

Seasonal Dynamics of Phytochemicals in *Cinnamomum tamala* (Buch.-Ham.) Nees and Eberm. (Tejpata)

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Abstract: *Cinnamomum tamala* (Buch.-Ham.) Nees and Eberm. (Family- Lauraceae) which is commonly known as 'Tejpata', is an evergreen tropical tree. *C. tamala* leaf and bark are very popular for their use as spices due to its spicy odour and are also used widely in pharmaceutical preparation because of therapeutic and nutritive efficacy against various diseases and disorders due to the presence of different phytochemicals and mineral nutrients. The present study reveals the seasonal dynamics [Early monsoon (EM)>Monsoon (M)>Late monsoon (LM)>Winter (W)] of primary metabolites (protein, carbohydrate, and fat), secondary metabolites [Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Tannin Content, Total Saponin Content (TSC)], pigments, minerals (N, P, K, S, Na, Fe), antioxidant and anti-inflammatory activities in ethanolic extract prepared from the leaves of *C. tamala*. All the bioactive components varied significantly ($P<0.01$) from season to season. In response to seasonal assay Carbohydrate, TPC, Flavonoid, Antioxidant activity possessed the same seasonal sequence as EM>LM>W>M and the average values of four seasons were determined to be 10.10%, 15.02%, 9.94mg/g, and the average IC_{50} value of scavenging activity was 46.02 μ g/ml respectively. Whilst Anti-inflammatory, Saponin, Sulphur contents and total foliar pigments followed the succession as EM>M>LM>W and the average quantities were estimated to be 48.88 μ g/ml in case of IC_{50} value and 4.73%, 0.605% and 1.163mg/g correspondingly. On the contrary, Protein, Nitrogen and Sodium contents followed the order as M>W>LM>EM and the average values were evaluated to be 10.3mg/g, 5.64% and 0.034% respectively. In contrast, Fat, Potassium and Iron contents maintained the sequence as M>LM>EM>W and the average amounts of four seasons were found to be 6.16%, 0.913%, 0.94% individually. Nevertheless, TTC and Phosphorus contents exhibited the series as LM>W>EM>M and the average quantities were measured to be 27.52mg/g and 0.06% respectively. The present study concludes that in respect to total bioactive components determined so far, **Early monsoon** is the best season for harvesting the leaves of *Cinnamomum tamala* following the sequence as EM>LM>W>M.

Keywords: Tejpata, Season, dynamics, phytochemicals.

1. INTRODUCTION

In Bangladesh, the spice plant *Cinnamomum tamala* (Buch.-Ham.) T. Nees and Eberm. (Family- Lauraceae) is commonly known as 'Tejpata,' which is also distributed in the Mediterranean region, West and Central Asia, South Asia, South East and East Asia, Africa, South East America, Australia, India, China and Myanmar. The genus *Cinnamomum* is represented by about 350 species worldwide. It is native to South-east Asia, some Pacific Islands and Australia, growing mainly in tropical rain forests at varying altitudes. It grows throughout Bangladesh but cultivated more in southern region as spice as well as for medicinal value. The leaves of this plant have been extensively used as spice in the food industry due to its special aroma and also as fodder. Historically, it is one of the oldest known and used spices. The essential oil from the leaves is also used as a flavouring agent ^[1].

Cinnamomum tamala (Tejpata) is a medium sized evergreen tree 2-10 m tall, leaves are staked, opposite, or sub opposite, elliptic-oblong, nerved from the base, shining, leathery, entire, long pointed, new leaves are slightly pinkish tinged, flowers are small, yellowish and blooming in the month of March to May ^[2]. Tejpata is generally harvested in dry and mild weather from October to December and in some places, the collection is continued till the month of March ^[3] (Krishnamurthy, 1996). On an average, a tree produces 10-25 kg of dry leaves and its 0.2- 0.4% oil can be extracted from leaves.

Timely collection of leaf is important since early and late collection may result in poor quality of the leaves or essential oil ^[4].

This plant has long been used as a traditional folk remedy for cold and cough, asthma, colic, blood dysentery, diarrhoea, constipation, flatulence, indigestion, jaundice, hyper acidity, anorexia, dysmenorrhoea, leucorrhoea, postpartum haemorrhage, high fever, skin diseases, sore throat, sexual weakness and tuberculosis ^[5]. The leaves are carminative, stimulant, diuretic, diaphoretic, lactagogue, deobstruent and aromatic ^[6]. The bark is used to treat gonorrhoea. Leaves and bark are mixed with tea which is believed to cure coughs and colds and is a very popular stimulant and diuretic drink in Indian subcontinent ^[7].

Phytochemical constituents are non-nutritive chemical present in plants that have disease preventive properties. Many literatures on preliminary phytochemical surveys ^[8-12] and its knowledge of herbal drugs and their preparations will be helpful to isolate and characterize the chemical constituents present in plant extracts. Therefore, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies. Phytochemicals possess various health-related effects such as antimutagenic, antibacterial, antifungal, antithrombotic, anticarcinogenic and vasodilatory activities ^[13]. The ability to inhibit the growth of pathogenic microorganisms, without harming the host, demonstrates their potential application as therapeutic agents.

Some experimental works have already been done on chlorophyll, TPC, TFC, TTC, PC, AO, AI and Nutrient contents (N, P, K, Ca, Na and Fe) of *Cinnamomum tamala* ^[14-18]. But no research work on seasonal variation (Early monsoon, Monsoon, Late monsoon and Winter) of bioactive components in *C. tamala* has yet been done in Bangladesh whilst it is an ever-demanding phenomenon to fill up the gap of knowledge in harvesting the plant parts to achieve the maximum amount of phytochemicals from *C. tamala*. With this view in mind, initially an extensive lab analysis was undertaken to evaluate the seasonal variation (Early monsoon, Monsoon, Late monsoon and Winter) of bioactive components in *C. tamala*.

2. MATERIALS AND METHODS

Collection of plant materials: The fresh leaves of *Cinnamomum tamala* was collected seasonally (Early monsoon, Monsoon, Late monsoon, and Winter) from the University of Chittagong Campus. The disease free and fresh plants were carefully chosen. The leaves of studied sample were used for the qualitative and quantitative estimation of secondary metabolites and their bioactivity test. These leaves of sample were thoroughly washed with water and dried in air, sun and at last in oven at 50°C for 72 hours for preserving chemical properties. Then that was ground into coarse powder by using grinding machine and stored in airtight container for further investigation. Mingling of one season sample with another was carefully avoided.

