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Effect of Season on *In vitro* Anti Oxidant Activity of A*ageratum conyzoides* L. Leaves

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Abstract

Effect of season on in vitro anti oxidant activity of A*ageratum conyzoides* Linn (*A. conyzoides* L.) leaves was studied. A. *conyzoides* L. leaves were collected in different seasons and its in vitro antioxidant activity was checked by superoxide anion generation with the help of xanthine-xanthine oxidase assay and with linoleic acid peroxidation assay as well as DPPH photometric assay. Total phenol, flavonoids, ascorbic acid and carotenoids contents of *A. conyzoides* L. leaves in different seasons were also estimated. Results showed that leaves of *A. conyzoides* L. of the month July and August had maximum in vitro anti oxidant activity.

Anti oxidant activity was related with high content of total phenol, flavonoid and ascorbic acid in the leaves .

Keywords: *Ageratum conyzoides* L. leaves, anti oxidant activity, xanthine-xanthine oxidase assay, linoleic acid peroxidation assay, DPPH photometric assay, total phenol, flavonoids

Introduction

A. conyzoides L. (family, Asteraceae) is a plant that grows commonly in the proximity of habitation, thrives in any garden soil and is very common in waste places and on ruined sites [1]. The plant is distributed throughout India, lower and middle hill in Sikkim and Darjeeling up to 6000 ft. *A. conyzoides* L. has erect hairy annual 30 – 90 cm high leaves. Different vernacular names are given to the plant. In Nepali the plant is called as 'Elame'; in Lepcha 'Namyew' and in English the plant is known as 'Goat weed'. Throughout the year the plant gives flower. Purple white flower appears.

A. conyzoides L. is a medicinal plant. The medicinal value of this plant in treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita [2]. Leaves, root, stem and flower of *A. conyzoides* L. are widely utilized in traditional medicine. Leaves are styptic effective in healing of wounds, used in boils and prevent tetanus. Leaf juice is also used as eye lotion. The root juice has antibiotic property. The plant is boiled with oil and applied externally in rheumatism.

Phytochemical screening showed that *A. conyzoides* L. contains tanins, saponin, resins, alkaloids, glycosides, flavonoids, ascorbic acid etc. It also contains many different compounds like caffeic acid, fumeric acid, kaempferol, quercetin, stigma-7-en-3-ol, scutellarein, chromane, pyrrolizidinic alkaloids, hexamethoxy flavone, coumarinic compoundssuch as licopsamine, benzopirone, licopsamine,disifropirrrolizidinic acid etc. Phenol, essential oil, friedolin, sitosterol, stigmasterol and unidentified esters are active components of *A. conyzoides* L. [3-6]

Modern researchers claimed that *A. conyzoides* L. has antibacterial [7] and wound

healing effect [8]. It has neurological activity [9] and possesses gastro protective effect [10]. The plant acts as analgesic [11] and has effect on circulation [12]. It gives protection against gamma radiation [13]. The plant has anti tumor activity [14] and has allopathic effects [15]. Ita *et al.* (2009) demonstrated hepato protective activity of this plant [16].

Anti oxidant activity of plant *A. conyzoides* L. is known in literature [17-19]. The present work was aimed to see effect of season on in vitro anti oxidant activity of *A. conyzoides* L. leaves.

Materials and methods

Collection of plant material

A. conyzoides L. leaves were collected in morning hours (9 - 10 AM) from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India randomly during the periods of January – February, March – April, May – June, July – August, September – October and November – December, 2012. Leaves were authenticated by the experts 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 In vitro Antioxidant activity

Leaves of *A. conyzoides* L. collected randomly in different seasons were shed dried and powdered. This powder was used to note its in vitro anti oxidant activity.

Antioxidant assays

In vitro anti oxidant activity of *A. conyzoides* L. leaves was assayed through superoxide anion generation by xanthine- xanthine oxidase assay [20], linoleic acid peroxidation assay [21] and by DPPH photometric assay [22].

Fig.1: *Ageratum conyzoides* L.

Flavonoids content

Flavonoids content of *A. conyzoides* L. leaves powder was determined using Aluminum chloride colorimetric method [23].

