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Impact of milk fat concentration and storage on the antioxidant activity of *Triphala Ghrita*

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Abstract
This study investigated the influence of milk fat concentration and storage duration on the antioxidant stability of *Triphala Ghrita*. Formulations were prepared using milk containing 1.5%, 3.0%, and 4.5% fat, with plain ghee as a control, and antioxidant activity was monitored over a 20-week period. While all samples exhibited a significant, time-dependent decline in antioxidant activity ($p<0.01$), the *Triphala Ghrita* formulations were substantially more stable than the control. The retention of this activity was directly correlated with the initial milk fat content. After 20 weeks, the *ghrita* made with 4.5% fat milk retained 87% of its initial antioxidant activity, compared to 82% (3.0% fat), 74% (1.5% fat), and only 56% for the plain ghee. These findings indicate that a higher lipid concentration enhances the preservation of bioactive compounds, likely by improving the solubility of polyphenols in *Triphala*. This validates the role of *Triphala* as a potent natural antioxidant, highlighting the potential of *Triphala Ghrita* as a functional therapeutic food and a viable alternative to synthetic antioxidants in fat-based products.

Keywords: Antioxidant activity, *Triphala Ghrita, Ayurveda,* milk fat, storage

Introduction
The antioxidant potential of milk fat is governed by the synergistic action of intrinsic antioxidants and antioxidant enzymes, as it is notably rich in fat-soluble vitamins such as vitamin A and vitamin E. Despite these natural defences, they are highly susceptible to oxidative degradation from reactive oxygen species (ROS) and free radicals, a process that significantly deteriorates food quality by causing off-flavors, discoloration, nutrient loss, and the formation of potentially harmful compounds (Chemat *et al.*, 2023; Böttcher *et al.*, 2015) [4, 3]. In ghee, this oxidation leads to rancidity and a shortened shelf life, while some lipid oxidation byproducts pose serious health risks, having been associated with neuromyopathic disorders and exhibiting mutagenic, carcinogenic, and cytotoxic properties (Ahmed *et al.*, 2016; Olpin, 2005; Keller *et al.*, 2015) [1, 12, 6].

Our bodies are constantly fighting off internal damage known as "oxidative stress," which is now seen as a major culprit behind many chronic illnesses like heart disease, cancer and arthritis. Although synthetic antioxidants have been extensively utilised, there are growing concerns about their possible toxicity and long-term safety. This has sparked a global shift toward finding safer, natural protectors in plants, which can effectively defend our cells without the risk of harmful side effects.

Triphala, meaning “three fruits” (*tri* = three, *phala* = fruit), is a classical *Ayurvedic* formulation composed of the dried fruits of *Terminalia chebula* (haritaki), *Terminalia bellirica* (bibhitaki), and *Emblica officinalis* (amalaki). Major ingredients in manufacture of *Triphala Ghrita* includes triphala (decoction and powder), ghee and milk. Recent research supports its effectiveness in managing various conditions, such as anemia, jaundice, constipation and chronic ulcers (Kumar *et al.*, 2016) [7]. In Ayurvedic tradition, it is classified as a *rasayana*, attributed with promoting overall health, enhancing immunity, supporting detoxification, improving digestion, and contributing to longevity (Srikanth, 2024) [14].

Triphala serves as a potent reservoir of bioactive compounds, including vitamin C, gallic acid, ellagic acid, chebulinic acid, β-sitosterol, bellericanin, flavonoids, alkaloids, tannins, saponins, glycosides and other phenolic compounds which collectively contribute to its

strong antioxidant and therapeutic potential (Nariya *et al.*, 2009) [11]. It has potential to restrain lipid peroxidation, promote endogenous antioxidant defence mechanisms and modify oxidative stress pathways is facilitated by these components. Recent *in vitro* studies have confirmed that ethanolic extracts of Triphala exhibit strong antioxidant potential, supporting its utility as an accessible, plant-based source of natural antioxidants. Given its safety profile, multifunctional benefits, and regulatory approval as a traditional medicine, Triphala holds significant potential for application as a nutraceutical, dietary supplement, or in the development of functional foods and phytopharmaceuticals (Babu *et. al*., 2013) [2].