Preparation of plant extract: 30 gm. of sample from each season were taken for analysis. 50 ml of ethanol was added to the 30 gm. of sample in a conical flask. Shaken very well for 30 minutes and then kept overnight and then shaken again and then filtered using Whatman filter paper. The process was repeated for 3 times with ethanolic extract, then rota-evaporated below 51°C and dried in water bath at 50°C. The dried sample was kept as crude sample for each season and stored at 4°C in a refrigerator. It was further used for phytochemical screening, quantitative analysis of Carbohydrate, Protein, lipid, Total phenols, Tannins, Flavonoids, antioxidant activity, and anti-inflammatory activities and Qualitative Analysis.

Qualitative analysis

Procedure for qualitative tests: Qualitative tests were carried out on the powdered specimens using standard procedures to identify different constituents such as: Anthroquinone, Quinine, Coumarin and Reducing sugar ^[19]; Phlobatannins and Flavonoids ^[20]; Tannins and Glycosides ^[8]; Terpinoids and Sterioids ^[21]; Phenols ^[22]; Saponins ^[23]; Cardiac glycosides: Keller-Killiani Test ^[24] and Protein-Biuret Test ^[25].

Quantitative analysis

Photosynthetic pigments: Foliar photosynthetic pigments were estimated by Wettstein, (1957) method ^[26].

Analysis of minerals: Foliar Nitrogen was determined by Micro-Kjeldahl distillation method ^[27]. Phosphorus was determined by spectrophotometric method ^[28]. Potassium, sodium and calcium were determined by flame photometric method ^[29]. Iron (Fe) was estimated by following spectrophotometric method ^[30-31]. Sulphur was determined through HPIC method ^[32].

Primary metabolites: Carbohydrate was evaluated by following the anthrone reagent method [33-34]. Total fat was estimated as described by Luo and Peng, 2012 [35]. The total Protein content of *Cinnamomum tamala* was determined by following Lowry's method [36].

Secondary metabolites

Total Phenols: Total phenols were determined by Folin Ciocalteu method [37]. 1 ml of each plant extract or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous 7.5% Na₂CO₃ (5 ml). The mixtures were allowed to stand for 20 min at 25^oC to complete the reaction. Then the absorbance of the solution was measured in spectrophotometer (USA) at 765 nm against blank. The standard curve was prepared using 50, 100, 150, 200, 250 mg L⁻¹ solutions of gallic acid in ethanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g⁻¹ of dry mass), which is a common reference compound.

Flavonoid: Aluminum Chloride Colorimetric Method was used for Flavonoids determination [38]. Quercetin was used to make the calibration curve. 10 milligrams of quercetin were dissolved in 80% ethanol and then diluted to 50, 100, 150, 200, 250 µg/ml. The diluted standard solutions and the extract solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance of their action mixture was measured at 415 nm with a Shimadzu UV-1800A spectrophotometer (Kyoto, Japan).

Tannin: The tannins were determined by Folin-Denis method [39]. About 1 ml (1mg/ml) of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (50, 100, 150, 200, 250) µg/ml were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Saponin: Determination of Saponin compound (Identification by analytical method). The samples were ground and 20 grams of each was put into conical flask and 100 ml of aqueous ethanol were added. The samples were heated over hot in water bath for 4 hours with continuous stirring at about 55o C. The mixture was filtered and the residue re extract with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml over water bath at about 90o C temperature was transferred into 250ml separators funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to constant weight and the saponins content was calculated [20].

Anti-oxidant activity: The antioxidant activities of the crude extracts of *C. tamala* and the standard antioxidant ascorbic acid were evaluated on the basis of the free radical scavenging effect of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH, MWt. 394.32) free radical activity according to the method described by Brand- William *et al.*, 1995 [40]. 3 ml of the DPPH solution was mixed with 3 ml of extract solution and standard solution separately. These solution mixtures were kept in dark for 30 minutes. The degree of DPPH purple de-colorization to DPPH yellow indicated the scavenging efficiency of the extracts. The absorbance of DPPH solution (control solution "A") was measured at 517 nm using UV-visible Spectrophotometer. Ascorbic acid served as positive control. Lower absorbance of the reaction mixture indicated higher free radical- scavenging activity. The scavenging activity against DPPH was calculated using the following equation as, Scavenging activity (%) = (A-B)/A x 100; Where, A= Absorbance of control (DPPH solution without the sample), B= Absorbance of DPPH solution in the presence of the sample (extract / ascorbic acid).

Standard and test solution preparation

Stock solution of plant extract and ascorbic acid of 10 mg/ml were prepared. In crude extract, 23 test tubes were taken. 15 test tubes for plant extract, 5 for standard and 3 for control, as control was done triplet. 50, 100, 150, 200 and 250 µg/ml were labeled in each test tubes. Added 3 ml of ethanol in each

test tube. Then added 50, 100, 150, 200 and 250µg/ml from the stock solution of conc. 10mg/ml according using micropipette.

Anti-inflammatory activity: The anti-inflammatory activity of *Cinnamomum tamala* was studied by using inhibition of albumin denaturation technique which was studied according to (Mizushima *et al.*, 1968) and (Sakat *et al.*, 2010) ^[41-42]. The reaction mixture was consisting of test extracts (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml and 300µg/ml concentrations) and 1% aqueous solution of egg albumin, pH (5.6 ± 0.2) of the all reactions mixtures was adjusted by 1N HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min, after cooling the samples the turbidity was measured at 660nm. (Spectrophotometer). The experiment was performed in triplicate ^[43]. The anti-inflammatory activity was calculated by using the following equation as, % of inhibition = (A-B)/A x100; Where, A = Absorbance of control (5% egg albumin solution and methanol), B= Absorbance of test group (5% egg albumin solution and plant extract) or, B= Absorbance of standard solution (5% egg albumin solution and acetyl salicylic acid)

Statistical analysis

All measurements were carried out in triplicate forms. Results of the parameter determined were expressed as a mean of the triplicate determination. The data were analyzed using the one-way analysis of variance (ANOVA) tool under the Statistical Package Social Science (SPSS) 16.0 software. The ANOVA, Correlation coefficient matrix and Duncan’s Multiple Range Test (DMRT) were completed to compare the mean values and standard deviation among the samples.

3. RESULTS AND DISCUSSION

It is obvious from the results that Early monsoon reveals the presence of highest (++++) amount of quinones, coumarins and protein, Moderate (++) amount of flavonoid, tannin, saponin, steroids, terpenoids and Mild (+) amount of phenol, phlobatannins, glycosides, cardiac glycosides. Whilst anthraquinone and reducing sugar were found to be absent in the early monsoon. The results indicate that the maximum (++++) amount of phenol, flavonoid, tannin, steroids, terpenoids, glycosides and protein; Moderate (++) amount of saponin, quinone, coumarins; and minimum (+) amount of phlobatannins were determined in monsoon. Anthraquinone, reducing sugar and cardiac glycosides were absent in monsoon (Table: 1).