Total phenols content

Total phenols content of *A. conyzoides* L. leaves powder was determined by Folin Ciocalteu reagent [24].

Ascorbic acid content

Ascorbic acid content of *A. conyzoides* L. leaves powder was determined by the method of Cakmak and Marschner [25].

Carotenoids content

Total carotenoids of *A. conyzoides* L. leaves powder were determined by the method of Jensen[26]

Chemicals

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

Statistical analysis

The statistical significance between antioxidant activity values of the powdered leaves of *A. conyzoides* L. was evaluated with a Duncan's multiple range test (DMRT). 5 % was considered to be statistically significant [27].

Results

Results on in vitro antioxidant activity of powdered leaves of *A. conyzoides* L. in different seasons through superoxide anion generation by xanthine- xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay were given in Table. 1

Table 1: Inhibitory activity of xanthine oxidation and linoleic acid peroxidation and scavenging capacity of DPPH by powdered leaves of A*ageratum conyzoides* L. in different seasons.

Leaves of A*ageratum conyzoides* L.	**Xanthine oxidase (% inhibition)**	**Linoleic acid peroxidation (% inhibition)**	**DPPH (% inhibition)**
January-February	22 ± 0.8	28 ± 0.9	25 ± 0.7
March-April	32 ± 1.0	37 ± 1.1	36 ± 1.1
May-June	45 ± 1.2	42 ± 1`.1	48 ± 1.3
July-August	97 ± 2.1*	95 ± 2.5*	96 ±2.3 *
September-October	48 ± 1.3	50 ± 1.3	49 ± 1.1
November-December	30 ± 0.9	39 ± 1.1	37 ± 0.9
Quercetin	100 ± 0.01	86 ± 1.1	100 ± 0.01

Concentration used : 100 μg / ml . Results were a mean of triplicate experiments ± SD .
*Significant

It appears from the table that powdered leaves of *A. conyzoides* L. of different seasons had more or less in vitro anti oxidant activity but maximum activity was found during July –

August. Inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH were found 97%, 95% and 96% respectively. Results were comparable with that of quercetin where both inhibition in xanthine oxidase and DPPH came 100%.

Table 2: Total phenol, flavonoids, ascorbic acid and carotenoids content of the powdered leaves of A*ageratum conyzoides* L. in different seasons.

Leaves of A*ageratum conyzoides* L.	**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)**
January-February	12 ± 0.7	25 ± 0.8	8 ± 0.5	7.8 ± 0.5
March-April	29 ± 1.0	33 ± 1.2	16 ± 1.1	7.8 ± 0.7
May-June	33 ± 1.2	47 ± 1.1	20 ± 1.0	7.7 ± 0.6
July-August	50 ± 1.3*	75 ± 2.4*	29 ±1.3 *	7.9 ± 0.8
September-October	31 ± 1.1	51 ± 2.1	15 ± 1.0	7.7 ± 0.8
November-December	20 ± 0.9	40 ± 1.1	10 ± 0.9	7.6 ± 0.5

Results were a mean of triplicate experiments ± SD .
*Significant

Table – 2 shows that total phenol content of the leaves of *A. conyzoides* L. was 50 ± 1.3 mg/mg dry wt of the leaves powder during July – August which was maximum in comparison to other time of the year. During January-February, March-April, May-June, September-October and November-December total phenol content of the leaves were 12 ± 0.7, 29 ± 1.0, 33 ± 1.2, 31 ± 1.1 and 20 ± 0.9 mg/mg dry wt respectively. Same trend was found in total flavonoids and ascorbic acid contents which were also maximum during July-August. Carotenoids content of the leaves of *A. conyzoides* L, however, was found more or less same throughout the year.

