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DRUG POTENTIATION WITH SUBSEQUENT EXTRACTION OF BIOMARKER: A CASE STUDY OF *BRAHMI GHRITA*

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Abstract: ***Introduction*** -To treat various neurological and psychological disorders, Ayurveda propounds use of lipids. One of the widely used internal lipoid dosage form is medicated ghee. It binds to various phytochemicals, minerals and effectively crosses blood brain barrier to make efficient drug delivery. However it's high dose, greasy nature, strong smell and taste lead to poor drug compliance. Present study was aimed at the potentiation of ghee using *Bacopa monnieri* Linn, and standardization using the biomarker *Bacoside-A*. ***Methods*** - Ghee procured from Indian Geer breed cow was processed using fresh *Bacopa* juice in three different proportions 1x, 5x and 10x; combined with three processing cycles single, five and ten respectively. For each repetition, Standard Operating Procedure (SOP) was strictly adhered to. Finished products were analyzed using HPTLC to calculate concentrations of *Bacoside A*. Results were compared. ***Results*** - SOP was established for potentiation of medicated ghee. Values for all the physicochemical parameters as per Ayurvedic Pharmacopeia of India were established for the samples. Concentration of Bacoside A increased as the processing cycles increased in 1x, 5x and 10x samples, but exact quantification of the marker in each sample is yet to be evaluated. ***Discussion and Conclusion*** - Method of potentiation using higher quantity of drug along with more processing cycles increases concentration of active biomarker in medicated ghee. Thus it opens the possibility of dose reduction and thereby it's encapsulation to increase drug compliance.

Key Words: *Avartana*, Biomarkers. *Brahmi Ghrita*, Drug Potentiation, *Siddha Ghrita*,
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1. INTRODUCTION

Siddha Ghrita (medicated ghee) is a novel and unique dosage form in Ayurved wherein active constituents from herbs, minerals or animal products are extracted into the lipids. It is recommended in the treatment of almost all the diseases elaborated in *Charaka Samhita* the best classics for Ayurvedic clinical practice.

Poor drug compliance of *siddha ghrita* owing to its high dose, greasy nature, strong smell and taste poses issues of palatability. To combat these issues; *avartana*, which literally means repetition of the procedure can prove helpful. A few *siddha sneha* (medicated ghee/oils) formulations are made using *Avartana* of *Paka* (boiling process) i.e. *Dashapaki/Shatapaki/Sahasrapaki bala taila* (Charaka Chikitsa 29/119-120 [1], *Shatapaki madhuka taila* (Charaka Chikitsa 29/115-118) [2], *Shatapaki ghrita* (Sushruta Chikitsa 29/115-118)[3] and a few more. Researches regarding *avartita sidhha sneha* have covered its conceptual review [4,5], physico-chemical analysis [6,7], various efficacy studies for its wound healing [8], anti-inflammatory and analgesic [9] and anticonvulsant activities [10]. All these studies gave values for physico chemical parameters and also concluded that the process of *avartana* of *sneha* increases the effect, enhances the action, eases the drug administration, packing and marketing.

However, to best the of authors knowledge no study has been reported that elucidates the process of *ghrita avartana*. It is challenging because the classical *ghrita* formulations are poly herbal. A single herb contains many bioactive compounds and if a formulation consists 4-5 herbs as ingredients, it can create complexity for analytical evaluation. A simpler version with a single herb could be helpful to evaluate effect of *avartana* on active extraction of bioactive compounds in the *ghrita*. Hence it was decided to work on *Brahmi Ghrita* (BG) where in Cow ghee was processed with single herb Brahmi. Conventional

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Brahmi Ghrita, which is advocated for treatment of *Apasmar* (~Epilepsy) in *Charak Samhita* contains four ingredients including *Brahmi* (Charaka Chikitsa 10/25) [11]. A comprehensive review of conventional *Brahmi Ghrita* [12], its physico-chemical profile [13], standardization [14, 15], acute and sub chronic toxicity [16], preclinical studies for scopalamine induced amnesia [17], CNS activity [18], learning and memory activity [19], anticonvulsant activity [20], nephroprotective effect [21], hepatoprotective activity [22] and anxiolytic activity [23] have been reported. It has also been evaluated clinically in patient of depression[24].

