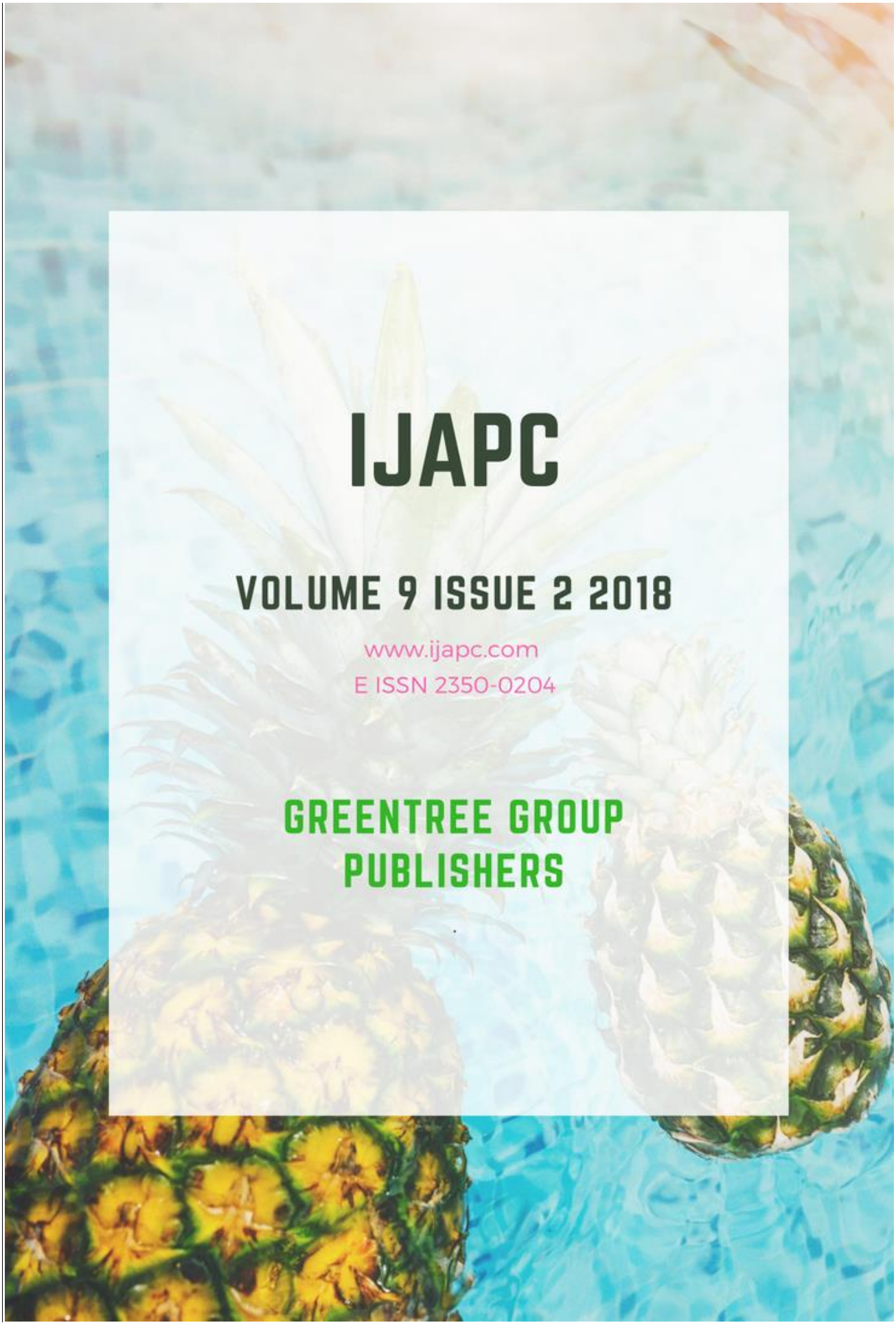

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Comparative Quality Control Evaluation of Market samples and Classically Prepared Samples of *Chyavanaprasa*

