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# Studies on *Ashwagandha Ghrita* with reference to *murcchana* process and storage conditions

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ABSTRACT

*Background:* *Withania somnifera* (L.) (family-Solanaceae), known as 'Indian ginseng' or '*Ashwagandha*' is acclaimed as an effective adaptogen, immunomodulator, aphrodisiac and sedative. *Ashwagandha ghrita* is a recognized ghee based Ayurvedic formulation. Few ancient texts suggest *murcchana* process for preparation of *Ashwagandha ghrita*.
*Objective:* The study was undertaken to evaluate probable effects of *murcchana* process on *ghrita* preparation with reference to time and storage conditions.
*Materials and Methods:* *Ashwagandha ghrita* samples were prepared separately using plain ghee (Indian cow's ghee) and *murcchana* ghee. These formulations were stored separately in different glass bottles at room temperature and 400C/75%RH. Organoleptic characters (colour, odour, taste, texture and touch) and physicochemical parameters (acid value, peroxide value, iodine value, saponification value, unsaponifiable matter, refractive index and specific gravity) were determined after 3, 6, 9 and 12 months. Plain ghee and prepared *ghrita* were subjected for antioxidant evaluation by various *in vitro* methods.
*Results:* Changes were observed in organoleptic characters and physicochemical parameters of plain ghee and *Ashwagandha ghrita* formulations. Alterations in these parameters were more pronounced at high temperature and on long storage. *Ashwagandha ghrita* prepared with *murcchana* process exhibited better antioxidant potential in all *in vitro* methods.
*Conclusion:* The *murcchana* process was found to be beneficial towards quality of *ghrita*. Hence, *Ashwagandha ghrita* may be prepared along with *murcchana* herbs and stored in a good quality glass bottle to ensure improved shelf life of *ghrita*.


## 1. Introduction

'*Panchgavya*' is a term used in *Ayurveda*, comprising five important substances obtained from cow, namely milk, *ghee* (clarified butter fat), curd, urine and dung. *Ayurveda* describes a good number of formulations containing '*panchgavya*' components either individually as well as conjointly with other substances of herbal, mineral or animal origin. Several formulations based on each one of these five components are reported in Ayurvedic texts with medicinal claims [1–5].

*Ghee*, widely considered as the Indian name for clarified butterfat, is usually prepared from cow milk or buffalo milk or combination thereof. '*Ghrita*', also known as clarified butter, is a traditional adjuvant/vehicle described in *Ayurveda* [6]. *Charaka* used word '*sahasraveerya*' for *ghee* and '*yogvahitwa*' for *ghrita* by way of which it enhances the therapeutic efficacy and potency of plant ingredients it is processed with [3–5,7].

*Withania somnifera* belongs to the family Solanaceae, popularly known as '*Ashwagandha*' is mentioned as herbal tonic and health food. It has been used in Ayurvedic and indigenous medicine for very long time to treat various kinds of diseases and human ailments. Among the Ayurvedic *rasayana* herbs (preparation that works as a health tonic to children, a medicine to middle aged persons and rejuvenator to the elderly), *Ashwagandha* holds an important place. It constitutes alkaloids and steroidal lactones

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namely; withanine, withananine, somnine, somniferine, somniferinine, pseudowithanine tropane, pseudo-tropine, choline, anaferine, anahydrine and isopelletierine [8–11]. *Ashwagandha* is widely claimed to have hepatoprotective [12,13], anxiolytic [14], antidepressant [14,15], nootropic [16], antimicrobial [17], anti-inflammatory [18,19], antioxidant [20], antistress [21], anticonvulsant [22], cardio-protective [23], antitumor [24–27], antigenotoxic [28], antiparkinsonian [29] and immunomodulatory [30] properties. *Ashwagandha ghrita* (AG) is an effective *ghrita* formulation beneficial for treatment of weakness, gynaecological disorders, general debility and infertility [31].

In *Ayurveda*, '*murcchana samskara*' i.e. processing of cow *ghee* with antioxidant *murcchana* herbs i.e. *Emblica officinalis* (Euphorbiaceae), *Cyperus rotundus* (Cyperaceae), *Curcuma longa* (Zingiberaceae), *Terminalia chebula* (Combretaceae) and *Terminalia bellirica* (Combretaceae) and *Citrus medicus,* is pondered as an important intermediate process in '*ghrit-kalpana*' to enhance the medicinal potency of *ghrita* and to get rid of bad odour and rancidity [32–37]. Despite the significance of *murcchana samskara* in the processing of *ghee*, no scientific and systematic studies were conducted till date to delineate the advantage of *murcchana samskara* with reference to storage conditions of *ghrita*. So, the present work was undertaken to verify some notions about incorporation of *murcchana* herbs in *ghee* by selecting *A. ghrita* as prototype of *ghrita*. Studies were also aimed towards investigation of 'effect of *murcchana* process and storage conditions' on *A. ghrita* to ensure its effect on formulations.

## 2. Materials and methods

### 2.1. Preparation of AG using plain ghee

Authentic samples of cow *ghee* and cow milk were purchased from *Go-vigyan Anusandhan Kendra*, Nagpur, Maharashtra, India. Herbal raw material was procured from experts at Shri-Shail Medifarms, Nagpur, identified and authenticated at Department of Botany, Rashtrasant Tukadoji Maharaj University, Nagpur, Maharashtra, India (Voucher Specimen No. 8961/21 to 8961/27) (Table 1). AG was prepared as per procedure described in *Ayurved Sarsangraha* [32]. Briefly, coarse powder of dried *Ashwagandha* roots (two parts) was boiled in water (sixteen parts) until four parts of water remained. This concentrate (*kwath*) was filtered through muslin cloth and kept aside. Then additional *Ashwagandha* roots (one part) were processed to prepare *Ashwagandha kalka* (fine paste). Subsequently cow *ghee* (one part) and cow milk (four parts) were mixed with *kwath* and *kalka* and treated on low to moderate fire in a big iron vessel with continuous stirring. The appearance of *ghee* as clear, transparent yellowish liquid and devoid of any froth indicated the preparation near to the end point. Evaporation of entire water was confirmed by burning a small quantity of the paste in fire and careful observation for the crackling sound when subjected to fire. Further, heating was stopped and the contents were separated before cooling by squeezing and filtering through two-fold muslin cloth. The filtrate i.e. AG was collected in clean autoclaved glass bottle and stored for further process.