Thus, this is the first and foremost attempt to facilitate understanding of drug potentiation by *avartana* with the possible resultant increase in percentage of marker compound Bacoside A. Here researchers studied preparation, comparative physico-chemical and HPTLC analysis of *Brahmi Ghrita* devoid of the ingredients other than *Brahmi* at three process intervals first, fifth and tenth.

2. MATERIALS AND METHODS

Brahmi (*Bacopa monnieri* Linn.), available throughout India especially in marshy lands, near ponds and streams is used to make *Brahmi Ghrita.* Fresh juice expressed of whole *Brahmi* plant and cow ghee are the two ingredients of the formulation. *Fresh Brahmi* was collected from natural habitat. A Sample was identified and confirmed based on morphological characters by botanist and the voucher specimen was confirmed and deposited in the herbaria of Medicinal Plants Conservation Centre, Pune (MPCC – 959). Fresh extracted *Brahmi swarasa* (juice) was subjected to various organoleptic and physico-chemical parameters. Results are reported. For the purpose of authentication, fresh *Brahmi* was shade dried and pulverized into fine powder. This *Brahmi* powder was used for physicochemical analysis.

Cow ghee was procured from an authentic source of Sai Gajanan dairy near Pune. Here, Geer breed cows are cradled in a natural environment and fed on natural feed. The ghee was prepared by *desi* method [25]. Fresh whole cow milk of fat percent 4.5 was boiled and allowed to cool naturally. Culture of curd in proportion of 1 percent was added to the milk and was incubated for 8 hours till it attained necessary sourness. It was then churned to separate butter. Obtained butter was boiled till all water content evaporated and some solids sedimented at the bottom of the vessel. This is ghee. Ghee was tested in State Public Health Laboratory, Government of Maharashtra, Pune. Values for physico-chemical parameters for Cow ghee complied with prescribed standards as per Food Safety & Standards Regulations 2011.

2.1 Potentiation of cow ghee:

2.1.1. Process optimization:

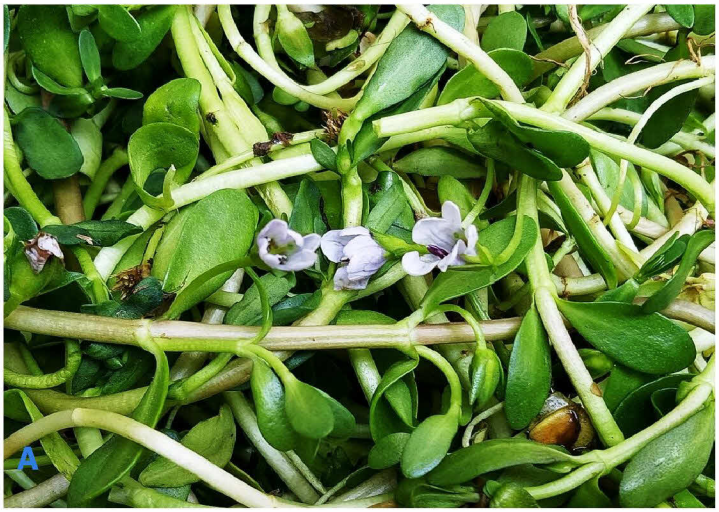
Development of SOP for the modified BG was the first step in standardization. The proportion of *Sneha Dravya* (Cow ghee) to *Drava Dravya* (*Brahmi* juice) was used as 1:4 (Sharagadhara Madhyam Khanda 9/10)[26]. *Brahmi Juice* was prepared using fresh whole *Brahmi* plant (Sharagadhara Madhyam Khanda 1/2)[27]. Cow ghee was heated in a thick bottom pan till appearance of fumes. *Brahmi juice* was added to it and the mixture was heated on low flame until the product was ready (Sharagadhara Madhyam Khanda 9/12-14)[28].