Mahesh S[1]*, Sheela Karalam B[2], Jacob M Titus[3] and Neethu S Pillai[4]

[1]Department of Rasa Shastra and Bhaishajya Kalpana, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

[2]Department of Indian System of Medicine, Trissur, Kerala, India

3Department of Rasa Shastra and Bhaishajya Kalpana, Govt.Ayurveda College, Trivandrum, Kerala, India

[4]Ayurithu Hospitals, Kozhikode, Kerala, India

ABSTRACT

Background: *Chyavanaprasa* is classified under the group of *Rasayana* (Rejuvenator), where the main purpose is to maintain the body's integrity for delaying the ageing process, enhances longevity and improves digestion. On account of increasing urbanization, the tendency is towards more and more dependence on readymade preparations. It is one of the most popular Ayurvedic OTC product purchased by the common public.

Aim: The aim of the present study is to compare the organoleptic, physicochemical, biochemical, microbial parameters of various market samples and a classically prepared sample of *Chyavanaprasa*.

Materials & Methods: Four market samples were collected by the month of July 2015, named $MSCP_1$ (Market sample of *Chyavanaprasa*), $MSCP_2$, $MSCP_3$ and $MSCP_4$ for comparative analysis. A standard sample named CP (*Chyavanaprasa*) was prepared and analyzed at Research and Development laboratory of Oushadi, The Pharmaceutical Corporation (I.M.) Kerala Ltd, Kerala.

Results and Conclusion: Analysis revealed there is a huge variation in the organoleptic characters among different samples and none of the market samples comply with all the physico chemical parameters as per API. Microbiological evaluation of all market samples and prepared samples are within the limit of API values except $MSCP_1$. CP shows the effective combination of ascorbic acid, carbohydrate, iron, protein and fat. HPTLC analysis shows the presence of ascorbic acid in all the samples. These analyses confirm the existence of quality issues in *Chyavanaprasa* marketed by various GMP Certified Companies and proves the need of standardization of market samples of *Chyavanaprasa.*

KEYWORDS *Chyavanaprasa, Quality control, GMP, OTC products, API, HPTLC*

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INTRODUCTION

Ayurveda represents the most ancient and classical knowledge base pertaining to Life Science, Health and Cure. According to WHO, over 80% of the world population relies on the traditional systems of medicines, largely plant based, to meet their primary healthcare needs[1]. Globalization of Traditional medicines is mainly through worldwide exports of these medicines and its knowledge. Today Ayurveda is gaining growing attention and acceptance all over the world. There is a growing demand for natural products including items of medicinal value, pharmaceuticals, food supplements and cosmetics in the international market[1]. The Indian pharmaceutical market was valued at an approximate of Rs. 145 billion as of 2008, and was growing at 8 to 9 percent annually[1]. The production from AYUSH sector in India during the corresponding period was approximately Rs 23 billion, which constitutes close to 6% of the pharmaceutical market[1].

In India there are thousands of companies producing Ayurvedic medicines as per the demand. It is estimated that the total value of products from the entire Ayurvedic production in India is on the order of one billion U.S dollars [2]. This herbal predominant system of medicine is now recognized not only in India, but also in the western world. Now a days Ayurvedic physicians depends mainly on the pharmaceutical industry and it is not easy to recognize the authenticity of a drug unless it is standardized. Safety, Efficacy, Stability and Palatability are the four basic requirements of a good drug dosage form[3]. *Avaleha kalpana*[4] (confection) is a semisolid preparation of herbal drugs prepared in decoction or extracts of different herbs by adding sweetening agents like jaggery, sugar or sugar candy. *Chyavanaprasa*[5] is a polyherbal formulation with a semisolid sticky nature. It is chocolate brown coloured having sweet taste with non-specific pleasant odour. *Chyavanaprasa* is one of the famous formulation under *Rasayana* (Rejuvinator) group, which helps in delaying the ageing process and hence enhances longevity. The preparation of *Chyavanaprasa* was described in Charaka Samhita[6], and references are available in Ashtanga Hridaya[7], Bhaishajyaratnavali[8] etc also. The reference from Charaka samhita was quoted in Ayurveda Pharmacopoeia of India (API) for preparing quality assured *Chyavanaprasa*. It has been regarded as one of the good promoter of *agni* and useful in the diseases like *Kasa* (Cough)[5], *swasa* (Dyspnoea/Asthma), *Kshata ksheena* (Debility due to chest injury), *Svarabheda* [5]

