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A Comparative Study on the Effect of Seasonal Variation in the Phenolic Compound Concentration and the Antioxidant Properties in Five Common Leafy Vegetables

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ABSTRACT: Metabolites in plants are the chemical compounds derived as the result of primary or secondary metabolism. Like that of primary metabolites the secondary metabolites also have greater importance in plant and human lives. Phenols, alkaloids, flavonoids, tannins, steroids, terpenoids and saponins are belong to this group, and which provide shelter to the plants from various Reactive oxygen species produce by the result of different environmental and biotic stresses. Reactive Oxygen Species (ROS) recognized as toxic byproducts of aerobic metabolism and also they played an important role in plants, controlling process such as growth development and especially response to biotic and abiotic environment stimuli. The cellular oxidation caused by ROS were alleviated in plants by producing non enzymatic and enzymatic ROS scavengers popularly known as antioxidants. The production of ROS were varied with respect to the seasons having different stressful conditions. Increased level of ROS were counter attacked by plants through the production of various antioxidants. Thus, their level also varied with respect to the concentration fluctuation of ROS and seasons. The major challenge of the study is to detect the variation in the concentration of phenols and identified the antioxidant property variation against the four different seasons such as winter, summer, south west monsoon and north east monsoon in five common leafy vegetables - *Amaranthus hypochondriacus* L., *Murraya koenigii* L.,*Cucurbita maxima* L., *Brassica oleracea* L. and *Moringa oleifera* L. The result of the study revealed that the maximum antioxidant activity and phenolic concentration was reported in all plants in summer and winter seasons than the monsoon. During these seasons the plants faced much more abiotic stresses in addition to the internal stresses. In some plant specimens, the winter or summer is the season for flowering or prepared for reproduction. Thus, that will also make stresses in them along with environmental stresses. *Moringa oleifera* and *Murraya koenigii* showed high phenolic concentration and antioxidant property among the five studied plant specimens in all the seasons. This type of studies give an awareness to the people about the concentration of various secondary metabolites in plants in different seasons. Since man used many leafy vegetables directly after cooking or not, their higher concentration may negatively affect the human health.

Keywords: Reactive Oxygen Species, Antioxidant compounds, *Amaranthus hypochondriacus* L., *Murraya koenigii* L.,*Cucurbita maxima* L., *Brassica oleracea* L. and *Moringa oleifera* L.

INTRODUCTION

Secondary metabolites in plants are bioactive chemical compounds that play a key role in defense and adaptation mechanisms. Among them carbon-based metabolites such as polyphenols and tannins have greater ecological and economic importance. Polyphenols are ubiquitous secondary metabolites present in 5 to 50 gm/kg dry weight of plants and were synthesized via phenyl propanoid pathway. They provide protection to the plants against pathogens, herbivores (Skarpe and Hester 2008) and UV radiations (Tharayil *et al*., 2011). These compounds are directly related to their pharmacological properties and have the greatest potential of being valuable to human health (Podsędek and Anna 2007). Epidermological evidence supported the role of polyphenols in diet which in turn play an important role in the prevention of cancers and cardiovascular diseases (Pandey *et al*., 2009). They also have antioxidant and anti-inflammatory properties could have therapeutic and prophylactic effects for neurogenerative diseases and these health benefits makes the phenolic compounds become the most explored secondary metabolic compounds (Cory *et al*., 2018).

Reactive Oxygen Species (ROS) recognized as toxic byproducts of aerobic metabolism. ROS plays an important role in plants, controlling process such as growth development and especially response to biotic and abiotic environment stimuli. The major members of the ROS family include free radicals like $O^{\cdot-}_2$, $OH^{\cdot}$ and non-radicals like H_2O_2 and $^1O_2$. Antioxidants, Reactive Oxygen Species (ROS) scavengers are phytochemicals which can inhibit the oxidation of biomolecules like DNA, proteins and lipids by scavenging the free radicals and alleviating stress (Raja *et al.*, 2017; Marcio and Isabel 2013). ROS are produced constantly due to aerobic respiration, various biotic and abiotic stresses in the chloroplast (Dietz *et al.*, 2016), mitochondria (Sako *et al.*, 2020; Honglin *et al.*, 2019), peroxisomes (Sandalio and Romero-Puertas 2015) and also by Apoplast (Jan *et al*., 2020).

