

Effect of Season on *In-vitro* Anti Oxidant Activity of *Astilberivularis* Buch – Ham. Ex D. Don leaves

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Abstract

Effect of season on *in vitro* anti oxidant activity of *Astilberivularis* Buch. – Ham. Ex D. Don leaves was studied. Leaves of *Astilberivularis* Buch. – Ham. Ex D. Don were collected in different seasons and *in vitro* anti oxidant activity of the leaves was measured by superoxide anion generation with the help of xanthine-xanthine oxidase assay and with linoleic acid peroxidation assay as well as by DPPH photometric assay. Total phenol, flavonoids, ascorbic acid and carotenoid contents in the leaves of different seasons were also estimated. Results showed that *in vitro* anti oxidant activity of the leaves of *Astilberivularis* Buch. – Ham. Ex D. Don was found maximum during rainy season (June – July). Anti oxidant compounds in leaves like total phenol, flavonoids, ascorbic acid and carotenoids were also present in maximum amount during rainy season. Anti oxidant activity, therefore, was related with high content of total phenol, flavonoid, ascorbic acid and carotenoids in the leaves. Leaves of *Astilberivularis* Buch. – Ham. Ex D. Don of rainy season should be used to get maximum anti oxidant activity.

Keywords: *Astilberivularis*, Buch. – Ham. Ex D. Don; Anti oxidant activity; Total phenol; Flavonoid; Ascorbic acid; Carotenoids; Effect of season

Introduction

As early as 1955 Fluck and Pharm showed influence of climate on the active principles in medicinal plants. Thereafter, numerous experiments were conducted in this direction showing influence of season on concentration of active compounds in plants [1-5]. We have also [1] undertaken series of experiments on seasonal variation in amounts of active metabolites in plants and noted that season can change concentration of bio active compounds in different parts of the plants [6-10]. *Astilberivularis*, Buch. – Ham. Ex D. Don (*A. rivularis*) is one of the medicinal plants of Sikkim Himalaya. The plant has different names like Buriokahti and Pango in Nepali and in Lepcha respectively [11]. *A. rivularis* is distributed at a range of 5000 – 9000 feet in Common Temperate of Himalaya and is also found on sloppy waste place. The plant has tall herb stem and leaves are covered with hairs [12].

A. rivularis has many medicinal uses. In traditional medicine juice of the plant is applied to sprains and muscular swelling. Rhizome of this plant is used in curing haemorrhages, diarrhoea, dysentery, headache, prolapse of uterus and to improve fertility [13]. The plant has anti viral activity [14]. We found anti peptic ulcer activity of *A. rivularis* leaves in experimental animals [15].

A. rivularis is known in literature for its anti oxidant activity too [16]. We also confirmed anti oxidant activity of *A. rivularis* grown in this region [17]. In this communication effect of season on *in vitro* anti oxidant activity of *A. rivularis* leaves is being reported.

Experimental

Plant material

A. rivularis leaves were collected from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India during Autumn (August – September), Post Autumn (October – November), Winter (December – January), Spring (February – March), Summer (April-May) and in rainy season (June – July) at about 10 am. Leaves were authenticated by the experts of the department of Botany of the said university. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of the Sikkim Manipal University, Gangtok, Sikkim, India for future references [2].

Test material

Collected leaves of *A. rivularis* were shade dried and powdered. The powder was used as test material.

Chemicals

Chemicals required for the study were purchased from Loba Chem. Lab, Himedia Lab, India and from Merck, Germany

Antioxidant assays

Antioxidant activity of *A. rivularis* leaves of different seasons was assayed by superoxide anion generation by xanthine-/xanthine oxidase assay [18], linoleic acid peroxidation assay [19] and by DPPH photometric assay [20].

Flavonoids content: Flavonoids content of *A. rivularis* leaves of different seasons was determined using Aluminum chloride colorimetric method [21].

Total phenols content: Total phenols content of *A. rivularis* leaves of different seasons was determined by Folin Ciocalteu reagent [22].

Ascorbic acid content: Ascorbic acid content of *A. rivularis* leaves of different seasons was determined by the method of Cakmak and Marschner [23].

Carotenoids content: Total carotenoids of *A. rivularis* leaves of different seasons was determined by the method of Jensen [24].

Statistical Analysis

The statistical significance between antioxidant activity values of the extracts was evaluated with a Duncan's multiple range test (DMRT) at 5 % were considered to be statistically significant [25].

