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## Comparative standardization and physicochemical evaluation of the fruits of *Tribulus terrestris* L. from different seasonal and geographical sources

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### ABSTRACT

The present investigation is based on a comparative standardization and physicochemical analysis of the dried fruits of *Tribulus terrestris* L. collected from three different geographical locations of South India viz., Ballery (Karnataka), Nakrekal (Telangana), Katummar koil (Tamilnadu) in June and December seasons. The various standardization parameters included determination of foreign matter, loss on drying, ash values, extractive values, fluorescence analysis and preliminary phytochemical screening was performed. The preliminary identification was done by using macroscopical characters and final identity was confirmed by botanist. The identification was also carried out with fluorescence analysis of the powdered fruits treating with various chemical reagents. The result from present study deals with the major variations observed in physicochemical characters in *Tribulus terrestris* due to change in season and region. The study proved that as the geographical and seasonal variation has direct influence with the variation in physicochemical constituents of the plants.

**Key words:** *Tribulus terrestris*, Zygophyllaceae, Comparative standardization, Seasonal and Geographical variations, South India.

### INTRODUCTION

*Tribulus terrestris* L. (Zygophyllaceae) is an annual creeping plant native of Mediterranean region, found around the world wide, especially in the subtropical regions [1]. *Tribulus terrestris* has been widely used as a traditional medicine in India, China, Bulgaria and South Africa to treat sexual impotency, abdominal dysfunction, cardiovascular diseases and edema [2, 3]. The season and geographic conditions has impact on active principles and other constituents of a variety of medicinal plants [4]. The phytoconstituents and active principles may differ during different seasons in a year. The specific morphological plant parts are usually collected in best season when the plant materials attain peak maturity and high concentration in active principles [5]. In ancient text of ayurvedic literature Charaka, Sushruta and others notified timely and seasonal collection of specific medicinal plant part, which is used for the preparation of ayurvedic medicine [6]. The evaluation of crude drugs has under gone different changes over the years. In olden days the crude drugs were identified by comparison with the standard description available in various reference books. Due to development of chemical, Physical and microscopical knowledge, the evaluation of crude drugs also include quantitative estimation of active constituents present in the crude drugs [7]. The various factors affecting the quality and quantity of bio active principles are age of the plant, time of harvesting, soil, climate, altitude, collector and post-harvesting conditions. In the present study, variation in physicochemical characters of the *Tribulus terrestris* due to the seasonal variation and geography has been carried out. Therefore, in the present study fruit samples of *Tribulus terrestris* were collected in June, October to study seasonal changes and

similarly geographical variations were studied on fruit samples collected from three states (Telangana, Karnataka, Tamilnadu) of South India.

## MATERIALS AND METHODS

### 2.1 Procuring of plant material

Fruits for the study were collected from their natural habitat [8]. The fresh fruits of *Tribulus terrestris* were collected from three different states (Telangana, Karnataka, Tamilnadu) of South India during June and December - 2015, when the most fruits were ripening and identified with authentication number PARC/2015/3214, PARC/2015/3215, PARC/2015/3216 by the botanist Prof. P. Jayaraman, Director, Institute of Herbal Botany, Tambaram, Chennai [9]. Fruits were immediately washed, shade dried and powdered in a mixer grinder. The powdered plant material was packed in air tight container and stored at room temperature for further study.

**Table 1: *Tribulus terrestris* samples from different regions of South India**

Samples	Authentication Number	Collected location	Geographical origin	Altitude
I	PARC/2015/ 3214	Nakrekal – Telangana State, India	Lat (DMS) 17°9'45N Long(DMS)79°25'39E	830 ft
II	PARC/2015/ 3215	Ballery – Karnataka State, India	Lat (DMS) 15°8'45N Long(DMS)76°55'3E	1669 ft
III	PARC/2015/ 3216	Katumnar koil – Tamilnadu state, India	Lat (DMS) 11°23'57N Long(DMS)79°41'28E	13 ft

### 2.2 Plant extracts chemicals and reagents

The each fruit powder of *Tribulus terrestris* was extracted successively with petroleum ether, chloroform, methanol, acetone and distilled water. The extracts were filtered and concentrated. All the extracts thus obtained, kept in desiccator for further studies. All the chemicals and reagents used for the present study were analytical grade.

