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SEASONAL EFFECT ON *IN VITRO* ANTIOXIDANT ACTIVITY OF *ABUTILON INDICUM* LINN LEAVES

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ABSTRACT

Effect of season on *in vitro* antioxidant activity of *Abutilon indicum* Linn *(A. indicum* L.*)* leaves was studied. Leaves of *A. indicum* L. of different seasons were collected from the medicinal plant garden of the North Bengal University and identified by the taxonomist. *In vitro* antioxidant activity of the leaves was measured by superoxide anion generation with the help of xanthine-xanthine oxidase assay, linoleic acid peroxidation assay and DPPH photometric assay. Amount of total phenols present in the leaves of different seasons was also estimated. Results showed that *in vitro* antioxidant activity of the leaves of *A. indicum* L. was maximum during summer (March – May) in comparison to other seasons of the year. Amount of total phenols present in the leaves was also found maximum in summer. *In vitro* antioxidant activity of *A. indicum* L. leaves, therefore, was due to high amount of total phenols in the leaves. Leaves of *A. indicum* L. of summer may be further investigated to get natural antioxidant compound.

KEYWORDS: *Abutilon indicum* Linn leaves, *in vitro* antioxidant activity; total phenols, effect of season.

1. INTRODUCTION

Biological activities of plants vary with seasons of the year. Qinxue *et al.* studied seasonal variations in the antioxidant activity of ground bamboo *Sasa argenteastriatus* Leaves. They noted that the highest antioxidant activity appeared in December and the lowest was in May.[1] Effect of seasonal variation on the antineoplastic activity of *Alstonias cholaris* R. Br. in HeLa cells was studied by Jagetia and Baliga. Highest cell killing effect was observed by

the plant of summer collection.[2] Osadebe *et al.* worked on seasonal variation for the antidiabetic activity of methanolic extract of *Loranthus micranthus* and noted that the activity is highest at the peak of the rainy season.[3] Ncube *et al*. studied seasonal variation in antimicrobial activity of frequently used medicinal bulbous plants from South Africa and noted that the activity was higher in spring and winter than in other seasons.[4] Effect of seasonal variation on the anti-inflammatory activity of *Sargassum wightii* was studied by Dar and coworkers. They found that the plant collected during winter was most effective in reducing carrageenan-induced edema in rats.[5] Report from our laboratory showed that *Cassia alata* leaves during the period of May – June had maximum protective effect on anti tubercular drugs induced hepatotoxicity in rats.[6] We also reported that UV absorption property of *Amaranthus spinosus* was maximum during autumn in comparison to other seasons of the year.[7]

A. indicum L. *(*Family: *Malvaceae),* commonly known as Abutilon, is a medicinal plant. The plant is used in traditional medicine for treatment of toothache, catarrhal bilious, bronchitis, diarrhoea, gonorrhoea and inflammation of bladder as well as in fever.[8] The plant contains various bioactive materials like terpenes, hydrocarbon, flavonoids, amino acids, ketone, aldehyde, fatty acids like stearic, palmitic linoleic, oleic acid, endesmol, α-pinene, caryophyllene, caryophyllene oxide, apigenin 7-O-beta-glucopyranoside, quercetin 3-O-beta-glucopyranoside, luteolin, chrysoeriol, luteolin 7-O-beta glucopyranoside, chrysoeriol 7-O-betaglucopyranoside, quercetin 3-Oalpha- rhamnopyranosyl (1 --> 6)-beta-glucopyranoside.[9] The plant has various pharmacological activities such as anti cancer, anti diabetic, antipyretic, anti oxidant, antifertility, anticholinestrase, anti bacterial, anti fungal, anti-inflammatory, antihelminthic, hepatopretective, hypolipidemic, adaptogenic activities etc.[10] Recently we have shown that ethanol extract of *A. indicum* L. leaves could exert maximum *in vitro* antioxidant activity (results are under communication). The aim of the present work was to see effect of season on *in vitro* antioxidant activity of *A. indicum* L. leaves.

