

Effect of Season on *In Vitro* Anti-Oxidant Activity of *Syzygium Cumini* L. Leaves



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Abstract

Syzygium cumini Linn (*S. cumini* L.) is a medicinal plant. Anti oxidant activity of *S. cumini* L. leaves is known in literature. In the present study we have examined effect of season on *in vitro* anti oxidant activity of *S. cumini* L. leaves. Leaves of *S. cumini* L. were collected in different seasons and *in vitro* anti oxidant activity of the leaves was measured by superoxide anion generation with the help of linoleic acid peroxidation assay, xanthine-xanthine oxidase assay and by DPPH photometric assay. Anti oxidant compounds like total phenol, ascorbic acid, flavonoids and carotenoid present in the leaves of different seasons were also estimated. Results showed that *in vitro* anti oxidant activity of *S. cumini* L. leaves was maximum during summer (March-May). Anti oxidant activity was related with high content of total phenol, ascorbic acid, flavonoid and carotenoids in the leaves. It is therefore concluded that leaves of *S. cumini* L. of summer should be used to get maximum anti oxidant activity.

Keywords: *Syzygium cumini* leaves; Anti-oxidant activity; Total phenol; Flavonoid; Ascorbic acid; Carotenoids; Effect of season

Introduction

Numerous medicinal plants are known possessing anti oxidant activity. Few of them are, *Piper longum* L., *Solanum nigrum* L., *Amaranthus caudatus* L., *Desmodium gangeticum* L., *Ocimum sanctum* L., *Eclipta alba* L., *Hyptis suaveolens* L., *Alpina calcarata* L., *Ocimum basilicum* L., *Jatropha multifida* L., *Hyptis suaveolens* L., *Solanum indicum* L., *Clitoria ternate* L. etc. [1,2]. *Syzygium cumini* L. (family Myrtaceae) is a tropical fruit tree of great economic importance. It is a large evergreen tree up to 30m height and a girth of 3.6m with a bole up to 15m. The plant is native to Nepal, Pakistan, Bangladesh, India, and Indonesia. In India the plant is found almost everywhere. In English the plant is known as Jambul tree. In Hindi, Bengali, Punjabi, Tamil, Gujrati and Malayalam the plant is called as Jamuna, Jaam, Jammun, Naval, Gambu and Njaval respectively [3].

S. cumini L. is known to possess a wide range of medicinal properties. Leaf has anti diabetic, anti allergic, anti viral, anti bacterial, anti DNA damage and anti oxidant activities. Fruit is anti-hyper lipidemic, possessing anti-cancer property. Seeds exert anti inflammatory and anti gastric ulcer activity. Bark and pulp of the plant are efficacious for diabetes [4].

Phytochemical studies showed that stem bark of *S. cumini* L. contains betulinic acid, β -sitosterol, β -sitosterol-D-glucoside, quercetin, myricetin, astragalol glycoside, kaempferol-3-O-glucoside,

friedelin, epi-friedelinol, eugenin and gallic acid. Leaves contain n-heptacosane, n-nonacosane, sitosterol, betulinic acid, crategolic (maslinic) acid, acid soxalic, citric acid, glycolic acids, n-hentriacontane, n-octacosanol, n-triacontanol, kaempferol 3-O- β -D-glucuronopyranoside, ellagitannin, nilocitin, myricetin 3-O- β -D-glucuronopyranoside and amino acids like glycine, alanine etc. Oleanolic acid, erategolic acid (maslinic acid), quercetin, kaempferol and myricetin flavonoids -isoquercitrin were found in the flowers of *S. cumini* [5,6].

Anti oxidant property of *S. cumini* L. leaf is known in literature. Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging and ferric-reducing antioxidant power (FRAP) assays Ruan et al. showed that that water, ethyl acetate, chloroform, n-hexane and methanol extracts of *S. cumini* L. leaf have anti oxidant activity [7]. By using the same methods Eshwarappa et al. also showed antioxidant activity of *S. cumini* leaf gall extracts [8]. In the present study effect of season on *in vitro* anti oxidant property of *S. cumini* L. leaf was investigated.

Methodology

Plant material

S. cumini L. leaves were collected from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India during Autumn (September-November), Winter

(December-February), Summer (March-May) and rainy season (June-August) at about 9 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, and India for future references.

Test material

Collected leaves of *S. cumini* L. were shade dried and powdered. The powder was used as test material.

Chemicals

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

Antioxidant assays

Antioxidant activity of *S. cumini* L. leaves of different seasons was assayed by superoxide anion generation by xanthine-/xanthine oxidase assay [9], linoleic acid peroxidation assay [10] and by DPPH photometric assay [11].

Flavonoids content

Flavonoids content of *S. cumini* L. leaves of different seasons was determined using Aluminum chloride colorimetric method [12].