### 2.3 Development of standard analytical parameters

The powdered samples and extracts of the fruits samples were standardized according to WHO guidelines and other pharmacopoeial specifications [10]. Physical parameters such as foreign matter, moisture content, loss on drying, total ash value, acid insoluble ash value, water soluble ash value, fluorescence analysis, extractive values in different solvents were analyzed as per the standard official methods [11, 12]. Successive hot extraction of fruit powder was carried out in soxhlet extractor by using various solvents of different polarity. Preliminary phytochemical investigation for all extracts was carried out with reference to the standard methods.

#### 2.3.1 Foreign organic matter

Foreign organic matter is the material not coming from the original plant. It includes insects, moulds and animals excretions, other parts of organ or organs from which the original is derived. The results of foreign organic matter for six *Tribulus terrestris* fruit samples were recorded in the form of % w/w (Table 2).

**Table 2: Foreign matter and microbial infestation of various *Tribulus terrestris* samples**

<i>Tribulus terrestris</i>	Season	Weight taken (in g)	Foreign matter ( % w/w)	Microbial infestation
Telangana	June	100	0.65	Nil
	December	100	0.93	Nil
Karnataka	June	100	1.35	Nil
	December	100	1.89	Nil
Tamilnadu	June	100	1.23	Nil
	December	100	0.97	Nil

#### 2.3.2 Ash values

The total ash, acid insoluble ash and water-soluble ash values were determined from air-dried samples using the procedure described in the IP. Ash value is used to determine the percentage of inorganic constituents present in the crude drug. These values were helping in determine the quality and purity of a crude drug. The ash of *Tribulus terrestris* fruit contains inorganic constituents like carbonates, phosphates and silicates of calcium, magnesium, potassium and sodium etc. The results of ash values of all samples were stated in Table 3.

**Table 3: Ash values of different samples of *Tribulus terrestris***

<i>Tribulus terrestris</i>	Season	Total ash (%w/w)	Water soluble ash (%w/w)	Acid-insoluble ash (%w/w)
Telangana	June	8.65	4.02	1.46
	December	11.23	5.34	2.87
Karnataka	June	7.76	3.23	1.34
	December	9.31	4.36	2.48
Tamilnadu	June	7.94	3.85	2.27
	December	10.97	4.71	2.56

### 2.3.3 Extractive values

An extractive value of the crude drugs determines the amount of active principles extracted with selected solvents from a particular amount of medicinal plant material/crude drug. For identifying extractive values the air dried, accurately weighed fine powdered drug was extracted with selected solvents like petroleum ether, chloroform, acetone, methanol and water. The extractive values of all fruit samples were reported in Table 4.

**Table 4: Successive solvent extractive values of different varieties of *Tribulus terrestris***

<i>Tribulus terrestris</i>	Season	Pet. ether (%w/w)	Chloroform (%w/w)	Acetone (%w/w)	Methanol (%w/w)	Water (%w/w)
Telangana	June	3.87	4.12	2.13	7.54	16.84
	December	4.23	4.85	2.90	5.02	14.26
Karnataka	June	3.16	4.67	1.85	5.68	14.97
	December	4.22	4.08	3.13	4.85	15.52
Tamilnadu	June	3.62	3.96	2.29	5.18	15.64
	December	4.13	4.03	1.60	4.67	13.08

### 2.3.4 Fluorescence analysis

1-2 gr of dried fruit powders of all samples were placed on microscopic slide and observed in day light as well as in UV light (Long UV 365 nm and Short UV 254 nm). All the powdered drugs were then treated with different reagents like 1 N sodium hydroxide (alcoholic), 1 N sodium hydroxide (aqueous), 1 N hydrochloric acid, ammonia, 5% ferric chloride, acetic acid, 1 N sulphuric acid, 1 N nitric acid and 5% iodine [13, 14, 15]. The change in colour was observed and the results were noted (Table 5, 6, 7, 8, 9, 10).

