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Research Article

EFFECT OF SEASONAL AND GEOGRAPHICAL CHANGES ON NUTRITIONAL YIELD OF FRUITS OF *TRIBULUS TERRESTRIS* LINN.

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ABSTRACT

The effect of seasonal and geographical changes on a nutritional yield of *Tribulus terrestris* fruits was investigated in the South Indian region. Principally important nutritive elements like carbohydrates, crude protein, and crude fiber were studied in this investigation. The study evaluated two seasonal and three geographically variable *Tribulus terrestris* fruits collected from South India (Karnataka, Tamilnadu and Telangana states) in June and December months during 2015 for analysis of crude protein, crude fiber, and carbohydrates. The values of the specifications determined were significantly greater for the December season than that of samples collected in June season. Fruits collected from Bellary (Karnataka state) region recorded higher quantity of nutritional elements than the fruits collected from Telangana and Tamilnadu regions. It was concluded that the variation in the nutritional quality of *Tribulus terrestris* fruits collected between the main June, December season and three different geographical locations is presented. The findings showed that the nutritive values of *Tribulus*fruits were remarkably altered as a result of seasonal and geographical variability.

KEY WORDS: *Tribulus terrestris* Linn fruits, Nutritive values, Geographical and Seasonal variations, South India

INTRODUCTION

Plant nutrients are necessary for plant growth and metabolism, because of these deficiencies the plant is unable to complete metabolic cycle. Majority soil conditions across the globe can contribute plants habituate to that atmosphere and soil with adequate nutrition for an entire life cycle, without the incorporation of nutritive compounds as fertilizers[1]. The maximum nutrients enforced in the individual human diet comes from plant sources. The nutritional conditions of plant-derived products can influence the person's healthy conditions. Globally, products from plants constitute the ampule majority of human meal intake, irrespective of financial status or location[2]. In some civilization, either by imperfection or design, plant-derived nutrition products absolutely compose 100% of the dietary intake.

In nature, plants are flourishing in a considerable diversity of habitats and are described by the noticeable changeability in terms of species distribution as a conclusion of climatic differences, biogeographical influences[3]. Nutritional element quality is impressed by biotic and abiotic environmental conditions, including soil type, climatic changes, floral composition and soil advancement practices[4]. Nutritional management is troublesome by the appreciable seasonal alteration which attributes their quality and yield. At the time of the wet season, there is a plenty of expeditiously thriving grasses consistently correlate with expeditious deterioration in quality and production is diminished as the season movement into the arid period. The season close to the end of the dry season, climate temperatures boost up, but soil humidity may be extremely low in order that all above-ground vegetation is wiped out. Those diversities in plant development and growth due to shift in environmental conditions[5].

Climatic conditions sometimes employ a direct management in the improvement and distribution of plant species. Certain plant growth is impressed by geographical and seasonal change as a few plants grow optimally at the time of the rainy season while others flourish better all along the dry season[6].The nutritional distribution of flora in India is also extremely fluctuating due to seasonal alteration in the geographical conditions and the maturity of the plant. An ample deviation in the content of plant nutrients endures not apart from area to area, however also even in the same field[7].

The fruits of *Tribulus terrestris*, belongs to the Zygophyllaceae family adapted to Mediterranean and semi-desert climates. It is a notorious traditional Indian, Chinese medicine. *Tribulus terrestris* L. is a well-established and extensively dispersed species of the genus *Tribulus*. *Tribulus terrestris* familiar with a few common names: devil's thorn, goat head, caltrop, puncture vine[8]. In spite of global dispersal of *Tribulus terrestris* and the evidence that the plant *Tribulus terrestris* collected from distinctive geographical regions has the disparate amount of the biologically active principles, most of the biological and phytochemical survey characterized in the literature point out to the *Tribulus terrestris* thriving in different American and European countries[9].