It is apparent from the results that the highest (++++) amount of steroids, terpenoids, and glycosides; Moderate (++) amount of phenol, coumarins and protein; Lowest (+) amount of flavonoid, tannin, saponin, and quinone were estimated in late monsoon. Whereas anthraquinone, phlobatannins, reducing sugar and cardiac glycosides were observed to be absent in late monsoon. In winter, the extreme (++++) amount of phenol, and tannin; Reasonable (++) amount of flavonoid, steroid, terpenoid, glycosides, and protein; and the least (+) amount of saponin, quinone, and coumarin were determined. Anthraquinone, phlobatannins, reducing sugar and cardiac glycosides were found to be absent (Table: 1).

According to Jain *et al.*, 2011, *C. tamala* leaf extract contained alkaloid, flavonoid, triterpenoid, glycosides and tannin. Negative results showed for the absence of carbohydrate and saponin ^[44]. By using various solvents (Ethanol, Aqueous, and Chloroform) for the preparation of *C. tamala* leaf extract, Raksha *et al.*, 2021 showed the presence of alkaloids, flavonoids, steroids, polyphenols, flavones and flavonols, tannins, saponins, glycosides, carbohydrates, proteins and amino acid in *C. tamala* ^[45]. However, hydroalcoholic fraction was found more suitable, because most of the bioactive components were found soluble in alcohol as alcohol group shows high polarity than most of the non-polar but lower polar than water. Previous studies also suggested that alcoholic extract contains more bioactive components as compared with other solvents extract ^[46]. This bears a close similitude with the results of the present experiment.

Table1. Qualitative status of phytochemicals of *Cinnamomum tamala* in four different seasons.

SL.No.	Phytochemicals	Seasons			
		Early monsoon	Monsoon	Late monsoon	Winter
1.	Phenol	+	++++	++	+++
2.	Flavonoids	++	+++	+	++
3.	Tannin	++	+++	+	+++
4.	Saponin	++	++	+	+
5.	Steroids	++	+++	+++	++

6.	Quinone	+++	++	+	+
7.	Anthraquinone	-	-	-	-
8.	Coumarins	+++	++	++	+
9.	Phlobatannins	+	+	=	=
10.	Terpenoids	++	+++	+++	++
11.	Reducing sugar	-	-	-	-
12.	Glycosides	+	+++	+++	++
13.	C. glycosides	+	-	-	-
14.	Protein	+++	+++	++	++

Quantitative analysis

Foliar pigments: All the pigments were found to be varied significantly with seasons. Maximum Chl-a was determined to be 0.740 mg/g in early monsoon whereas minimum Chl-a was determined to be 0.415 mg/g in winter. The highest amount of Chl-b was estimated to be 0.443 mg/g in early monsoon whilst the lowest amount of Chl-b was estimated to be 0.231 mg/g in winter. Maximum amount of carotenoid was evaluated to be 0.341 mg/g in early monsoon while the lowest amount of carotenoid was evaluated to be 0.209 mg/g in winter. The results also indicate that the maximum concentration of plant pigment (1.524 mg/g) was found in the early monsoon and the lowest concentration (0.855 mg/g) in the winter following the sequence as EM>M>LM>W (Table: 2,8,9; Fig. 1).

Table2. Seasonal variation of foliar pigments (mg/g) of *C. tamala* in a full production year.

Seasons	Total pigment(mg/g)						
	Chl-a	Chl-b	Total chl.	Carotenoid	Total pig.	chl-a:chl-b	Chl/Car.
EM	0.740±0.48	0.443±0.17	1.183	0.341±0.87	1.524±0.33d	1.670:1	3.469:1
M	0.589±0.58	0.398±1.48	0.987	0.309±.008	1.296±0.36b	1.480:1	3.194:1
LM	0.443±0.78	0.252±.055	0.695	0.281±.038	0.976±0.14a	1.758:1	2.473:1
W	0.415±0.46	0.231±1.21	0.646	0.209±0.89	0.855±0.29c	1.797:1	3.091:1

It is obvious from the result that the highest ratio of chlorophyll-a and chlorophyll-b was observed in winter and (1.797:1) whilst the lowest ratio of chlorophyll-a and chlorophyll-b was observed in monsoon (1.480:1). On the other hand, maximum ratio of chlorophyll and carotenoid was found to be 3.469:1 in early monsoon whilst minimum ratio was found to be 2.473:1 in late monsoon (Table: 2,8 and 9; Fig. 1).

In an extensive experiment among the phytochemical composition and content of *C. verum*, total chlorophyll content of leaves ranged between 0.67 and 1.08 mg/g [47]. Rawat *et al.*, (2009) reported that the leaves of normal and variant plant seedlings of *C. tamala* did not differ much in Chl a, Chl b, total Chl, and carotenoid content. They also reported that Chl a, Chl b and total Chl were 0.4, 0.1 and 0.5 (mg/g fr. wt.) in normal leaves wherein variant contents were 0.4, 0.07, and 0.5 respectively [48]. The normal and variant leaves did not differ much in their carotenoid contents which were recorded to be 0.33 and 0.30 respectively. According to Wagay *et al.*, 2009 the results of Chlorophyll-a, chlorophyll-b and chlorophyll-a+b concentrations using 95% Ethanol as solvent were 0.0187, 0.0245, 0.0432 mg/g [49]. Fahrettin *et al.*, (2020) demonstrated the seasonal change of chlorophyll content in both May and October and the values showed statistically significant differences (0.49 mg/g and 0.39 mg/g) of different species at 99% confidence level [50].

Primary metabolites

Protein: The result reveals that total protein content was found to vary significantly from season to season. The result also indicates that *C.tamala* contained the highest amount of total protein content in monsoon and it was found to be 14.00 mg/g. The lowest amount of total protein content was found to be to 8.02 mg/g in early monsoon. In case of total protein content *C. tamala* followed the sequence as Monsoon>Winter>Late Monsoon> Early Monsoon (Table: 3,8 and 9; Fig. 1). In an experiment, Protein content was determined to be 8.5 ± 0.5g/100g in *C. tamala* [51]. Kuna *et al.*, 2020 estimated that Protein content was also higher in fresh bay leaf (19.84±0.73 mg/g) compared to Bay leaf tea powder (12.91 ± 0.81mg/g) and Bay leaf spice cube (10.35 ± 0.76 mg/g) [18]. As reported earlier, the total protein content of leaf extracts of *C. tamala* was 7.62% of nutritional values [52]. Protein content of the present experiment is analogous to these research findings.

