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Nutritional Evaluation of *Satwa*, an Ayurvedic Formulation of Three *Tinospora* Species from India

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ABSTRACT

Guduchi (*Tinospora sp.*) is referred to as one of the most versatile rejuvenating herb in Ayurvedic system of medicine. Although the literature is available for various medicinal uses of *Tinospora sp.*, the systematic nutritional analysis of the plant has not yet been reported. In the present study, we report the nutritional characteristics of *Satwa*, an ayurvedic formulation, prepared from three different species of *Tinospora* viz. *T. cordifolia*, *T. sinensis* and *Neem-guduchi* (*T. cordifolia* growing on *Neem* Tree). As per ayurvedic literature, the *satwa* is regarded as aqueous extractable solid substances with starchy material. In the present studies, *satwa* from *Neem-guduchi* was found to be significantly rich in carbohydrates while *satwa* from *T. sinensis* was found to be rich in starch. The HPTLC profiling revealed characteristic concentration of one of the constituents of satwa from *Neem-guduchi*. The HPTLC profile and the nutritional parameters can be used for quality control procedures to prevent adulterations and to encourage the use of most potent species of *guduchi*. Based on the earlier studies from the authors' laboratory, it is recommended to use the *satwa* made from *Neem-guduchi* and a supplement of omega 3-fatty acids for enhanced hepatocorrective and hepatoprotective actions of the formulation.

Keywords: *Guduchi satwa; HPTLC; Nutritional analysis; Tinospora*

Introduction

Medicinal plants are the most important source of life saving drugs for the majority of the World's population [1]. Rig-Veda which was written between 4500-1600 BC is the first record of use of herbs or plants as medicine. India has a wide biodiversity including the rich botanical wealth and considered as goldmine of herbal medicine and popularly called as "medicinal garden of the world" [2-4]. There are many different plant products which are prescribed for various preventive as well as curative roles, as evident from their established efficacy and safety [5]. *Tinospora*, known by the local name *Guduchi* or *Gulwel*, is one of such plants which are prescribed as tonic [6] or as a medicine against various ailments [7-10].

There is huge amount of literature available of *Tinospora* and its various health promoting or disease preventing properties [3, 11-15]. Four different species of *Tinospora* occur in India viz. *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms, *Tinospora sinensis* (Lour.) Merr., *Tinospora crispa* (L.) Miers ex Hook. f. & Thoms and *Tinospora glabra* (Burm f.) Merrill. *Tinospora cordifolia* has been used by traditional practitioners for various therapeutic purposes like treating jaundice, emaciations, skin ailments, diabetes, anaemia, dyspepsia, chronic, diarrhoea and dysentery, urinary problems and various diseases [3, 11]. The description of *Guduchi* in ayurvedic literature matches with that of *T. sinensis* and *T. cordifolia*, but later is commonly used

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owing to its easy availability. In our earlier work, we have reported the hepatoprotective activities of *satwa*, an ayurvedic preparation, from three different species of *Tinospora* [16]. There are only a few reports which mention the systematic nutritional analysis of *Tinospora* [17-18]. There are no reports of nutritional analysis of the three most commonly observed species from India. In view of this, the present analysis was undertaken to identify the nutritionally rich species of *Tinospora*.

Materials and Methods

Collection of plant material

The stem pieces of *Tinospora cordifolia*, *Tinospora sinensis* and *Neem-guduchi* (Guduchi plant growing on *Azadirachta indica* (Neem) tree) were collected from Pune and Dapoli, Maharashtra, India. The plant material was identified and voucher specimen was deposited at the herbarium of Medicinal Plants Conservation Centre (MPCC), Pune (*Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms (MPCC 3483), *Tinospora sinensis* (Lour.) Merr. (MPCC 3529) and *Neem-guduchi* (*T. cordifolia* (Willd.) Miers ex Hook. F. & Thoms) (MPCC 3526).

Preparation of Guduchi Satwa

Fresh stems of the three *Tinospora* species were used for the preparation of *Guduchi* Satwa. The starchy sediment extract is defined in Ayurvedic literature [19] as Satwa. The fresh stem pieces were washed with water and the stem peel was removed. The stems were made into small pieces and pounded briefly. The stem pieces were soaked in the volume of water 4 times the weight of the stem pieces. After 24 hours, the mixture was homogenized and filtered through layers of sterile muslin cloth. The resulting filtrate was again kept at room temperature for 24 hours. The sediment was collected, suspended in 500ml of water and was again kept at room temperature for 2 hours. The sediment was collected as Satwa after decanting the water. It was dried and stored in air-tight containers till further use.

Nutrient analysis of the extract

Estimation of protein, carbohydrate, starch, total lipid, crude fibre and total ash contents of the satwa of three *Tinospora* species was done by following standard techniques.