Tribulus terrestris is well practiced as folk medicine in India, Bulgaria, China and South Africa to treat sexual impotence, eye trouble, abdominal dysfunction, cardiovascular diseases and oedemas. A substantial number of pharmaceutical formulations and food supplements from *Tribulus terrestris* fruits are on marketing world-wide. *Tribulus terrestris* also incorporated in several dietary supplements declared to possess a biostimulating action[10]. *Tribulus terrestris* formulations are on sale in United States of America as food supplements with the assertion of a natural stimulating response on motor activity, restorative tonic

for vigor and muscle tone. Absolutely, *T. terrestris* formulations are principally used to enhance performance in sports[11].

The objective of this investigation was accordingly to determine the difference in the nutritional content of *Tribulus terrestris* fruits influenced by the geographical and seasonal variations, over the period (June and December) of the year 2015, in South Indian regions. Therefore, preserving the aforesaid details in view, the research was prepared to initiate by analyzing and quantifying the essential nutritive values, and determining their variation in the South Indian region.

MATERIALS AND METHODS

The fresh fruits of *Tribulus terrestris* Linn. Were collected from their natural habitat in the South Indian region; especially from Nakrekal (Telangana state), Bellary (Karnataka state), Katumnar Koil (Tamilnadu state) in June and December season. The identity was confirmed by Prof. Jayaraman, Botanist and specimens were preserved in Department of Pharmacy, Annamalai University (PARC/2015/3214, PARC/2015/3215, PARC/2015/3216). The collected fruit samples were washed thoroughly under running tap water and dried in shade. The fully dried fruit samples were transferred to grinding mill to obtain coarse powder material. The powder was preserved in a clean, well closed transparent glass container in research work.

Estimation of carbohydrates

Materials required: Quartz cuvettes, class A 10ml standard flasks, boiling tubes and class A pipettes (10ml).
Reagents required: Glucose, Sulfuric acid, Anthrone, and HCl.

Method

1. Preparation of Reagents
Anthrone reagent: Accurately weighs 200mg of enthrone and dissolved in 95% ice cold Sulfuric acid. The prepared Anthrone reagent was valid for only 10 days.

2. Preparation of standard: Take 100mg of Glucose, transferred to a 100ml standard flask. Make up to 10ml using distilled water. 1mg/mL is taken as 1000ppm solution. From above preparation, 0.01ml, 0.02ml, 0.04ml, 0.06ml, 0.08ml, 0.1ml and 0.12ml (corresponding to 10, 20,40,60,80, 100 and 120ppm) are pipetted out into a clean boiling tube.

3. Preparation of sample: 100mg of the powdered sample is taken in a boiling tube and then add 5ml of 2.5N HCl to the boiling tube. The above solution was kept in a water bath for 3 hours to establish entire hydrolysis of the sample. The whole mixture was subjected to cooling at room temperature, then neutralized with sodium carbonate solution until the breakoff effervescence. From the supernatant 0.5ml and 1ml is pipetted out into clear boiling tubes.

4. Measurement
a. Calibration curve: The standard solution in boiling tubes are made up to 1ml by using distilled water. To above, 4ml of freshly prepared Anthrone reagent is added and then the boiling tubes were kept in a water bath. Heat the water bath for 8 minutes. The boiling tubes were rapidly cooled and absorbance is measured at 630nm against blank which containing Anthrone reagent. The calibration curve is plotted as concentration (X axis) against absorbance (Y axis). From the calibration curve, Slope and Y- intercept were calculated.

b. Unknown Sample: The boiling tubes containing the sample solution is made up to1ml using distilled water. Freshly prepared anthrone reagent (4ml) is added and the boiling tubes are kept in a water bath, heated for 8 minutes. Then boiling tubes were cooled rapidly and absorbance is measured at 630nm against blank which containing anthrone reagent.

5. Calculation: The concentration of the unknown triplicates was calculated by plotting the absorbance values of the unknown sample in the calibration curve[12].

Estimation of proteins

1. Material required: Folin-C reagent, sodium hydroxide, sodium potassium tartarate, sodium carbonate, bovine serum albumin, test tubes, class A standard flask and distilled water.