Carbohydrate: The result indicates that carbohydrate content was found to be differed significantly within the seasons and maximum amount of carbohydrate content was estimated to be 13.18% in early

monsoon whilst minimum amount was estimated to be to 8.38% in monsoon. In case of carbohydrate content *C. tamala* followed the progression as Early Monsoon>Late Monsoon>Winter>Monsoon (Table: 3,8 and 9; Fig. 1). While investigating the nutritional values, Dandapat *et al.*, (2015) found that *C. tamala* leaves contained $9.5 \pm 0.5\text{g}/100\text{g}$ carbohydrate^[53]. Rawat *et al.*, (2009) said that soluble sugar content was found maximum in variant plant seedlings (9.53 mg/g) in comparison to normal plant (7.8 mg/g) in *C. tamala*^[48]. Sukumar *et al.*, (2013) calculated the value of carbohydrate as $9.5 \pm 0.5\text{g}/100\text{g}$ in *C. tamala*^[51]. These research results are consistent with the Carbohydrate status of present experiment.

Table3. Seasonal variation of primary metabolites of *C. tamala* in a full production year.

Season	Total protein equivalent (mg/g)	BSA	Total Carbohydrate (%)	Fat (%)
Early monsoon	8.02±1.03a		13.18±0.76d	5.91±0.73a
Monsoon	14.00±1.28c		8.38±0.23a	7.04±0.28c
Late monsoon	8.93±1.28a		12.19±0.39c	6.51±0.41b
Winter	10.25±1.23b		10.23±0.77b	5.19±0.13a

Fat: The result reveals that fat content was determined to be varied significantly with seasons. It is noticeable from the result that *C. tamala* contained the highest amount of fat content (7.04%) in monsoon whereas the lowest amount of fat content (5.19%) in winter. In case of fat content *C. tamala* followed the sequence as Monsoon >Late Monsoon>Early Monsoon>Winter (Table: 3,8 and 9; Fig. 1). Dandapat *et al.*, (2015) evaluated the fat content $6.0 \pm 0.5\text{g}/100\text{g}$ in *C. tamala*^[53]. Nutritional analysis of the leaves of *C. tamala* revealed that *C. tamala* contained $6.0 \pm 0.18\text{g}/100\text{g}$ fat^[51]. Due to moderate level of crude fat in the leaves, people suffering from overweight or obesity can consume it in diet. Kuna *et al.*, 2020 found that crude fat content reduced in Bay leaf tea powder ($1.30 \pm 0.38\%$) and Bay leaf spice cube ($2.73 \pm 0.20\%$), when compared to fresh bay leaf ($4.58 \pm 1.18\%$)^[18]. These findings substantiate with the finding of the present experiment.

Total Phenol content: The results indicate that total phenol content was found to be varied significantly from season to season. It is obvious from the results that *C. tamala* contained the peak amount of phenol content in Early Monsoon (18.08%) and the lowest amount of phenol content was found to be to 11.29% in Monsoon. In case of total phenol content *Cinnamomum tamala* showed the following sequence as: Early monsoon>Late Monsoon>Winter> Monsoon (Table: 4,8 and 9; Fig. 1). Total phenolic content of *C. tamala* was found to be $11.35 \mu\text{g GAE}/\text{mg}$ dry weight of extract^[54]. Hartanti *et al.*, (2019) estimated total phenolic content of ethanol extract of bay leaves by percolation method which ranged between 53.6ppm to 193.7ppm^[55]. The results of phytochemical analysis of the leaf samples of *C. tamala* and *A. marmelos* by Dandapat *et al.*, 2014 revealed that polyphenol was higher ($16.7 \pm 0.7 \text{ g}/100\text{g}$) in *C. tamala* than *A. marmelos* ($6.7 \pm 0.61 \text{ g}/100\text{g}$)^[56] and these research results are comparable to the present experiment.

Flavonoid: Flavonoid content was also found to diverge significantly with seasons. The results designate that *C. tamala* possessed the maximum flavonoid content in early monsoon and it was found to be 14.34 mg/g whereas the lowest amount of total flavonoid content was found to be to 6.62 mg/g in monsoon and showed the succession as: Early Monsoon> Late Monsoon>Winter>Monsoon (Table: 4,8 and 9; Fig. 1). Dandapat *et al.*, (2014) reported that flavonoids occurred in the lowest quantity ($1.0 \pm 1.01 \text{ g}/100\text{g}$) in *C. tamala* among all the studied phytochemicals^[56]. According to Raksha *et al.*, (2021) the total flavonoid content in the hydroalcoholic extract of *C. tamala* was 22.1 mg QE/g ^[45]. According to Kuna *et al.*, 2020 *C. tamala* fresh leaf contains $23.82 \pm 0.84 \text{ mg/g}$ flavonoid^[18]. Total flavonoid content of *C. tamala* was found to be $712.85 \mu\text{g QE}/\text{mg}$ dry weight of extract^[54]. The quantitative assessment of *C. tamala* revealed that leaf extract contains flavonoid content 23.8 (mg QE/g) and total flavones and flavonols 0.67 (mg QE/g) ^[57]. Ethanol and water extracts showed higher total flavonoid content with the value $153.33\pm 3.59 \text{ mg equivalent rutin/g}$ dry extract in *C. tamala*^[58]. These research results are corresponding to the Flavonoid status of present experiment.

Table4. Seasonal variation of secondary metabolites of *C. tamala* in a full production year.

Season	TPC Gallic acid equivalent (%)	Flavonoid quercetin equivalent (mg/g)	Tannin Tannic acid equivalent (mg/g)	Sapopnin (%)
Early monsoon	18.08±1.75d	14.34±2.42d	25.63±0.69b	6.14 ±0.45d
Monsoon	11.29±1.78a	6.62±1.09a	18.48±0.84a	5.07±0.37c

Late monsoon	16.67±2.43c	10.49±1.2c	36.59±0.81c	4.43±0.26 b
Winter	14.04±2.90b	8.3±3.12b	29.39±0.62b	3.28±0.48a

Tannin: It is eminent from the result that maximum amount of tannin content was estimated to be 35.59 mg/g in Late Monsoon whilst minimum amount was estimated to be to 18.48 mg/g in Monsoon. In case of total tannin content *C. tamala* followed the progression as Late Monsoon>Winter>Early Monsoon>Monsoon (Table: 4,8 and 9; Fig. 1). The tannin content was found to be 9.28±0.76 mg/g in bay leaf tea powder [18]. The earlier study of Dandapat *et al.* (2013) showed that the average tannin content in Indian Bay Leaf ranged from 2.50 - 4.0% [59]. Silva *et al.* (2019) determined the tannin amount to be 14.58±1.48 mg EAT/g in *C. triplinerve* bark extract [60].

Saponin: *C. tamala* contained the highest amount of saponin content (6.14%) in early monsoon whereas the lowest amount of saponin content (3.28%) in winter. In case of saponin content *C. tamala* followed the sequence as Early Monsoon>Monsoon>Late Monsoon> Winter (Table: 4,8 and 9; Fig. 1). The results of phytochemical analysis of the leaf samples of *C. tamala* revealed 2.3g/100g saponin content as stated by Dandapat *et al.*, 2014 [56]. Total saponin content was detected to be 71.25mg/g by Sivapriya and John, 2020 in *C. zeylanicum* [61]. Ambrosy *et al.*, (2014) reported that the extract of *C. zeylanicum* contained 6.04±0.02% saponin [62].