(Hoarseness of voice), *Hridroga* (Heart disease), *Agnimandya* (Digestive impairment), *Uroroga* (Disease of thorax), *Vatarakta* (Gout), *Pipasa* (Thirst), *Mutraroga* (Urinary diseases), *Sukra dosa* (Vitiation of semen), *Jara* (Senility/Progeriasis), used as *Rasayana, Medhya* (Improves memory) [5].

On account of increasing urbanization, the tendency is towards more and more dependence on readymade preparations. The major source of *Chyavanaprasa* to the consumers is from GMP certified Companies (GCC). Nowadays *Chyavanaprasa* is a Fast Moving Consumer Good (FMCG) and a money maker for Ayurvedic manufacturers. Branding and modern science must make *Chyavanaprasa* attractive in the eyes of consumers[3]. It is one of the most popular Ayurvedic OTC product purchased by the common public.

Due to the increased demand from market and the shortage of authentic raw materials have made it incumbent that some sort of uniformity is needed in the manufacture of *Ayurvedic* medicines. So quality control validation should be done to maintain uniform standards. Many of these industries have deviated from the traditional preparatory methods to decrease the lead time of preparation. Now different value added forms of *Chyavanaprasa* are also available in market. The aim of the study is to conduct a market sample analysis with existing standardization parameters for *avalehas* and to compare it with classically prepared sample.

MATERIALS AND METHODS

Procurement of raw materials

All raw materials used in *Chyavanaprasa* were procured from the raw material store of Oushadi The Pharmaceutical Company Ltd. Kerala in July 2015. The material was examined for probable adulterations and foreign matter adhering to the surface was removed. Morphological and microscopical evaluation were done and authenticated by Pharmacognosy Department of Oushadi . Potable water was used for preparation of sample of *Chyavanaprasa*.

Pharmaceutical preparation of *Chyavanaprasa* as per Classical reference

Raw materials required for sample preparation (Table No.1) were weighed and taken for preprocessing as in table no.1. All of the drugs were taken in dried form except *amalaki*, which is taken in fresh form. A standard sample as per API reference was prepared and named CP at Research and Development laboratory of Oushadi. Quantity of fresh *amalaki* mentioned in the reference of *Chyavanaprasa* for preparing one batch was standardized as 5 kilogram as per API. Fresh clean riped *amalaki's*