2. Reagent preparation
a. Alkaline sodium carbonate solution (A): Accurately weigh 2g of sodium carbonate and dissolved in a 0.1N NaOH solution (Dissolve 0.4g of NaOH in 100ml of distilled water).
b. Copper sulfate solution: Weigh 1.56g of copper sulfate and dissolved in 100ml of distilled water.
c. Sodium potassium tartarate solution: Sodium potassium tartarate (2.37g) is dissolved in 100ml of distilled water.
d. Solution (B): Copper sulfate solution (10ml) is mixed with sodium potassium tartarate (10ml) and stored the solution in a clean reagent bottle.
e. Biuret reagent: Take 2ml of solution (B) and mix with 100ml of solution (A), stored in a clean transparent reagent bottle.
f. Folin C reagent: Folin C reagent is purchased from SpectroChem and diluted 1:1 ratio with distilled water before use. (Freshly prepared)

3. Preparation of standard: Weigh 100mg of BSA, transferred to a 100ml standard clean flask and make up to the volume 10ml by using distilled water. 1mg/mL is taken as 1000ppm from above solution. Pipette out 0.01ml, 0.02ml, 0.04ml, 0.06ml, 0.08ml, 0.1ml and 0.12ml (corresponding to 10, 20,40,60,80, 100 and 120ppm) into clean test tubes.

4. Sample preparation - 10mg of the powder sample is dissolved in 10ml of distilled water. From above preparation pipette out 0.1ml, 0.25ml and0.5ml into standard clean test tubes.

5. Measurement
a. Standard curve: The test tubes containing the corresponding volume of standard BSA solution are made up to 5ml by addition with distilled water. 2ml of the freshly prepared Biuret reagent is added to the test tubes and the test tubes were incubated at RT for 10 minutes. To above, 0.2ml of Folin C reagent is added to each test tube and the test tubes were again incubated for 30 minutes. The absorbance of the above solution is measured at 660nm against blank consists all reagents except Bovine serum albumin. The calibration curve is plotted against concentration (X axis) vs Absorbance (Y axis). From this, the Y Slope and intercept were calculated.
b. Sample: Test tubes containing the requisite volume of sample solution are made up to 5ml with distilled water. Biuret reagent (2ml) is added to the test tubes and the test tubes were incubated at RT for 10 minutes. To the test tubes add, 0.2ml of Folin C reagent and the test tubes were again incubated for 30 minutes. The absorbance of this whole solution is measured at 660nm against blank consists all reagents except Bovine serum albumin[13].

6. Calculation
The concentration of the unknown triplicates is determined by plotting the absorbance values of the unknown sample in the calibration curve.

Table 1: Percent concentration of carbohydrates present in *Tribulus terrestris* fruit powder

Tribulus terrestris	Amount of carbohydrate (%)					
	Nakrekal (Telangana)		Bellary (Karnataka)		Katumnar Koil (Tamilnadu)	
	June	16.7	June	18.4	June	14.2
	December	18.6	December	20.4	December	11.6

*Carbohydrate values are expressed as an average percentage, (n=3).

Table 2: Percent concentration of crude protein present in *Tribulus terrestris* fruit powder

Tribulus terrestris	Amount of crude protein (%)					
	Nakrekal (Telangana)		Bellary (Karnataka)		Katumnar Koil (Tamilnadu)	
	June	1.87	June	1.62	June	0.86
	December	2.83	December	2.54	December	1.92

*Crude protein values are expressed as an average percentage, (n=3).

Table 3: Percent concentration of crude fiber present in *Tribulus terrestris* fruit powder

Tribulus terrestris	Amount of crude fiber (%)					
	Nakrekal (Telangana)		Bellary (Karnataka)		Katumnar Koil (Tamilnadu)	
	June	4.6	June	5.1	June	3.8
	December	3.7	December	4.2	December	3.1

*Crude fiber values are expressed as an average percentage, (n=3).