Observations and Results

Table 1: Effect of seasons on inhibitory activity of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by powdered leaves of *A. rivularis*.

Leaves of <i>A. rivularis</i> of different seasons	Xanthine oxidase (% inhibition)	Linoleic acid peroxidation (% inhibition)	DPPH (% inhibition)
Summer (April – May)	51 ± 1.2	57 ± 1.4	48 ± 1.1
Rainy (June - July)	93 ± 1.8*	98 ± 2.0*	96 ± 1.9*
Autumn (August - September)	48 ± 1.1	46 ± 1.1	50 ± 1.2
Post Autumn (October – November)	35 ± 0.9	39 ± 1.0	38 ± 0.8
Winter (December – January)	30 ± 0.8	30 ± 0.9	31 ± 1.0
Spring (February – March)	37 ± 0.9	40 ± 1.1	40 ± 0.9
Quercetin	100 ± 0.01	87 ± 1.0	100 ± 0.01

Effect of seasons on *in vitro* antioxidant activity of powdered leaves of *A. rivularis* through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay was given in (Table 1). Concentration used: 100 µg / ml. Dose was fixed based on our earlier report [17]. Results were a mean of triplicate experiments ± SD. (Table 1) shows that powdered leaves of *A. rivularis* of different

seasons had more or less *in vitro* anti oxidant activity but maximum activity was found during rainy season (June – July). Inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH were found 93%, 98% and 96% respectively. Results were comparable to that of quercetin, a known anti oxidant compound, where inhibition in both xanthine oxidase and DPPH came 100%.

Table 2: Effect of seasons on total phenol, flavonoids, ascorbic acid and carotenoids content of the powdered leaves of *A. rivularis*.

Leaves of <i>A. rivularis</i> of different seasons	Total phenol content (mg/mg dry wt)	Total flavonoids content (mg/mg dry wt)	Ascorbic acid content (mg/g dry wt)	Carotenoids content (mg/g dry wt)
Summer (April – May)	33 ± 1.0	54 ± 1.0	27 ± 0.8	12.8 ± 0.8
Rainy (June - July)	51 ± 1.2*	76 ± 1.2*	32 ± 1.0*	17.8 ± 1.0*
Autumn (August - September)	38 ± 1.1	60 ± 1.1	25 ± 0.7	11.7 ± 0.7
Post Autumn (October – November)	30 ± 0.8	45 ± 0.9	20 ± 0.5	8.9 ± 0.7
Winter (December – January)	23 ± 1.0	40 ± 0.9	15 ± 0.4	7.0 ± 0.5
Spring (February – March)	25 ± 0.9	48 ± 1.0	20 ± 0.5	10.6 ± 0.9

Results were a mean of triplicate experiments ± SD.

Table 2 shows that during rainy season (June – July) total phenol content of the leaves of *A. rivularis* was 51 ± 1.2 mg/mg dry wt of the leaves. This was maximum when compared to total phenol content of the leaves in other seasons of the year. During August – September (Autumn), October – November (Post Autumn), December – January (Winter), February – March (Spring), April – May (Summer) total phenol content of the leaves were 38 ± 1.1, 30 ± 0.8, 23 ± 1.0, 25 ± 0.9 and 33 ± 1.0 mg/mg dry wt respectively. Same trend was found in total flavonoids, ascorbic acid and in carotenoids content of *A. rivularis* leaves. All these anti oxidant chemicals were found in maximum amount in leaves during rainy season [5].

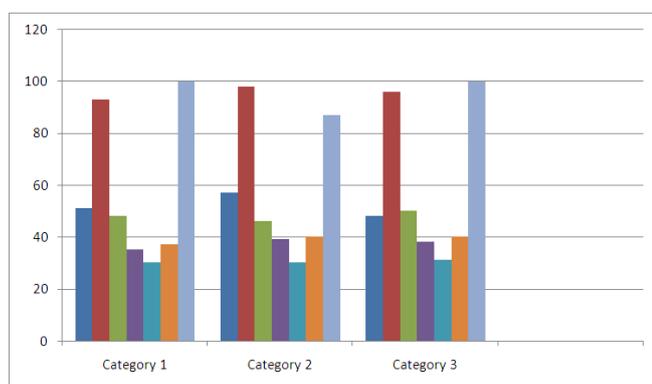
Discussion

Many plants are known possessing anti oxidant activity [26] and the anti oxidant activity is influenced by season [27]. Recently we have noted *in vitro* anti oxidant activity of *A. rivularis* leaves [17]. In present study effect of season on *in vitro* anti oxidant activity of *A. rivularis* leaves was studied (Figure 1). Results showed that *in vitro* anti oxidant activity of *A. rivularis* leaves in terms of inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH was maximum during rainy season (June – July) and the anti oxidant activity was comparable to that of synthetic anti oxidant quercetin (Figure 2). Aysel and Sevcan in 2014 showed that antioxidant activity of *Prunus amygdalus L.* reached the highest value in April

for leaves whereas in October for stems [28]. Bahmanzadegan et al. [28] demonstrated the best antioxidant activity of *Laurusnobilis* was in spring and the lowest one was in winter [29].