Phenol are widely used in household products and as intermediatesfor industrial synthesis. Serotonin, a neuro transmitter and urushiol, an irritant secreted by poison to prevent animals from eating its leaves etc. are the examples of naturally occurring phenols. Many of the more complex phenols used as flavoring and aromas are obtained from essential oils of plants. Phenols are largely dispersed in plant kingdom and are the most abundant secondary metabolites of plants. Plant phenolics are generally involved in defense against ultraviolet radiations or aggression by pathogens, parasites and predators, as well as contributing to plants colours. They are present in all plant organs. Phenolics are wide spread constituents of plant food. Phenolic compounds are widely found in various amounts in fruits, vegetables, cereals, and beverages such as wine, coffee, cocoa, and tea (Papuc *et al*., 2017).

The present study is focussed to a comparative assay of change in phenolic concentration and antioxidant properties in five common leafy vegetables *Amaranthus hypochondriacus* L, *Murraya koenigii* L., *Cucurbita maxima* L., *Brassica oleracea* L. and *Moringa oleifera* L. with respect to varied seasons.

MATERIALS AND METHODS

Plant Materials. Five leafy vegetables such as *Amaranthus hypochondriacus* L. (family, Amaranthaceae), *Murraya koenigii* L. (family, Rutaceae), *Cucurbita maxima* L. (Cucurbitaceae), *Brassica oleracea* L. (Brassicaceae) and *Moringa oleifera* L. (Moringaceae) are selected for the present study (Fig. 1). The leaves of such plants were collected in four seasons: South west monsoon, from June to September (Average temperature is 19 to 30°C and 2250 to 2500 mm precipitation) North east monsoon, from October to November (Average temperature of 29 to 30°C and 450 to 500mm precipitation), Winter, from December to the beginning of February (Average temperature of 18 to 28°C and 25mm precipitation) and Summer, from February to May (Average temperature of 32 to 36°C and 135mm precipitation). Then the leaves of plants were collected was dried inshade and powdered. The methanol extract of the samples was used for the study.

Analytical Methods. Preliminary Qualitative Screening of Secondary Metabolites

(1) Add a few drops of ferric chloride solution in a small amount of extract which is diluted with distilled water in the ratio 1:4. The presence of tannin were indicated by the appearance of blue or green colour.

(2) The extract was treated with a few drops of 5% neutral ferric chloride solution, appearance of an intense blue colour resulted the presence of phenol.

(3) The extract was treated with 1N aqueous NaOH, resulted yellow orange colour revealed the presence of flavonoids.

(4) The addition of wagner's reagent (1.27g of iodine and 2g of potassium iodide dissolved in 5ml of water and made up to 100ml withdistilled water) to a fraction of extract, appearance of reddish-brown precipitate indicates the presence of alkaloid

(5) A small amount of plant extract was vigorously shaken, formation of persistent foam indicates the presence of saponins.

(6) A few drops of acetic anhydride followed by a little drops of conc. H_2SO_4 were added to the extract (1ml) dissolved in chloroform producing dark green colour confirms the presence of terpenoids.

(7) To the extracts evaporated to dryness, add a little drops of conc: H_2SO_4 and acetic anhydride; an array of colour changes from yellow, green, brown to black detects the presence of steroids.

Quantification of Phenols. Total phenols content were valued by the method of Mayer *et al.* (1995). An aliquot of the extracts of four samples in 80% methanol were pipetted out separately and made up to 3 ml with the solvent. Add 0.5 ml Folin-ciocalteau reagent in to it. Kept for 3 minutes. Boiling the mixture in a water bath for 1 minute after adding 2 ml of 20% Na_2CO_3 and remove the white precipitate by centrifuging it for few min The absorbance of the clear light blue solution was recorded at 650 nm against the reagent blank containing 80% methanol (3ml), 0.5 ml Folin's reagent and 2 ml of 20% Na_2CO_3. Blue complex formation is due to the reaction between phenols and an oxidizing agent phosphomolyb date in Folin-ciocalteau reagent. A standard graph of phenols was constructed with pyrocatechol by taking absorbance against concentration. The total phenols/gm tissue was determined from the standard graph.