2. METHODOLOGY

2.1 Collection of plant materials

Leaves of *A. indicum* L. were collected from 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, during summer (March – May), rainy season (June – August),

autumn (September – November) and winter (December – February). Leaves were authenticated by the experts of the department of Botany of the said University**.** A voucher specimen (No. SM-MB-012/19) 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.

***Abutilon indicum* Linn.**

2.2 Preparation of Test drug

Leaves of *A. indicum* L. of different seasons were washed thoroughly under tap followed by distilled water. Leaves were then separately shed dried and powered. The powder, used as test drug, was stored desiccated at 4 ^{0}C until further use.

2.3 Extraction of the test drug

Test drugs (100g each) obtained from leaves of *A. indicum* L. of different seasons were separately extracted with 500 ml of ethanol in soxhlet at 37^{0}C for 15 minutes. Ethanol was used as solvent because we have noted earlier (results are under communication) that ethanol extract of *A. indicum* L. leaves had maximum *in vitro* antioxidant activity. Extracts obtained were filtered and the solvents of all extracts were evaporated separately to dryness *in vacuo* with rotary evaporator at 40 – 50 ^{0}C. Obtained brown mass was used for antioxidant assays as well as well as for the determination of total phenols.

2.4 Antioxidant assays

In vitro antioxidant activity of powdered leave extracts of *A. indicum* L. of different seasons was assayed through superoxide anion generation by xanthine-xanthine oxidase assay[11], linoleic acid peroxidation assay[12] and by DPPH photometric assays.[13]

2.5 Determination of Total phenols

Total phenols were determined following the method of McDonald *et al.*[14]

2.6 Chemicals

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

2.7 Statistical analysis

All experiments were performed triplicate. The results were expressed as mean ± SE. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A p-value of <0.05 was considered statistically significant.[15]

3. RESULTS

Effect of seasons on inhibitions of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by leave extracts of *A. indicum* L. was shown in Table – 1. Results show that ethanol extracts of leaves of *A. indicum* L. of different seasons had *in vitro* antioxidant activity but maximum activity was found during Summer (March – May). Inhibitions of xanthine oxidase, linoleic acid peroxidation and DPPH scavenging capacity of summer sample were 94.1%, 77.5% and 85.3% respectively. Values were statistically significant in comparison to the values obtained for leave extracts of autumn, winter and rainy seasons. Results were also comparable to that of quercetin, a known antioxidant compound, where inhibition in xanthine oxidase, linoleic acid peroxidation and DPPH came 100%, 87.5% and 96.1% respectively.

Table 1: Effect of seasons on inhibitions of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by ethanol extracts of *A. indicum* L. leaves.

Powdered leaves of *A. indicum* L. of different seasons	Xanthine oxidase (% inhibition)	Linoleic acid peroxidation (% inhibition)	DPPH (% inhibition)
Summer (March – May)	94.1 ± 1.1*	77.5 ± 0.8*	85.3 ± 0.6*
Rainy season (June - August)	23.7 ± 1.0	18.3 ± 0.7	16.5 ± 0.5
Autumn (September - November)	17.2 ± 1.0	29.6 ± 1.3	26.7 ± 1.1
Winter (December – February)	20.7 ± 1.7	20.4 ± 1.5	20.5 ± 0.6
Quercetin	100 ± 0.01	87.5 ± 0.5	96.1 ± 0.02

Concentration used: 100 µg / ml. Results were mean of triplicate experiments ± SE.

*Significant.

Table – 2 showed effect of season on total phenols content of the powdered ethanol extracts of *A. indicum* L. leaves. Leaves extract of summer sample of *A. indicum* L. contained maximum amount of total phenols (69.8 mg/mg dry wt.) while ethanol extracts of *A. indicum* L. leaves for autumn, winter and rainy seasons contained 28.9 mg/mg dry wt., 37.3 mg/mg dry wt. and 21.5 mg/mg dry wt. of total phenols respectively. Results were found statistically significant.