### 2.2. Preparation of murcchana ghee (MG)

The MG was prepared as per the procedure described in reference texts and published reports [32–34]. Briefly, initially stated amount of plain *ghee* (sixteen parts, 768 g) was melted in a vessel with moderate heating. A mixture of coarsely powdered five herbs; *T. chebula* (Combretaceae) fruits, *Terminalia belerica* (Combretaceae) fruits, *C. rotundus* (Cyperaceae) rhizomes, *E. officinalis* (Euphorbiaceae) fruits and *C. longa* (Zingiberaceae) rhizomes, in equal quantities (one part, 48 g) was ground with juice of *C. medicus* (one part) to form a smooth paste (*kalka*). The *kalka* was added to the molten *ghee* along with water (3.072 lit) and boiled on slow fire till complete evaporation of water. It was then strained through muslin cloth and stored in a well closed autoclaved glass bottle.

### 2.3. Preparation of AG using MG

During the study, additional sample of AG was also prepared at once using MG instead of plain *ghee*, following the same procedure and denoted as AGM i.e. *A. ghrita* processed with *murcchana ghee* [32]. Here also iron vessel was used during preparation. It was worthy to prepare separate formulations so the effects of *murcchana* herbs on composition of *ghee* during storage at different temperatures could also be estimated.

### 2.4. Evaluation of plain ghee, AG, MG and AGM

The *ghrita* preparations were denoted as MG, AG and AGM for *murcchana ghee, A. ghrita* prepared with plain *ghee*, *A. ghrita* prepared with *murcchana ghee* respectively. The use of two different *ghee* samples i.e. plain *ghee* and *murcchana ghee* for preparation of AG allowed comparative assessment between AG and AGM. The samples were examined for various organoleptic properties, physicochemical parameters and antioxidant activity and then stored separately in well closed glass containers at room temperature (RT) and 40 °C/75%RH in humidity chamber (Newtronic, NEC 212 ET). Subsequently physicochemical and organoleptic evaluation of all samples was done after 3, 6, 9 and 12 months of storage. All studies were performed in triplicate and mean values were recorded.

#### 2.4.1. Organoleptic evaluation

Sensory (organoleptic) characters play an important role towards suitability of *ghee* containing formulations and are indicative of formulations rancidity [38]. These characters comprise of color, odour, taste, texture and touch. The plain *ghee* and *ghritas* were allowed to reach normal temperature for proper crystallization before testing. Initial specific observations and further changes in sensory characters after 3, 6, 9 and 12 months of storage were noted carefully.

**Table 1**
Herbal composition of *Ashwagandha ghrita*.

| Name | Family | Common name | Voucher Specimen No. | Part of plant |
|---|---|---|---|---|
| *Withania somnifera* (L.) Dunal | Solanaceae | *Ashwagandha* | 8961/21 | Roots |
| *Emblica officinalis* Gaertn. | Euphorbiaceae | *Amlaki* | 8961/22 | Pericarp of fruits |
| *Terminalia chebula* Retz. | Combretaceae | *Haritaki* | 8961/23 | Pericarp of fruits |
| *Terminalia bellirica* Roxb. | Combretaceae | Bibhitaki | 8961/24 | Pericarp of fruits |
| *Cyperus rotundus* Linn. | Cyperaceae | *Musta* | 8961/25 | Rhizomes |
| *Curcuma longa* Linn. | Zingiberaceae | *Haridra* | 8961/26 | Rhizomes |
| *Citrus medica* var. *Acidica* | Rutaceae | *Matulunga* | 8961/27 | Juice |

*2.4.2. Physicochemical evaluation*

The plain *ghee*, MG, AG, and AGM samples were analysed for various physicochemical parameters like acid value, peroxide value, iodine value, saponification value, unsaponifiable matter, refractive index (RI) and specific gravity as per standard procedures described in reference books [39,40].

*2.4.3. Anti-oxidant evaluation*

Antioxidant activity of freshly prepared plain *ghee*, MG, AG and AGM was evaluated using various *in-vitro* methods [41–43]. The antioxidant potential was expressed as $IC_{50}$, which denoted the concentration of test samples that inhibited the formation of free radicals by 50%. Ascorbic acid was used as reference standard in all methods.

*2.4.3.1. DPPH radical scavenging assay.* The radical-scavenging or hydrogen-donating ability of plain *ghee*, MG, AG and AGM was measured using the established 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method [41]. Briefly, 3.0 mL of 10–100 $\mu g.mL^{-1}$of *ghee*/*ghrita* solutions and 1.0 mL of 0.1 mM solution of DPPH in ethanol were mixed together and after 30 min the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture specifies higher free radical-scavenging activity.

*2.4.3.2. Nitric oxide radical scavenging assay.* Nitrite detection method i.e. Greiss reaction to measure NO generated from sodium nitroprusside was used to assess radical scavenging activity of test samples [41,42]. Briefly, 3.0 mL of *ghee*/*ghrita* solutions at the concentration of 10–100 $\mu g\ mL^{-1}$ were mixed with sodium nitroprusside (5 mM) in phosphate-buffered saline and allowed to incubate at 25 °C for 150 min. Further these samples were reacted with Greiss reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% napthylethylenediamine dihydrochloride). The chromophore formed during the diazocoupling of nitrite with sulphanilamide and napthylethylenediamine was subjected for absorbance measurement at 546 nm. The reaction mixture (without test sample) with equivalent quantity of distilled water served as control.