Antioxidant essay by DPPH Method. The antioxidant property of the samples in different seasons was identified by DPPH method. It is a rapid, simple inexpensive method of antioxidant property analysis. DPPH method was discovered by Goldsmith and Renn in 1920. DPPH stands for 2, 2 – diphenyl-1-1-picryl hydrazin. It is a stable free radical with purple colour which turn yellow when scavenged. DPPH is used for measuring the total antioxidant capacity. Antioxidant capacity is the overall ability of organism or food to catch free radicals and prevent their harmful effect. 100 µl (0.1ml) sample is taken in a test tube and make up to 3 ml by using methanol. Then add 1 ml of 0.004% (4 mg/100 ml methanol) ml DPPH in to it, incubate it in darkness for 30 minutes at room temperature. Take the absorbency at 517 mm, 80% methanol (5ml) is used as blank. The percentage of total antioxidant capacity of the sample (TAC) can be calculated by

$$\text{TAC} = \frac{\text{Absorbancy of Control} - \text{Absorbancy of Sample}}{\text{Absorbancy of Control}} \times 100$$

Statistical Analysis. The data obtained were analyzed statistically by one-way analysis of variance (ANOVA) and t- test ($p < 0.05$). The results were average of five replications and represented as mean ± SD.

RESULTS AND DISCUSSION

Preliminary qualitative assay. The qualitative screening of various phytochemicals in 20 samples of 5 plant materials in four seasons revealed the presence of phenols, alkaloids, tannins, steroids, flavonoids, terpenoids and saponins (Table 1a and 1b). Phenols, flavonoids, and alkaloids showed its strong presence in all the samples. Terpenoids revealed its moderate presence in *Cucurbita maxima and Brassica oleracea* but strongly positive in other three plant materials in all the seasons. Tannins are strong positive in all seasons in *Amaranthus hypochondriacus, Murraya koenigii* and *Moringa oleifera* but its presence is moderate in Cucurbita and Brassica in south west and north east monsoon seasons but strong in winter and summer seasons. Presence of saponins is strong in Murraya, Moringa and Amaranthus in winter and summer seasons, moderate in southwest and north east monsoon seasons. Saponins are absent in Brassica during southwest, north east monsoon and winter seasons. Terpenoids and steroids aremoderately noticed in all the four seasons in Brassica and Cucurbita but strong in its presence in other three plants.

Total Antioxidant Activity. Antioxidant activity of 20 samples in four seasons by DPPH method showed good results. In *Amaranthus hypochondriacus* L., the maximum antioxidant property were noticed in summer season (68%) which is followed by winter (61%). This is the result of flowering and pre-flowering stresses in addition to the environmental stresses. Whereas comparatively low antioxidant properties were noticed in south west and north east monsoon seasons (36% and 47%) (Table 2).

In *Murraya koenigii* L., 80% is the antioxidant activity in summer season and 78% in winter. 51 and 60 are the percentage of antioxidant property in south west monsoon and north east monsoon seasons respectively (Table 2).

Cucurbita maxima L., also revealed its maximum antioxidant property in summer and winter seasons and that was 64% and 49% respectively due to the plant passed through more stressful conditions during these seasons. South west monsoon season have only 29% and north east monsoon have 27% antioxidant properties (Table 2).

Winter season makes more antioxidant activity in *Brassica oleracea* L. (69%), then in summer (62%). 26% and 22% are the antioxidant activity in south west and north east monsoon seasons respectively (Table 2).

***Moringa oleifera* L.** showed remarkable antioxidant activity in all seasons and maximum in winter (86%) which is followed by summer (84%). The property not showed drastic decrease in monsoon seasons like that of other plant samples (Table 2).