Table 2: Effect of season on total phenols content of the leaves extracts of *A. indicum* L.

Powdered leaves of *A. indicum* L. of different seasons	**Total phenol content (mg/mg dry wt.)**
Summer (March – May)	69.8 ± 0.4*
Rainy season (June - August)	21.5 ± 0.5
Autumn (September - November)	28.9 ± 0.9
Winter (December – February)	37.3 ± 0.6

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

4. DISCUSSION

Secondary metabolites of plants have no direct role in growth of the plant but are responsible to defend themselves against exogenous biotic / abiotic constraints. Different types of secondary metabolites present in plants are, terpenoids, phenols, alkaloids and sulphur containing compounds. These secondary metabolites are responsible for pharmacological activities of plants like anti diabetic, anti inflammatory, anti microbial, anti allergic, anti cancer, anti oxidant, anti gastric ulcer etc.[16]

It is reported that amount of secondary metabolites present in plant varies with season. Influence of climate on secondary metabolites in medicinal plants was first studied by Fluck and Pharm.[17] Thereafter, many investigators have shown that accumulation of secondary metabolites in leaves, stem and roots of plants varies with season.[18-22]

In the present study effect of season on *in vitro* antioxidant activity of *A. indicum* L. leaves was studied. Antioxidant activity of the plant leaves, measured by inhibitions in xanthine oxidase, linoleic acid peroxidation and scavenging capacity of DPPH, was found maximum during summer ie, March – May (Figure – 1). Perhaps, this is the first time we are reporting that in vitro antioxidant activity of *A. indicum* L. leaves is maximum during summer.

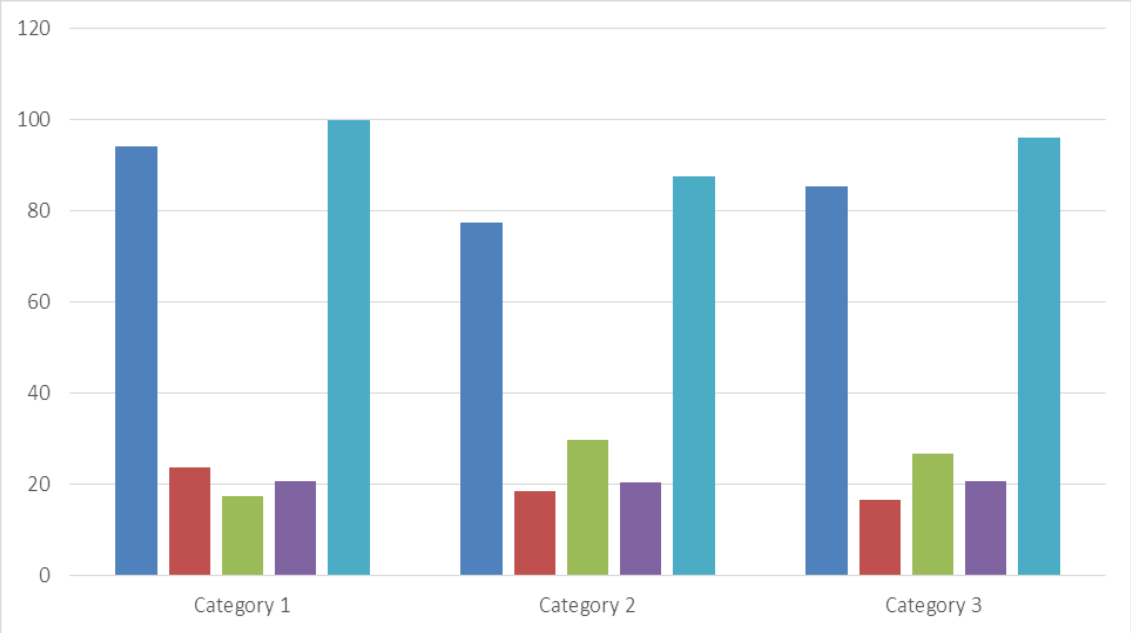

Figure 1: Showing % inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by *A, indicum* L. leaves extracts of different seasons.