Thus 1x potency BG was prepared in a 150 g quantity in three different batches namely BG I, BG II and BG III. Addition 66 ml of water for one kg *brahmi* was required to convert it in to paste form. For all three samples heating was carried out for two days. Heating was discontinued after one hour on the first day which was resumed on next day till process completion to comply with textual method of *Ushita Paka* (Sharagadhara Madhyam Khanda 9/18)[29].

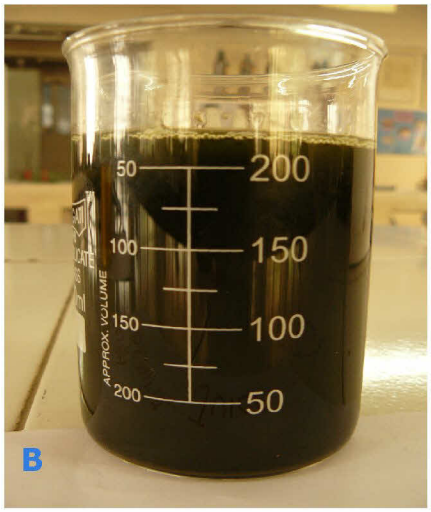
2.1.2. Observations during preparation of three batches of BG:

Out of the150g ghee, the yield of BG I was 132g whereas in case of BG II and BG III it was 134g each. The time required for completion of process was 1.25 hr for BG I sample and 1.20 hr in BG II and BG III. Initial temperature was between 28°C-29°C, during process temperature ranged between 96°C-98°C and final stage temperature between 100°C-103°C. Percentage loss in BG I sample was 12% and in BG II and BG III samples it was 10.6 % each.

All the three samples were subjected to organoleptic testing and physico-chemical analysis. The mean value for each physico-chemical parameter was calculated. Results are reported in **table 1**, the SOP was developed.

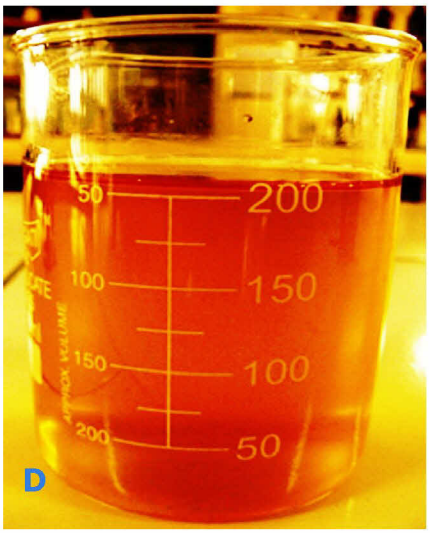


[A] *Bacopa monnieri* Linn



[B] Juice of *Bacopa monnieri* Linn

[D] Preparation process for 10 x BG



[C] Cow ghee

[E] 1x BG in solidified form

[F] 5x BG in solidified form

[G] 10x BG in solidified form

Figure 1. Photographs showing raw material, ingredients, process and finished products for 1x, 5x & 10x BG

2.1.3. Preparation of study drug in required quantity using different concentrations and processing cycles:

After development of SOP mentioned above, study drug was prepared following the SOP. *Brahmi* was hand cleaned to remove physical impurities and washed thoroughly with water till it was free from mud. It was weighed, chopped and converted in to a paste using multi mill. The paste was squeezed through a clean cotton cloth to obtain juice.

Six kilogram of Cow ghee was taken by extrapolating mean loss of 11.06% to make 10x potency BG. *Ghrita murchhna* was not carried out. It was heated in 80 l capacity stainless steel thick bottom vessel till it completely melted and fumes aroused. *Brahmi juice* was slowly added in to melted ghee. The mixture was heated on low and constant flame with continuous stirring till achievement of the testing criteria i. e. ghee became completely devoid of moisture which was tested by soundless burning of cotton weak soaked in the ghee, ghee acquired the green color and bitter taste of *Brahmi* [28].

After completion of first *paka* (one processing cycle), ghee was filtered; and was used as a base for next *paka*. Every time freshly prepared juice was added in four times quantity as that of the obtained weight of ghee from the previous *paka*.