Extraction and estimation of proteins

Five hundred mg of each satwa from three *Tinospora* species was homogenized with 5 ml of phosphate buffer separately. The homogenates were centrifuged at 2000rpm for 10min. The supernatant was collected and used for protein analysis [20]. Proteins estimation was done by following Lowry method [21-22]. Bovine serum albumin was used as a standard.

Extraction and estimation of total carbohydrates

The satwa (100mg) from each species was suspended in5 ml of 2.5N Hydrochloric acid and was hydrolyzed in a boiling water bath for 3 hours. After cooling to room temperature, it was neutralized with solid sodium carbonate until there was no effervescence and the volume was made to 20 ml with distilled water. It was then centrifuged at 2000 rpm for 10minutes. The supernatant was used for the carbohydrate analysis [23]. Total carbohydrates were estimated by following phenol sulphuric acid method [24-25].

Extraction and estimation of starch

The satwa (500 mg) from three *Tinospora* species was homogenized in hot 80 % ethanol to remove sugars. The homogenate was centrifuged at 2000 rpm for 10 minutes and the residue was washed with hot 80 % ethanol till the washings were colourless with anthrone reagent. The residue was dried in water bath and 5ml of 52 % Perchloric acid was added to the residue. Starch was extracted at 0 °C for 20 minutes. The suspension was centrifuged at 2000 rpm at 4 °C for 10 minutes and the residue was retained. The extraction was repeated with fresh 52 % Perchloric acid. The extracts were pooled after centrifugation and the volume was made up to 100 ml with 52 % Perchloric acid [23]. Estimation was starch was done by using anthrone reagent [23, 26].

Estimation of total lipids

Total lipids were estimated by a method modified from Folch method [27]. The satwa (1gm) of three *Tinospora* species was suspended and thoroughly mixed in 10 ml of chloroform: methanol (2:1 v/v) mixture and left undisturbed at room temperature for 3 days. The suspensions were then filtrated through muslin cloth and centrifuged at 3000 rpm for 5 min by. The upper layer of methanol was removed and the interface was washed three times with chloroform: methanol: water (3:48:47) without disturbing the lower phase. The suspensions were then centrifuged at 3000 rpm for 5 minutes. The lower layer containing the lipids was transferred to a pre-weighed glass tube. The solution was dried at 60 °C and the difference in the weights of the tubes was estimated as crude lipid content.

Estimation of crude fibre

The satwa (1gm) of three *Tinospora* species was suspended in 100 ml of 1.25 % sulphuric acid. The mixture was boiled for 30 minutes keeping the volume constant by addition of water at frequent intervals. The mixture was filtered through a muslin cloth and the residue was washed with hot water till free from acid. The material was then treated with 1.25% 100 ml boiling sodium peroxide. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and residue washed with hot water till free from alkali, followed by a single alcohol wash. It was then transferred to a crucible, weighed (W1) and dried for 3 to 4 hours at 60-80 °C and again weighed (W2) in an electric balance. The crucible was heated in a muffle furnace at 450 °Cfor 4 to 5 hours, cooled and weighed again (W3). Crude fibre was determined using fat-free samples. Crude fibre content was determined using the following Equation:

% crude fibre in ground sample = (Loss in weight on ignition (W2-W1)-(W3-W1)/Weight of sample)*100

Estimation of total ash

The satwa of three *Tinospora* species (1mg) was added to a pre-weighed crucible. The samples were placed in a muffle furnace at 450 °C for about 4 hours, cooled in desiccators and reweighed. The ash content was determined by the equation as follows:

% ash = weight of ash/weight of sample x 10 [28].

HPTLC characterization of *T. cordifolia, T. sinensis* and *Neem-guduchi* aqueous satwa

HPTLC fingerprinting of aqueous satwa of *T. cordifolia, T. sinensis* and *Neem-guduchi* was done with a Camag HPTLC system. Pre-coated Silica gel plates (Silica gel 60 F_{254}, Merck) were used for HPTLC analysis. The aqueous satwa (10µl) of *T. cordifolia, T. sinensis* and *Neem-guduchi* was applied onto the plates as 6mm band, 8 mm from the bottom edge with a 50µL Hamilton microsyringe. The separation was achieved in a solvent system containing Chloroform and methanol in 9:1 ratio. The chromatogram was developed in glass twin-trough chambers (10 cm × 10 cm, with metal lids; Camag, Switzerland) previously saturated with mobile phase vapour for 20 min. The development distance was 80 mm. The developed plates were dried and scanned on Camag TLC scanner III with absorbance at 366 nm with deuterium beam as a light source.