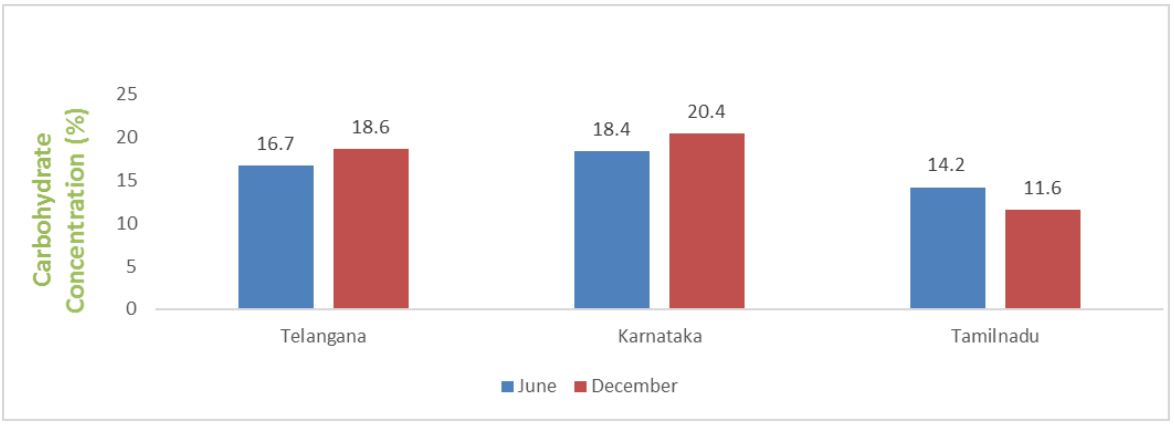

Figure 1: Analysis of carbohydrate content of *Tribulus terrestris* fruit. Percentage values are expressed as an average, (n=3)

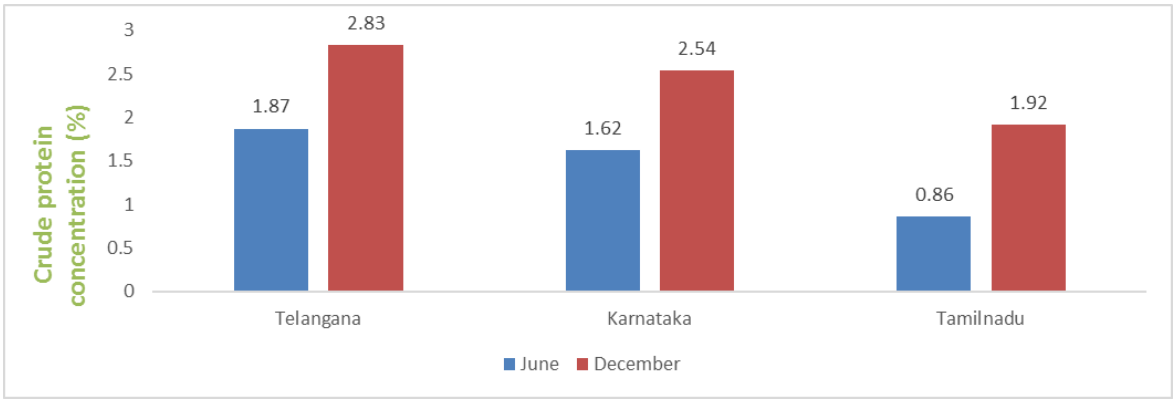

Figure 2: Analysis of crude protein content of *Tribulus terrestris* fruit. Percentage values are expressed as an average, (n=3)