Total phenols content

Total phenols content of *S. cumini* L. leaves of different seasons was determined by Folin Ciocalteu reagent [13].

Ascorbic acid content

Ascorbic acid content of *S. cumini* L. leaves of different seasons was determined by the method of Cakmak and Marschner [14].

Carotenoids content

Total carotenoids of *S. cumini* L. leaves of different seasons was determined by the method of Jensen [15].

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 [16].

Results and Findings

Effect of seasons on *in vitro* antioxidant activity of powdered leaves of *S. cumini* L. through superoxide anion generation by linoleic acid peroxidation assay, xanthine-/xanthine oxidase assay and by DPPH photometric assay is tabulated in Table 1.

Concentration used: 100µg/ml Dose was fixed based on our earlier report [17]. Results were a mean of triplicate experiments ±SEM. *In vitro* anti oxidant activity of the powdered leaves of *S. cumini* L. was notices in all seasons but maximum activity was found during summer (March-May). Inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH were found 91%,

78% and 90% respectively. Results were comparable to that of quercetin, a known anti-oxidant compound, where inhibition in linoleic acid peroxidation came 87% but for both xanthine oxidase and DPPH inhibition was 100% (Table 1). Results were a mean of triplicate experiments ±SE.

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

Leaves of <i>S. cumini</i> L. of different seasons	Xanthine oxidase(% inhibition)	Linoleic acid peroxidation (% inhibition)	DPPH (% inhibition)
Summer (March-May)	91±1.3*	78±1.9*	90±1.7*
Rainy season (June-August)	49±1.2	45±1.2	56±1.3
Autumn (September-November)	33±1.0	31±1.0	30±1.0
Winter (December-February)	39±0.9	36±1.4	41±1.1
Quercetin	100±0.01	87±1.0	100±0.01

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

Leaves of <i>S. cumini</i> L. 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 (March-May)	62±1.3*	74±1.2*	38±0.9*	16.8±1.5
Rainy season (June - August)	41±1.2	56±1.1	29±1.0	15.8±1.3
Autumn (September - November)	33±1.0	43±1.0	20±0.6	13.7±1.4
Winter (December-February)	21±1.0	29±0.9	15±0.5	12.9±1.1

Table 2 shows that total phenol content of the leaves of *S. cumini* L. in summer was 62±1.3mg/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 autumn, winter and rainy seasons total phenol content of *S. cumini* L. leaves were 33±1.0, 21±1.0 and 41±1.2mg/mg dry wt respectively. Same trend was found in total flavonoids, ascorbic acid content of *S. cumini* L. leaves. Carotenoids content in *S. cumini* L. leaves, however, did not show any significant change in different seasons of the year.

Discussion

Fluck and Pharm in the year 1955 showed influence of climate on the active principles in medicinal plants. Thereafter, researchers

conducted experiments in this direction and demonstrated influence of season on concentration of active compounds in plants [18-22]. We have also undertaken experiments on seasonal

variation in concentrations of active metabolites in plants and noted that season can change amount of bio active compounds in different parts of the plants [23-27](Figure 1).



Figure 1: *S. cumini* L. leaves

In present study effect of season on *in vitro* anti oxidant activity of *S. cumini* L. leaves was studied. Results showed that anti oxidant activity of *S. cumini* L. leaves in terms of inhibitions in linoleic acid peroxidation, xanthine oxidase and DPPH was maximum during summer season (March-May) 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. in 2015 demonstrated the best antioxidant activity of *Laurus nobilis* L. was in spring and the lowest one was in winter [29].

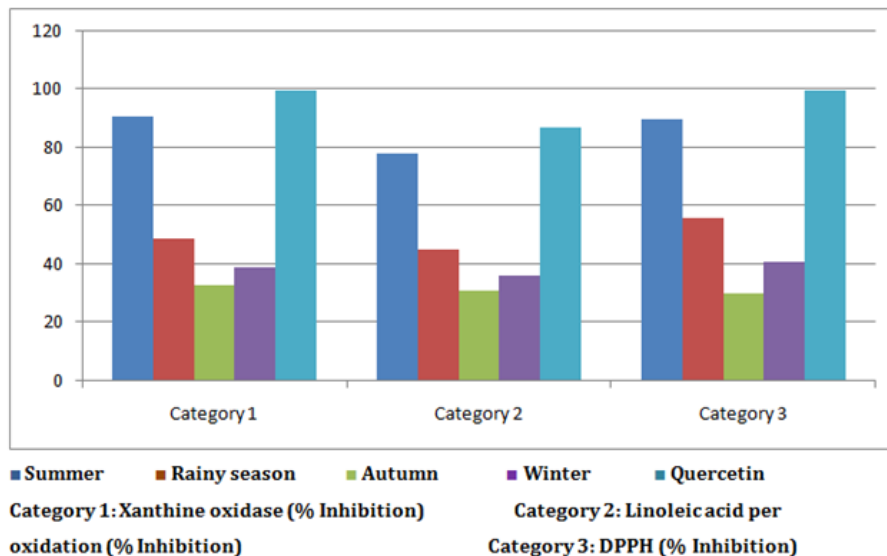


Figure 2: Showing % inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by powdered leaves of *S. cumini* L. in different seasons.