The temperature was recorded at three stages, initial stage, mid-stage and end stage of each *paka*. At the end of first (1x BG) and fifth (5x BG) *paka*, 250g sample each was kept aside. **Figure 1** displays photographs showing raw material, ingredients, process and finished products for 1x, 5x and 10 x BG. **Figure 2** displays the original quantity, loss and obtained quantity of ghee after completion of each processing cycle.

2.1.4. Observations during pharmaceutical preparation:

Amount of herb:

Amount of *Brahmi* required for 10x BG preparation was 255 kilogram. Total water required for converting the drug in to paste form was

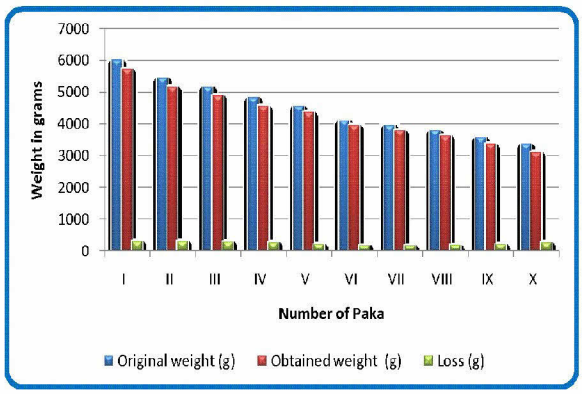


Figure 2. Graph showing weight record over the duration of preparation of 10x BG

17 Litre. The total quantity of *juice* used was 178.896 Litre.

Temperature:

Initial temperature (before ignition) was 29°C for the first *paka*, from second *paka* onwards it was ranging between 50°C-54°C immediately after ignition. The temperature at the mid-stage was ranging between 96°C-98°C. At end stage, temperature ranged between 100°C-103°C.

Duration of Making:

Every *paka* was completed in three nights and four days span. Thus it took total thirty-one days for completion of 10x BG.

2.2. Organoleptic Tests:

All the three samples namely 1x BG, 5x BG and 10x BG were subjected to organoleptic evaluation.

2.3. Physico-chemical Parameters:

1x BG, 5x BG and 10x BG were subjected to evaluation of various physical as well as chemical parameters. All the tests were performed as per guidelines mentioned in Ayurvedic Pharmacopea

Table 1. Physico-chemical analysis of three samples of *Brahmi Ghrita* prepared for developing SOP

Sr. No.	Parameter	BG I	BG II	BG III	Mean value ± SD
1.	pH	3.43	4.93	2.6	3.7 ± 1.6
2.	Moisture content @ 110° C	0.12%	0.66%	0.06%	0.2% ± 0.003
3.	g/ml	0.8332	0.8593	0.8598	0.850 ± 0.015
4.	Specific Gravity	1.01	0.998	0.7	0.9 ± 0.1
5.	Refractive Index	1.45	1.45	1.45	1.45
6.	Acid Value	2.49	2.88	2.72	2.69 ± 0.19
7.	Peroxide Value	6.55	6.79	6.8	6.71 ± 0.14
8.	Iodine Value	31.87	32.15	32.29	32.10 ± 0.21
9.	Saponification Value	224.31	227.84	228.03	226.7 ± 2.49

Table 2. Physico-chemical Analysis of 1x BG, 5x BG and 10x BG

Sr. No.	Parameter	1x BG	5x BG	10x BG
1.	pH	5.6	4.6	4.21
2.	Melting Point	36˚ C	36˚ C	36˚ C
3.	Moisture content @ 110˚ C	0.28% ± 0.005	0.12%± 0.005	0.22 % ± 0.01
4.	g/ml	0.8428 ± 0.0002	0.8363 ± 0.0001	0.8656 ± 0.0002
5.	Specific Gravity@300C	1.14 ± 0.01	0.95 ± 0.001	0.88 ± 0.005
6.	Refractive Index	1.4534	1.4539	1.4541
7.	Acid Value	3.08 ± 0.005	3.09 ± 0.005	3.09 ± 0.005
8.	Peroxide Value	7.34 ± 0.005	7.83 ± 0.01	7.59 ± 0.005
9.	Iodine Value	34.07 ± 0.01	35.44 ± 0.005	37.12 ± 0.005
10.	Saponification Value	230.68 ± 0.005	229.17 ± 0.005	229.46 ± 0.005