**Table 5: Fluorescence analysis of *Tribulus terrestris* fruit powder collected from Telangana in June**

Treatment	<i>Tribulus terrestris</i> (Telangana)		
	Day light	UV Light	
		Short UV (264 nm)	Long UV (366 nm)
Powder as such	Pale Brown	Green	Pale Green
Powder + 1N NaOH (alc.)	Dark Yellow	Dark Green	Pale Green
Powder + 1N NaOH (aq.)	Dark Green	Dark Green	Brownish Green
Powder + 1N HCl	Pale Brown	Dark Green	Green
Powder + 1N H <sub>2</sub> SO <sub>4</sub>	Dark Brown	Black	Pale Black
Powder + 1N HNO <sub>3</sub>	Dark Yellow	Black	Dark Green
Powder + NH <sub>3</sub>	Brownish Green	Blackish Green	Black
Powder + 5% FeCl <sub>3</sub>	Dark Green	Blackish Green	Brownish Green
Powder + acetic acid	Pale Green	Dark Green	Brownish Green
Powder + 5% iodine	Dark Green	Blackish Green	Purplish Green

**Table 6: Fluorescence analysis of *Tribulus terrestris* fruit powder collected from Karnataka in June**

Treatment	<i>Tribulus terrestris</i> (Karnataka)		
	Day light	UV Light	
		Short UV (264 nm)	Long UV (366 nm)
Powder as such	Pale Brown	Dark Green	Pale Green
Powder + 1N NaOH (alc.)	Pale Yellow	Dark Green	Pale Green
Powder + 1N NaOH (aq.)	Dark Green	Pale Green	Greenish Brown
Powder + 1N HCl	Dark Brown	Pale Green	Light Green
Powder + 1N H <sub>2</sub> SO <sub>4</sub>	Greenish Brown	Black	Pale Black
Powder + 1N HNO <sub>3</sub>	Yellow Brown	Dark Green	Black
Powder + NH <sub>3</sub>	Brownish Green	Dark Green	Brownish Black
Powder + 5% FeCl <sub>3</sub>	Dark Green	Greenish Black	Brownish Green
Powder + acetic acid	Pale Brown	Dark Brown	Greenish Brown
Powder + 5% iodine	Dark Green	Pale Green	Purplish Green

**Table 7: Fluorescence analysis of *Tribulus terrestris* fruit powder collected from Tamilnadu in June**

Treatment	<i>Tribulus terrestris</i> (Tamilnadu)		
	Day light	UV Light	
		Short UV (264 nm)	Long UV (366 nm)
Powder as such	Pale Green	Dark Brown	Pale Brown
Powder + 1N NaOH (alc.)	Pale Yellow	Pale Green	Dark Green
Powder + 1N NaOH (aq.)	Dark Green	Greenish Brown	Pale Green
Powder + 1N HCl	Dark Yellow	Pale Green	Light Green
Powder + 1N H <sub>2</sub> SO <sub>4</sub>	Brownish Green	Pale Black	Black
Powder + 1N HNO <sub>3</sub>	Yellowish Brown	Greenish Brown	Black
Powder + NH <sub>3</sub>	Brownish Green	Brownish Black	Dark Green
Powder + 5% FeCl <sub>3</sub>	Brownish Green	Greenish Black	Dark Green
Powder + acetic acid	Greenish Brown	Dark Brown	Pale Brown
Powder + 5% iodine	Dark Green	Pale Green	Purplish Green