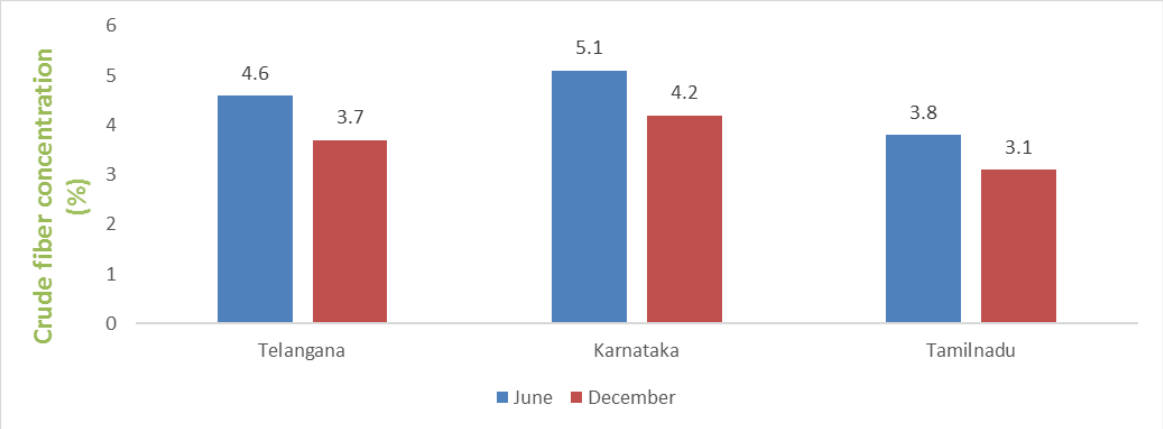

Figure 3: Analysis of crude fiber content of *Tribulus terrestris* fruit. Percentage values are expressed as an average, (n=3)

Estimation of fiber

Extract 2g of powder material with petroleum ether to remove fatty materials at initial boiling temperature is 35 -38°C and a final temperature of maximum 52°C. After completion of extraction with ether, boil 2g of dried material with 200mL of boiling dilute H_2SO_4 (1 in 78) for 30min with using bumping chips. Filter the contents through a muslin cloth and wash the residue with boiling water repeatedly until washing is no longer acidic. Collect the residue and rinse the residue with 200mL of boiling sodium hydroxide solution and reflux the contents for 30min. Filter the whole content through the muslin cloth and wash the residue with boiling water until the washings reach to the neutral condition.Collect the residue and the dry the residue at a constant temperature at 110°C and cool it until constant weight obtained. Collect fully dried residue and subjected to incinerate till the conversion of fine ash, by gradually increasing the heat not to exceed 450°C. Collect the total ash obtained by incineration process and cool the ash in a desiccator and weigh the whole content[14].

RESULTS AND DISCUSSION

Data achieved reveal the plant *Tribulus terrestris* nutrients from different geographical and seasonal variations of the South Indian region are given in table 1, 2, 3 and shown in fig 1, 2, 3. It was observed that seasonal and geographical variations directly influencing the concentration of nutritive values of *Tribulus terrestris* fruits. It was noticed that a greater nutritional content was reported during the December season (except fruits from Katumnar Koil for carbohydrates, Table 1) with compared to fruits collected in June season (except for crude fiber concentration which was maximum in June season, Table 3).

It was identified that *Tribulus terrestiris* fruits collected from Bellary (Karnataka state) showed the maximum amount of carbohydrates and crude fiber. The maximum crude protein concentration was identified in the fruits of Telangana region. Fruit samples which collected from Katumnar Koil (Tamilnadu state) resulted in the lower amount of nutritive values. *Tribulus terrestris* fruit samples collected from Nakrekal (Telangana state) showed median concentrations of nutritive values.

The highest amount of carbohydrates (20.4 %) was from Bellary (Karnataka state) in December and lowest concentration of carbohydrate (11.6 %) is identified in December from Katumnarkoil (Table. 1). The amount of protein is high in December and low in June. Highest protein content (2.83 %) is observed from the fruits from Nakrekal (Telangana state) in December and *Tribulus terrestris* fruits from Katumnar Koil showed the low amount of crude protein in June (Table. 2). The amount of crude fiber is maximum in June and reported low levels in December. *Tribulus* fruits from Bellary reported the highest (5.1 %) amount and fruits from Katumnar Koil showed the low amount of crude fiber (Table. 3).

From the outcome, it was noticed that seasonal and geographical changes affect the nutritional content of *Tribulus terrestris* fruits. This data agrees with the research work of I. Mountousisa et al., Nutritional analysis of *Tribulus terrestris* fruits revealed that highest amount of carbohydrates (20.4 %) was observed in December and the highest concentration of crude fiber (5.1 %) in June from Bellary. Crude protein concentration was maximum (2.83 %) from Telangana in December. This may due to the ability of *Tribulus terrestris* fruits to retain nutritional content with the influence of seasonal and geographical variability.

CONCLUSION

In conclusion, the nutritive concentration of *Tribulus terrestris* fruits directly influenced by the seasonal and geographical variations in South India. The nutritional content was adequate during the December season, but the nutritional content was declined towards the June season. However, *Tribulus terrestris* fruits collected from Bellary recorded the maximum concentration of nutritive values.

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