Table 1 Quantity of raw drugs taken for preparation of *Chyavanaprasa*

Sl.No.	Ingredients	Latin Name / English term	Quantity
1	*Bilva*	*Aegle marmelos* L.	48 gm
2	*Agnimantha*	*Premna integrifolia* Linn.	48 gm
3	*Shyonaka*	*Oroxylum indicum* L.	48 gm
4	*Kashmari*	*Gmelina arborea* Roxb.	48 gm
5	*Paatala*	*Stereospermum suaveolens* Roxb	48 gm
6	*Bala*	*Sida cordifolia* L.	48 gm
7	*Prisniparni*	*Uraria picta* Jacq.	48 gm
8	*Shaliparni*	*Desmodium gangeticum* L.	48 gm
9	*Mashaparni*	*Teramnus labialis* L.	48 gm
10	*Mudgaparni*	*Phaseolus trilobus* L.	48 gm
11	*Pippali*	*Piper longum* L.	48 gm
12	*Gokshura*	*Tribulus terrestris* L.	48 gm
13	*Brihati*	*Solanum indicum* L.	48 gm
14	*Kantakari*	*Solanum surattense* Burm.	48 gm
15	*Shringi*	*Pistacia chinensis* Bunge	48 gm
16	*Tamalaki*	*Phyllanthus fraternus* L.	48 gm
17	*Draksha*	*Vitis vinifera* L.	48 gm
18	*Jeevanthi*	*Leptadenia reticulate* Retz.	48 gm
19	*Pushkaramoola*	*Inula racemosa* Hook.	48 gm
20	*Agaru*	*Aquilaria agallocha* Roxb.	48 gm
21	*Abhaya*	*Terminalia chebula* Retz.	48 gm
22	*Amritha*	*Tinospora cordifolia* Miers.	48 gm
23	*Riddhi*	*Habenaria intermedia* D.Don	48 gm
24	*Jeevaka*	*Malaxis acuminate* D.Don	48 gm
25	*Rshabhaka*	*Malaxis muscifera* (Lindl.)Kuntze	48 gm
26	*Shati*	*Hedychium spicatum* Buch Ham.	48 gm
27	*Musta*	*Cyperus rotundus* L.	48 gm
28	*Ela*	*Elettaria cardamomum* L.	48 gm
29	*Chandana*	*Santalum album* Linn.	48 gm
30	*Utapala*	*Nymphaea stellate* Wild.	48 gm
31	*Vrishamula*	*Adhatoda zeylanica* Nees.	48 gm
32	*Kakoli*	*Lilium polyphyllum* D.Don	48 gm
33	*Punarnava*	*Boerhavia diffusa* L.	48 gm
34	*Medha*	*Polygonatum cirrhifolium* Mill.	48 gm
35	*Kakanasika*	*Martynia annua* L.	48 gm
36	*Utpala*	*Nymphaea stellata* Wild.	48 gm
37	*Amalaki*	*Phyllanthus emblica* L.	5 kg
38	*Water*		12.288 L reduced to 3.072 L
39	*Thugaksheeri*	*Bambusa bambos* L.	192 gm
40	*Pippali*	*Piper Longum* L.	96 gm
41	*Ela*	*Elettaria cardamomum* L.	48 gm
42	*Twak*	*Cinnamomum zeylanicum* Blume	48 gm
43	*Patra*	*Cinnamomum tamala* Buch.Ham.	48 gm
44	*Kesara*	*Mesua ferrea* L.	48 gm
45	*Thila thaila*	Oil of *Sesamum indicum* L.	288 ml
46	*Ghrita*	Cow's ghee	288 ml
47	*Matsyandika*	Sugar candy	2.4kg
48	*Madhu*	Honey	288ml

were washed properly and bundled in a piece of cloth. Ingredients one to 36 were powdered coarsely for *kwatha* preparation and is mixed with required quandity of water taken in vessel and the *amalaki* bundle was immersed fully in water and boiled till the water is reduced to one forth. The *amalaki* bundle is then taken out and the seeds were removed, and the decoction is strained and kept separately. Pulp of amalaki was prepared by grinding and was fried with *ghritha* and *taila*, till the moisture evaporates. Sugar candy was added to the decoction and boiled till attaining *leha paka*, at which stage the fried pulp is added and boiled again. Fine powders of ingredients 39 to 44 are added to the l*eha* at *leha paka* and stirred well and allowed to cool, and then *thugaksheeri* and *madhu* was added and mixed well.

Collection of Market samples

Chyavanaprasa samples prepared by 4 GMP certified companies by the month of July 2015, identified from the label (the month in which the research carried out) were purchased from their respective outlets. The bottles were examined for any damage or leakage. Market samples selected were named as $MSCP_1$ (Market Sample *Chyavanaprasa*), $MSCP_2$, $MSCP_3$, $MSCP_4$. The analysis was conducted at Research and Development laboratory of Oushadi.

