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Analytical evaluation of *Tinospora cordifolia* extract and capsule

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Abstract
Tinospora cordifolia, commonly known as guduchi or giloy is an immune system boosting herb used in Ayurveda to enhance vitality. It can provide the benefit for people with diabetes, urinary tract infection (UTI), anemia, jaundice, asthma, cardiac disorders and so on. We tested *Tinospora cordifolia* extract and single ingredient Tinosporine capsule formulation. General tests were carried out in extract and capsule formulation as well. Focus of the quality evaluation was on the estimation of tannins, bitters and polysaccharides. Extract used was standardized for 2.00% tannins, 2.00% bitters and 15.00% polysaccharides. Corresponding values in capsule formulation were proposed to be not less than 10.00mg/ capsule, for tannins and bitters and not less than 75mg/capsule for polysaccharides. HPTLC fingerprinting was used to confirm the presence of *Tinospora cordifolia* in the final capsule formulation. Quantitative evaluation of heavy metals and microbiological test parameters enhances the quality of the product.

Keywords: *Tinospora cordifolia*, tannins, bitters, polysaccharides

1. Introduction
Now days because of industrialization and increasing pollution, people are losing their immunity. Hence they need supplementary dieter appropriate medicines to enhance the immunity level. Herbal formulations are becoming more and more popular because of their lesser side effects. Herbs of "Natural" origin can be considered as "Safer". Natural products with medicinal values are gradually gaining importance in clinical research due to their well-known property of no side effects as compared to drugs. Saha and Ghosh [1] in their review article explained about the genetic diversity of the plant, active components isolated from the plant and their biological role in disease targeting. *Tinospora cordifolia* commonly known as "Guduchi" has immune modulating [2] properties which directly stimulates the immune system through various mechanisms to ward off invading harmful microbes. *Tinospora cordifolia* shows protective effect by immune – modulation including effects on polymorpho nuclear cells, phagocytes [3] and on macrophage function. It contains a polysaccharide which is responsible for immune stimulant property. It functions by boosting the phagocytic activity of macrophages, production of reactive oxygen species (ROS) [4] in human neutrophil cells, enhancement in nitric oxide (NO) production. Extensive experimental and clinical work has shown the immune enhancing effects of *Tinospora cordifolia*. Treating UTI with herbal medications is a safe second line treatment. Chemical composition [5] of *Tinospora cordifolia* is alkaloids, berberine, bitters-columbine, chasmanthin, palmarin, tinosporon, tinosporic acid, tinosporol and so on. *Tinospora cordifolia* extract powder can be used as a major component in the capsule formulation. When we use it as an "Ayurvedic drug" or a "dietary supplement", analytical evaluation is to be carried out to determine the quality of the product. Complete analysis of extract powder used in the final capsule formulation and the finished good- capsule was carried out. On the basis of composition, theoretical values of components like content of tannins, bitters and content of polysaccharides were calculated in final capsule formulation. Appropriate methods of analysis were applied to both the formulations and results were recorded. HPTLC fingerprinting was used to confirm the presence of *Tinospora cordifolia* extract in the final capsule formulation. Microbial load was evaluated in both extract as well as in capsule formulation. Evaluation of heavy metals in the final capsule formulation provides additional support to ensure the quality of the product.

2. Materials and Methods
2.1 Samples
Four different lots of *Tinospora cordifolia* extract were procured from M/s Kisalaya Herbals Ltd. Single ingredient Tinosporine capsule was manufactured using the stated composition.

Four different lots of Tinosporine capsule formulation were manufactured in our own laboratory. Each lot of extract powder as well as final capsule formulation was subjected to quality testing.

2.2 Chemicals and Reagents
All the chemicals and reagents used in different processes were procured from M/s Merck India and M/s Qualigens.

2.3 Equipments and Instruments
All the glass-wares used were well calibrated and were procured from M/s Borosil Glass Works Ltd. Instruments used were weighing balance (M/s Schimadzu Corporation), Electric oven (M/s Pathak Electrical Works Ltd.), pH meter (M/s Control dynamic Lab.), Disintegration Machine (M/s Thermonik), HPTLC (M/s Anchrome and Camag Ltd.),

2.4 Methods
General test parameters of capsule formulations such as average weight and disintegration time were carried out as per standard pharmacopoeial method [6]. Other physicochemical test parameters such as loss on drying, total ash, acid insoluble ash, pH, water and alcohol soluble extractives in active raw materials as well as in final capsule formulation were carried out as per the Ayurvedic Pharmacopoeia of India [7].