Results are mean values ± SD from three experiments

of India. Each test for each sample was performed in triplicate and mean value was taken **(Table 2).**

2.4. Qualitative analysis of active ingredient by high-pressure thin-layer chromatography (HPTLC):

Reference standard biomarker Bacoside A was purchased from Natural remedies Pvt. Ltd. Bangalore. Camag HPTLC instrument with Linomat V (automated TLC scanner) along with Win cats software version 4 for data integration and Camag scanner III for scanning of plate at scanning wavelength of 196 nm were used for qualitative estimation of *Bacoside A*.

Sample and standard solutions were applied on silica gel GF 254 (20 × 10 cm) pre-coated TLC plates using Camag Linomat V sample applicator. Twin trough chamber were subjected for pre-saturation with 20 ml mobile phase for 20 min at room temperature. Development was carried out using mobile phase Dichloromethane: methanol: water (4.5:1.0:0.1 v/v/v) in 20 x 10 cm twin trough chamber. After development when solvent reached up to 90 cm, plates were removed and air dried in a current of air and further drying was achieved using hair dryer. Qualitative analysis for presence of Bacoside A in different samples of BG was performed with Camag TLC scanner 3 at a wavelength of 196 nm.

Accurately weighed 5 g formulations were extracted with an equal amount of hexane and methanol (20 ml each) by means of separating funnel. It was shaken vigorously and allowed to stand for 5 min for separating the two layers. The methanolic layer was again treated with 10 ml hexane till it was free from fat. Hexane layers were discarded. The volume was made with methanol up to 25 ml by using volumetric flask and filtered through 0.22 micron filter paper and used for further procedure. Bacoside A peak height and area under the peak obtained from densitograms for 1x, 5x and 10x BG are reported **(Table 3)**. Densitograms for the standard (Bacoside A) and 1x, 5x and 10x BG are shown in **Fig. 3.**

3. RESULTS

3.1. Organoleptic and Physico-chemical parameter of Brahmi Juice:

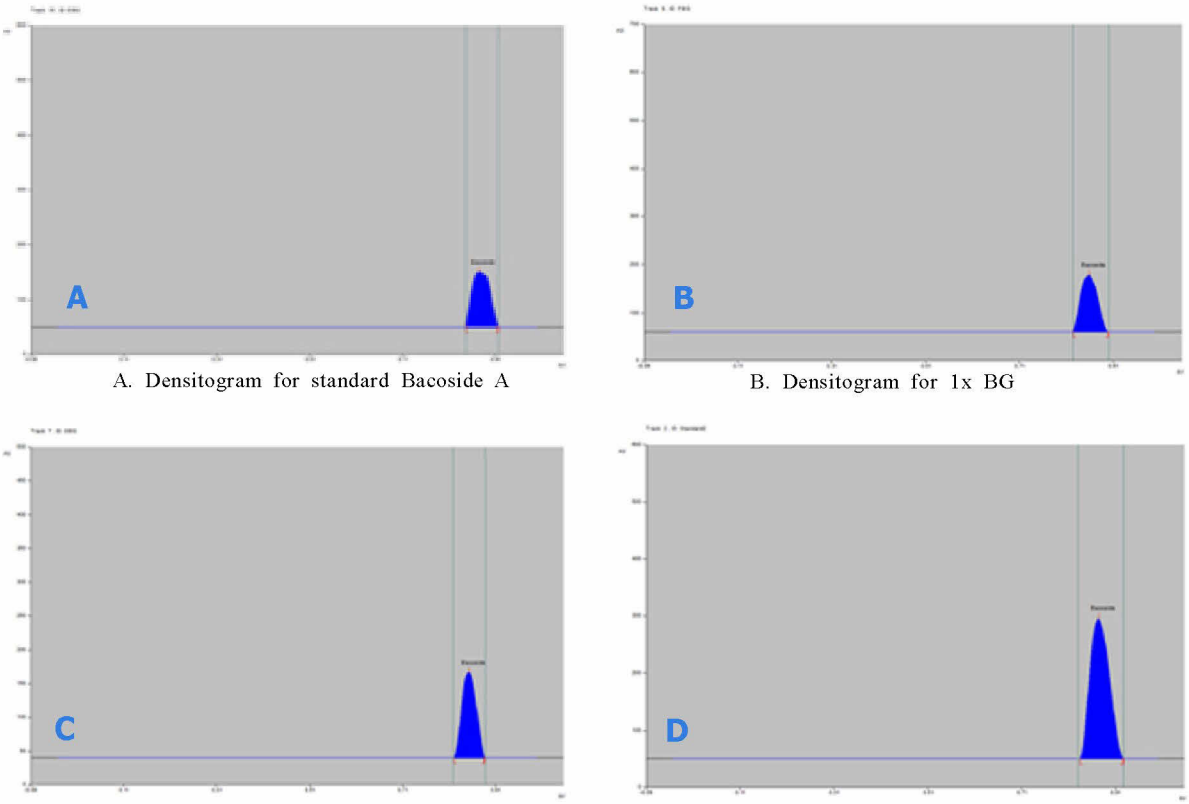
Brahmi juice was green in color and bitter in taste with mild aromatic odor specific to *Brahmi*. Its pH was 6.5, Refractive index 1.3302, viscosity 0.0112 CPS and specific gravity was 1.019g/ml