The present study reports are an agreement to the inventions of previous studies of Sandalio *et al.* (2015).

Total Phenolic Content (TPC).

Phenolic compounds are strong antioxidant secondary metabolites which alleviate the injury caused by ROS produced by the effect of environmental stresses. Change of season with varied environmental conditions affect the normal plant cells that will stimulate or reduce cellular oxidation by ROS. Thus the production and action of antioxidant phenolic compounds are very valuable in plants and that will support the human health also.

In *Amaranthus hypochondriacus* L., the phenolic concentration is more during the summer season (1.4990 ± 0.114) than the monsoon and winter seasons because of high temperature, high light intensity and water scarcity. Comparatively low phenolic concentration is reported in monsoon seasons (0.3474 ± 0.19 in south west monsoon and 0.5626±0.220 in north east monsoon). The winter season is also stressful period to the plant because it is a pre-flowering season. Thus the plant prepared for flowering and that will create more stress along with abiotic factors. This resulted more phenolic concentration (0.9127 ±0.71) than monsoon (Table 3).

In the case of *Murraya koenigii* L. the phenolic concentration is quite less during South west monsoon season (0.9663 ± 0.048) and north east monsoon season (1.0038 ±0.912). But TPC is high in winter season (1.8689±0.412). This is because the flowering stresses along with abiotic. Maximum TPC were reported in the summer sample (1.9256 ± 0.109) because of high temperature, high light intensity and water scarcity (Table 3).

The study of total phenolic concentration in *Cucurbita maxima* L. revealed the fact that it will varied along with seasonal fluctuations. Phenolic concentration is maximum during the winter season (0.8467±0.67) compared to the monsoon and summer seasons. Comparatively low TPC identified in monsoon seasons, south west monsoon (0.2520± 0.011) and in north east monsoon (0.2991±0.012). Like that of winter sample, the summer season sample also showed high (0.7197±0.62) phenolic concentration due to high environmental stresses (Table 3).

The sample of *Brassica oleracea* L. in summer season contains more phenolic content (0.550±0.118) than that in monsoon and winter seasons due to high temperature, high Intensity of Light and Less availability of Water. In south west monsoon, comparatively very low phenolic concentration is observed (0.0977±0.02) than that of north East Monsoon (0.1426±0.17). The winter season is also a stressful period tothe plant because it is the flowering season. Thus the plant faced internal stress along with abiotic stresses, resulted maximum phenolic concentration (0.607±0.017) (Table 3).

During Monsoon seasons phenolic concentration in *Moringa oleifera* L. is low (0.9387±0.213 in South West monsoon and 0.8653±0.002 in North West monsoon) compared to summer and winter seasons. The high temperature, high light intensity and water scarcity in summer resulted high phenolic concentration (2.4430±0.018). The winter season is also a stressful season to the plant that was also reflected in the phenolic concentration in *Moringa oleifera* L. (1.6571±0.093) (Table 3). The result of the study have a correlation with the work of Shalaj *et al.* (2023).

Table 1a: Preliminary phytochemical analysis using methanol extracts of (A) *Amaranthus hypochondriacus* L. (B) *Murraya koenigii* L. (C) *Cucurbita maxima* L. (D) *Brassica oleracea* L. and (E) *Moringa oleifera* L. leaves in two seasons [South West monsoon: North East monsoon) [+++; Strong positive, ++; Moderately positive, +; Low positive, -; Negative].

Phyto Chemical	South west monsoon					North east monsoon				
	A	B	C	D	E	A	B	C	D	E
Phenols	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Tannins	+++	+++	++	++	+++	+++	+++	++	++	+++
Alkaloids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Terpenoids	++	+++	++	++	+++	+++	+++	++	++	+++
Steroids	+++	+++	++	++	+++	+++	+++	++	++	+++
Saponins	++	++	-	-	++	++	++	+	-	++

Table 1b: Preliminary polyphenol analysis using methanol extracts of (A) *Amaranthus hypochondriacus* L. (B) *Murraya koenigii* L. (C) *Cucurbita maxima* L. and (D) *Brassica oleracea* L. (E) *Moringa oleifera* L. leaves in two seasons (Winter and Summer) [+++; Strong positive, ++; Moderately positive, +; Low positive, -; Negative].