2.4.1 Content of tannins
Tannins were evaluated by titration method[8].Appropriate quantity of sample was dissolved in water and titrated against 0.1N Potassium permanganate using 25ml Indigo carmine solution to golden yellow colored end point. Similarly blank was performed omitting the sample. The difference between the two titrations (TR) represents the quantity of potassium permanganate solution required to oxidise the tannins. Each ml of N/10 $KMnO_4$ is equivalent to 0.004157g of Tannin (as gallotannic acid).
Content of tannins (%w/w) = {(TR) X 0.004157 x 100 x NF of KMnO4} / Wt. of the sample (g)
Content of tannins (mg/capsule) = %w/w X Filled average weight in g x 1000/100
For estimating content of bitters [9], the method of analysis was derived from Indian Pharmacopoeia. Whereas, the method for estimation of polysaccharides was derived from standard reference book [10].

2.4.2 Content of bitters
Weighed accurately about 1g to 2g of *Tinospora cordifolia* extract / 2g to 3g of capsule blend and 0.5g $CaCO_3$ in a 250 ml beaker. Extracted with boiling distilled water. Allowed the powder to settle and filtered the supernatant liquid through cotton in another flask. Performed extraction 4 times till the extract becomes pale yellow. Collected all the extracted liquid in a 250 ml beaker. Boiled to concentrate upto 10 ml to 15 ml on a hot plate or on a burner. Extracted with 20 ml of rectified sprit (alcohol) in hot condition. Repeated the extraction till extract (alcohol layer) becomes pale yellow to colorless. Collected all these filtered extracts and allowed to concentrate to dryness on water bath in a big evaporating dish. To the residue, added about 15 ml distilled water. Dissolved residue was extracted with ethyl acetate (about 4-5 times) to complete extraction. All ethyl acetate extracts were collected together and thoroughly washed with distilled water. Allowed to separate two layers. Water layer was discarded and ethyl acetate layer was collected gently in a previously weighed clean, dry conical flask. Evaporated ethyl acetate completely on water bath or on steam bath. Dried the residue in an oven at 105 °C. Cooled it in a desiccator to room temperature and weighed to constant weight.
Bitter content was calculated in percentage and mg/capsule in *Tinospora cordifolia* extract powder and Tinosporine capsule formulation respectively by applying following formula
Content of bitters (%w/w) = weight of the residue in g X 100 / weight of the sample in g
Content of bitters in (mg/capsule) = bitters in %w/w X filled average weight in g X 1000/100

2.4.3 Content of polysaccharides
Weighed accurately about 1 g to 2 g of extract powder / blend of the capsule sample in a conical flask. 100ml distilled water was added and left it overnight after shaking well for two hours. Next day filtered and concentrated upto 10ml. Then 50ml 90%methanol was added gently with constant stirring and left it for half an hour. Filtered the solution and dried the residue on filter paper at 80^0C under vacuum till constant weight. Calculation was carried out as given below
Total polysaccharides in (%w/w) = Wt. of the residue in g X(100 / Wt. of the sample in g)
Total polysaccharides (mg/capsule) = % w/w X average filled wt in g X 1000/100.
Heavy metals viz. arsenic, mercury, lead and cadmium were estimated as per AYUSH guidelines using ICP. Whereas, microbiological testing was carried out as per USP / BP.
For carrying out HPTLC fingerprinting in capsule formulation, *Tinospora cordifolia* extract powder and blend of the Tinosporine capsule were extracted individually in methanol, 10 microlitres of each was spotted on TLC Aluminium sheet silica gel 60 F 254.The plate was allowed to run in (9:1) Chloroform: Methanol system previously saturated for 30 minutes. The plate was subjected to air drying after attaining the level of the solvent front upto the mark. Then it was scanned at 254 and 366nm. Also sprayed with vanillin sulphuric acid reagent and the presence of active claimed ingredient was photo documented using camag power shot camera unit.

3. Results and Discussion
Composition of the final capsule formulation is given in table 1

Table 1: Composition of Tinosporine capsule

Composition : Label Claim		
Each capsule contains	*Tinospora cordifolia*(Guduchi extract)	500.00mg
	Excipients	q.s.