3.2. Organoleptic and Physicochemical parameters of **Brahmi** *Powder:*

Brahmi powder was greenish-brown in color with mild aromatic odor specific to *Brahmi* with a bitter taste. Obtained values for physicochemical tests complied with the values

Table 3. Bacoside A peak height and area under peak in 1x BG, 5x BG and 10x BG

Sr. No.	Sample name	Peak Height	Area under Peak
1.	1x BG	100.42	3996.65
2.	5x BG	117.14	4272.35
3.	10x BG	133.74	4508.49



A. Densitogram for standard Bacoside A

B. Densitogram for 1x BG

C. Densitogram for 5x BG

D. Densitogram for 10x BG

Figure 3. Densitograms for Standard *Bacoside-A* and 1x BG, 5x BG and 10x BG

quoted in API. (Foreign matter –Nil, Total ash – 13.5%, Alcohol soluble extractive – 16%, water soluble extractive -18.4%)

3.3. Organoleptic characters of three samples of Brahmi Ghrita:

All the three samples namely BG I, BG II and BG III were dull green in color with a bitter taste and mild aromatic odor. All three were *snigdha* (greasy) in touch.

3.4. Physico-chemical analysis of Brahmi Ghrita

Table 1 contains Physico-chemical analysis of three samples of *Brahmi Ghrita* prepared for developing SOP

3.5. Organoleptic characters of 1x BG, 5x BG and 10x BG:

All three samples were *snigdha* (greasy) in touch. 1x BG was dull green with solid consistency. 5x BG was dark green in color with solid consistency. 10x BG was blackish green in color with partially liquid consistency. 1x BG was bitter in taste, the intensity of bitterness successively increased in 5x BG and 10x BG. 1x BG and 5x BG possessed mild aromatic smell specific to *Brahmi*. 10x BG possessed strong bitter odor.

3.6.: Physico-chemical Analysis of 1x BG, 5x BG and 10x BG (Table 2)

3.7. HPTLC Analysis:

Rf value of *Bacoside-A* was 0.86. The height of the peak and area under peak in the densitogram of the three samples display that both are directly proportionate to the number of *paka* carried out. Bacoside A area found highest in case of 10x BG and lowest in case of 1x BG. Concentration of Bacoside A (highest to lowest) in different *Brahmi Ghrita* samples considering height and area was found as 1x BG >5x BG>10x BG.

4. DISCUSSION

Cow ghee is the base material for medicated ghee formulations. In Indian household it is prepared by *desi* method which is proved superior to direct cream method mostly practiced in industry [25,

30]. Comparative analysis of the two samples of ghee, for fatty acid composition revealed that *desi* ghee contains a higher amount of DHA; Omega-3 long-chain polyunsaturated fatty acids, which is a major component of retinal and brain tissues and remains important in the prevention of various diseases[30].

