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STUDIES ON STANDARDIZATION OF GUDUCHI SATVA

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ABSTRACT

Guduchi (Tinospora cardifolia Linn.) is an Ayurvedic herb where as Guduchi Satva is a water extractable solid substance collected from the drug Guduchi and is used in the management of pyrexia, Immuno-modulatory and Rasayana etc.. conditions. On basis of Ayurvedic textual indications and recent scientific studies, the satva was selected for studying its effect clinically as immunomodulatory. Before conducting clinical trials this satva was subjected to certain chemical studies to find out its characterization.

The observations and results shows that size of the granules measured around 4µ and is readily soluble in dil. HCl, Conc. Sulphuric acid and cold solution of KOH and produces white, brown and white gelatinized precipitations respectively. The acid soluble ash of the product has reported not more than 1% w/w, where as alcohol soluble extract has more than 2% w/w. The other chemical tests confirms that the Satva is a specific starch of Tinospora Cardifolia.

Key Words: *Guduchi, Satva, immunomodulatory, Standardization specific starch.*

INTRODUCTION:

Ancient traditional system is called "Ayurveda" It advocates the use of herbs and herbal preparations similar to other civilizations of the world. In Ayurveda, the pharmaceutics is dealt under the heading of Bhaishajya Kalpana. It deals wide ranges of medicinal preparations, they are, primarily Pancha vidha kashaya kalpana, namely Swarasa, Kalka, Kashaya, Hima and Phanta and secondary preparations like Sneha, Asavarishta, Avaleha and Satva etc.. Kalpanas.

Satva kalpana is one of the most important pharmaceutical preparation of Ayurveda. The nomenclature of Satva kalpana is sum of words Satva and Kalpana, where Satva means a product of water extractable solid substance collected from the drug (s) and kalpana means a process[1]. This process ensures extraction of starch of the plant which is active therapeutic property of ingredient (s) used.

Historically, the word 'Satva' is known from vedic period. According to 'Shabda Kalpa Druma' Satva means' 'SatobhAva[2]' 'Prakrute Gunavishesha[3]' i.e. 'most potent' or 'active principle' and 'natural superior property'). But, in Ayurveda, the preparation Satva Kalpana is first coined by the author of Yogaratnakara[4].

Guduchi (Tinospora cardifolia Linn.) satva is a water extractable solid substance collected from the drug Guduchi[5] and is used widely by the Ayurvedic physicians in their day to day practice in a large scale for different ailments. It has strong anti Pyretic, Immuno-modulatory and Rasayana properties[6] etc... As it is tasteless in taste and required less in quantity and more effective as compared to raw form of Guduchi.

Pharmaceutically, the preparation Guduchi Satva is a tremendous task, this involves lots of adulterants mixed with it. So it is very difficult to get the genuine form of drug from the market. Hence, there is need to standardize the drug according to the newly available standardization methods. Keeping these facts in view, the present study has been planned to standardize the product analytically.

MATERIALS AND METHODS:

The present study was under taken at National Institute of Ayurveda, Jaipur by following the reference of A.F.I. part.I[7].

SOURCE OF DATA:

Procurement of raw drug:

The herbal raw drug (Guduchi stem) was obtained from the forest area of the Chitrakoot, Satna.

Authentification of plant material: The Raw drug collected in a fresh state and was subjected for authentication.

PREPARATION OF SAMPLE:

1. **Formula used for preparation[8]:**

Guduchi Stem : 1Part (49.703kg) (After removing Stem cover)
Water: 21 Part (1043.763 lit)

Method of preparation:

The Guduchi Stem was rinsed with tap water and removed dust, foreign particles adhered to it, then the drug was wiped out with cotton cloth and removed outer layer so as to avoid interference during preparation of satva. The stem was subjected to pounding machine for proper crushing then added specified quantity of water, rubbed well with hands thoroughly and kept overnight for soaking.