Specifications for functional groups can be calculated theoretically.
Tinospora cordifolia (Guduchi) extract standardized for…. 2%w/ wtannins, 2% bitters and 15%w/w polysaccharide was used.
Capsule is filled with 555mg blend including excipients, where 500mg of *Tinospora cordifolia* extract was used.
2% tannins as well as bitters each= 10mg in 500mg = 10mg in 555mg = 10mg per capsule

Content of tannins as well as bitters …….. Not less than 10.00mg per capsule
Similarly for polysaccharides
15% polysaccharides = 75mg in 500mg = 75mg in 555mg = 75mg per capsule

Content of polysaccharides …… Not less than 75.00mg per capsule

Hence, specifications of finished goods that is capsule formulation finalized for tannins and bitters are "not less than 10mg/capsule", whereas that for polysaccharides is "not less than 75mg/capsule" respectively.

Analytical findings of *Tinospora cordifolia* extract are reported in table 2

Table 2: Analytical findings of *Tinospora cordifolia* extract

Sr. No.	Test Parameters	Lot 1 Te/14/1051	Lot 2 Kg 475	Lot 3 Kg 154	Lot 4 Kg173
1	Description	Brown colored powder	Brown colored powder	Brown colored powder	Brown colored powder
2	Loss On Drying at 105 °C (%w/w)	3.35	3.88	2.41	5.59
3	Total Ash (%w/w)	11.82	6.42	6.43	6.68
4	Acid Insoluble Ash (%w/w)	1.37	0.71	0.53	1.49
5	Water Soluble Extractive on d/b (%w/w)	88.62	96.18	89.83	93.42
6	50%Alcohol Soluble Extractive (%w/w)	81.68	83.05	81.26	86.72
7	pH of 1.00%w/v solution	5.54	5.26	4.21	5.62
8	Content of Tannins (%w/w)	2.50	2.58	2.53	2.66
9	Content of Bitters on d/b (Not less than 2.00%w/w)	2.29	2.60	2.65	2.12
10	Content of Polysaccharides (Not less than 15%w/w)	22.13	22.13	23.50	22.20
11	Microbiological Testing: → (As per USP / BP Specifications)				
i	Total Aerobic Microbial Count (cfu/g)	50	110	160	20
ii	Total Combined Yeast/Moulds Count (cfu/g)	<10	<10	<10	<10
iii	Bile-Tolerant Gram Negative Bacteria (cfu/g)	<10	<10	<10	<10
iv	*Escherichia coli (should be abs. /10g)*	Absent	Absent	Absent	Absent
v	*Salmonellae spp.(should be abs. /10g)*	Absent	Absent	Absent	Absent
vi	*Staphylococcus aureus (should be abs.)*	Absent	Absent	Absent	Absent
vii	*Pseudomonas aeruginosa (should be abs.)*	Absent	Absent	Absent	Absent
viii	*Clostridium spp.(should be absent)*	Absent	Absent	Absent	Absent

Note: cfu/g = colony forming unit

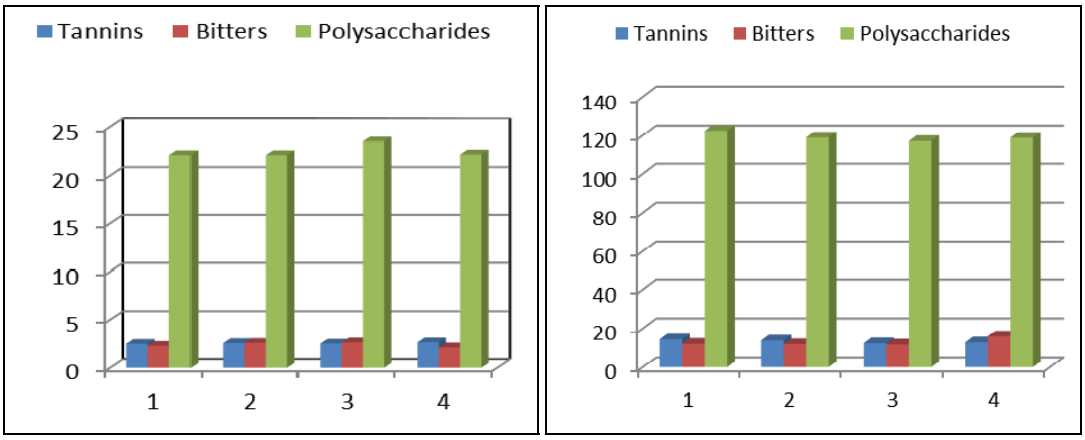
From table 2 it can be seen that, all the four lots of extract powder are complying with respect to the general physicochemical parameters. *Tinospora cordifolia* extract was standardized for mimimum2% tannins, minimum 2% bitters and minimum 15% polysaccharides. All our respective findings are more than 100% of the minimum expected value. Also all four lots are found to comply the microbiological specifications as per USP / BP. This approved extract powder was used for manufacturing the capsule formulation. After mixing appropriate excipients, diluents to the approved extract, the blend of the capsule was prepared as mentioned in table 1, then this blend was filled in "00" size capsule. Complete analytical testing of final capsule formulations were carried out. Analytical findings of four different lots of final capsule formulation were recorded in table 3.