Analysis

1. *Organoleptic analysis*:- Parameters like colour, odour, taste and consistency was noted.

2. *Physicochemical and Bio-chemical analysis*

Physico chemical and Biochemical parameters like pH[9] , Loss on Drying[9], Water Soluble Extractive[9], Alcohol Soluble Extractive[9], Total Ash[9], Acid Insoluble ash[9], Water soluble ash[9], Reducing sugar and Non-reducing sugar[10], Crude Fat content[11], Ascorbic acid estimation[11], Carbohydrate estimation[11], Calcium estimation[12], Iron estimation[12] and Protein estimation[12] of *Chyavanaprasa* samples were performed and results were mentioned in Table no.3 and 4.

3. *Microbial limit tests* [12]

The medium used for the bacterial count was Nutrient Agar (NA) and Yeast Glucose Chloramphenicol agar (YGC agar) was used for determining the fungal count. All the plates were observed for bacterial and fungal colonies. Then the number of colonies in the plates was subjected for counting. The number of microorganisms per ml of the original suspension was calculated using the formula. Organisms per ml = number of colonies / amount plated × dilution. And the results were mentioned in Table no. 5.

4. *High Performance Thin Layer Chromatography (HPTLC)* [13]

Instrumentation**:-**Chromatographic conditions:

Application mode: CAMAG Linomat V Hamilton Syringe

Development chamber: CAMAG Twin trough chamber (20x10 cm2)

Plates: Precoated silica gel GF254 plates

Chamber saturation: 30 min

Development distance: 10 cm

Development time: 30 min

Scanner: CAMAG TLC Scanner III

Scanning mode: Linear at 254 nm and 366 nm

Detection: Deuterium lamp, Mercury lamp

Photo documentation: CAMAG reprostar

Data system: CATS software (Ver. 3.17)

Drying device : Oven

UV Spectrum: 200 nm to 800 nm

Solvent System: Ethanol: Glacial acetic acid: Toluene (11:2:3)

Spray reagent: Anisaldehde Sulphuric Acid reagent

Method:- An ideal solvent system was devised first by TLC which enables maximum separation of components. After numerous trial and errors a mixture of ethanol, glacial acetic acid and toluene in the ratio 11:2:3, respectively was found to be suitable for separation. The Standards used were 1% Vitamin C and 1% Celin tablet (vitamin c tablet). Samples were numbered, 1% Vitamin C as sample 1, 1% Celin tablet as sample 2, MSCP1 as 3, MSCP2 as 4, MSCP3 as 5, MSCP4 as 6, CP as sample 7. Thus, a total of seven samples were prepared for spotting into the silica gel plate. Sample application volume was 6 micro liters, which was done using LINOMAT 5 of CAMAG. The solvent and the prepared plate were fed in to the automatic developing chamber of CAMAG. After separation, the plate was viewed in ultraviolet light and the photograph of the chromatogram was also captured.

RESULTS AND DISCUSSION

Results:

1. *Organoleptic evaluation of market samples of Chyavanaprasa*

The colour and consistency of each samples was different from one another. The colour was varying from brown to blackish brown (Table no.2).

Odour: All of the market samples possess pleasant aromatic smell without much difference.

Taste:- In API the recommended taste for *Chyavanaprasa* is sweet while on observing the market samples they have predominant sweet taste with sour and astringent. Sample prepared as per classical textbooks of *Ayurveda* had sweet taste and

sour taste predominantly followed by astringent taste.

2. *Physico chemical, biochemical and microbial evaluation of market samples and prepared sample.*

The Physico-chemical, biochemical and microbial evaluation of market samples and prepared sample as per API reference were done and results were shown in Table no.3, table no.4 and table no.5 respectively.