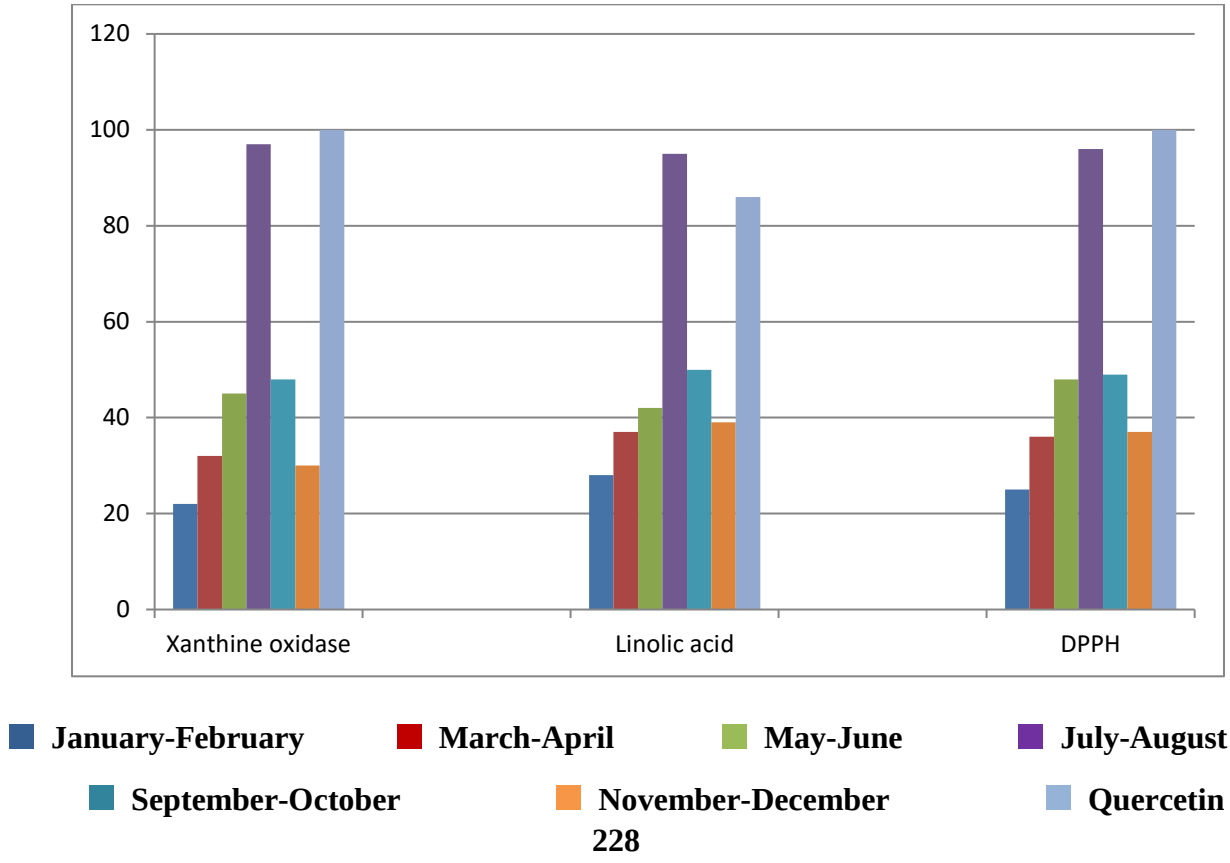
Discussion

Fluck and Pharm [28] for the first time showed influence of climate on the active principles in medicinal plants. Thereafter, series of experiments were conducted in this

direction. Now a days numerous reports are available in literature which suggest that accumulation of chemical compounds in roots, stem and leaves of plants varies with season [29-33].