Category 1: Xanthine oxidase (% inhibition) Category 2: Linoleic acid peroxidation (% inhibition) Category 3: DPPH (% inhibition)

■ Summer ■ Rainy season ■ Autumn ■ Winter ■ Quercetin

It is known that phenolic compounds are responsible for antioxidant activity thereby exert multiple biological effects like free radical scavenging abilities, anti inflammatory, anti carcinogenic anti diabetic, anti gastric ulcer activities etc.[23] We estimated total phenols content in *A. indicum* L. leaves in different seasons of the year. Amount of total phenols in the leave extracts was found maximum in summer (Figure – 2). Antioxidant activity of *A. indicum* L. leaves during summer was, therefore, due to maximum accumulation of total phenols in the plant leaves.

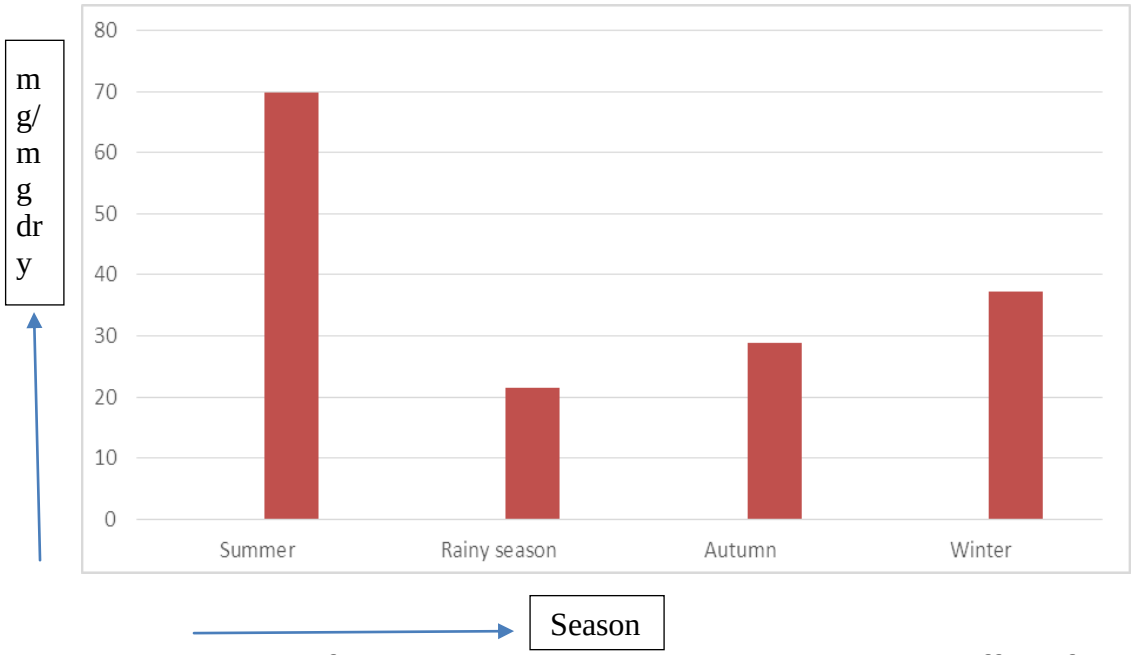

Figure – 2: Amount of phenolic compounds in *A. indicum* L. leaves: Effect of season.

There is high demand of natural antioxidant as synthetic antioxidant such as butylated hydroxyanisole and butylated hydroxytoluene, though commercially available, are not safe.[24] As *A. indicum* L. leaves of summer showed maximum *in vitro* antioxidant activity we are now planning to isolate natural antioxidant compound from the summer sample of *A. indicum* L. leaves. Work in this direction is presently in progress in our laboratory.

5. CONCLUSION

In the present study summer variety of *A. indicum* L. leaves showed maximum *in vitro* antioxidant activity. These leaves, therefore, may be used as natural antioxidant.

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Conflict of interest: Nil.

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