3. *HPTLC analysis:–To find out the presence of ascorbic acid in the samples.*

Chyavanaprasa samples of four GCC and one prepared sample were selected for sample application. The concentration of the component can be compared by analyzing the area of samples. Here ascorbic acid is being compared. (Figure no.1 and Figure no.2)

DISCUSSION

Globalization has brought many new opportunities to developing countries like greater access to developed country markets and technology transfer which has resulted in improved productivity and higher standard of living[14]. It is a process of increasing the connectivity and interdependence of the world's markets and businesses [14].

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assign
1	1	0.53 Rf	12.1 AU	0.68 Rf	364.7 AU	100.00 %	0.73 Rf	8.8 AU	21211.1 AU	100.00 %	Auto
2	1	0.53 Rf	14.3 AU	0.63 Rf	242.0 AU	100.00 %	0.69 Rf	0.5 AU	12783.3 AU	100.00 %	Auto
3	1	0.57 Rf	0.9 AU	0.63 Rf	30.6 AU	100.00 %	0.68 Rf	3.2 AU	777.0 AU	100.00 %	Auto
4	1	0.61 Rf	4.4 AU	0.64 Rf	24.5 AU	100.00 %	0.67 Rf	4.0 AU	501.6 AU	100.00 %	Auto
5	1	0.60 Rf	1.6 AU	0.64 Rf	25.7 AU	100.00 %	0.69 Rf	1.6 AU	593.3 AU	100.00 %	Auto
6	1	0.61 Rf	0.0 AU	0.64 Rf	24.1 AU	100.00 %	0.69 Rf	0.2 AU	496.7 AU	100.00 %	Auto
7	1	0.61 Rf	1.1 AU	0.65 Rf	22.8 AU	100.00 %	0.71 Rf	1.0 AU	578.7 AU	100.00 %	Auto

Fig 1 Peak table of standards and samples showing presence of ascorbic acid

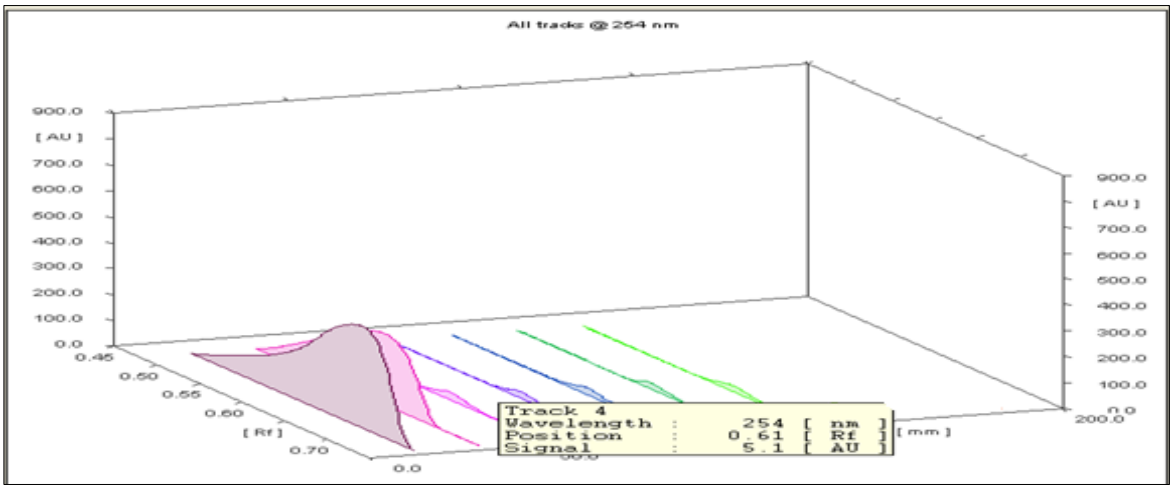

Fig 2 Dimensional view of chromatogram obtains through HPTLC viewed at a wavelength of 254 nm showing the presence of ascorbic acid