*2.4.3.3. Hydrogen peroxide scavenging assay.* The plain *ghee*, MG, AG and AGM were subjected for Hydrogen peroxide ($H_2O_2$) scavenging assay based on replacement titration [41,43]. Briefly, 1.0 mL of 0.1 mM $H_2O_2$, 1.0 mL of 10–100 $\mu g.mL^{-1}$of *ghee*/*ghrita* solutions, 2 drops of 3% ammonium molybdate, 10 mL of 2 M $H_2SO_4$, and 7.0 mL of 1.8 M KI were mixed together and the resultant solution was titrated with 5.09 mM $Na_2S_2O_3$ till complete disappearance of yellow color. Hydrogen peroxide scavenging potential was calculated as

$$\% \text{ Inhibition} = (V_0 - V_1)/V_0 \times 100$$

where, $V_0$ was volume of sodium thiosulphate solution used to titrate the control sample in the presence of hydrogen peroxide (without *ghee*/*ghrita*) and $V_1$ was the volume of sodium thiosulphate solution used in the presence of the *ghee*/*ghrita*.

## 3. Results

*3.1. Organoleptic evaluation*

The plain *ghee* and *ghrita* preparations exhibited some specific peculiarities. The sensory characteristics were noted as soon as formulations were made. The taste and odour (aroma) were best observed when sample was warm and melted. Sensory characters of these samples of plain *ghee* and *ghrita* preparations were carefully observed initially (first day of preparation) and after 3, 6, 9 and 12 months of storage. During the course of study, it was observed that organoleptic characters demonstrated by plain *ghee* vary with that of *ghrita* formulations.

*3.1.1. Color*

It was observed that during storage of *ghee*, color got fade. Golden yellow color of plain *ghee* was changed to pale yellow after storage for 12 months at higher temperature whereas MG showed same color throughout investigation at both temperatures. The AG samples exhibited yellowish color while AGM appeared slightly brownish. On storage, color of *ghee* and *ghritas* showed changes which were mainly established after 6–9 months.

*3.1.2. Odour and taste*

Aromatic, pleasant and characteristic odour and taste of plain *ghee* and AG were gradually changed to slightly fragrant and bitter taste on storage at high temperature for 12 months duration. The MG and AGM revealed nearly identical odour (characteristic, aromatic and pleasant) and slightly astringent taste throughout study and at all storage conditions.

*3.1.3. Touch and texture*

*Ghritas* showed formation of small granules and loose layers when stored at room temperature. These layers and granule arrangements were hindered at high temperature. The observations revealed that the smooth, soft touch and texture of *ghee*, MG, AG and AGM samples were nearly unaffected during the study.

*3.2. Physicochemical evaluation*

*Ghee* undergoes physico-chemical changes, dependent primarily on the temperature of storage [44,45]. Thus, it was thought worthwhile to study different physicochemical parameters of *ghee* and prepared *ghritas* which reflect corresponding changes in '*ghee*' composition and are summarized in Table 2.

*3.2.1. Acid value*

Initially, acid values of AG and AGM were found to be more as compared to plain *ghee* and MG. All samples showed rise in acid value on storage. Samples of AG and AGM showed slow and gradual rise in acid values during period of storage. The rate of increase in acid value in sample containing *murcchana* herbs was slower. The AGM samples stored at both temperatures were found to be protected to some extent as indicated by acid values.

*3.2.2. Peroxide value*

In case of plain *ghee*, AG, MG and AGM, peroxide value showed gradual increase during storage at both temperatures. Results clearly revealed that plain *ghee* showed steady and maximum oxidative damage (1.581–5.982), mainly at high temperature and after twelve months storage. MG and AGM also showed rise in peroxide value at high temperature during storage period.

*3.2.3. Iodine value*

Iodine value of plain *ghee*, MG, AG and AGM samples showed gradual decrease on storage. Changes in iodine value were strikingly different at various temperatures. It was observed that initially iodine value dropped slowly but declined significantly at higher temperature, mainly with samples stored for 9–12 months duration. The AG showed large fall in iodine value (42.10–32.34) as compared to AGM (45.71 To 38.34) on storage for 12 months duration at 40 °C/75%RH.

**Table 2**
Physicochemical evaluation of Plain *ghee*, MG, AG and AGM after 0, 3, 6, 9 and 12 months at RT and 40 °C temperature.