Statistical analysis

The data values are an average of three independent replicates and are represented as mean ± standard error. The Dunnett test was performed to check if the difference between the observed values was significant. *Tinospora cordifolia* was taken as a control for the comparisons.

Results and Discussion

In the present studies, *Guduchi Satwa* was prepared as per the ayurvedic guidelines. The ayurvedic texts describe this *satwa* as aqueous extractable solid substances with starchy material [29]. Several nutritional parameters were measured for *Tinospora* species. Data on nutritional analysis of *Tinospora* species is shown in Table 1. One gram of *T. cordifolia* satwa contains 21.06mg/g of protein, 272.28 mg/gm of carbohydrate, 18.67 mg/g of lipid, 16.33 µg/gm of starch, and 0.20% of crude fibre and 88.42 mg/g of ash. One gram of *T. sinensis* satwa contains 36.31 mg/g of protein, 333.16 mg/gm of carbohydrate, 11mg/g of lipid, 41.35 µg/gm of starch, 0.43% of crude fibre and 96.07 mg/g of ash. The protein, carbohydrate, lipid, starch, crude fibre, and ash contents in one gram of *Neem-guduchi* satwa were found as 18.48 mg/gm, 797 mg/gm, 21.33 mg/g, 10.88 µg/gm, 0.22 % and 98.21 mg/g respectively. The lipid and carbohydrate content of *Neem-guduchi* were greater than that of *T. cordifolia* and *T. sinensis* (Fig. 1 and Fig. 2).

The ash content was found to be greater in *Neem-guduchi* as compared to *T. sinensis* and *T. cordifolia*. *T. sinensis* shows higher amount of protein than *Neem-guduchi* and *T. cordifolia* (Fig 2).

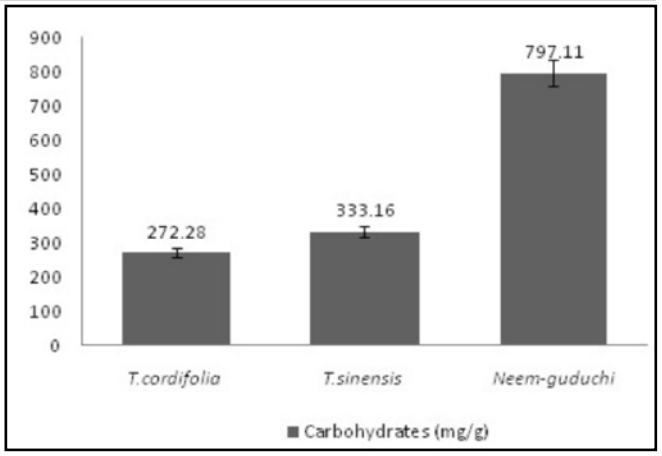

Figure 1: Levels of carbohydrate in *T. cordifolia, T. sinensis* and *Neem-guduchi*

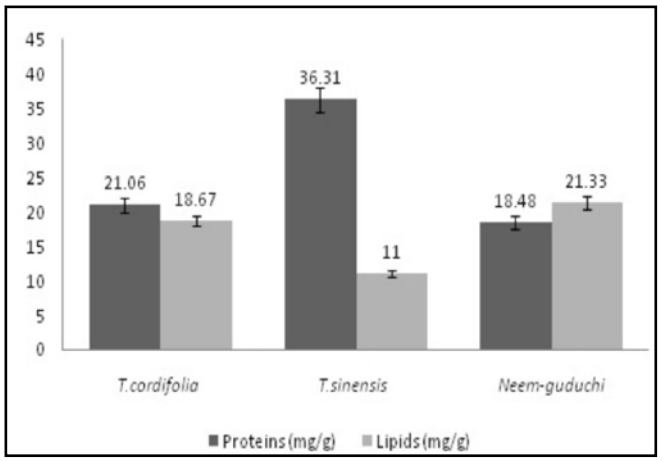

Figure 2: Levels of protein and lipid in *T. cordifolia, T. sinensis* and *Neem-guduchi*

HPTLC fingerprints of all the three *satwa* were found to be similar except for the higher concentration of a peak at Rf 0.78 in *Neem-Guduchi* aqueous satwa (Fig. 3). The recent report of HPTLC analysis of *satwa* from *T. cordifolia* indicates similar pattern of peaks as in the present study [30]. The earlier studies from our laboratory have indicated that the *satwa* from *Neem-Guduchi* has exceptional hepatocorrective activity with targeted effect on normalization of liver architecture, as compared to other two *satwa*. This variation in HPTLC profile of *Neem-Guduchi* may be responsible for the specific action of the *satwa*. Based on the work from this laboratory [16, 31], the authors recommend satwa prepared from *Neem-Guduchi* and a supplement of omega 3-fatty acids for hepatoregenerative and hepatocorrective treatments. The unique HPTLC fingerprint of the aqueous *satwa* from *Neem-Guduchi* may serve as a tool in light of increasing interest for quality control in research and production of medicinal plants [32].