4. MINERALS

Nitrogen: The average value of Nitrogen (average of four seasons) was estimated to be 5.64%. It is apparent from the result that *C. tamala* contained the highest amount of nitrogen content (6.9%) in Monsoon whereas the lowest amount of nitrogen content (3.46%) in Early Monsoon and followed the succession as Monsoon>Winter>Late Monsoon>Early Monsoon (Table: 5,8 and 9; Fig. 1). By using in situ litter decomposition method, to examine the decomposition characteristics of leaf litter of *C. glanduliferum*, Liu *et al.*, 2022 suggested that *C. glanduliferum* litter increased the contents of soil organic carbon, soil total nitrogen, and soil hydrolyzed nitrogen which contains 16.38 ± 0.74 g/kg litter total nitrogen [63]. The contents of nitrogen in *C. verum* was found to be 2.49% [64]. The earlier study showed that the nitrogen content in *C. porrectum* ranged from 1.17-2.36% [65].

Maximum foliar nitrogen content of *Andrographis paniculata* was observed to be 10.35 mg/g in monsoon (June-August) and minimum was observed to be 5.24 mg/g in winter (December-February) respectively [66]. Foliar Nitrogen contents in *Vitex negundo* fluctuated from 4.39% (Monsoon- June to August) to 3.14% (Late Monsoon- Sept to Nov) [67]. Twig nutrient status of tea was found to change with agrotypes and periods whilst N contents ranged from 4.33% to 5.76%, 6.07% to 7.51% and 3.77% to 5.00% in early monsoon, monsoon and late monsoon respectively [68]. These opinions are in full agreement with the seasonal impact on the nitrogen status of the present experiment.

Table5. Seasonal variation of minerals of *Cinnamomum tamala* in a full production year.

Season	N (%)	P (%)	K (%)	Na (%)	Fe (%)	S (%)
Early monsoon	3.46± 0.16a	0.05±00b	0.74±00b	0.023±0.056a	0.83±0.02b	0.70±0.05c
Monsoon	6.9± 0.24c	0.04±00a	1.27±00d	0.046±0.036c	1.39±0.004d	0.66±0.06b
Late monsoon	5.64± 0.03b	0.07±00d	1.05±00c	0.027±0.032a	1.08±0.003c	0.61±0.03b
Winter	6.56±0.19c	0.06±00c	0.59±00a	0.039±0.026b	0.46±0.003a	0.45±0.02a

Phosphorus: *C. tamala* contained the peak amount of phosphorus content in late monsoon and it was found to be 0.07% whilst the bottommost amount of Phosphorus content was found to be to 0.04% in monsoon. The average value of phosphorus (average of four seasons) was estimated to be 0.06%. In case of Phosphorus contents *C. tamala* maintained the progression as Late Monsoon>Winter>Early Monsoon> Monsoon (Table: 5,8 and 9; Fig. 1). Dandapat *et al.*, (2014) estimated that *C. tamala* carried out 62.10 ± 4.2 mg/100g phosphorus in mineral composition [56]. Liu *et al.*, 2022 estimated litter total phosphorus 0.91±0.03 g/kg in *C. glanduliferum* [63]. According to USDA (2019) phosphorus value of *C. tamala* is 113mg/100g [69]. The earlier study shows that the average phosphorus content in *C. tamala* is 0.112% [52]. Haider *et al.*, 2018 detected the concentration of P (106.83 mg 100 g-1) significantly higher in Uttarakhand bark than Uttarakhand leaf (99.47 mg 100g-1) and Market leaf (63.60 mg 100 g-1) of *C. tamala* [17]. These findings substantiate with the finding of the present experiment.

Potassium: The result discloses that potassium content was found to vary significantly with seasons. Potassium contents were found to be 0.74%, 1.27%, 1.05% and 0.59% at early monsoon, monsoon, late monsoon and winter respectively. Potassium content was found to be maximum at Monsoon and minimum at Winter and showed the following sequence as Monsoon>Late Monsoon>Early Monsoon>Winter (Table: 5,8 and 9; Fig. 1). Among the trace mineral elements, Dandapat *et al.*, (2014) determined 13.4 ± 2.7 mg/100g potassium in *C. tamala* ^[56]. Liu *et al.*, (2022) assessed litter total potassium 02.14 ± 0.01 g/kg in *C. glanduliferum* ^[63]. According to USDA (2019) potassium content of *C. tamala* was estimated to be 529 mg/100g ^[69]. Al-Hashimi and Mahmood (2016) quantified that among macro minerals *C. tamala* possessed 0.55% phosphorus ^[52]. These findings corroborate with the present results of potassium.

Sodium: Sodium content was found to be 0.023%, 0.046%, 0.027% and 0.039% at early monsoon, monsoon, late monsoon and winter respectively. Sodium content was found to be Maximum at Monsoon and minimum at Early Monsoon and maintained the following sequence as Monsoon>Winter> Late Monsoon> Early Monsoon (Table: 5,8 and 9; Fig. 1). Dandapat *et al.*, (2014) carried out a nutritional analysis and reported that *C. tamala* leaves contained 0.6 ± 1.4 mg/100g sodium ^[70]. According to USDA (2019) report, Sodium content was found to be 23 mg in bay leaf ^[69]. *C. cassia* has higher value of sodium (30mg/100g) compared to 10mg/100g in *C. camphora* ^[71]. Haider *et al.*, (2018) found that the concentration of Na in *C. tamala* Uttarakhand leaf is 6.43 mg/100g which is higher than the market leaf (1.31 mg/100 g) and Uttarakhand bark (5.44mg/100g) ^[17]. Maximum foliar sodium content of *Andrographis paniculata* was observed to be 5.83 mg/g in monsoon (June-August) and minimum was observed to be 4.07 mg/g in winter (December-February) respectively ^[66]. Chowdhury and Alam, (2001) reported that the average sodium content in ten clonal varieties of Bangladesh tea ranged from 0.071-0.118% ^[68]. Twig nutrient status was found to change with agrotypes and periods. Na content ranged from 0.068% to 0.090%, 0.084% to 0.184% and 0.058% to 0.092% in early monsoon, monsoon and late monsoon respectively ^[67]. These reports are comparable to the seasonal impact on the sodium status of the present experiment.