One of the most crucial dimensions of globalization and liberalization is export business[14]. Traditional medicines are now globalizing slowly through worldwide exports of these medicines and its knowledge[15]. People in developed countries are now aware about the adverse effects of chemical drugs and they have also started showing preference for traditional medicines [1]. Globalization of Ayurveda and Ayurvedic medicines has created tremendous opportunities for the manufacturers of these medicines[1]. Due to the high demand for safer drugs, attention has been drawn to the standardization, safety and efficacy of Ayurvedic formulations[1]. By the process of standardization, the final products become more reliable in terms of quality, efficacy and safety and in turn help to avoid unwanted adulteration and improper substitution.

The method of preparation of *Chyavanaprasa* on an industrial scale has considerable difference compared to the textual reference and API. There is non-uniformity in the production of *Chyavanaprasa* among GMP Certified companies[17]. As per API recommendations, fresh *amalaki* was used in production of *Chyavanaprasa* and it should be boiled along with *kashaya*. But some GMP Certified Companies (GCC) uses dried *amalaki's* for the production of *Chyavanaprasa*[28]. Some of the GCC's were using jaggery in the production of *Chyavanaprasa* instead of sugar candy. Because jaggery is cheaper compared to sugar candy, which in turn reduce the cost of production. Some of the companies avoid frying of amalaki and they add amalaki powder at *Leha paka* [17].

The organoleptic analysis of market samples to identify the quality issues in *Chyavanaprasa* from the market, revealed that there is a huge variation in the organoleptic characters particularly colour, consistency and taste among different samples. The colour change may be due to the change of substrate (sugar base) or avoiding frying of *amalaki* paste. Consistency of *avaleha kalpanas* varies with ratio of Sugar base and *prakshepa choorna*[16]. The consistency of market samples of *Chyavanaprasa* varies from each other. In API the recommended consistency for *Chyavanaprasa* is sticky while on observing the market samples, $MSCP_4$ have a free flowing nature and others having sticky nature. The sample CP was prepared as per classical reference, dark brown and free flowing which comply with API parameters (Table no.2).

Table 2 Colour and consistency observed in Market samples and prepared sample

Sr.	Sample	Colour	Consistency
1.	**$MSCP_1$**	Blackish brown	Non sticky compared to other samples
2.	**$MSCP_2$**	Dark brown	Sticky semisolid
3.	**$MSCP_3$**	Brown	Sticky semisolid but not to the extent of free flowing.
4.	**$MSCP_4$**	Chocolate brown	Sticky with free flowing nature
5.	**CP**	Dark brown	Sticky, semisolid

There is no uniformity observed in physicochemical parameters between various samples. All the samples were acidic with pH in range with API reference except $MSCP_1$ which was less acidic than the reference. As per API standards loss on drying should be not more than 9%. All of the samples have value within the limit except $MSCP_4$.Water soluble extractives of all market samples were within the limit of API standards. Alcohol soluble extractives of all four samples were less than the limit of API. Total ashes of all samples were within the limit of API standards except sample $MSCP_1$, may be due to presence of foreign matters. Acid insoluble ash values of all samples were within the limit. None of the market samples comply with all the physico chemical parameters as per API (Table no.3).

Table 3 Comparison of physicochemical evaluation of samples

Sr.No.	**Parameters**	**API parameters**	**$MSCP_1$**	**$MSCP_2$**	**$MSCP_3$**	**$MSCP_4$**	**CP**
1.	**Loss on drying**	Not more than 9 %	8.40%	9%	8.5%	10%	8%
2.	**Total solids**	-	55%	63%	55%	57%	74%
3.	**Water soluble extractives**	Not less than 50 %	60%	65%	64.70%	68%	66.70%
4.	**Alcohol soluble extractives**	Not less than 50 %	20%	29%	25%	40%	53.01%
5.	**Total ash**	Not more than 2 %	2.30%	2%	1.2%	1.3%	1.29%
6.	**Acid insoluble ash**	Not more than 2 %	0.90%	0.40%	0.41%	0.50%	0.51%
7.	**Water soluble ash**	-	1%	0.83%	0.60%	0.36%	0.60%
8.	**pH (1% aq. soln.)**	3.82 –4.23	4.37	3.3	4.2	3.4	3.4
9.	**Reducing sugar**	-	55%	65%	58%	60%	68%