| P | ↓ T M→ | Plain *ghee* | | | | | MG | | | | | AG | | | | | AGM | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 | 0 | 3 | 6 | 9 | 12 | 0 | 3 | 6 | 9 | 12 | 0 | 3 | 6 | 9 | 12 |
| AV | RT | 0.335 | 0.336 | 0.468 | 0.538 | 0.649 | 1.307 | 1.310 | 1.315 | 1.382 | 1.423 | 1.450 | 1.451 | 1.452 | 1.680 | 2.250 | 2.342 | 2.343 | 2.345 | 2.351 | 2.362 |
| | 40 | – | 0.427 | 0.586 | 0.639 | 0.722 | – | 1.385 | 2.245 | 2.522 | 2.614 | – | 1.458 | 1.460 | 2.905 | 3.651 | – | 2.400 | 2.405 | 2.411 | 2.412 |
| PV | RT | 1.581 | 1.581 | 1.586 | 1.592 | 3.590 | 1.339 | 1.341 | 1.386 | 1.410 | 1.416 | 1.822 | 1.826 | 1.845 | 1.921 | 2.87 | 2.062 | 2.072 | 2.075 | 2.252 | 2.260 |
| | 40 | – | 2.450 | 5.210 | 5.630 | 5.982 | – | 1.464 | 1.865 | 3.369 | 4.625 | – | 1.978 | 1.990 | 2.005 | 3.05 | – | 2.983 | 3.022 | 3.225 | 3.345 |
| IV | RT | 38.99 | 38.12 | 36.92 | 35.26 | 30.28 | 36.97 | 36.52 | 33.50 | 31.08 | 30.52 | 42.10 | 42.02 | 41.01 | 40.19 | 38.11 | 45.71 | 44.32 | 44.20 | 42.56 | 40.22 |
| | 40 | – | 33.20 | 32.03 | 31.16 | 28.35 | – | 35.20 | 30.26 | 26.85 | 25.85 | – | 39.12 | 38.95 | 37.58 | 32.34 | – | 45.71 | 43.45 | 41.22 | 38.34 |
| SV | RT | 227.1 | 227.2 | 220.3 | 218.3 | 219.6 | 217.8 | 216.2 | 213.3 | 211.5 | 209.6 | 188.9 | 188.6 | 188.4 | 188.1 | 180.2 | 198.9 | 199.6 | 198.5 | 195.1 | 191.3 |
| | 40 | – | 224.1 | 217.6 | 215.5 | 216.5 | – | 213.9 | 210.2 | 208.3 | 205.7 | – | 186.5 | 185.5 | 184.3 | 178.0 | – | 200.8 | 196.2 | 194.2 | 188.6 |
| UM | RT | 0.201 | 0.201 | 0.196 | 0.196 | 0.196 | 0.102 | 0.102 | 0.102 | 0.102 | 0.102 | 0.101 | 0.099 | 0.099 | 0.10 | 0.11 | 0.105 | 0.112 | 0.112 | 0.11 | 0.111 |
| | 40 | – | 0.202 | 0.201 | 0.201 | 0.201 | – | 0.104 | 0.104 | 0.104 | 0.104 | – | 0.099 | 0.100 | 0.100 | 0.110 | – | 0.114 | 0.114 | 0.110 | 0.111 |
| RI | RT | 1.4448 | 1.4448 | 1.4449 | 1.4449 | 1.4449 | 1.453 | 1.4530 | 1.453 | 1.453 | 1.4532 | 1.4550 | 1.455 | 1.4552 | 1.4555 | 1.4557 | 1.4549 | 1.455 | 1.454 | 1.454 | 1.455 |
| | 40 | – | 1.4449 | 1.4448 | 1.4448 | 1.4448 | – | 1.4532 | 1.453 | 1.453 | 1.4532 | – | 1.455 | 1.4553 | 1.4558 | 1.4565 | – | 1.455 | 1.454 | 1.454 | 1.455 |
| SG | RT | 0.925 | 0.926 | 0.930 | 0.940 | 0.949 | 0.941 | 0.950 | 0.966 | 0.972 | 0.981 | 0.941 | 0.940 | 0.955 | 0.968 | 0.986 | 0.952 | 0.964 | 0.978 | 0.983 | 1.250 |
| | 40 | – | 0.930 | 0.937 | 0.941 | 0.953 | – | 0.971 | 0.988 | 0.991 | 1.097 | – | 0.953 | 0.968 | 0.973 | 0.993 | – | 0.981 | 0.994 | 1.101 | 1.471 |

Note: All values are mean of three replications.
P- Parameter, M– Months, T- Temperature, RT- Room Temperature, AV- Acid Value, PV- Peroxide Value, IV- Iodine Value, SV- Saponification Value, UM- Unsaponifiable Matter, RI- Refractive Index, SG- Specific Gravity, MG- *Murcchana ghee*, AG- *Ashwagandha ghrita*, AGM- *Ashwagandha ghrita* processed with *murcchana ghee*.

### 3.2.4. Saponification value

The results indicate the changes in saponification value of *ghee* and *ghrita* formulations during storage. In case of plain *ghee*, saponification value was decreased up to nine months but then surprisingly it showed upward shift in some samples. In AG and MG samples stored in glass container, saponification value got decreased at all temperatures throughout the period of study. As shown in Table 2, AGM stored at both temperatures initially showed increase in saponification value after three months i.e. 198.8 to 199.6, followed by gradual decline on further storage for long duration.

### 3.2.5. Unsaponifiable matter

This parameter was evaluated after determination of saponification value which indicates quantity of residual unsaponified matter. It is clearly observed that changes in unsaponifiable matter of samples stored at both temperatures throughout study were insignificant.

### 3.2.6. Refractive index

During the present study it was observed that the RI of plain *ghee* was lower i.e. 1.4448 than MG, AG and AGM. AG sample exhibited sharp rise in RI (1.4550–1.4565) at high temperature for 12 months storage whereas MG and AGM showed slight changes in RI after nine months storage.

### 3.2.7. Specific gravity

All *ghrita* samples i.e. MG, AG and AGM showed gradual increase in specific gravity with time at both temperatures. Comparatively plain *ghee* showed less increase in specific gravity after three months to 12 months at 40 °C i.e. 0.925 to 0.953. In case of AGM, sample kept at 40 °C showed sudden rise in specific gravity i.e. 0.952 to 1.471 after twelve months storage.

### 3.3. Antioxidant evaluation

In case of *in-vitro* antioxidant evaluation, all test samples i.e. plain *ghee*, MG, AG and AGM exhibited concentration dependent (10–100 μg $mL^{-1}$) free radical scavenging activity. The $IC_{50}$ of AGM by the DPPH method was found to be 22.97 μg $mL^{-1}$, whereas plain *ghee*, MG and AG showed $IC_{50}$ values as 40.76, 26.42 and 33.23 μg $mL^{-1}$, respectively. In NO method, $IC_{50}$ for AGM was found to be 24.56 μg $mL^{-1}$, whereas plain *ghee*, MG and AG showed $IC_{50}$ value as 41.43, 28.22 and 35.12 μg $mL^{-1}$ respectively. Plain *ghee*, MG, AG and AGM demonstrated dose dependent $H_2O_2$ scavenging activity with the $IC_{50}$ 43.13, 31.03, 38.76 and 25.98 μg/ml respectively (Table 3). Ascorbic acid revealed excellent antioxidant activity in all *in-vitro* methods.