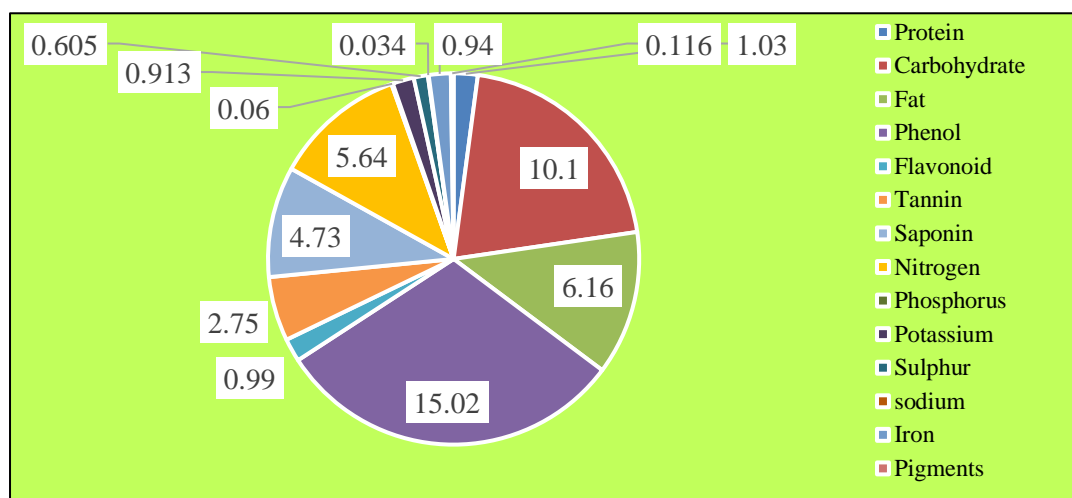


Fig1. Average bioactive components (average of four seasons) of *C. tamala* in a full production year.

Iron: It is apparent from the result that iron content was found to be diverged significantly from with seasons. The average value of iron (average of four seasons) was estimated to be 0.94%. Iron content was found to be Maximum at Monsoon period (1.39%) and minimum at Winter period (0.46%). In case of seasonal variation iron content showed the following succession as Monsoon>Late Monsoon>Early Monsoon>Winter (Table: 5,8 and 9; Fig. 1). Dandapat *et al.*, (2014) reported that *C. tamala* leaf contained 10.7 ± 1.3 mg/100g iron ^[70]. Fe concentration for bay leaf was found to be 43 mg in the USDA (2019) report ^[69]. Al-Hashimi and Mahmood (2016) reported that the average iron content in *C. tamala* was 0.45% ^[52]. Haider *et al.*, (2018) reported that iron content (19.15 mg 100g-1) was significantly higher in Uttarakhand leaf than Market leaf (10.60 mg 100g-1) of *C. tamala* ^[17].

Sulphur:

Sulphur content was found to be 0.70%, 0.66%, 0.61% and 0.45% at early monsoon, monsoon, late monsoon and winter respectively. The average value of sulphur content (average of four seasons) was

estimated to be 0.605%. The result also designates that sulphur content was found to be maximum at Early Monsoon and minimum at Winter and maintained the following sequence as Early Monsoon> Monsoon>Late Monsoon>Winter (Table: 5,8 and 9; Fig. 1). Sulfur operates as cofactor of several enzymes critically involved in the regulation of oxidative processes. Guo *et al.*, (2012) claimed that the average sulfur content in *C. camphora* leaves was 0.2160%. It had a positive correlation with the sulphur content in the air. It had a negative correlation with diameter at breast height. It was also significant between seasons, higher in spring and autumn, lower in summer and winter ^[72]. So, these findings substantiate with the finding of the present experiment.

Antioxidant activity: The results of DPPH free radical Scavenging activity of Ascorbic acid (used as standard) show that it varied markedly with different concentrations. The scavenging activity of ascorbic acid was found to be 51.20, 66.23, 78.12, 84.30 and 93.57% at the concentration of 50, 100, 150, 200 and 250µg/ml respectively. The highest scavenging activity of Ascorbic acid was found to be 93.57% at the concentration of 250µg/ml and the lowest scavenging activity was found to be 51.20% at the concentration of 50µg/ml. It is evident from the result that the IC₅₀ value of standard ascorbic acid, early monsoon, monsoon, late monsoon, and winter was observed to be 29.96, 36.19, 49.71, 41.20, 56.99µg/ml respectively. It is obvious from the result that the IC₅₀ value of early monsoon was observed to be the closest to the IC₅₀ value of standard ascorbic acid which indicates that the 50% antioxidant inhibition of *Cinnamomum tamala* was occurred in the ethanolic crude extract of early monsoon. It is also apparent from the result that the ethanolic crude extracts of early monsoon revealed highest anti-oxidant activity in compared with the standard (Table: 6 and 8; Fig. 3).

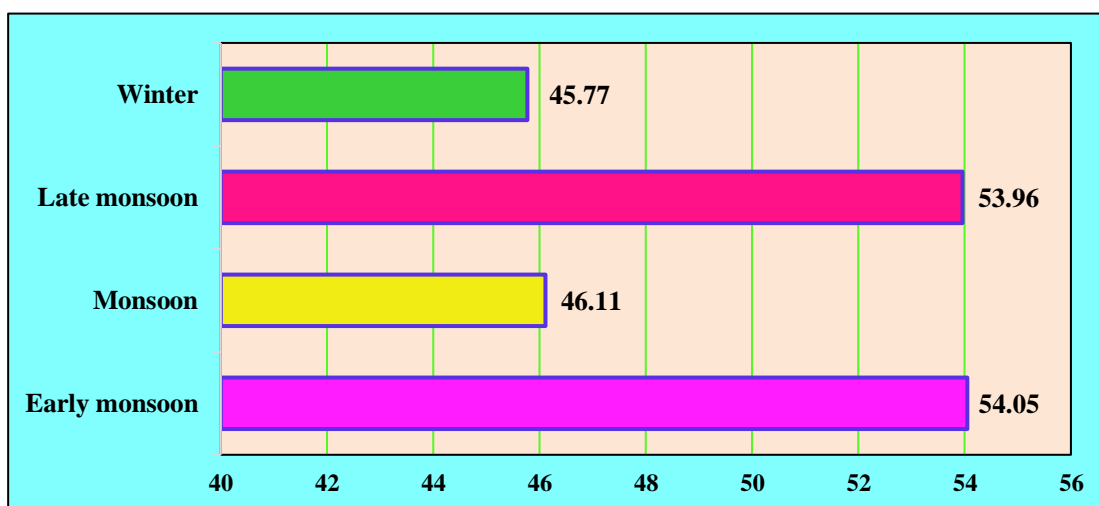


Fig2. Total bioactive components in each season of *C. tamala* in a full production year.

Table6. Seasonal variation of % of scavenging activity (Mean±SEM) of *Cinnamomum tamala* in a full production year.