The bio-chemical analysis of samples shows, Ascorbic acid content and fat content is more in $MSCP_1$ than CP (Table no.4). The increased ascorbic acid content in sample may be to the usage of dry *amalaki* powder without any processing like boiling of *amalaki* etc. and increased fat content may be due to avoiding the frying step of *amalaki* and addition of ghee and oil at *leha paka*[17]. Vitamin C, also known as ascorbic acid, is a water soluble vitamin found in amalaki[18]. It is an antioxidant that is very essential for human nutrition and proper functioning of the body. The human body cannot synthesize vitamin C endogenously, so it is an essential dietary component. At high temperature, in the presence of sunlight and

oxygen in air, vitamin C reacts and it is oxidized[18]. Boiling in high temperature also destroys vitamin C since it easily leaches into the cooking water being a water-soluble vitamin[18]. So better recommended to boil it in low heat.

Iron content is more in CP sample than the other samples (Table no.4), which is produced as per the unique methodology said in Charaka Samhita/API. These biochemical tests may help to find out the quality of the finished product. While comparing with market samples, CP shows the effective combination of ascorbic acid, carbohydrate, iron, protein, fat etc. (Table no.4). Change in those values reflected in market samples may be due to the deviations made in preparatory methods.

The permissible limit of total microbial plate count as per API is up to 10^5 cfu/ml. Here all the samples had below countable number of colonies in 10^4dilutions itself, except $MSCP_4$ sample (Table no.5). In that sample microbial count is more than the limit, which confirms some quality issues in production. Total fungal counts of all samples were within the permissible limits. Microbiological evaluation of market samples are within the limit of API values except $MSCP_1$. (Table no. 5).

Table 5 Microbiological evaluation of market samples of *Chyavanaprasa*
**NA: Nutrient agar; YGC: Yeast Glucose Chloramphenicol

Microbiological analysis		
Market Samples	**NA**	**YGC**
	Total microbial plate count (cfu/ml)	**Total fungal count (cfu/ml)**
$MSCP_1$	2.14×10^4	Nil
$MSCP_2$	1.56×10^4	Nil
$MSCP_3$	0.18×10^4	Nil
$MSCP_4$	Too numerous to count(TNTC)	Nil
CP	0.28×10^4	Nil

HPTLC analysis has been done in all market samples and a prepared sample with Ascorbic acid standard shows the presence of ascorbic acid in all the samples(Figure no.1 and Figure no.2). Samples 1 (1% Vitamin C) and sample 2 (1% Celin-Vitamin C tablet) show high peak being the standard samples. All other samples show the presence of ascorbic acid. HPTLC study reveals the presence of ascorbic acid in all of the 4 Market samples and prepared sample. It proves the antioxidant activity of *Chyavanaprasa* (Figure no.1 and Figure no.2).

CONCLUSION

The results of organoleptic, physicochemical, biochemical study concluded that all market samples ($MSCP_1$, $MSCP_2$, $MSCP_3$ and $MSCP_4$) were not found within the standard range of API and with remarkable variations when compared with standard sample CP. HPTLC analysis shows the presence of ascorbic acid in all the samples and Microbial analysis of all

samples are within the limit of API values except $MSCP_1$. This confirms the existence of quality issues in *Chyavanaprasa* marketed by various GCC and proves the need of standardization of market samples of *Chyavanaprasa*.

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