International Journal of Food Science and Nutrition
ISSN: 2455-4898; Impact Factor: RJIF 5.14
Received: 01-10-2020; Accepted: 15-10-2020; Published: 02-11-2020
www.foodsciencejournal.com
Volume 5; Issue 5; 2020; Page No. 01-04

Comparative nutrients analysis of leave, stem, fruit and seed of *Tinospora cordifolia*

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Abstract
The *Tinospora cordifolia* (Thunb.) Miers, commonly known as "Giloe" plant; is reported to possess notable medicinal properties in the traditional systems of medicine. Usually the leaves and stems parts of this plant are being exploited by pharmaceutical industries for preparation of various herbal healthcare formulations. However, the fruits and seeds are still less-explored in terms of detailed phyto-constituents analysis due to rare fruiting of this plant. This research paper highlights the comparative nutritive phytochemical (primary and secondary metabolites, essential mineral content, dietary fibres, calorific value *etc*) analysis in different parts (leaves, stem, fruit and seed) of cultivated female plant of *T. cordifolia*. Our results suggest that *T. cordifolia* fruits and seeds are rich source of primary and secondary metabolites (protein, carbohydrate, reducing sugars, total phenol, tannins *etc*), antioxidants (Vitamin C, D *etc*), essential mineral content (Na, Fe, *etc*) and calorific energy (Kcal/100 gm) value as compared to leaves and stem parts of this plant. However, total dietary fibre content was found maximum in stem. Therefore, the ripened fruits can be best suited for commercial exploitation for the development of giloe based healthcare supplements.

Keywords: dioecy, giloe, phytonutrient analysis, *Tinospora cordifolia*, therapeutic phyto-constituents

Introduction

Tinospora cordifolia (Thunb.) Miers (Fam. Menispermaceae) is a dioecious creeper, commonly called as "Giloe" with significant medicinal importance in Ayurvedic and Homeopathic systems of medicine (Khan *et al*., 2017; Sharma *et al*., 2019) [1, 2]. This plants is widely distributed throughout India and neighbouring South-East Asian tropical countries. Usually almost all the parts of the plant is reported to possess notable medicinal properties such as antispasmodic, anti-inflammatory, anti-allergic, anti-diabetic, anti-periodic, anti-spasmodic, anti-arthritic, anti-oxidant, anti-stress, anti-leprotic, anti-malarial, antipyretic, hepato-protective, immunomodulatory, anti-neoplastic activities, radio-protective and many more (Goel *et al*., 2004; Joshi and Kaur, 2016; Singh and Chaudhuri 2017; Ahmad *et al*., 2020) [3-6]. But, the use of mature leaves and stem of thickness equivalent to little finger is more common in Ayurvedic medicine system, which led to exploit by the pharmaceutical industries for preparation of various herbal healthcare formulations (Pradhan *et al*., 2013; Namdev and Gupta, 2015; Garg and Garg, 2018) [7, 9]. However, the use of other parts of this plant like fruits and seeds are still less explored in terms of phyto-constituents that needs scientific attention for sustainable use of this plant (Khan *et al*., 2011a, b; Bhan, 2016) [10, 12]. The male and female plants are quite similar one and only at the time of flowering it distinguished. The male and female plants bears distinct staminate (comes in clusters) and pistillate (usually solitary) flowers on separate plants during monsoon-winter seasons (August-January), respectively (Choudhry *et al*., 2014) [13]. Fruiting on female plants is less common since, usually the mode of propagation is asexual one (stem and root cutting), and the chance of sexual propagation is possible if both male and female plants are found in close vicinity so that pollination took place through insects or winds. Therefore, phytochemical analysis of fruits and seeds of this plant is not much studied in detailed due to rare fruiting of this plant and only fewer reports are available (Khan *et al*., 2011a, b) [10, 11]. Also, the different pharmacological actions of *T. cordifolia* like other medicinal plants can be attributed to the presence of various secondary metabolites *viz*. alkaloids, flavonoids, phenols, steroids, saponin, glycosides *etc*. The biosynthesis of these secondary metabolites although controlled by genetic factors is affected by other reasons also such as, geo-climatic factors, developmental stages of the plants, function, and activities of different plant parts (Choudhary *et al*., 2013) [14]. These will result in fluctuations in the concentration and quantities of secondary metabolites throughout the year and growing stages of the plants. It is generally assumed that the material is best collected when the organ in question has reached its optimal state of development; based on such assumptions, generally herbs are collected at the flowering and fruiting stage (Gholamreza *et al*., 2002; Panchabhai *et al*., 2008) [15, 16]. Therefore, the aim of the present research work was to evaluate comparative nutritive phytochemical (primary and secondary metabolites, essential mineral content, dietary fibres, calorific value *etc*) analysis in different parts (leaves, stem, fruit and seed) of cultivated female plant of *T. cordifolia*.