Next day the material again well rubbed, till the disappearance of stickiness, the fibers were removed and the remaining material was stained through sterile cloth. The strained material was collected in a flat bottom stainless steel container and allowed for the sedimentation. When the solid particles of materials were found sedimented, the supernant liquid portion was decanted carefully. After decantation the starch obtained was again mixed with little quantity of water and allowed again for sedimentation and then liquid portion was removed by decantation process. This decantation process was done for 7 times and finally clear white powder was obtained. The process was repeated for three times and each sample coded as G1, G2 , G3. and were capsulated 500 mg, stored in a air tight glass container and used for further studies.

Quantity of Satva Obtained : G_1: 1.208 Kg (2.4%), G_2: 1.070 Kg. (2.18%) and G_3 : 0.995 Kg (2.1%)

The samples were studied for organoleptic characters and physico-chemical properties viz. Microscopical, particle size, characterization, color, taste, odor, texture, pH, Ash, Acid and water insoluble ash, Alcohol and water soluble extractive values and TLC[9] etc…

OBSERVATIONS AND RESULTS:

A. MICROSCOPIC IDENTIFICATION[10]:

Slide Preparation: For the microscopic studies, slides were prepared by dusting Guduchi Satva samples and were observed under electrical microscope to determine the exact shape of starch grains. The same slides were studied after exposing with Iodine. The results were shown in figure 1 & Table No. 2.

B. DETERMINATION OF GRAINS SIZE OF STARCH IN GUDUCHI SATVA: (fig. 2 & Table No. 3.)

10 part of ocular = 4 part of stage
1 part of ocular = 4/10 part of stage.
(1part of stage = 0.1 mm) (1μ = 1000 mm)
Hence, 1 part of ocular = 4/10×0.01mm=0.004mm or 4 μ = 4μ

C. DETERMINATION OF QUANTITATIVE DATA : WEIGHT VARIATION [11:]

The content of the final pack shall not be less than 95 percent of the declared net content. Randomly, 20 capsules of Guduchi Satva were taken and weighed collectively and separately, the variation in weight observed were not more than ± 25 mg.

D. DETERMINATION OF TOTAL ASH[12]:

About 5 gr. of the ground air-dried material was placed in a previously ignited and weighed platinum crucible. The material was spread in an even layer and ignited by gradually increasing the heat to 450°C, until free from carbon, cooled and weighed. The charred mass exhaust with hot water, the residue collected on an ashless filter paper, the residue and filter paper incinerated, filtrate added, evaporated

to dryness, and ignited at a temperature not exceeding 450°c. The percentage of ash calculated with reference to the air-dried drug. Thus the total ash was shown in table no. 4.

E. DETERMINATION OF ACID-INSOLUBLE ASH[13]:

The total ash was collected and boiled with 25 ml of dil HCL for 5 minutes. This solution was filtered with the Whatman (No. 41) filter paper along with the insoluble ash, the filter paper was burnt in a Gooch crucible. Heating, cooling and weighings of the crucible was done until the weight of the crucible comes constant. The percentage of acid insoluble ash was calculated with reference to the air-dried drug (5 gr.) Thus the results were shown in table no. 5.

F. DETERMINATION OF WATER SOLUBLE ASH[14]:

Ash was boiled for 5 minutes with 25 ml of water, the insoluble matter collected in on an ash-less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash, the difference in weight represents the water-soluble ash. Percentage of water-soluble ash was calculated with reference to the air-dried drug. The results were shown in table no. 6.

G. DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVE[15]

5 g of the air dried drug mixed with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaked frequently during six hours and allowed to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105^0 C, till to constant weight and then weighed. Percentage of alcohol-soluble extractive calculated with reference to the air-dried drug. The results are shown in table no.7.

H. DETERMINATION OF WATER SOLUBLE EXTRACTIVE[16]: Proceeded as directed for the determination of alcohol-soluble extractive, using chloroform-water instead of ethanol. The results were shown in table no. 8.

I. CHEMICAL TESTS FOR IDENTIFICATION OF SATVA[17]:

Various chemicals are added to the Guduchi Satva samples and randomly tested in hot and cold chemicals to determine presence of material in all the samples. The result were shown in table no. 9.