## 4. Discussion

Multicomponent formulations are a common practice in *Ayurveda*. Generally, many ingredients in different forms are processed together/separately to get maximum collaborative therapeutic effect (sometimes additive effects) or to minimize side effects or to make it more acceptable by patient. For instance, AG contains *Ashwagandha* roots processed with cow *ghee*. In some Ayurvedic scripts it is mentioned that before making any *ghrita*, *ghee* should be processed with '*murcchana kriya*', with the use of some herbs to supress, if any rancid (bad) smell present in *ghee* [32]. This renders the *ghee* clear, devoid of any bad effects and smell and prevents *ghee* from spoilage [33].

In present study, AG was selected as a prototype. In *Ayurved Sarsangraha*, MG is mentioned for AG preparation [32]. Some other reference texts lack mention of specific process i.e. '*murcchana* process' to be carried out during preparation of AG. Therefore, this study was an attempt to unlock the ambiguity regarding use of *murcchana* herbs during preparation of AG.

### 4.1. Organoleptic evaluation

Sensory evaluation is a scientific discipline used to measure, analyse and interpret reactions to organoleptic characteristics of foods and materials perceived by the senses of sight, smell, taste, touch and hearing [46]. Timely discussions with Ayurvedic experts revealed that some sensory properties of *ghee* and *ghrita* formulations manifest themselves optimally at different temperatures.

Temperature of storage of fat (*ghee*) is an important factor towards sensory acceptability and oxidative deterioration. The cow *ghee* contains carotenoids which are responsible for its distinctive golden yellow color [47,48]. Color of *ghee*/*ghrita* is also dependent on processing, duration of heating and temperature, nature of herbal ingredients, possible degradation of carotenoids and other fat-soluble pigments present in *ghee* etc. [48,49]. Addition of *murcchana* herbs to yellowish AG must be responsible for brownish color of AGM.

The characteristic flavour of '*ghee*' is because of a complex mixture of compounds produced during the various stages of

**Table 3**
*In-vitro* antioxidant evaluation of Plain *ghee*, MG, AG and AGM.

| $IC_{50}$ Values ($\mu g.mL^{-1}$) | | | | | |
|---|---|---|---|---|---|
| Method | Plain *ghee* | MG | AG | AGM | Ascorbic Acid |
| DPPH Method | 40.76 (0.9877) | 26.42 (0.9904) | 33.23 (0.9556) | 22.97 (0.9822) | 14.38 (0.9762) |
| NO Method | 41.43 (0.9732) | 28.22 (0.9621) | 35.12 (0.9859) | 24.56 (0.9459) | 17.82 (0.9826) |
| $H_2O_2$ Method | 43.13 (0.9839) | 31.03 (0.9732) | 38.76 (0.9733) | 25.98 (0.9912) | 20.21 (0.9458) |

Note: All values are mean of three replications, Values in bracket indicate r* i.e. regression coefficient.
MG- *Murcchana ghee*, AG- *Ashwagandha ghrita*, AGM- *Ashwagandha ghrita* processed with *murcchana ghee*.

processing [44,48–50]. The plain *ghee* possesses a characteristic aroma which is due to presence of free fatty acids, carbonyls and lactones contributing to *ghee* flavour [51]. The taste and aroma were altered due to presence of various phytoconstituents in MG, AG and AGM. The tannin rich *murcchana* herbs seemed to be the important compounds influencing the astringent taste and flavour of MG and AGM samples at both temperatures.

Organoleptic evaluation of touch and texture of plain *ghee* and all *ghrita* formulations failed to reveal any significant inference.

### 4.2. Physicochemical evaluation

Acid value is a measure of the amount of carboxylic acid groups in fatty acid compounds. As oil/fat rancify, over a period of time, triglycerides get converted into fatty acids and glycerol, causing an increase in amount of acids. It can be considered as catalytic effect of iron (from manufacturing vessel) or presence of acidic phytoconstituents or generation of free fatty acids from triglycerides present in *ghee* or all these may be responsible for higher acid values of *ghrita* preparations as compared to plain *ghee* [52].

Lipid peroxidation depends on fatty acid composition and storage conditions of fat or oil [53]. Extent of lipid peroxidation i.e. auto-oxidation is measured in terms of peroxide value, giving initial evidence of rancidity in unsaturated fats and oils. Free unsaturated acids are oxidized more easily and quickly than the same acids in intact glycerides and thus high acidity signifies high peroxide value [54]. It can be assumed that catalytic action of iron and/or high temperature must be responsible for maximum oxidative deterioration of *ghee* [52,55]. The *murcchana* herbs seemed to be offering some protection against this catalytic and oxidative damage or peroxide formation at room temperature only.

Iodine value indicates quantity of iodine absorbed at unsaturation which expresses amount of unsaturation in a fat. Large fall in iodine value of AG as compared to AGM can be corroborated with more oxidation due to high unsaturation in AG whereas in AGM *murcchana* herbs might have shown their protective antioxidant effect.

Saponification value is an index of mean molecular weight (or chain length) of all the fatty acids present and is directly proportional to the fatty matter content. It indicates the number of reactive terminal acid groups in the fat. More the fatty matter content or more the carboxylic functional group per unit mass, there will be more chances of rancidity factor and less will be the shelf life and therapeutic value [55–58]. In present investigation, changes in saponification value failed to reveal any significant inference and these variations could be attributable to interactions between different *ghee* components and phytoconstituents.

The unsaponifiable matter consists of substances (lipids of natural origin such as sterols, pigments, vitamins, higher aliphatic alcohols and hydrocarbons as well as any non-volatile foreign organic matter) present in oils or fats which are not saponifiable by alkali hydroxides. Possible interactions between phytoconstituents and *ghee* components might have resulted in insignificant changes in unsaponifiable matter of samples stored at both temperatures throughout study.