**Table 8: Fluorescence analysis of *Tribulus terrestris* fruit powder collected from Telangana in December**

Treatment	<i>Tribulus terrestris</i> (Telangana)		
	Day light	UV Light	
		Short UV (264 nm)	Long UV (366 nm)
Powder as such	Pale Brown	Dark Brown	Pale Green
Powder + 1N NaOH (alc.)	Pale Green	Pale Yellow	Dark Green
Powder + 1N NaOH (aq.)	Greenish Brown	Dark Green	Pale Green
Powder + 1N HCl	Yellowish Green	Pale Green	Dark Green
Powder + 1N H <sub>2</sub> SO <sub>4</sub>	Brownish Green	Black	Pale Black
Powder + 1N HNO <sub>3</sub>	Greenish Brown	Yellowish Brown	Pale Black
Powder + NH <sub>3</sub>	Brownish Green	Yellowish Brown	Pale Green
Powder + 5% FeCl <sub>3</sub>	Greenish Brown	Greenish Black	Dark Green
Powder + acetic acid	Brownish Green	Pale Brown	Dark Brown
Powder + 5% iodine	Dark Green	Purplish Green	Pale Green

**Table 9: Fluorescence analysis of *Tribulus terrestris* fruit powder collected from Karnataka in December**

Treatment	<i>Tribulus terrestris</i> (Karnataka)		
	Day light	UV Light	
		Short UV (264 nm)	Long UV (366 nm)
Powder as such	Light Green	Pale Brown	Pale Green
Powder + 1N NaOH (alc.)	Dark Green	Pale Yellow	Pale Green
Powder + 1N NaOH (aq.)	Dark Green	Greenish Brown	Pale Green
Powder + 1N HCl	Greenish Yellow	Pale Green	Dark Green
Powder + 1N H <sub>2</sub> SO <sub>4</sub>	Yellowish Green	Black	Pale Yellow
Powder + 1N HNO <sub>3</sub>	Greenish Brown	Brownish Yellow	Blackish Yellow
Powder + NH <sub>3</sub>	Brownish Green	Pale Green	Yellowish Brown
Powder + 5% FeCl <sub>3</sub>	Greenish Black	Greenish Brown	Dark Green
Powder + acetic acid	Brownish Green	Dark Brown	Pale Brown
Powder + 5% iodine	Dark Green	Pale Green	Purplish Green

**Table 10: Fluorescence analysis of *Tribulus terrestris* fruit powder collected from Tamilnadu in December**

Treatment	<i>Tribulus terrestris</i> (Tamilnadu)		
	Day light	UV Light	
		Short UV (264 nm)	Long UV (366 nm)
Powder as such	Pale Green	Pale Brown	Dark Green
Powder + 1N NaOH (alc.)	Dark Green	Pale Green	Pale Yellow
Powder + 1N NaOH (aq.)	Greenish Brown	Dark Green	Pale Green
Powder + 1N HCl	Dark Green	Pale Green	Greenish Yellow
Powder + 1N H <sub>2</sub> SO <sub>4</sub>	Pale Yellow	Yellowish Black	Yellowish Green
Powder + 1N HNO <sub>3</sub>	Brownish Green	Yellowish Brown	Blackish Yellow
Powder + NH <sub>3</sub>	Dark Green	Pale Green	Yellowish Green
Powder + 5% FeCl <sub>3</sub>	Dark Green	Greenish Brown	Pale Green
Powder + acetic acid	Brownish Green	Pale Brown	Dark Brown
Powder + 5% iodine	Dark Green	Purplish Green	Pale Green

**2.3.5 Determination of moisture (Loss on drying) content**

The percentage of active constituents present in the crude drugs was determined on dry weight basis. So the moisture content in the crude drugs must be determined. The easiest method to determine moisture content is to heat

the required quantity of drug till its get constant weight at 100 °C. Crude drugs which undergo physicochemical change with subsequent loss of weight at a temperature of 100 °C, other methods were employed [16]. The loss on drying of fruit samples were reported in Table 11.