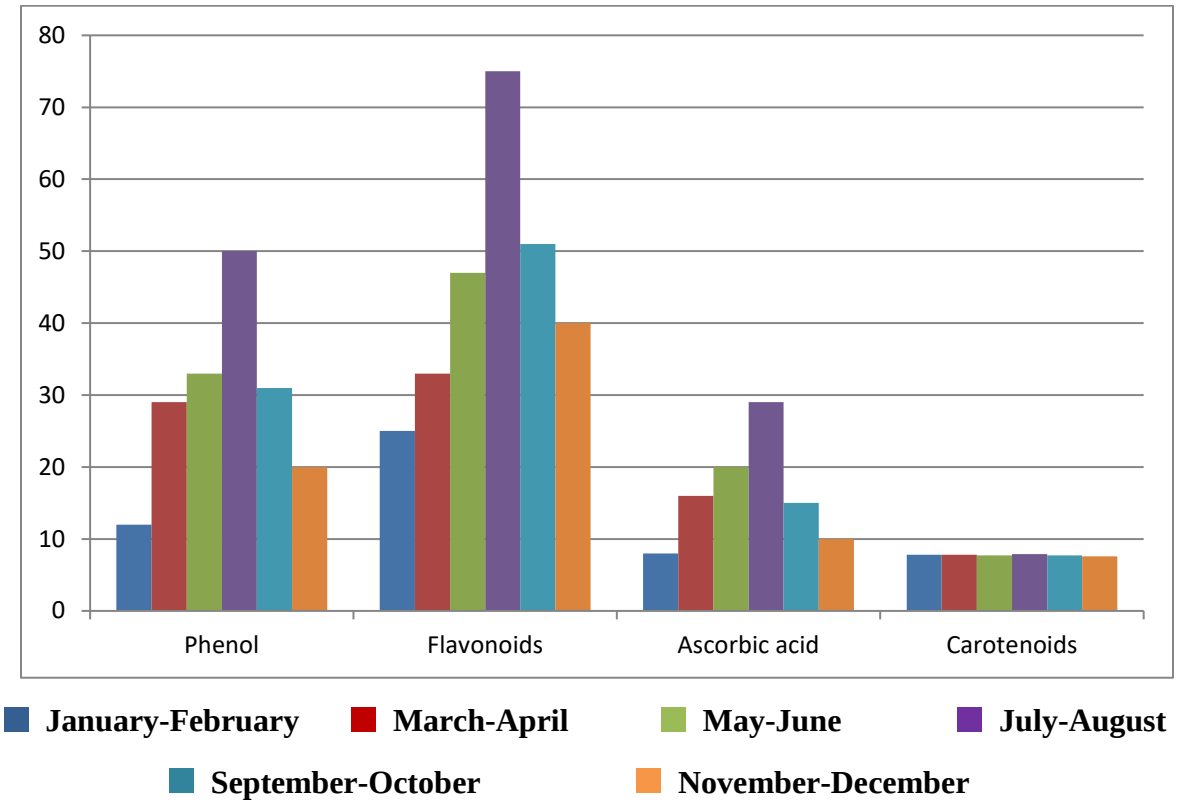
In the present study effect of season on in vitro anti oxidant activity of A. *conyzoides* L. leaves was studied. Results showed that in vitro anti oxidant activity of A. *conyzoides* L. leaves in terms of inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH was maximum during July-August 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 [34]. Bahmanzadegan et al. in 2015 demonstrated the best antioxidant activity of *Laurus nobilis* was in spring and the lowest one was in winter [35].

Figure 2: Showing % inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by powdered leaves of A*ageratum conyzoides* L. 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 [36]. We, therefore, studied effect of season on these chemical compounds in *A.conyzoides* L. leaves. Results showed that amounts of phenolic compounds, flavonoids and ascorbic acid in the plant leave were maximum during July-August. In 2014 Aysel and Sevcan found that highest level of total phenolic compounds in *Prunus amygdalus* L.was in January for stems while in October for leaves [34] . We, however, noted that concentration of carotenoids in *A. conyzoides* L. leaves remained same throughout the year (Figure – 3). In vitro anti oxidant activity of *A.conyzoides* L. leaves during July-August was, therefore, due to accumulation of maximum amount of phenolic compounds, flavonoids and ascorbic acid in the plant leaves.

Figure 3: Total phenol, flavonoids, ascorbic acid and carotenoids content of the powdered leaves of A*geratum conyzoides* L. in different seasons.



There are high demands for naturally occurring anti oxidants as synthetic anti oxidants like butylated hydroxyanisole and butylated hydroxytoluene, 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 [37]. As the present study indicates that July – August is the period when *A. conyzoides* L. 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. conyzoides* L. leaves was studied. It revealed that in vitro anti oxidant activity of *A. conyzoides* L. leaves was maximum during July –August. The anti oxidant effect was due to presence of maximum amount of phenols, flavonoids and ascorbic acid in the leaves during that period. *A. conyzoides* L. leaves of July – August may be used as natural antioxidant.

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