Ghrita murchhana is not carried out in this research work. The significance of *ghrita murchhana* prior to preparation of the *sidhha ghrita* is aimed at controlling the probable rancidity factors (*aama dosha*) and bad odor of the ghee (Bhaishajyaratnavali, Jwara Chikitasa 5/1285) [31]. Six herbal ingredients are used for *ghrita murchhana* process. Each herb contains numerous bioactive compounds which are likely to be extracted in to the ghee. To avoid the interference of these compounds with extraction of the selected biomarker Bacoside A the authors decided to omit the process. Instead, preparation of cow ghee was done at 120 C which resulted to produce most desirable flavor with minimal moisture content as 0.038% to compensate the above mentioned objectives of *murchhana*.

There are 17 formulations of *Brahmi* for intellect enhancer effect listed in *Charak Samhita*, *Sushruta Samhita* and *Ashtanghridaya*. Out of those, six formulations specify use of *Brahmi* juice (*juice*), two use paste (*kalka*) and one uses decoction (*quatha*). Remaining formulations are silent on the form of the herb. Use of *Brahmi* paste (*kalka*) is avoided in making the ghee as per selected guideline [26]. Secondly, use of paste along with juice or decoction to manufacture medicated ghee incurs 15 to 25 percent loss in one processing cycle. Average percent loss was 11.06 with use of only juice during development of SOP. This study includes 10 processing cycles. Estimated starting amount of ghee with use of paste was 28 kg as against the amount 6 kg we used for this study. Based on the above findings and also easy availability of fresh *Brahmi* throughout the year; it was decided to use only *Brahmi* juice for the study.

The manufacturing steps of modified *Brahmi Ghrita* were confirmed by 3 times repetition. The mean of the obtained values of the physico-chemical parameters, BG I, BG II & BG III served as pharmaceutical standards for main study drug.

To convert drug into paste, potable water was added in the proportion of 1liter for 15 kg *Brahmi*. The good amount of foam that was observed during paste making was due to saponins present in the *Brahmi* [32]. During maceration and grinding the saponins separated from the plant cells mix with water to form foam. Tasteless and semi-dry nature of remnant paste complies with the standard practice of juice expression. While manufacturing main study drug each process cycle was completed over the duration of three nights (Vaidyak Paribhasha Pradeep Tritiya 26/45) [33] thus total thirty-one days were required for preparation of 10x BG. Subsequent heating and cooling may have some positive effect to extract active phyto chemicals into the ghee. Comparison of quantity of a phytochemical in the samples withdrawn at pre heating, after first heating and subsequent cooling may be able to throw more light in the direction. It was out of scope of this study. However, qualitatively it may be said that proportionate increase in the intensity of green color and bitter taste with each process cycle could be due to increased extraction of active components from *Brahmi* into the ghee.

pH is one of the most important factors affecting the stability of a product. [34]. It is the measurement of concentration of hydrogen ions in the sample. pH for *Brahmi* juice was found to be near neutral. As against that; pH of 1x BG, 5x BG and 10x BG samples were found to be higher and higher acidic in nature successively. This could mean that hydrogen ion concentration in the drug increases with the process.

Melting point is one of the physical properties employed for checking the presence of foreign fats. Adulteration in ghee with animal fat or hydrogenated fat leads to change in its melting point. Ghee has a melting range of 28 to 44°C [25]. All the three samples displayed melting point as 36°C, suggestive of no any addition of foreign fat.

Maximum moisture content recommended in ghee is 0.3% [35]. Moisture content of all the three samples was within the specified limit which ensures shelf life of two years of the same [36].

Refractive index is the ratio of the velocity of light in the vacuum to the velocity of light in the medium. The pure ghee has the refractive index of 1.45. If any adulteration in the ghee is present, the refractive index of ghee either decreases or increases. Refractive index of all the three samples viz. 1x BG, 5x BG & 10x BG complied with the

standard value mentioned which displayed the unadulterated nature the base drug ghee.