**Table 11: Loss on drying of different varieties of *Tribulus terrestris***

<i>Tribulus terrestris</i>	Season	Weight of sample taken	Loss of weight on drying (% w/w)	Moisture content (% w/w)
Telangana	June	10	34.26	11.28
	December	10	28.14	8.62
Karnataka	June	10	36.58	13.41
	December	10	29.62	9.24
Tamilnadu	June	10	39.26	15.26
	December	10	22.17	6.74

### 2.3.6 Preliminary phytochemical analysis

The different extracts of fruits of *Tribulus terrestris* Linn were subjected to preliminary phytochemical studies to identify the various phytoconstituents like carbohydrates, phenolic compounds, flavonoids, alkaloids, triterpenoids, proteins, saponins, lipids, steroids and tannins using different standard methods [17]. The results of Preliminary phytochemical screening were stated in Table 12.

**Table 12: Preliminary phytochemical screening of methanolic extract of different varieties of *Tribulus terrestris***

Test	<i>Tribulus terrestris</i>					
	Telangana		Karnataka		Tamilnadu	
	June	December	June	December	June	December
Alkaloids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Triterpenoids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Proteins	-	-	-	-	-	-
Aminoacids	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	-
Phytosterols	-	-	-	-	-	-
Fixed oils	-	-	-	-	-	-
Gums/Mucilage	-	-	-	-	-	-
Volatile oils	-	-	-	-	-	-

## RESULTS AND DISCUSSION

The present investigation reported in a detailed comparative standardization and physicochemical analysis of the three varieties of dried fruits of *Tribulus terrestris* procured from three different geographical locations of South India viz., Ballery (Karnataka), Nakrekal (Telangana), Katumnr koil (Tamilnadu) in June and December seasons. From current investigation assessed the differentiation of the three varieties of *Tribulus terrestris* fruits based on their physicochemical standardization parameters.

Physicochemical parameters included determination of foreign matter, moisture content; ash values, extractive values and fluorescence analysis were conducted to determine quality, purity and identity. The highest foreign matter was found to be in the fruits of *Tribulus terrestris* collected from Ballery with a value of 1.89% w/w in December and the lowest in the fruits from Nakrekal with the value of 0.65% w/w. The value of moisture content determined through Loss on drying, it was high in the plants collected in June and it was gradually decreases with the season. It was found to be the maximum (in June) and minimum (in December) in Tamilnadu variety i.e., 15.26% w/w, 6.74% w/w respectively.

The total ash values were estimated to identify percentage of inorganic salts present in the drug and to determine adulteration by adhering added inorganic materials. The total ash and acid insoluble ash vales found to be maximum in the Telangana variety in December with the value of 11.23% w/w and 2.87% w/w respectively. Minimum in the Karnataka variety with a value of 7.76% w/w and 1.34% w/w respectively. The water soluble ash values was found to be maximum in Telangana variety in December and minimum in Karnataka variety in June with the value of 5.34% w/w and 2.2% w/w respectively.

Preliminary phytochemical screening was carried out by using methanolic extracts of all varieties of *Tribulus terrestris* fruit samples resulted in presence of wide range of phytoconstituents like alkaloids, saponins, tannins, steroids, triterpenoids and flavonoids. Fluorescence analysis was carried out to fruit powder by using various chemical reagents in day light and UV light (long UV and short UV) for identification of plant material.

### CONCLUSION

From the current study it is easy to differentiate the three varieties of *Tribulus terrestris* fruits collected from different seasonal and geographical conditions based on their standardization and physicochemical parameters. The various physicochemical parameters like foreign matter, ash values, extractive values, loss on drying and preliminary phytochemical screening as well as fluorescence analysis supported the identity, purity, quality and authenticity of the three varieties of *Tribulus terrestris* fruits. From the study it is concluded that the seasonal and geographical variation has direct influence with the variation in physicochemical properties of the plants. The results may be helpful in obtaining the good quality products in manufacturing of various *Tribulus terrestris* based products.

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