Figure 1: *Astilberivularis* Buch. - Ham. Ex D. Don.



■ April - May; ■ June - July; ■ August - September; ■ October - November; ■ December - January; ■ February - March; ■ Quercetin; Category 1: Xanthine oxidase (% Inhibition); Category 2: Linoleic acid per oxidation (% Inhibition); Category 3: DPPH (% Inhibition)

Figure 2: Showing % inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by powdered leaves of *A. rivularis* in different seasons.

Anti oxidant activity of medicinal plant is mainly due to presence of phenolic compounds, flavonoids, ascorbic acid and carotenoids. These chemicals are responsible for multiple biological effects like free radical scavenging abilities, anti inflammatory and anti carcinogenic activities [30]. We, therefore, studied effect of season on these anti oxidant compounds in *Arivularis* leaves. Results (Figure 3) showed that amounts of phenolic compounds, flavonoids, ascorbic acid and carotenoids in the plant leaf were maximum during rainy season (June - July). In 2014 Aysel and Sevcin found that highest level of total phenolic compounds in *Prunusamygdalus L.* was in January for stems while in October for leaves [27]. *In vitro* anti oxidant activity of *A.rivularis* leaves during June - July was, therefore, due to accumulation of maximum amount of phenolic compounds, flavonoids ascorbic acid and carotenoids in the plant leaves though commercially available and commonly used in

processed food, are not safe. Their toxicity is also matter of concern. It is often claimed that these synthetic anti oxidants have many side effect including carcinogenic activity [30]. Therefore, there are high demands for naturally occurring anti oxidants. As the present study indicates that June - July (Rainy season) is the period when *A.rivularis* leaves showed maximum *in vitro* anti oxidant activity, plant leaves of that period may be used as natural anti oxidant.

Conclusion

Effect of season on *in vitro* anti oxidant activity of *A.rivularis* leaves was studied. Results showed that *in vitro* anti oxidant activity of *A.rivularis* leaves was maximum during rainy season (June - July). The anti oxidant effect was due to presence of maximum amount of phenols, flavonoids, ascorbic acid and carotenoids in the leaves during that period. *A.rivularis* leaves of rainy season may be used as natural antioxidant.

References

- H Fluck, M Pharm (1955) The influence of climate on the active principles in medicinal plants. *J Pharm Pharmacol* 7: 361-383.
- P L Gupta (1977) Variation in morphological characters and active principle constituents of *E. prostrata* Linn. under different seasonal and soil conditions. *JRIM* 12(1): 80-84.
- J C Schultz, P J Nothnagle, I T Baldwin (1982) Seasonal and individual variation in leaf quality of two northern hardwoods tree species. *American Journal of Botany* 69(5): 753-759.
- Y Mauffette, W C Oechel (1989) Seasonal variation in leaf chemistry of the coast live oak *Quercusagrifolia* and implications for the California oak moth. *Phryganidia californica* *Oecologia* 79: 439-445.
- Atefeh Bahmanzadegan, Vahid Rowshan, Faraneh Zareian, Reza Alizadeh, Mohammad Bahmanzadegan (2015) Seasonal Variation in Volatile Oil, Polyphenol Content and Antioxidan Activity in Extract of *Laurusnobilis* Grown in Iran. *Journal of Pharmacy and Pharmacology* 3: 223-231.
- Prasanta Kumar Mitra (2014) *In vitro* antibacterial activity of leaves of *Amaranthus spinosus* L: Seasonal variation. *World Journal of Pharmaceutical Sciences* 2(12): 1702-1706.
- Tanaya Ghosh, Prasenjit Mitra, Dilip Kumar Jha, Prasanta Kumar Mitra (2015) A study on body weight loss in rats by the leaves of *Abrusprecatorius* Linnaeus: Effect of season. *International Journal of Pharmacy & Therapeutics* 6: 64-68.
- Prasenjit Mitra, Tanaya Ghosh, Prasanta Kumar Mitra (2016) Seasonal variation in anti ulcerogenic activity of *ageratum conyzoides* l. leaves. *International Journal of Biopharmaceutics* 7(2): 63-68.
- Prasanta Kumar Mitra, Tanaya Ghosh, Prasenjit Mitra (2016) *In vitro* anti thiamine effect of *murrykoenigii* (linn.) sprengwettst leaves: effect of season. *International Journal of Biopharmaceutics* 7(2): 69-72.
- Prasenjit Mitra, Tanaya Ghosh, Prasanta Kumar Mitra (2017) Effect of Season on *In vitro* Anti Oxidant Activity of *Aageratumconyzoides L.* Leaves. *SMU Medical Journal* 4(2): 222-233.
- Bejoy Gurung (2002) The medicinal plants of Sikkim Himalaya. Pub. BejoyGurung, East Sikkim, P. 55.
- A P Das, Chandra Ghosh (2009) Germplasm collection in garden of medicinal plants. University of North Bengal, Siliguri, West Bengal, P. 8.
- N P Manandhar (2002) Plants and people of Nepal. Timber Press, Inc. Oregon, USA, P.29
- M Rajbhandari, M R Mentel, P K Jha, R P Chaudhary, S Bhattarai, M B Gewali, N Karmacharya, U Hipper Mand, R Lindequist (2009) Antiviral activity of some plants used in Nepalese traditional medicine. *eCAM* 6(4): 517-522.