The male and female Giloe plants were cultivated in close vicinity at Defence Institute of Bio-Energy (DIBER), DRDO, Haldwani (1400' asl), Uttarakhand, India (Fig.1a). The mature leaves, stem, fruits and seeds samples were collected from female Giloe plant for comparative nutritive phytochemical analyses (Fig.1b).

The phyto-nutrients (primary and secondary metabolites) were determined as per the standard biochemical methods described by the Association of Official Analytical Chemists (AOAC) (Maynard, 1970; Harborne, 1984; AOAC, 1996) [17-19]. Mineral analysis was carried out after digestion of 2 g of the grounded sample with 10 mL of a mixture of nitric acid, sulphuric and perchloric acid (4:1:1, v/v) untill a clear solution was obtained. The digest was allowed to cool and then transferred into a 100 mL volumetric flask and made up to mark with de-ionized water. The mineral elements like sodium, calcium and iron were analysed using atomic absorption spectrophotometer (Perkin elmer) equipped with air-acetylenes flame. The total dietary fibre and calorific values of samples was estimated according to the study conducted by Pandey *et al*. (2016) [20]. All phytochemical analysis was performed in triplicate and data obtained were also verified for genuineness by third part evaluation by reputed NABL accredited lab (EKO PRO Engineers Pvt. Ltd, Ghaziabad).

T. cordifolia fruits and seeds contains significantly higher amount of primary metabolites (total protein carbohydrate, reducing sugar) as compared to leaves and stem parts of Giloe. However, the total fat content was found maximum in stem (6.04%) followed by fruit (5.21%) and leaves (3.12%). The healthiest type of fatty acid profiles including MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid) and saturated fat contents was found only in Giloe fruits with values of 1.8, 0.6 and 2.1%, respectively, that may help in reducing low-density lipoprotein (LDL; Bad cholesterol) level and maintains high-density lipoprotein (HDL; Good cholesterol) level. However the trans-fat (worst type of fatty acid profile) and cholesterol contents was found negligible in all studied part of Giloe (Table 1). Giloe fruits contains significantly higher amount of secondary metabolites *viz*. total phenol, DPPH, ABTS and tannin content as compared to other studied parts of Giloe. However, the flavonoids content was found maximum in stem (0.47%) followed by leaves (0.25%), fruits (0.16%) and seeds (0.02%) (Mahima *et al*., 2014) [21]. Among antioxidants, Vitamin C contents was found maximum in seeds (88.1 mg/100g) followed by leaves (56 mg/100g), stem (48.5 mg/100g), and fruits (43 mg/100g). Vitamin A content (0.30 mg/100g) was observed only in leaves, while Vitamin D content was found negligible in all studied parts of Giloe (Table 1). Among essential minerals, the seeds contain significantly higher amount of sodium (Na) and iron (Fe) contents with values of 40 and 17.6 mg/100g, as compared to other studied parts of Giloe, respectively. However, the calcium (Ca) content was found maximum in leaves (85.25 mg/100g) followed by seeds (33 mg/100g), stem (10.22 mg/100g) and fruits (4.23 mg/100g), which is in accordance with Pandey *et al*. (2016) [20]. The total dietary fibre content was found maximum in stem (56.42 %) and minimum in fruits (1.28%). However, calorific energy of seeds was found maximum (374.1 Kcal/100 gm) followed by fruits (353.3 Kcal/100 gm), stem (146.6 Kcal/100 gm) and leaves (88.6 Kcal/100 gm) (Table 1).