J. DETERMINATION OF MOISTURE CONTENT (LOSS ON DRYING[18]:

About 10 gr. of drug taken in a tared evaporating dish was dried at 105 ^oC for 5 hours, and weighed. The drying and weighing continued at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight was reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desicator, show not more than 0.01 gr. difference. The results were shown in table no.10.

K. THIN-LAYER CHROMATOGRAPHY (TLC)[19]:

Sample preparation:

Exactly 3 gr. of sample was refluxed with 3 x 50 methanol for 1hour**,** filtered and concentrated to form a residue and made to 10 ml. with methanol, 5 µl was spotted.

Stationary phase (application): The prepared samples were applied over the pre coated silica-gel plate of 0.2mm thickness.

Development (Mobile phase): The samples were develop with the help of mobile phase, i. e. chloroform: methanol (9:1).

Visualization:

For visualization, the plates were dried at 100^0 C and scanned at 254 nm UV.

Observations:

The phases of UV 254 nm and UV 366 nm indicates that the plates examined under an ultra-violet light having a maximum output at about 254 (single spots) and at about 366 nm (four spots of each). The results were shown in table No. 11.

L. Starch test (Iodine test)[20]:

1gm of each Guduchi Satva sample was taken in a glass dish and added few drops of iodine solution into it. The color of samples turns to violet blue. This confirmed presence of starch in all the sample.

Table No. 1. Showing organoleptic properties of Gudchi Satva samples.

Characteristics	G_1	G_2	G3
Rupa (Color)	Chalky white	Chalky white	Chalky white
Rasa (Taste)	Tasteless	Tasteless	Tasteless
Gandha (Odor)	Odorless	Odorless	Odorless
Sparsha(Consistency)	Fine powder	Fine powder	Fine powder

Table No. 2. Showing microscopic structure of Guduchi satva samples.

Samples	G1	G2	G3
Structure	Starch grains were ovoid, irregular ovoid or eleptical, hilum was in center with concentric strips	Same as G1	Same as G1
Size	16 to 36 µ	Same as G1	Same as G1
After iodine filling	Well stained voilet blue cloured	Same as G1	Same as G1

Table No. 3. Showing Starch Grain Size of Guduchi Satva samples.

Sl.no.	G_1	G_2	G_3
1	5 × 4 = 20	4 × 4 = 16	5×4=20
2	9 × 4 = 36	7 × 4 = 28	9×4=36
3	7 × 4 = 28	5 × 4 = 20	4×4=16

Table No. 4. Showing total Ash % of Guduchi Satva samples.

Samples	G_1	G_2	G_3
Initial Wt.	48.26368	47.689	49.157
Final Wt.	48.25848	47.6823	49.1509
Wt. of Ash	0.0052	0.0067	0.0061
Total % Ash	0.25	0.32%	0.30%

Table No. 5. Showing total Acid insoluble ash in Guduchi satva samples.

Samples	G_1	G_2	G_3
Initial wt.	42.5871	41.0082	40.9821
Final wt.	42.57742	40.9881	40.9532
Weight of ash	0.00968	0.0201	0.0289
% Acid insoluble Ash	0.19%	0.4294%	0.5909%

Table No.6. Showing Total water soluble Ash in Guduchi satva Samples.

Samples	G_1	G_2	G_3
Initial wt	32.4553	35.9661	41.0454
Final wt	32.4690	35.9789	41.0690
Weight of ash	0.0137	0.0128	0.0136
% water soluble Ash	0.4501%	0.4298%	0.4531%

Table No. 7. Showing alcohol soluble extractive of Guduchi Satva of all samples.

Samples	G_1	G_2	G_3
Wt of Pt.dish	32.7829	34.1179	34.9051
Wt of Pt.dish+dried	32.7960	34.1392	34.9219
Wt of extract	0.0131	0.0223	0.0168
Ext. in 100ml	0.0508	0.0683	0.590
% of Alcohol soluble Ext.	1.0521%	1.120%	1.267%

Table No.8. Showing water soluble extractive of Guduchi satva Samples.