Table 3: Analytical findings of *Tinosporine* capsule

Sr. No.	Test Parameters	Analytical findings of LOT 1	Analytical findings of LOT 2	Analytical findings of LOT 3	Analytical findings of LOT 4
1	Description ("00" size capsule filled with brown colored powder)	Complies	Complies	Complies	Complies
2	Disintegration Time (minutes)	16.00	13.00	20.00	15.00
3	Average weight (mg)	684.80	678.10	661.87	665.72
4	Filled average weight (mg)	568.27	561.02	543.86	550.30
5	Loss On Drying at 105 °C (%w/w)	7.38	7.36	5.56	4.12
6	Total Ash (%w/w)	13.75	14.75	9.59	10.46
7	Acid Insoluble Ash (%w/w)	0.89	1.77	4.15	3.62
8	Water Soluble Extractive on d/b (%w/w)	81.40	78.65	71.31	73.69
9	Alcohol Soluble Extractive (%w/w)	3.85	3.77	2.62	2.51
10	pH of 1.00%w/v solution	5.65	5.68	5.38	5.00
11	Content of Tannins	14.37	13.80	12.37	12.82
12	Content of Bitters (mg/capsule)	12.08	11.92	11.76	15.96
13	Content of Polysaccharides (mg/capsule)	122.40	118.94	117.15	118.82
14	Heavy Metals as per Department of AYUSH				
i	Arsenic (as As) (ppm)	< 0.20	< 0.20	< 0.20	< 0.10
ii	Mercury (as Hg) (ppm)	< 0.20	< 0.20	< 0.20	< 0.10
iii	Lead (as Pb) (ppm)	< 0.20	< 0.20	< 0.20	< 0.10
iv	Cadmium (as Cd) (ppm)	< 0.20	< 0.20	< 0.20	< 0.10
15	Microbiological Testing: → (As per USP / BP Specifications)				
i	Total Aerobic Microbial Count (cfu/g)	30	60	40	110
ii	Total Combined Yeast/Moulds Count (cfu/g)	<10	<10	<10	20
iii	Bile-Tolerant Gram Negative Bacteria (cfu/g)	<10	<10	<10	<10
iv	*Escherichia coli*	Absent	Absent	Absent	Absent
v	*Salmonellae spp.*	Absent	Absent	Absent	Absent
vi	*Staphylococcus aureus*	Absent	Absent	Absent	Absent
vii	*Pseudomonas aeruginosa*	Absent	Absent	Absent	Absent
viii	*Clostridium spp.*	Absent	Absent	Absent	Absent

Note: ppm= parts per million, cfu/g = colony forming unit

Table 3 shows the analytical findings of four different lots of Tinosporine capsule formulation. As per the data mentioned in the table, tannin content is found to vary from 12.37mg/capsule to 14.37mg/capsule. Bitter content is found to be in the range of 11.76mg/capsule to 15.96 mg/capsule and content of polysaccharides is found in the range of 117.15 mg/capsule to 122.40mg/capsule. Observed values of tannins, bitters and polysaccharides in different lots of *Tinospora cordifolia* extract and Tinosporine capsules are well illustrated in Figure 1.

