Extraction of Leaf Pigments, Carbohydrate and Protein content from Fresh Leaves of *Tinospora cordifolia* (Willd.)

Masrat Jan[1*] and Mukta Shrivastava[2]

Department of Botany, Govt. M. L.B. Girls P.G. (Autonomous) College, Bhopal-462002, M.P. India.

Abstract:

During the past decades, spice and medicinal plants gained a more important role in agronomy production, pharmacy, and exportation because of their increased use as a raw material for the pharmaceutical industry and pharmaceutical preparations and in the everyday life of the general population .They are the sources of many bioactive compounds and primary metabolites like leaf pigments, carbohydrates. Proteins, starch and total phenols etc that are used as cosmetics, herbal food and also as remedy for many diseases. In view of this, fresh leaves of *Tinospora cordifolia* were evaluated for the estimation of leaf Pigments, Carbohydrate and Protein content. The results showed that the leaves of *Tinospora cordifolia* contained a substantial amount of leaf pigments including Chloropyhll a, b, total chlorophyll, carotenoid, carbohydrate and proteins. The values of these constituents are as: Chlorophyll 'a' 1.63; Chl.'b' 0.72; total chlorophyll 2.36; carotenoid 0.68; Carbohydrate 3.57 and protein 1.89 mg\g of fresh weight. The data obtained from this study clearly indicates that *Tinospora cordifolia* can be used as herbal food as well as therapeutic agent due the presence of rich quantity of primary metabolites.

Key words: *Tinospora cordifolia*; Chlorophyll; Carbohydrate; protein

Introduction:

The World Health Organization reported that 80% of the world population relies chiefly on traditional medicines involving the use of plant extracts or their active constituents. India with its mega-biodiversity and knowledge of rich ancient traditional systems of medicine (Ayurveda, Siddha, Unani, Amchi and local health traditions) provide a strong base for the utilization of a large number of plants in general healthcare and alleviation of common ailments of the people (Pandey *et al.*, 2008). Miers Hook F. and Thoms repoted *Tinospora cordifolia* (wild), is widely used in folkflore and Ayurvedic system of medicine, belonging to the family Menispermaceae (Kashyap *et al.*, 2015). It is a glabrous climbing shrub with heart shaped leaves and distributed throughout the tropical Indian subcontinent, Bangladesh, Srilanka, South Africa (The wealth of India, 1959) The plant is commonly known as Guduchi, Giloy or Amritha, which are Hindu

mythological terms that refer to the heavenly elixir that have saved celestial beings from old age and kept them eternally young. *Tinospora cordifolia* (TC) is a large extensively spreading glabrous, perennial deciduous twiner with succulent stems and papery bark; leaves simple, alternate, cordate, entire, 7-9 nerved; flowers in clusters, female flowers usually solitary; fruits drupes, red when ripe. The leaves are heart shaped and rich in protein (11.2%) and are fairly rich in calcium and phosphorus. Root is a powerful emetic and used for visceral obstruction; its watery extracts is used in leprosy (Mahesh and Satish, 2008). The surface of the stems appears to be closely studded with warty tubercles and the surface skin is longitudinally fissured. On removal of the surface skin the dark greenish mucilaginous stem is seen. The plant is sometimes cultivated for ornamental value and is propagated by cuttings. The leaves afford a good fodder for cattle.The bitter principle present shows adaptogenic, antispasmodic, antiinflammatory, antipyretic, anti-neoplastic, hypolipidemic, hypoglycemic, antioxidant, immunopotentiating and hepatoprotective properties (Adhvaryu *et al.*, 2007) It contains tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodane furano diterpene, diterpenoid furanolactone tinosporidine, columbin and ß-sitosterol (Adhvaryu, 2008). Tinospora Cordifolia is a plant prescribed in Ayurveda as a Rasayana or general tonic (Singh *et al.*, 2010). The combination of Tinospora cordifolia and turmeric extract is effective in preventing the hepatotoxicity which is otherwise produced as a side effect of conventional pharmaceutical treatments for tuberculosis using drugs such as isoniazid and rifampicin (Uddin *et al.*, 2011).