Samples	G_1	G_2	G_3
Wt. of Pt. dish	37.8296	38.3298	43.9812
Wt of Pt. dish+dried	37.8968	38.3899	44.0594
Wt of ext	0.0672	0.0601	0.0782
Wt. of Ext in 100ml	0.1902	0.1631	0.2038
% of water soluble Ext	0.5008%	0.4389%	0.5678%

Table No. 9. Showing chemical identifications tests for Guduchi satva samples.

Sl.No	Chemicals		G_1	G_2	G_3
1	**Distilled H2O**	**Cold**	Soluble, milky white	Soluble, milky white	Soluble, milky white
		Hot	Gelatinized	Gelatinized	Gelatinized
2	**Dil HCL**	**Cold**	Soluble, creamish white	Soluble, creamish white	Soluble, creamish white
		Hot	Yellow colored	Yellow colored	Yellow colored
3	**Conc HCL**	**Cold**	Insoluble, white ppt.	Insoluble, white ppt.	Insoluble, white ppt.
		Hot	Soluble, brown colored sol.	Soluble, brown colored sol.	Soluble, brown colored sol.
4	**Dil H2SO4**	**Cold**	Soluble, milky white sol.	Soluble, milky white sol.	Soluble, milky white sol.
		Hot	Gelatinized	Gelatinized	Gelatinized
5	**Conc H2SO4**	**Cold**	Insoluble, yellow ppt	Insoluble, yellow ppt	Insoluble, yellow ppt
		Hot	Charred sol.	Charred sol.	Charred sol.
6	**Dil HNO3**	**Cold**	Soluble, milky white sol.	Soluble, milky white sol.	Soluble, milky white sol.
		Hot	Charred sol.	Charred sol.	Charred sol.
7	**Conc HNO3**	**Cold**	Insoluble, white ppt	Insoluble, white ppt	Insoluble, white ppt
		Hot	Yellow sol & foam deposited on inner wall of test tube.	Yellow sol & foam deposited on inner wall of test tube.	Yellow sol & foam deposited on inner wall of test tube.
8	**NaOH**	**Cold**	Initially soluble, then gelatinized.	Initially soluble, then gelatinized.	Initially soluble, then gelatinized.

		Hot	More gelatinized	More gelatinized	More gelatinized
9	**KOH**	**Cold**	Soluble, white ppt.	Soluble, white ppt.	Soluble, white ppt.
		Hot	Gelatinized	Gelatinized	Gelatinized
10	**KI**	**Cold**	Soluble, milky white,	Soluble, milky white,	Soluble, milky white,
		Hot	Semisolid gelatin formed.	Semisolid gelatin formed.	Semisolid gelatin formed.
11	**Fecl3**	**Cold**	Soluble, yellow solution.	Soluble, yellow solution.	Soluble, yellow solution.
		Hot	Yellow, gelatinized.	Yellow, gelatinized.	Yellow, gelatinized.
12	**Iodine Solution**		Blue color	Blue color	Blue color
13	**Ethyl alcohol**		Milky white	Milky white	Milky white

Table No. 10. Showing Total Moisture content (LOD) in Guduchi satva samples.

Samples	G_1	G_2	G_3
Initial wt	35.8931	38.3698	37.8291
Final wt.	35.2168	38.1009	37.2089
Change	0,06763	0.2697	0.6202
LOD in %	13.0055	8.1310	12.3995

Table No. 11. Showing Rf values of samples of Guduchi Satva.

Sr. No.	Samples	254nm		365nm		After Spraying with 10% ferric chroride	
		No of spots	**Rf value**	**No of spots**	**Rf value**	**No of spots**	**Rf value**
1	**G_1**	0	--	4	0.1, 0.27 ,0.8	0	--
2	**G_2**	0	--	4	0.37, 0.4 ,0.82	0	--
3	**G_3**	0	---	4	0.12, 0.29, 087	0	--

Table No. 12. Showing pH of Guduchi Satva samples.

Sample ⇨ / Dilution ⇩	G_1	G_2	G_3
5%	7.66	7.68	7.62
10%	7.81	7.83	7.83

GUDUCHI SATVA MICROSCOPIC STUDY PHOTOGRAPHS

Fig. 1.