Table 1: Comparative phyto-nutrients analysis of leave, stem, fruit and seed of *Tinospora cordifolia* female plant.

S. No.	Nutrient parameters	Leaves	Stem	Fruits	Seeds
1.	Total protein (%)	2.30	7.74	36.97	19.56
2.	Carbohydrate (%)	25.78	26.35	39.62	73.04
3.	Reducing Sugars (%)	0.13	0.73	4.1	0.25
4.	Total fat (%)	3.12	6.04	5.21	0.41
5.	Fatty acid Profile: MUFA (%)	BLQ*	BLQ*	1.8	BLQ*
6.	Fatty acid Profile: PUFA (%)	BLQ*	BLQ*	0.60	BLQ*
7.	Fatty acid Profile: Saturated fat (%)	BLQ*	BLQ*	2.10	BLQ*
8.	Fatty acid Profile: Trans-fat (%)	BLQ*	BLQ*	BLQ*	BLQ*
9.	Cholesterol (mg/100g)	BLQ*	BLQ*	BLQ*	BLQ*
10.	Total Phenol (%)	0.06	0.07	0.34	0.02
11.	DPPH (% inhibition of free radicals)	2.64	39.8	86.43	2.53
12.	ABTS(% inhibition of free radicals)	82.76	72.8	98.14	49.14
13.	Tannin (%)	2.73	1.44	12.27	0.56
14.	Flavonoids (%)	0.25	0.47	0.16	0.02
15.	Vitamin C (mg/100g)	56.0	48.5	43.0	88.10
16.	Vitamin A (mg/100g)	0.30	BLQ*	BLQ*	BLQ*
17.	Vitamin D (mg/100g)	BLQ*	BLQ*	BLQ*	BLQ*
18.	Sodium (mg/100g)	BLQ*	BLQ*	0.40	40.0
19.	Calcium (mg/100g)	85.25	10.22	4.23	33.0
20.	Iron (mg/100g)	5.87	2.61	0.46	17.6
21.	Total Dietary Fibre (%)	11.32	56.42	1.28	3.42
22.	Energy (Kcal/100g)	88.6	146.6	353.3	374.1

*BLQ - Below limit of quantification

Fig 1: (a) Cultivated *Tinospora cordifolia* (Giloe) male and female plants at DIBER, Haldwani (1400' asl), Uttarakhand; (b) selected leaves, stem, fruits and seeds samples of female giloe plant for comparative phyto-nutrient analysis.

Conclusions

It is evident from this study that different biochemical attributes varied significantly in different part of the plant. The results of present study suggest that *T. cordifolia* fruit can be a rich source of bioactive nutrients and antioxidants as compared to other parts of this plant. The ripened fruits may contain other bioactive pharmaceuticals as well that will be best suited for commercial exploitation for future healthcare supplement development. However, suitable agronomic strategies may be needed for sustainable fruit yield of *T. cordifolia*. The outcome of the studied comparative nutraceutical analysis of various parts of this plant is expected to create interest among researchers and the industry for development of potent healthcare supplements.

Acknowledgments

Financial and technical assistance received from Defence Research and Development Organization (DRDO), Ministry of Defence, Government of India under project SARHAD (TD/17-18/DIB-78) is duly acknowledged. The technical support received from team members of project SARHAD is also acknowledged.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author's contribution: MB conceived the study and helped in critical proofreading of the manuscript. SMG executed the studies and wrote the manuscript. BB, JR, OP and AA helped in phyto-nutrient data analyses and proofreading of the manuscript.

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