In Ayurveda, it is designated as Rasayana drug recommended to enhance general body resistance, promote longevity and as anti-stress and adaptogen (Patil *et al.*, 1997; Patwardhan Gautam, 2005).The whole plant is used in Ayurvedic “Rasayanas” to improve the immune system and the body resistance against infections and root is known for its anti-stress, anti-leprotic and anti-malarial activities. The fact that it is called “Amrita” signifies its use for revitalization and its importance in Ayurveda and in Unani as Ikseere Badan. This significant plant is also mentioned in important Pharmacopoeias(Zhao *et al.*, 1991; BPC 1994)Guduchi has been reported to be active against throat cancer in man and it has been reported to be non-toxic in acute toxicity studies in vivo, with almost no side effects. . Primary metabolite analysis is necessary for knowing the nutritional potential of plants and then also from the precursors for the synthesis of secondary metabolites. (Tatsuta and Hosokawa, 2006; Vijayvergia and Kumar, 2007), Primary plant metabolites are simple molecules or polymers of simple molecules

synthesized by plants, generally do not possess therapeutic as such but essential for the life of plants and contain high-energy bonds. These are used up for the biosynthesis of secondary metabolites (Harada and Fukusaki, 2009) .The aim of the present study was to extract and estimate the primary metabolites content like leaf pigments, carbohydrate and protein contents from the fresh leaves of *Tinospora cordifolia.*

Materials and Methods:

The healthy seeds of *Tinospora cordifolia* were purchased from Vindhya Herbals at Sanjeevani Ayurveda, Bhopal. Seeds were sterilized with 1% Hgcl2 for 10 min, then washed several times with distilled water. The pot experiment was conducted at Govt. M.L.B. Girls P.G (Autonomous) College Bhopal. The homogenous mixture of sandy soil collected from the field of Vindhya Herbals Bhopal. The seeds were sown in pots filled with soil. These pots were placed in green house and regularly watered to allow germination. After reaching the maturity stage, the plants were used for further analysis.

Methods of extraction and Estimation:

Total Chlorophyll: The total chlorophyll was estimated by the method of Arnon, 1949 with slight modification. 100 mg of fresh leaf material was ground in a prechilled mortar and pestle with 2 ml of 80 per cent acetone. The homogenate was centrifuged at 1000 rpm for 10 min. The supernatant was saved and the pellet was again centrifuged at 1000 rpm for 10 min with 2 ml of 80 per cent acetone. The supernatant was saved and the optical density (OD) of solution was read at 645 and 663nm using a UV spectrophotometer

The chlorophyll a, chlorophyll b and total chlorophyll contents were estimated
and expressed in mg g-1 fresh weight basis.

Chlorophyll ‘a’ (mg g -1 FW) = 12.7 (A663) - 2.69 (A645) x **V**/W×1000

Chlorophyll ‘b’ (mg g -1 FW) = 22.9 (A645) - 4.68 (A663) x V/W×1000

Total Chlorophyll (mg g -1 FW) = 20.2 (OD645) + 8.02 (OD663) x V/W×1000

Where

A 645 = optical density at 645 nm, A 663 = optical density at 663 nm

A 480 = optical density at 480 nm, A 510 = optical density at 510 nm

V = final volume (ml) of chlorophyll extract in 80% acetone

W = fresh weight (g) of leaves used for chlorophyll extraction

Estimation of protein:

Sample preparation: Protein estimation was done according to the method described by Lowry and others (1951). Hundred mg of fresh leaf material was weighed and ground with 2ml of 20% trichloroacetic acid. The homogenate was centrifuged at 1000 rpm for 15 minutes. The supernatant was discarded. To the pellet, 1 ml of 0.1 Sodium hydroxide was added and centrifuged for 10 min. The supernatant was saved and made up to 2 ml with 0.1 N NaOH. The absorbance of the solution was estimated at 660nm. Bovine serum albumin 1mg/ml was used as a standard. The total protein content was expressed in $mg^{-1}FW$.

Estimation: One ml of the extract was taken in a 10 ml test tube and 5 ml of reagent 'C' was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 ml of Folin-phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm using a UV spectrophotometer.

Estimation of Carbohydrates:

Carbohydrate estimation was done by using Anthrone reagent. 100mg of plant material was weighed and extracted by ethanol. The sample was hydrolysed with 5ml of 2.5N Hcl by keeping it in water bath for 3hrs at 100 0C. After cooled it to room temperature, the sample was neutralized with sodium carbonate powder. The volume to 10ml was made and then centrifuged for 10 minutes at 8000 r.p.m. The supernatant was collected and 0.1-to 1ml was used for analysis. 4ml of anthrone reagent was added and heat for 10 minutes 1n water bath. Absorbance was read at 630nm by using spectrometer. Glucose-D was used as standard. The total carbohydrate content was expressed in $mg^{-1}FW$.