Refractive index, the ratio of the velocity of light in vacuum to its velocity in the substance, is a fundamental physical property of a substance. The RI is often used to ascertain a particular substance, check its purity, or measure its concentration. If RI is more, there will be more concentration of light which facilitates rancidification of *ghrita* i.e. decomposition of *ghrita* [55–58]. Slight changes in RI of AGM and MG showed reduction in rancidity indicating the antioxidant effect of *murcchana* herbs.

Specific gravity of *ghrita* is an indication of the solid to liquid ratio in *ghrita*. In case of liquid and semi-solid preparations, ongoing chemical processes change their consistency and are responsible for conversion of liquid contents into solid or *vice-versa* [55]. Sudden rise in specific gravity of AGM after twelve months storage could be due to the solid extractives originated from the added herbs during the formulation process. Increase in specific gravity revealed increase in solid contents compared to liquid contents with respect to all *ghrita* formulations. Less proportion of liquid contents in preparation increases life span of formulations [55–58].

Antioxidant potential of test samples by all *in-vitro* methods was found in increasing order i.e. plain *ghee* < AG < MG < AGM. Various tannin-rich herbs used in preparation of MG might be responsible for potent antioxidant activity. There could be synergistic effect of antioxidant herbs from MG and *Ashwagandha*, which resulted in highest antioxidant potential of AGM.

From the physicochemical evaluation of plain *ghee* and all *ghrita* samples, it can be interpreted that plain *ghee* and AG had undergone certain major physicochemical changes during storage as compared to MG and AGM (Table 2). It can be assumed that oxidation is the main reason towards alterations in physicochemical properties and ultimately rancidity of *ghee*-based formulations. *Murcchana* herbs i.e. *T. chebula*, *T. belerica*, *C. rotundus*, *E. officinalis* and *C. longa* are sources of polyphenolic compounds comprising phenolic acids (gallic acid, ellagic acid, chebulinic acid), flavonoids, coumarins, tannins with proven free radical scavenging potential [59–63]. These antioxidant principles of *murcchana* herbs must have contributed towards protection against oxidative damage and hence AGM performed better as compared to all other *ghrita* preparations. Our findings confirm the earlier reports of significance of *'murcchana samskara'* of *ghrita* for minimizing rancidity and increasing stability.

## 5. Conclusion

In a nutshell, the observations and results suggest that, *murcchana* process is prerequisite for preparation of *A. ghrita* to ensure its maximum acceptability, stability and better shelf life. However, it is also established that *murcchana* herbs are not much effective against oxidative damage occurred at high temperature.

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## Conflicts of interest

None.