The densities of fats in their liquid form are commonly determined and expressed, as specific gravities. Proportionate decrease of specific gravity with increasing process cycles marks the sequential lightness of the sample. This may have happened due to the effect of more amount of heat induced over the duration of successive *paka*.

Acid value is also called as neutralization number[37]. It is a measure of amount of free fatty acids present in the sample. Free fatty acids are liberated from hydrolysis of triglycerides and are responsible for development of objectionable flavor and odor in ghee. Freshly prepared cow ghee has almost negligible acid value [38]. Acid value for 1x BG, 5x BG and 10x BG was ranging between 3.08-3.09. The lower range for acid value of rancid samples of cow ghee was found as 5.3 [38], thus we may say that though all the three samples displayed a slightly higher acid value due to prolonged duration of heating; it is lesser than the value for a rancid sample. It is a known fact that liberation of fatty acids is also a result of hydrolysis and thermal effects.

Peroxide value is the measure of oxidative rancidity of the sample. Peroxides (R-OOH) are primary reaction products formed in the initial stages of oxidation, and therefore give an indication of the progress of lipid oxidation. Higher the peroxide value more rancid is the sample. Peroxide value of the three samples was ranging between 7.34 – 7.83. Freshly prepared cow ghee possesses negligible peroxide value [38]. Though the three samples displayed a higher peroxide value it is still lesser than the value for the rancid samples which was found as 15[38].

Iodine value is the measure of degree of unsaturation of constituent fatty acids. Unsaturated fatty acids contain either one or more carbon-carbon double bonds. This value could be used to quantify the amount of double bond present in the fat which reflects the susceptibility of the sample to oxidation [39]. Iodine value for all the three samples (1x BG, 5x BG & 10x BG) was within the normal range[38].

The saponification value is the index of the mean molecular weight of fatty acids of glycerides comprising of fat. Less is the saponification value, more the molecular weight of fatty acids and triglycerides and vice-versa [40]. Saponification number for cow ghee is not less than 220[25]. In case of our samples it was ranging between 229-230.68. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat and therefore high molecular weight.

For qualitative and partial quantitative analysis of the prominent bio marker from the herb *Brahmi* in the samples of *Brahmi Ghrita* HPTLC technique was used. Bacoside A was selected as a biomarker to be evaluated in the samples because the major chemical entity shown responsible for neuropharmacological effects of *Bacopa monniera* is *Bacoside-A* (64.28%) and *Bacoside-B* (27.11%) [32]. Bacoside A which was used is a mixture of Bacoside A, Bacopacide II, Jujubogenin isomer of bacopasaponin C and Bacopasaponin C. Results displayed that as the concentration of *Brahmi* juice was increased in successive samples 1x BG<5x BG<10xBG, the concentration of *Bacoside A* extracted was also increased. Thus 10x BG showed the maximum concentration of the biomarker among the three samples. Theoretically 5x and 10x BG should display concentration of extracted Bacoside A as five times and ten times of 1x BG respectively. But in this study we found that concentration of the extracted biomarker was not proportionate to the potentiation carried out. This can be because *avartana* is a complex pharmaceutical procedure where in intricate chemical reactions occur and thus probably mathematical rules may not be applicable. Study lacks in exact quantification of Bacoside A in the three samples due to limitation of sample preparation technique.

Future scope of the study is exact quantification of Bacoside A in the three samples using a different sample preparation technique. *Ghrita* formulations are not preferred by a class of patients. To develop better compliance with smaller dose, potentiation may be the solution.

In future it may be good to conduct a separate experiment to find out whether juice and paste together increase the amount of Bacoside A extracted in ghee. Knowing the concentration of biomarker in the end product opens up possibility of use of alternate technique which may reduce the tedious and long process of *avartana*.

5. CONCLUSION

In the present study, SOP for making modified *Brahmi Ghrita* was developed to analyze the process of potentiation. Physico-chemical constants and HPTLC standardization using Bacoside A as the marker at three levels of the process confirm that repetition of processing cycles with addition of *Brahmi* juice in each cycle increases concentration of the active biomarker in medicated ghee, but the exact quantitative increase is needed to be evaluated in future.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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