% of Scavenging activity (Mean±SEM) of ethanolic crude extracts of <i>Cinnamomum tamala</i> and standard					
Concentration (µg/ml)	Early monsoon	Monsoon	Late monsoon	Winter	Ascorbic acid
50	50.66±0.50a	49.37±0.08a	50.42±0.36a	51.03±0.08a	51.20±0.72
100	64.76±0.13b	55.13±0.06a	59.11±0.32b	55.22±0.08a	66.23±0.47
150	74.97±0.21c	68.31±0.12b	68.22±0.11b	64.98±0.13b	78.12±0.56
200	86.35±0.20d	75.30±0.12c	72.42±0.10c	70.29±0.15c	84.30±0.49
250	92.05±0.28e	82.73±0.03d	81.13±0.03d	78.30±0.28c	93.57±0.23

Means (n=3) in table followed by a common letter are not significantly different (P > 0.05) according to DMRT.

Pandey *et al.*, 2012 showed that a different extract of *C. tamala* (Petroleum ether, Benzene, Chloroform, Ethyl acetate, Acetone, ethyl alcohol, water) has antioxidant activity of 66.3%, 34.8%, 38.9%, 5.2%, 31.1%, 28.5%, 4.0% respectively ^[73]. Kalauni *et al.*, 2021 reported strong antioxidant activity was found

in methanolic extract of young leaves of *C. tamala*, and its IC₅₀ value was estimated as (67.19±14.96 µg/mL) at a concentration range of 31.25-500 µg/mL while IC₅₀ value of standard ascorbic acid was found to be 33.53±0.97 µg/mL at the concentration range of 10-50 µg/mL [74]. The IC₅₀ value of ethanolic extract of *C.tamala* (leaves) was estimated to be 13.55µg/ml and that of standard ascorbic acid was 5.35 µg/ml [75]. Preeti *et al.*, (2021) demonstrated that the DPPH free radical assay revealed that methanolic extract of *C.tamala* at a 100µm/ml concentration showed the % inhibition activity as 92.29± 0.89 [57]. In *C. tamala* hydroalcoholic leaf extract at 100 µm/ml concentration, the % inhibition activity was 96.99± 0.99 [45]. Silva *et al.*, (2019) concluded that the extract from the barks of *C. triplinerve* showed remarkable antioxidant activity (IC₅₀ 11.42 ± 0.41 µg/mL) [60]. These research outcomes validate the present investigation.

Anti-inflammatory activity: The results of anti-inflammatory activity of acetyl salicylic acid (used as standard) showed that it varied distinctly at different concentrations. Inhibition (%) activity of salicylic acid was found to be 51.24, 66.33, 75.46, 82.90 and 92.33 % at the concentration of 50, 100, 150, 200 and 250 µg/ml respectively. The highest Inhibition (%) activity of acetyl salicylic acid was determined to be 92.33% at the concentration 250 µg/ml and the lowest Inhibition (%) activity was determined to be 51.24% at the concentration of 50µg/ml. It is obvious from the result that the IC₅₀ value of standard acetyl salicylic acid, early monsoon, monsoon, late monsoon, and winter was observed to be 30.24, 38.62, 42.96, 49.70, and 64.25µg/ml respectively. The IC₅₀ value of early monsoon was observed to be the closest to the IC₅₀ value of standard acetyl salicylic acid which indicates that the 50% anti-inflammatory inhibition of *C. tamala* was occurred in the ethanolic crude extract of early monsoon. It is also ostensible from the result that the ethanolic crude extracts of early monsoon revealed the highest anti-inflammatory activity in compared with the standard (Table: 7 and 8; Fig. 4).

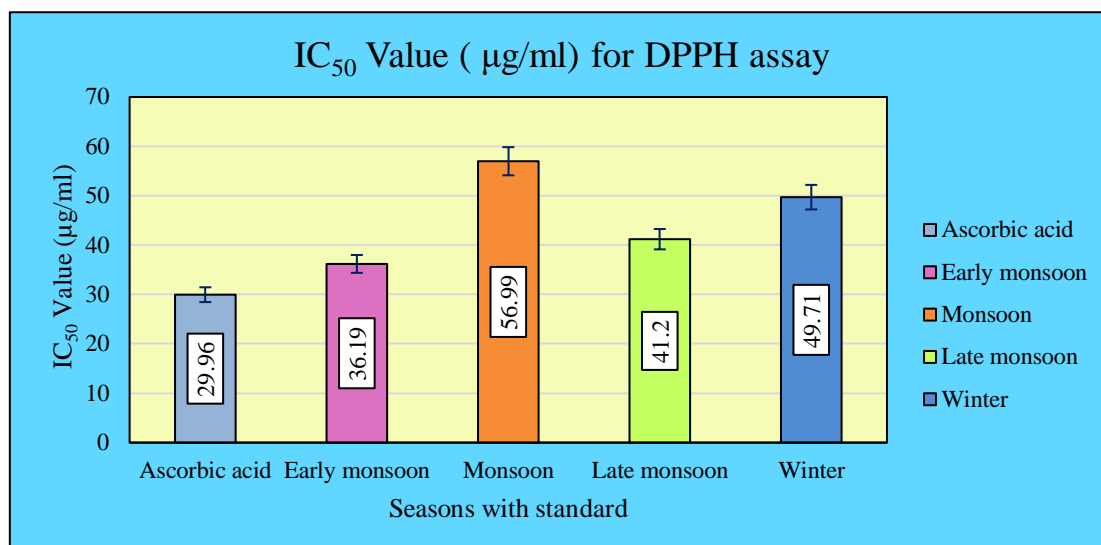


Fig3. IC₅₀ values of standard and ethanolic crude extracts of *Cinnamomum tamala* in four different seasons for antioxidant test.

Table7. Seasonal variation of albumin protein denaturation assay of anti-inflammatory activity in *Cinnamomum tamala*.

Conc. (µg/ml)	(%)† Inhibition of albumin denaturation of <i>Cinnamomum tamala</i>				
	Early monsoon	Monsoon	Late monsoon	Winter	ASA(Standard)
50	51.49±0.89a	52.20±1.04a	48.95±0.91a	47.88±0.52a	51.24±0.54
100	57.95±1.15a	59.52±1.78b	56.8±0.15b	55.22±0.14b	66.33±0.03
150	65.77±2.22b	68.83±0.50c	65.35±1.08c	59.85±0.72c	75.46±2.42
200	71.20±2.00c	75.02±0.81d	69.80±1.06d	68.31±0.15d	82.90±0.99
250	78.49±2.63d	88.30±0.99e	75.60±1.07e	72.95±0.87e	92.33±0.76

Means (n=3) in table followed by a common letter are not significantly different (P > 0.05) according to DMRT.