15. P Mitra, P K Mitra (2008) Use of *Astilberivularis* Buch. – Ham. Ex D. Don as anti peptic ulcer agent, *Pleione* 2(1): 74-76.
16. Palash Mandal, T K Sen (2009) Remove from marked Records *Invitro* antioxidant potential of *Astilberivularis* rhizome. *Canadian Journal of Pure & Applied Sciences* 3(1): 649-654.
17. Prasanta Kumar Mitra, Tanaya Ghosh, Prasenjit Mitra (2016) *Invitro* anti oxidant activity of chromatographically separated fractions from the leaves of *Astilberivularis* buch. – Ham. Ex D. Don. *SMU Medical Journal* 3(2): 226-239.
18. W S Chang, Y H Chang, F J Lu, H C Chiang (1994) Inhibitory effects of phenolics on xanthine oxidase. *Anticancer Res* 14: 501-506.
19. W Chang, a b Choi, C Sei, a b Kim, S Soon, et al. (2002) Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science* 163: 1161-1168.
20. L L Mensor, F S Menezes, G G Leita, A S Reis, T C dos Santos, et al. (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res* 15: 127-130.
21. C Chang, M Yang, H Wen, JChern (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Analysis* 10: 178-182.
22. S McDonald, PD Prenzler, M Autolovich, K Robards (2001) Phenolic content and antioxidant activity of olive extracts. *Food Chemistry* 73: 73-84.
23. I Cakmak, H Marschner (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant Physiol* 98:1222-1227.
24. A Jensen (1978) Chlorophyll and carotenoids. In: Hallebust JA, Craigie JS. (eds). *Handbook of Physiochemical and Biochemical Methods*. Cambridge University Press, Cambridge, UK, pp. 5-70.
25. C I Bliss (1967) *Statistics in biology, Statistical methods for research in the natural sciences*, Vol. 1, McGraw Hill Book Company, NY, pp. 558.
26. Nithya Narayana swamy, K P Bala krrishnan (2011) Evaluation of some medicinal plants for their antioxidant properties. *Int J Pharm Tech Research* 3(1): 381-385.
27. AyselSivaci, Sevcan Duman (2014) Evaluation of seasonal antioxidant activity and total phenolic compounds in stems and leaves of some almond (*Prunusamygdalus L.*) varieties. *Biological Research* 47(9) : 1-5.
28. Atefeh Bahmanzadegan, Vahid Rowshan, Faraneh Zareian, Reza Alizadeh, Mohammad Bahmanzadegan (2015) Seasonal Variation in Volatile Oil, Polyphenol Content and Antioxidan Activity in Extract of *Laurusnobilis* Grown in Iran. *Journal of Pharmacy and Pharmacology* 3: 223-231.
29. A L Miller (1996) Antioxidant flavonoids: structure, function and clinical usage. *Alt Med Rev* 1: 103-110.
30. A L Branen (1975) Synthetic anti oxidants. *Journal of American Oil Chemist Society* 52: 59-63. Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal



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