Guduchi is referred to as one of the most versatile rejuvenating herb in Ayurvedic system of medicine [33]. It has been reported that methanolic extract from *T. cordifolia*

Table 1: Nutritional analysis of three *Tinospora* species.

Species	Proteins (mg/g)	Carbohydrates (mg/g)	Lipids (mg/g)	Starch (µg/g)	Crude Fiber (gm%)	Ash (mg/gm)
T. cordifolia	21.06±0.02	272.28±27.67	18.67±2.01	16.33±1.20	0.2±0.04	88.43±0.01
T. sinensis	36.31±0.66***	333.16±8.11^{ns}	11±0.97**	41.35±2.95**	0.43±0.05**	96.07±0.02**
Neem-guduchi	18.48±0.45**	797.11±11.30**	21.33±0.92^{ns}	10.88±2.58^{ns}	0.22±0.01^{ns}	98.21±0.01**

*The values are a mean of three replicates and are represented as Mean±SE. Dunnett test was performed to test the significance. Tinospora cordifolia was taken as a control for comparison. *P≤0.05; **P≤0.01; ns: Non-significant*

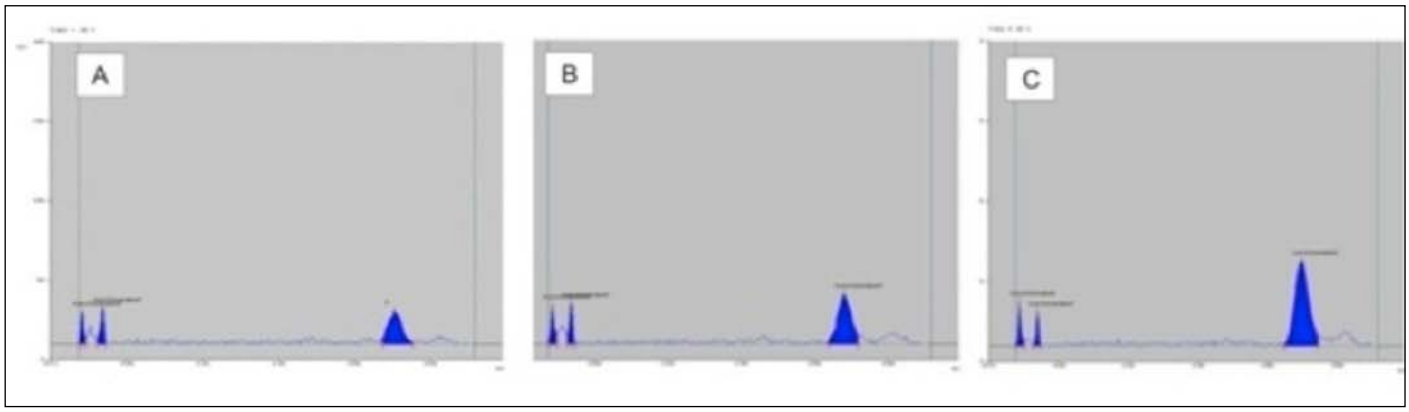

Figure 3: HPTLC fingerprints of aqueous extracts of satwa from *T. cordifolia* (A), *T. sinensis* (B) and *Neem Guduchi* (C)

increases leukocyte count and ablate neutropenia, protecting against induced infections in mice and rat [15]. The earlier studies on *T. cordifolia* indicate crude protein and fibre as principal components of the plant [34]. The reports of hepatoprotective potential of *T. cordifolia*, *T. sinensis* and *Neem-guduchi* include normalization of altered liver functions and liver histology [16]. The present study, in accordance with the available literature, points towards the *satwa* as a potential rich source of nutrition which may prove beneficial for boosting the immune-response [17, 33]. The variations in nutritional composition of medicinal plants might depend, in addition to the intrinsic variations, on the environmental and physiological conditions when plant species are harvested [35-37]. To the best of author's knowledge, this is the first report of the nutritional analysis of the *satwa* prepared from *T. cordifolia*, *T. sinensis* and *Neem-guduchi*. The present study supplements the available information to further the research of *Tinospora* in ayurvedic system of medicine.

Conclusion

These data suggest that dietary and medicinal use of *Neem-guduchi* and *T. sinensis* are good source of nutrients, especially proteins, carbohydrates, and starch. These results also indicate that the studied guduchi species have good nutritive value. Further research is warranted to investigate the anti-nutritive, enzymatic and molecular effects of *Neem-Guduchi* on human as well as animal health.

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