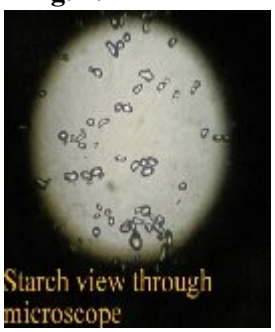


Fig. 2

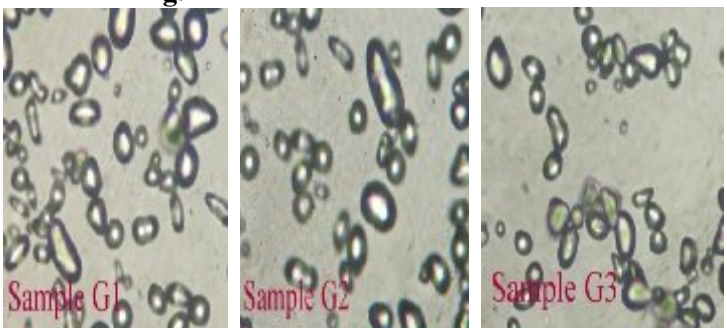
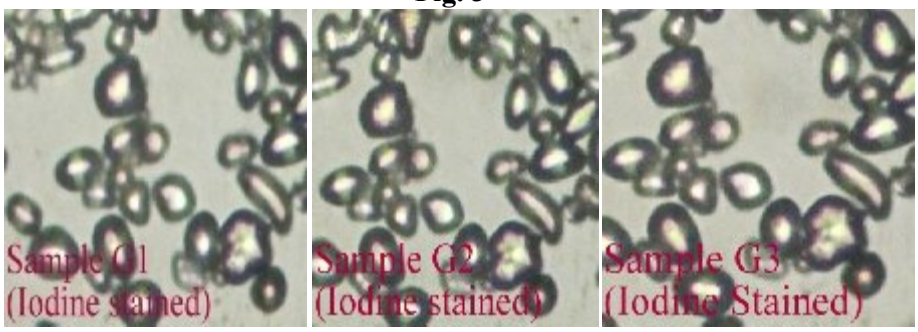


Fig. 3



DISCUSSION:

Satva kalpana is one the most important dosage form in the Ayurvedic medicine. Gduchi satva was frist described by Yogaratnakara in the context of Guduchi modak, later it was adopted by A.F.I. During the pharmaceutical study, Matured Guduchi stem was used so as to get good amount of satva and the process was carried out under GLP (Good laboratory practices) conditions. The final weight obtained were 1.208 Kg.,(2.4%), 1.070Kg.(2.18%) and 0.995Kg.(2.11%) in the samples of G1, G2 and G3 respectively.

The organoleptic properties of Guduchi satva is white in color, tasteless, smooth and fine powder in form and no significant difference found between the prepard samples. Grain size of Sample G1, G2, G3 starch ranges from 16 to 36µ.

The percentage of ash value was found below 1% in all the samples. If it is more than 1% indicates addition of inorganic substances. The percentage of acid insoluble ash and water insoluble ash are found less than 2% in all the samples.

The alcohol and water soluble extractives are found below 2% where as moisture content of all samples rangew between 8-13%, this is because, the samples were found highly hygroscopic in nature. All the chemical identification tests showed the presence of starch and the pH of all the Guduchi Satva samples are found to be weak basic in nature.

The TLC plates (chloroform : methanol 9:1) examined under an ultra-violet light having a maximum output at about 254 and at about 366 nm. and confirms presence of material.

CONCLUSION:

Satva kalpana is an important dosage form and Gduchi satva is frist mentioned by Yogaratnakara later it has been considered as formula by A.F.I.

The GLP conditions of this preparation yeilds 2.4%.The organoleptic properties of Guduchi satva are white in color, tasteless, smooth powder in form. The Grain size ranges from 16 to 36µ. The ash value has less than1% and acid

insluble and water solubles are less than 2%

The alcohol and water soluble extractives are found below 2% and the moisture content ranges between 8-13% where as pH is weak basic in nature.

Overall, the chemical tests indicating that the drug is of a specific starch in nature.

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