Phyto Chemical	Winter					Summer				
	A	B	C	D	E	A	B	C	D	E
Phenols	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Tannins	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Alkaloids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Terpenoids	+++	+++	++	++	+++	+++	+++	++	++	+++
Steroids	+++	+++	++	++	+++	+++	+++	++	++	+++
Saponins	+++	+++	+	-	+++	+++	+++	++	+	+++

Table 2: Percentage of total antioxidant activity in five leafy vegetablesin four seasons.

Sr. No.	Plant Material	South West Monsoon	North East Monsoon	Winter	Summer
1.	*Amaranthus hypochondriacus* L.	36	47	61	68
2.	*Murraya koenigii* L.	51	60	78	80
3.	*Cucurbita maxima* L.	29	27	49	64
4.	*Brassica oleracea* L.	26	22	69	62
5.	*Moringa oleifera* L.	61	70	86	84

Table 3: Total phenolic content in *Amaranthus hypochondriacus* L., *Murraya koenigii* L., *Cucurbita maxima* L., *Brassica oleracea* L. and *Moringa oleifera* L. (mg/gm fresh tissue) in four different seasons. [Values are mean ±SD of five independent replications].

Plant Materials	South west monsoon	North east monsoon	Winter	Summer
Amaranthus hypochondriacus L	0.3474±0.19	0.5626±0.220	0.9127±0.71	1.4990±0.114
Murraya koenigii L.	0.9663 ±0.048	1.0038 ±0.912	1.8689±0.412	1.9256 ±0.109
Cucurbita maxima L.	0.2520±0.011	0.2991±0.012	0.8467±0.67	0.7197±0.62
Brassica oleracea L.	0.0977±0.02	0.1426±0.17	0.607±0.017	0.550±0.118
Moringa oleifera L.	0.9387±0.213	0.8653±0.002	1.6571±0.093	2.4430±0.018

Fig. 1. Plant materials; *Cucurbita maxima* L., *Amaranthus hypochondriacus* L., *Murraya koenigii* L., *Brassica oleracea* L. and *Moringa oleifera* L.

CONCLUSIONS

Plants are blessed with a large number of secondary metabolites that are produced by them as the part of their adaptations and defence against various biotic and abiotic factors. These phytochemicals with antioxidant properties have a plenty of uses in human life also. Phenols, alkaloids, flavonoids, tannins, terpenoids, saponins, steroids etc. are the common metabolites in plants, of these carbon based metabolites such as phenolic compounds are one of the major antioxidant in plants. The production and action of these antioxidant compounds varied with the variation of seasons having different environmental conditions. The present study aimed to identify the total phenolic content and antioxidant property in five leafy vegetables such as *Amaranthus hypochondriacus*, *Murraya koenigii*, *Cucurbita maxima*, *Brassica oleracea* and *Moringa oleifera* with respect to varied seasons. The result of the study revealed that the antioxidant activity and phenolic compound concentration is altered in different seasons. In all the plant materials, the antioxidant property and TPC are maximum during summer and winter seasons dueto the impact of environmental factors and the stress behind the reproductive changes. Among the five plant materials, *Murraya koenigii* L. showed the high TPC and antioxidant property; *Cucurbita maxima* L., *Brassica oleracea* L. revealed lowest content of phenol and antioxidant property.

FUTURE SCOPE

Since the man used the leafy vegetables directly after cooking or not, the knowledge about the concentration of secondary metabolites in different seasons is very important. The direct use of some phytochemicals in higher concentration is dangerous to the human health. So this study have a greater importance to educate the people to know them that a particular leafy vegetable not use in which season, higher phenolic content negatively affect the health.

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Conflict of Interest. None.

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