Results and Discussion:

Table.1: Isolated leaf pigments, carbohydrate and protein contents from fresh leaves of *Tinospora cordifolia*

CONTENTS	Values
Chlorophyll 'a' content (mg/g) f.w. in leaf	**1.63±0.008**
Chlorophyll 'b' content (mg/g)f.w. in leaf	**0.72±0.003**
Total chlorophyll content (mg/g)f.w. in leaf	**2.36±0.005**
Carotenoid content (mg/g) f.w. in leaf	**0.68±0.003**
Carbohydrate content (mg/g) f.w. in leaf	**3.57±0.04**
Protein content (mg/g)f.w. in leaf	**1.89±0.04**

Values are means of three replicates with S.E. (±)

Plants are the sources of many bioactive compounds containing many primary metabolites like, carbohydrates (starch, sugar), proteins, phenols, ascorbic acid etc. are useful for flavoring, fragrances, insecticides, sweetener and natural dyes (Kaufman *et al.*, 1998). They are used as herbal food and therapeutic agents. Keeping in view the importance of these primary metabolites the present studies for biochemical evaluation of primary metabolites from leaves of *Tinospora cordifolia* was undertaken . The results are depicted in (Table 1). The results showed that the leaves of *Tinospora cordifolia* contained a substantial amount of leaf pigments including Chloropyhll a, b, total chlorophyll, carotenoid , carbohydrate and proteins. The values of these constituents are as: Chlorophyll 'a'1.63 ; Chl.'b' 0.72 ; total chlorophyll 2.36 ; carotenoid 0.68 ; Carbohydrate 3.57 and protein 1.89 mg\g of fresh weight. It has been stated that regular intake of chlorophyll keeps digestive and circulatory system healthier. Chlorophyll is bioavailable, constituting an important prerequisite for antioxidant and longevity-promoting activities inside the body (Wang and Wink, (2016).Chlorophylls are also mainly used in food industry as natural colorants as well as to give green colour to alcoholic drinks. Pathan *et al.*, (2015) found chlorophyll content in leaves of Tinospora cordifolia by routine extraction method with values like Chlorophyll a 1.41,Chlorophyll b 0.36 Total chlorophyll1.76 and Carotenoids 0.34 $mg^{-1}g$. Sharna and Batra, (2016) found maximum Chlorophyll 'a' 5.37 gm/g DW. Chlorophyll b 4.67 gm/g dry weight.

Data regarding carbohydrates and proteins as given in table-1 pointed out that *T. cordifolia* leaf has substantial quantity of carbohydrates and proteins . Carbohydrate content is higher than protein content. The results related to carbohydrate and protein content of *Tinospora cordifolia* were supported by the findings of Nile and Khobragade, (2009). They Determined the nutritive value and mineral elements of some important medicinal plants from western part of India and find that the shoot of *Tinospora cordifolia* have high nutritive value,rich in carbohydrate (61.66%), Enough in protein(4.5%) and low in fat. Another study carried out by Chauhan *et al.*, (2014) on analysis of stem of *Tinospora cordifolia*, leaves of *Andrographis paniculata* and root and leaves of Boerhaavia diffusa for nutritional and phytochemical composition. Retarding the results related to Mineral content of *Tinospora cordifolia* of this study It was found that *Tinospora cordifolia* had hidh level of carbohydrates but low protein content with values 9.05% of carbohydrate and 1.85% of protein. According to WHO (2007) efficient dietary provision of protein is must along with energy providing bio-molecules for normal cellular and tissue

function. A diet with adequate amino acid is essential to fulfil the demand for protein synthesis and other metabolic pathways in a healthy individual. Proteins are the primary components of living beings. Proteins constitute the second most abundant substance in the body, after water, and are responsible for the formation and maintenance of body tissues and cells, which are being continually renewed. They form the enzymes (that catalyze the biochemical reactions in the body), the blood haemoglobin, the tissue collagen, and the defence antibodies.

Conclusion

Primary metabolite analysis is necessary for knowing the nutritional potential of plants and their role as precursors for the synthesis of secondary metabolites. Therefore The data obtained from this study clearly indicates that *Tinospora cordifolia* can be used as herbal food as well as therapeutic agent due the presence of rich quantity of primary metabolites.

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