## References

[1] Achliya GS, Wadodkar SG, Dorle AK. Neuropharmacological actions of Panchagavya formulation containing *Emblica officinalis* Gaerth and *Glycyrrhiza glabra* Linn in mice. Indian J Exp Biol 2004;42:499–503.
[2] Jarald EE, Edwin S, Tiwari V, Garg R, Toppo E. Antidiabetic activity of cow urine and a herbal preparation prepared using cow urine. Pharm Biol 2008;46(10–11):789–92. https://doi.org/10.1080/13880200802315816.
[3] Gupta SK, Baghel MS, Bhuyan C, Ravishankar B, Ashok BK, Patil PD. Evaluation of anti-urolithiatic activity of Pashanabhedadi Ghrita against experimentally induced renal calculi in rats. Ayu 2012;33(3):429–34. https://doi.org/10.4103/0974-8520.108860.
[4] Achliya GS, Wadodkar SG, Dorle AK. Evaluation of sedative and anticonvulsant activities of Unmadnashak ghrita. J Ethnopharmacol 2004;94(1):77–83. https://doi.org/10.1016/j.jep.2004.04.020.
[5] Achliya GS, Wadodkar SG, Dorle AK. Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride-induced hepatic damage in rats. J Ethnopharmacol 2004;90(2–3):229–32. https://doi.org/10.1016/j.jep.2003.09.037.
[6] Sachdeva S. Quality evaluation of Butter and *Ghee*, advances in fat-rich dairy products. Karnal: Lecture compendium at National Dairy Research Institute, ICAR; 2002. p. 153–7.
[7] Singh N, Bhalla M, de Jager P, Gilca M. An overview on Ashwagandha: a rasayana (rejuvenator) of Ayurveda. Afr J Tradit, Complementary Altern Med 2011;8(5 Suppl):208–13. https://doi.org/10.4314/ajtcam.v8i5S.9.
[8] Mirjalili MH, Moyano E, Bonfill M, Cusido RM, Palazon J. Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. Molecules 2009;14:2373–93. https://doi.org/10.3390/molecules14072373.
[9] Misra L, Mishra P, Pandey A, Sangwan RS, Sangwan NS, Tuli R. Withanolides from *Withania somnifera* roots. Phytochemistry 2008;69(4):1000–4. https://doi.org/10.1016/j.phytochem.2007.10.024.
[10] Misra L, Mishra P, Pandey A, Sangwan RS, Sangwan NS. 1,4-Dioxane and ergosterol derivatives from *Withania somnifera* roots. J Asian Nat Prod Res 2012;14(1):39–45. https://doi.org/10.1080/10286020.2011.622719.
[11] Bhattacharya A, Ramanathan M, Ghosal S, Bhattacharya SK. Effect of *Withania somnifera* glycowithanolides on iron-induced hepatotoxicity in rats. Phytother Res 2000a;14(7):568–70. https://doi.org/10.1002/1099-1573(200011)14:7<568::AID-PTR663>3.0.CO;2-Q.
[12] Devkar ST, Kandhare AD, Zanwar AA, Jagtap SD, Katyare SS, Bodhankar SL, et al. Hepatoprotective effect of withanolide-rich fraction in acetaminophen-intoxicated rat: decisive role of TNF-α, IL-1β, COX-II and iNOS. Pharm Biol 2016;54(11):2394–403. https://doi.org/10.3109/13880209.2016.1157193.
[13] Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. Phytomedicine 2000b;7(6):463–9. https://doi.org/10.1016/S0944-7113(00)80030-6.
[14] Bhattacharya SK, Muruganandam AV. Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 2003;75(3):547–55. https://doi.org/10.1016/s0091-3057(03)00110-2.
[15] Wadhwa R, Konar A, Kaul SC. Nootropic potential of Ashwagandha leaves: beyond traditional root extracts. Neurochem Int 2016;95:109–18. https://doi.org/10.1016/j.neuint.2015.09.001.
[16] Girish KS, Machiah KD, Ushanandini S, Harish Kumar K, Nagaraju S, Govindappa M, et al. Antimicrobial properties of a non-toxic glycoprotein (WSG) from *Withania somnifera* (Ashwagandha). J Basic Microbiol 2006;46(5):365–74. https://doi.org/10.1002/jobm.200510108.
[17] Sahni YP, Srivastava DN. Anti-inflammatory activity of *Withania somnifera:* possible mode of action. J Appl Anim Res 1993;3(2):129–36. https://doi.org/10.1080/09712119.1993.9705964.
[18] Gupta M, Kaur G. Aqueous extract from the *Withania somnifera* leaves as a potential anti-neuroinflammatory agent: a mechanistic study. J Neuroinflammation 2016;13(1):193. https://doi.org/10.1186/s12974-016-0650-3.
[19] Jayaprakasam B, Strasburg GA, Nair MG. Potent lipid peroxidation inhibitors from *Withania somnifera* fruits. Tetrahedron 2004;60(13):3109–21. https://doi.org/10.1016/j.tet.2004.01.016.
[20] Kaurav BP, Wanjari MM, Chandekar A, Chauhan NS, Upmanyu N. Influence of *Withania somnifera* on obsessive compulsive disorder in mice. Asian Pac J Trop Med 2012;5(5):380–4. https://doi.org/10.1016/S1995-7645(12)60063-7.
[21] Kulkarni SK, George B. Anticonvulsant action of *Withania somnifera* (Aswaganda) root extract against Pentylenetetrazol-induced kindling in mice. Phytother Res 1996;10(5):447–9. https://doi.org/10.1002/(SICI)1099-1573(199608)10:5<447::AID-PTR869>3.0.CO;2-M.
[22] Mohanty IR, Arya DS, Gupt SK. *Withania somnifera* provides cardioprotection and attenuates ischemia reperfusion induced apoptosis. Clin Nutr 2008;27(4):635–42. https://doi.org/10.1016/j.clnu.2008.05.006.
[23] Mondal S, Roy S, Maity R, Mallick A, Sangwan R, Misra Bhattacharya S, et al. Withanolide D, carrying the baton of Indian Rasayana herb as a lead candidate of antileukemic agent in modern medicine. Adv Exp Med Biol 2012;749:295–312. https://doi.org/0.1007/978-1-4614-3381-1_20.
[24] Gu M, Yu Y, Gunaherath GM, Gunatilaka AA, Li D, Sun D. Structure-activity relationship (SAR) of withanolides to inhibit Hsp90 for its activity in pancreatic cancer cells. Investig New Drugs 2013;32(1):68–74. https://doi.org/10.1007/s10637-013-9987-y.
[25] Mohan R, Hammers H, Bargagna-Mohan P, Zhan XH, Herbstriit CJ, Ruiz A, et al. Withaferin A is a potent inhibitor of angiogenesis. Angiogenesis 2004;7(2):115–22. https://doi.org/10.1007/s10456-004-1026-3.
[26] Zhang H, Samadi AK, Cohen MS, Timmermann BN. Antiproliferative withanolides from the solanaceae: a structure-activity study. Pure Appl Chem 2012;84(6):1353–67. https://doi.org/10.1351/PAC-CON-11-10-08.
[27] Panjamurthy K, Manoharan S, Menon VP, Nirmal MR, Senthil N. Protective role of withaferin-A on 7,12-dimethylbenz(a)anthracene-induced genotoxicity in bone marrow of Syrian golden hamsters. J Biochem Mol Toxicol 2008;22(4):251–8. https://doi.org/10.1002/jbt.20235.
[28] Rajasankar S, Manivasagam T, Surendran S. Ashwagandha leaf extract: a potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. Neurosci Lett 2009;454(1):11–5. https://doi.org/10.1016/j.neulet.2009.02.044.
[29] Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B. Studies on the immunomodulatory effects of Ashwagandha. J Ethnopharmacol 1996;50(2):69–76. https://doi.org/10.1016/0378-8741(95)01318-0.
[30] Vaidya PY. Vaidya khadiwale: Yashashvi Ayurvediya Aushadikaran- Shastra ani Vyawahar- Chalisvarshe mage valun baghtana (Marathi). 2nd ed. Pune: Vaidya Khadiwale Vaidyak Research Centre; 2008. p. 18–9.
[31] Acharya YT. Charaka Samhita*: Ayurveda Dipika Commentary* with Chakrapaanidatta. 1st ed. Varanasi: Chaukhambha Orientalia; 1984. p. 166.
[32] Ayurved Sarsangraha: Ghrit Prakaran (Hindi). Shri Baidyanath Ayurved Bhavan Pvt. Ltd; 2014. p. 647–57.
[33] Dorle AK, Biyani DM, Fulzele SV, Pande DM, Gore CV. A novel process for preservation of ghee in pharmaceutical, ayurvedic and nutraceutical formulations. Indian Patent No. 196260 (2002).
[34] Anonymous. The Ayurvedic pharmacopeia of India, part-II. Ministry of health and family welfare, government of India, department of Indian systems of medicine and homeopathy New Delhi. 1st ed., vol. 1; 2007. p. 258–9.
[35] Rai P. Role of *murchhana* sanskara in the preparation of medicated ghrita w.s.r. to Panchtikta ghrita. Int. Ayurvedic Med. J. 2015;3(8):2386–90.
[36] Kadibagil VR, Sarashetti RS. Assessment of the significance of murchana samskara of ghrita by GC-MS. Int J Res Ayurveda Pharm 2017;8(2):166–9. https://doi.org/10.7897/2277-4343.082105.
[37] Shrestha S, Bedarkar P, Chaudhari S, Galib R, Patgiri B. Facets of sneha murchhana sanskara–a review. J Ayurveda Holist Med 2017;5(1):52–9.
[38] Ranganna S. Sensory evaluation. In: Ranganna S, editor. Handbook of analysis and quality control for fruit and vegetable products. New Delhi: Tata Mc Graw Hill Publishing; 1986. p. 594–645.
[39] Anonymous. Indian Pharmacopeia. Ministry of Health and Family Welfare, Govt. of India; 2014.
[40] Lohar DR. Protocol for testing of 'ayurvedic, Siddha and Unani medicines'. Pharmacopeial Laboratory for Indian medicines. Department of AYUSH, Govt of India; 2008.
[41] Gurav SS, Deshkar NS, Tilloo SK, Duragkar NJ, Burade KB. Antimicrobial and antioxidant evaluation of *Flacourtia ramontchi* L. Herit. J Herbs, Spices, Med Plants 2013;19:76–95. https://doi.org/10.1080/10496475.2012.743107.
[42] Green LD, Wagner MW, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131–8. https://doi.org/10.1016/0003-2697(82)90118-X.
[43] Zhang XY. Principles of chemical analysis. Beijing: China Science Press; 2000. p. 275–6.
[44] Mehta BM. Butter, butter oil, and ghee. In: Moreau RA, Kamal-Eldin A, editors. Gourmet and health-promoting specialty oils. Academic Press and AOCS Press; 2009. p. 527–59. https://doi.org/10.1016/b978-1-893997-97-4.50027-9.
[45] Singh S, Ram BP. Effect of ripening of cream, manufacturing temperature and packaging materials on flavour and keeping quality of ghee. J Food Sci Technol 1978;15:142–5.
[46] Stone H, Sidel JL. Introduction to sensory evaluation. In: Stone H, Sidel JL, editors. Sensory evaluation practices. California: Elsevier Academic Press; 2004. p. 1–17. https://doi.org/10.1016/B978-0-12-672482-0.50008-3.
[47] Bailey AE, Swern D, Mattil KF. Bailey's Industrial oil and fat products. 3rd ed. New York: Wiley-Interscience Publishers; 1964.
[48] Sserunjogi ML, Abrahamsen RK, Narvhus J. A review paper: current knowledge of ghee and related products. Int Dairy J 1998;8(8):677–88. https://doi.org/10.1016/s0958-6946(98)00106-x.
[49] Achaya KT. Ghee, vanaspati and special fats in India. In: Gunstone FD, Padley FB, editors. Lipids technologies and applications. New York: Marcel Dekker Inc.; 1997. p. 369–90.

