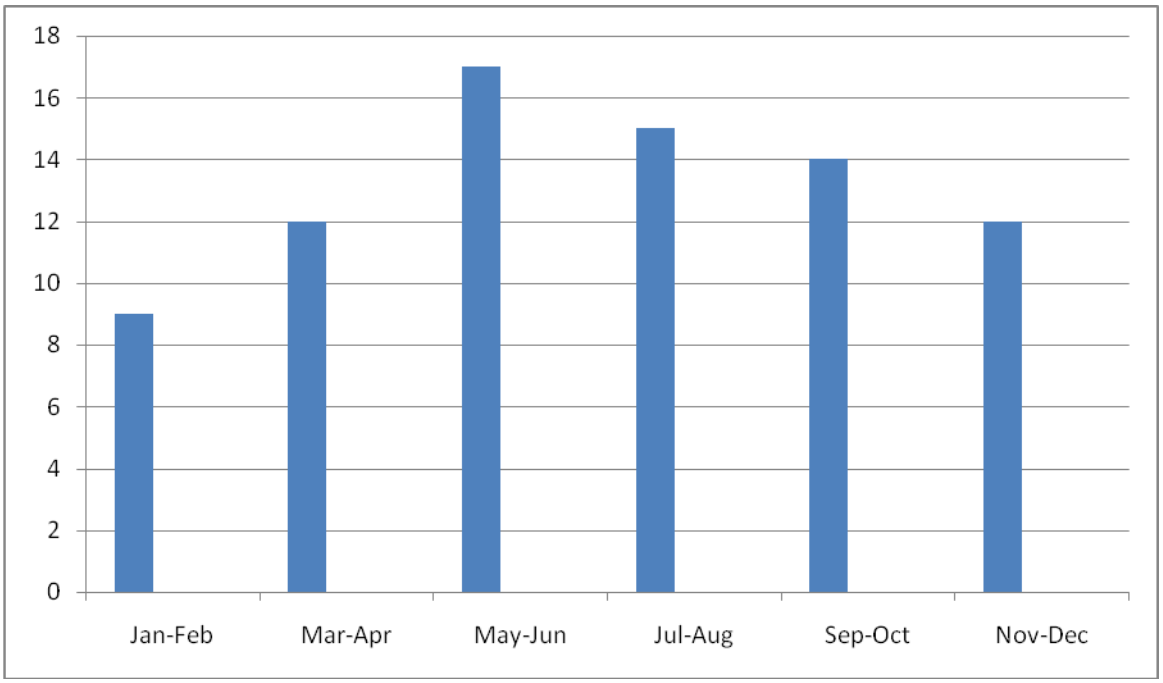
Gram positive bacteria: ■*Bacillus subtilis* ■*Bacillus megaterium* ■*Staphylococcus aureus* ■*Streptococcus pyogenes*
Gram negative bacteria: ■*Escherichia coli* ■*Shigella dysenteriae* ■*Pseudomonas aeruginosa* ■*Salmonella typhi*

Fig 2 : Anti bacterial effect in terms of zone of inhibition in mm.by *Amaranthus spinosus* L. leaves against four Gram positive and four Gram negative bacteria. Maximum and minimum zones of inhibition were found in *Staphylococcus aureus* and *Escherichia coli* respectively.



■Anti bacterial effect in terms of zone of inhibition in mm;

Fig 3 : Seasonal variation in *in vitro* anti bacterial effect of *Amaranthus spinosus* L. leaves Against *Staphylococcus aureus*



■Anti bacterial effect in terms of zone of inhibition in mm.

Fig 4 : Seasonal variation in *in vitro* anti bacterial effect of *Amaranthus spinosus* L. leaves Against *Escherichia coli*

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