Miller in 1996 found that anti oxidant activity of medicinal plant is mainly due to presence of flavonoids, ascorbic acid, phenolic compounds 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 *S. cumini* L. leaves. Results (Figure 3) showed that amounts of phenolic compounds, ascorbic acid

and flavonoids in the plant leaf were maximum during summer (March-May). 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 [28]. *In vitro* anti oxidant activity of *S. cumini* L. leaves during summer was, therefore, due to accumulation of maximum amount of phenolic compounds, ascorbic acid and flavonoids in the plant leaves.

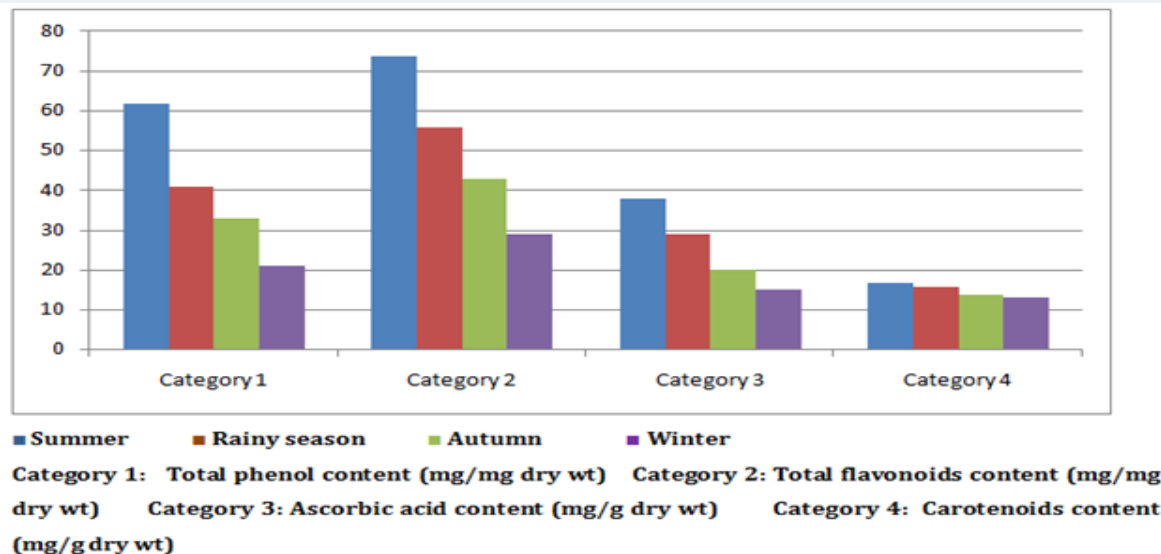


Figure 3: Total phenol, flavonoids, ascorbic acid and carotenoids content of the powdered leaves of *S. cumini* L. in different seasons.

Oxidative process develops free radicals in living systems. These free radicals are major causative factors for induction of many chronic and degenerative diseases including diabetes mellitus, cancer, atherosclerosis, ischemic heart disease, neurodegenerative diseases, ageing; immunosuppressant etc. [31]. Anti oxidants exert protective effects against oxidative stress in biological systems [32]. Therefore, search of anti oxidants are of utmost importance to combat oxidative stress and the related diseases.

Synthetic anti-oxidants like butylated hydroxyanisole and butylated hydroxytoluene are commercially available. They are commonly used in processed food also. But, their toxicity is a matter of concern. It is often claimed that these synthetic anti oxidants have many side effect including carcinogenic activity [33]. Therefore, there are high demands for naturally occurring anti oxidants. As the present study indicates that March-May (Summer season) is the period when *S. cumini* L. leaves showed maximum *in vitro* anti oxidant activity, *S. cumini* L. leaves of summer may be used as the source of natural anti oxidant.

Conclusion

Effect of season on *in vitro* anti oxidant activity of *S. cumini* L. leaves was studied. Results showed that *in vitro* anti oxidant activity of *S. cumini* L. leaves was maximum during summer season (March-May). The anti oxidant effect was due to presence of maximum amount of phenols, flavonoids and ascorbic acid in the leaves during summer.

Recommendation

S. cumini L. leaves of summer season may be used as the source of natural antioxidant.

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