[50] Abraham MJ, Srinivasan RA. Effect of ripening cream with selected lactic acid bacteria on the quality of ghee. J Dairy Res 1980;47:411–5. https://doi.org/10.1017/S0022029900021300.
[51] Wadhwa BK, Jain MK. Chemistry of ghee flavour - a review. Indian J Dairy Sci 1990;43(4):601–7.
[52] Unnikrishnan V, Rao MB. Copper and iron in ghee and their influence on oxidative deterioration. J Food Sci Technol 1977;14:164–9.
[53] Kirk RS, Sawyer R. Pearson's composition and analysis of foods. 9th ed. England: Longman Scientific and Technical; 1991. p. 607–17.
[54] Ganguli NC, Jain MK. Ghee: its chemistry, processing and technology. J Dairy Sci 1972;56(1):19–25. https://doi.org/10.3168/jds.S0022-0302(73)85109-4.
[55] Mortensen G, Andersen U, Nielsen JH, Andersen HJ. Chemical deterioration and physical instability of dairy products. In: Skibsted LH, Risbo J, Andersen ML, editors. Chemical deterioration and physical instability of food and beverages, Cambridge: Woodhead Publishing Series in Food Science, Technology and Nutrition; 2010; 2010. p. 726–62. https://doi.org/10.1533/9781845699260.3.726.
[56] Hiremath BS, Hiremath VV, Mohapatra S, Sarashetti RS. Effect of murchhana samskara in the preparation of Hingutriguna Taila - an analytical study. Biomed Pharmacol J 2013;6(2):371–4. https://doi.org/10.13005/bpj/428.
[57] Gosewade S, Gandhi K, Ranvir S, Kumar A, Lal D. A study on the physico-chemical changes occurring in ghee (butter oil). Indian J Dairy Sci 2017;70(1):81–8.
[58] Yadav KD, Reddy KR, Agarwal A. Preliminary physico-chemical profile of brahmi ghrita. Ayu 2013;34:294–6. https://doi.org/10.4103/0974-8520.123130.
[59] Nadkarni KM. Indian materia medica, vol. 2; 1994. Mumbai: Popular Prakashan.
[60] Anonymous. The Ayurvedic Pharmacopoeia of India, Part–I. 1st ed. New Delhi: Ministry of Health and Family Welfare, Government of India, Department of Indian Systems of Medicine and Homeopathy; 2007.
[61] Variya BC, Bakrania AK, Patel SS. *Emblica officinalis* (Amla): a review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. Pharmacol Res 2016;111:180–200. https://doi.org/10.1016/j.phrs.2016.06.013.
[62] Lydia J, Sundarsanam D. Phytoconstituents of *Cyperus rotundus* L. that attribute to its medicinal value and antioxidant property. Int J Pharm Sci Res 2012;3(9):3304–8. https://doi.org/10.13040/IJPSR.0975-8232.3(9).3304-08.
[63] Zeynep K, Isil G, Erim FB. Comparison of antioxidant, anticholinesterase, and antidiabetic activities of three curcuminoids isolated from *Curcuma longa* L. Nat Prod Res 2017;31(24):1–4. https://doi.org/10.1080/14786419.2017.1299727.