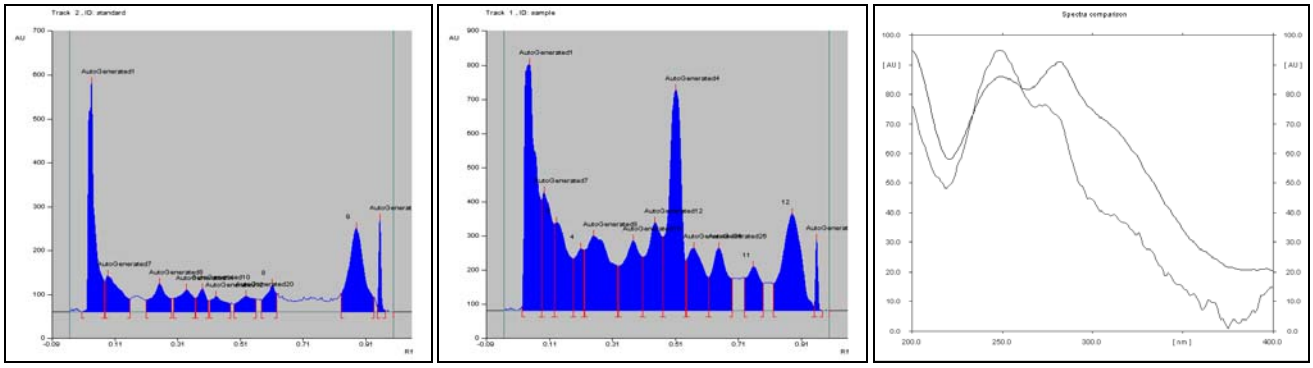


Tinospora cordifolia extract Tinosporine capsule

Fig 1: Graphical presentation of Concentration of tannins, bitters and polysaccharides

Findings of arsenic, mercury, lead and cadmium all lie within the limit specified by AYUSH guidelines (as not more than 3ppm for arsenic, not more than 1ppm for mercury, not more than 10ppm for lead and not more than 0.3ppm for cadmium).Also microbiological test results recorded in the table assure all these lots are safe with respect to microbial loads and pathogens as per USP and BP.

Presence of *Tinospora cordifolia* extract in the capsule formulation is confirmed by HPTLC fingerprinting as shown in figure 2.



Tinospora cordifolia extract Tinosporine capsule Overlapping spectrum

Fig 2: HPTLC Fingerprinting of Tinosporine capsule

Tinospora cordifolia **extract (Under 254nm)**	**Tinosporine capsule (Under 254nm)**	**Rf value**	***Tinospora Cordifolia*** **extract (Under 366nm)**	**Tinosporine capsule (Under 366nm)**	**Rf value**	***Tinospora Cordifolia*** **extract (After spraying)**	***Tinosporine capsule (After spraying)***	**Rf value**
		Dark spot at Rf about 0.25			Yellow spot at Rf about 0.08			Blue spot at Rf about 0.25

After scanning the plate at 254 nm, overlapping spectrum observed at Rf about 0.25 in extract and capsule. After observing the plate under 254 nm, dark colored spot is observed at Rf about 0.25 in both extract and capsule. After observing the plate under 366 nm, yellow colored spot is observed at Rf about 0.08 in both extract and capsule. After spraying the plate with Vanillin Sulphuric acid Reagent and observing under white light, blue colored spot is seen at Rf

about 0.25, in both extract and capsule. This indicates the presence of *Tinospora cordifolia* extract in the Tinosporine capsule formulation.

Sharma *et al*. [11] evaluated and reported the immunomodulatory activity of three polysaccharide enriched fractions. Raveendran Nair *et al*. [12] isolated the novel polysaccharide which is non cytotoxic and non proliferating to normal lymphocytes as well as tumor cell lines at 0-1000 µg/ml. Mehra *et al*. [13] standardized and evaluated parameters of *Tinospora cordifolia* tablet formulation. Durai *et al*. [14] carried out qualitative and quantitative analysis of phytochemicals in crude extract of big- leaf mahogany and observed high content of tannins, alkaloids and flavonoids in seed extract and high phenols in leaf extract. Sharma *et al*. [15] have reported *Tinospora cordifolia* as a preventive and curative antidiabetic herb, which are substantiated by clinical trials. Reddy *et al*. [16] summarized the information concerning the chemical constituents and medicinal aspects of the *Tinospora cordifolia* plant. Salkar *et al*. [17] carried out phagocytosis assay to demonstrate the immunomodulatory potential of *Tinospora cordifolia* extract.

4. Conclusions

Though *Tinospora cordifolia* that is Guduchi has many active components, we have quantified few functional groups and showed the relative values in extract powder and capsule formulation. Qualitative testing by HPTLC method is ensuring the presence of *Tinospora cordifolia* extract in the capsule formulation. Altogether test results indicate the compliance of raw material that is *Tinospora cordifolia* extract and the final product that is Tinosporine capsule with respect to the quality parameters. More than 10mg /capsule of tannins and bitters as well as considerable amount of polysaccharides, about more than 100mg/ capsule impart additional quality level to the capsule formulation.

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