In an extensive study, Thamizhselvam *et al.*, (2012) evaluated that the methanolic extract of *C. tamala* has anti-inflammatory activity in the carrageenan induced paw edema in Wistar albino rats and the

percentage inhibition of edema formation was 66.75% and 73.71% at 250 and 500 mg/kg dosage respectively [76]. At the dose of 400 mg/kg body weight, the extract of *C. tamala* showed a significant anti-inflammatory activity both in the carrageenan and histamine-induced oedema test models in rats showing 60.84% and 59.48% reduction in the paw volume comparable ($P < 0.01$) to that produced by the standard drug indomethacin (63.63% and 66.01%) respectively [77]. In an extensive experiment Kusumastuti and Jaya, (2022) concluded that ethanolic extract of bay leaves had an anti-inflammatory effect on male white rats at all doses and the most effective dose of ethanolic extract at a concentration of 70% was at a dose of 150 mg/kg [78]. As reported by Gunawardena *et al.*, (2015) *C. cassia* exhibited IC_{50} value 55.09 for NO and IC_{50} value 63.3 for TNF- α as anti-inflammatory activity [79]. Budiastuti *et al.*, (2021) reported that IC_{50} value of anti-inflammatory activity in ethanolic extract of *C. brumannii* bark was 69.45 $\mu\text{g/mL}$ [80]. These research outcomes are comparable with the present investigation.

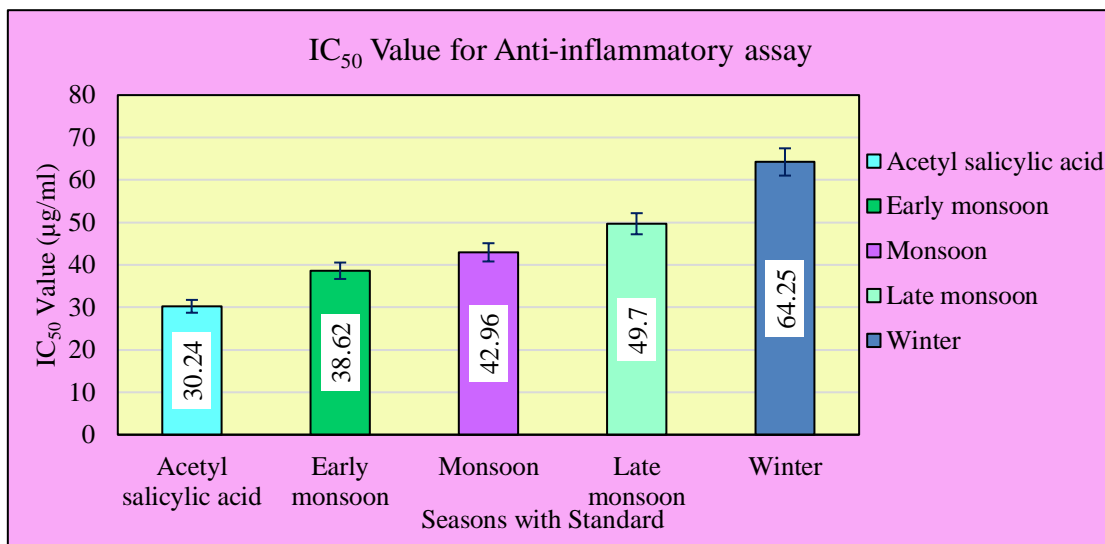


Fig4. Variation of IC_{50} Value ($\mu\text{g/ml}$) of Anti-inflammatory assay including Standard and ethanolic crude extracts of *C. tamala* at early monsoon, monsoon, late monsoon and winter.

Table8. Analysis of variance of pigments, minerals, primary metabolites and secondary metabolites of *C. tamala* estimated in four different seasons (Early monsoon, Monsoon, Late monsoon and Winter).

S. of variables	D. of freedom	F-Values																	
		Total pigments			Minerals					Protein	Carbohydrate	Fat	TPC	TFC	TTC	Saponin	AOA	AIA	
		Chl-a	Chl-b	Carot.	N	P	K	S	Na										Fe
Seasons	3-1=2	25.27*	33.21*	95.04*	102.26*	5.94**	413.83***	117.19*	132.67**	18.77*	28.62**	30.69*	8.38*	106.73*	10.37**	113.98**	26.10*	5.00*	4.27*

**Denotes significant at 1% level ($p < 0.01$), * Denotes significant at 5% level ($p < 0.05$).

Table9. Correlation co-efficient matrix analysis of average (average of four different seasons: Early monsoon, Monsoon, Late monsoon, Winter) bioactive components (Protein, Carbohydrate, Fat, Total Phenolic content, Total Flavonoid Content, Total Tannin Content, Saponin, Nitrogen, Phosphorus, Potassium, Sulphur, Sodium, Iron, and Pigments) of *Cinnamomum tamala*.

	Protein	Carb.	Fat	TPC	TF C	TT C	Saponin	N	P	K	S	Na	Fe	Pig
Protein	1													
Carb.	-0.91	1												

Fat	0.817	-0.98	1											
TPC	-0.69	0.37	-0.23	1										
TFC	0.93	-0.99	0.966	-0.47	1									
TTC	-0.94	0.82	-0.70	0.60	-0.81	1								
Saponin	-0.55	0.40	-0.36	0.82	-0.52	0.30	1							
N	0.28	0.03	-0.14	-0.88	0.08	-0.15	-0.82	1						
P	-0.07	0.06	0.00	-0.30	0.04	0.37	-0.74	0.57	1					
K	0.60	-0.68	0.72	-0.47	0.75	-0.32	-0.83	0.37	0.67	1				
S	0.46	-0.78	0.88	0.249	0.72	-0.37	-4.2E-	-0.5	-0.0	0.53	1			
Na	0.64	-0.30	0.14	-0.99	0.39	-0.58	-0.76	0.88	0.23	0.37	-0.34	1		
Fe	0.64	-0.78	0.83	-0.37	0.83	-0.39	-0.71	0.19	0.55	0.98	0.69	0.25	1	
Pig.	0.55	-0.78	0.81	0.21	0.69	-0.60	0.24	-0.63	-0.51	0.21	0.88	-0.26	0.39	1

5. CONCLUSION

All the studied parameters of *C. tamala* were found to be varied significantly with seasons. In response to seasonal assay Carbohydrate, Total polyphenol, Flavonoid, and Antioxidant activity possessed the same seasonal sequence as EM>LM>W>M. Whilst the highest amount of Anti-inflammatory activity, Saponin, Sulphur and Foliar pigments were estimated in Early monsoon and followed the succession as EM>M>LM>W. On the contrary, Protein, Nitrogen and Sodium contents followed the order as M>W>LM>EM. In contrast, Fat, Potassium and Iron contents maintained the sequence as M>LM>EM>W. Furthermore, Tannin and Phosphorus contents exhibited the series as LM>W>EM>M. Therefore, the present study concludes that in respect to total bioactive components so far studied, Early monsoon was found to be the best season for collecting the leaves of *Cinnamomum tamala* to achieve the maximum medicinal benefits and could be followed the seasonal harvesting sequence as EM>LM>W>M.

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