Despite the recognized therapeutic benefits of *Triphala Ghrita*, a significant gap exists in the scientific literature regarding its optimal formulation and stability. To date, no systematic investigation has been conducted to evaluate the influence of varying milk fat concentrations during its preparation or the impact of storage duration on its antioxidant efficacy. This study was designed to investigate how varying the fat concentration in milk affects the antioxidant activity of *Triphala Ghrita*. Furthermore, the research evaluated the stability of the formulation's antioxidant potential during storage.

Materials and Methods

Three distinct formulations of *Triphala Ghrita* were prepared (as per *Ashtanga Hridaya*) using *Triphala* powder, *Triphala Kashaya* (decoction), ghee and raw milk with varying fat concentrations: 1.5%, 3.0%, and 4.5%. A sample of pure ghee, prepared without Triphala served as the experimental control. Following preparation, all samples were packaged in sealed plastic bottles for a 20-week storage stability trial under ambient conditions. To evaluate the influence of milk fat content and storage duration, the antioxidant activity of each sample was systematically assessed on the day of preparation (0 weeks) and at subsequent intervals of 4, 8, 12, 16, and 20 weeks using DPPH radical scavenging activity. A slightly modified version of the Mishra *et al.* (2012) method was used to measure antioxidant activity. 5 mL of 100 µM DPPH (2, 2-diphenyl-1-picrylhydrazyl) in methanol solution was vortexed with an aliquot of ImL of the sample (1g sample in 10mL of 50mM phosphate buffer pH7.5)/Trolox standard solution (10-100µg/mL). The absorbance at 515 nm was measured after the reaction mixture was exposed to the dark for 30 minutes. Methanol served as the blank.

Per cent scavenging activity = $[(A_o-A_1)/A_o]*100$

where, A_o = Absorbance of DPPH in the absence of sample/antioxidant standard

A_1 = Absorbance of DPPH in the presence of the sample/ standar

Trolox equivalent antioxidant capacity (TEAC) was used to express the sample's DPPH radical scavenging activity.

Results

A statistically significant, time-dependent decline in antioxidant activity was observed across all the treatments (T1-1.5% fat, T2-3% fat and T3- 4.5% fat) and during the 20-week storage period ($p<0.01$) (Table 1). It is also observed that this degradation was progressive, with each storage interval showing a significant reduction in antioxidant potential compared to the preceding one. Throughout the study, all three *Triphala Ghrita* formulations exhibited significantly higher antioxidant activity than the control (C), emphasizing the substantial enhancement of oxidative stability conferred by the incorporation of Triphala. The retention of this activity was directly correlated with the milk fat concentration used in the preparation. The formulation with 4.5% fat consistently demonstrated the highest preservation of antioxidant potential, followed by the 3.0% and 1.5% fat formulations, respectively.

Quantitatively, at the conclusion of the 20-week trial, the 4.5% fat *Triphala Ghrita* retained approximately 87% of its initial antioxidant activity, compared to 82% for the 3.0% fat formulation and 74% for the 1.5% fat formulation. In contrast, the control ghee retained only 56% of its initial activity. This suggests that a higher lipid concentration more effectively preserves bioactive compounds, likely due to the improved solubility and dispersion of Triphala's polyphenols within the ghee matrix.

Table 1: Effect of milk fat content and storage on antioxidant activity of *Triphala ghrita*

Treatment	Weeks of storage						F value
	0 weeks	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	
T1	1894.13± 7.94 ^{e}	1783.40± 21.74^{d}	1666.9±8.24^{c}	1649.99±26.14^{c}	1555.46±24.079^{b}	1408.65±42.50^{a}	47.15***
T2	1892.33± 17.14^{e}	1810.37±.94^{d}	1728.93± 20.24^{c}	1768.65± 9.31^{cd}	1616.97± 17.19^{b}	1557.22±14.90^{a}	70.57***
T3	1916.03±.031^{d}	1813.82±2.59^{c}	1781.88±19.19^{b}	1801.68± 2.66^{bc}	1691.61±3.36^{a}	1662.37± 13.73^{a}	86.091***
C	1527.13±3.28	1508.01±.78	1264.72±.32782	1029.6954±2.43	1003.09±6.6	851.90 ±6.09	4838.518***

T1- *Triphala Ghrita* made of milk with 1.5% fat
T2- *Triphala Ghrita* made of milk with 3% fat
T3- *Triphala Ghrita* made of milk with 4.5% fat
C-Normal ghee

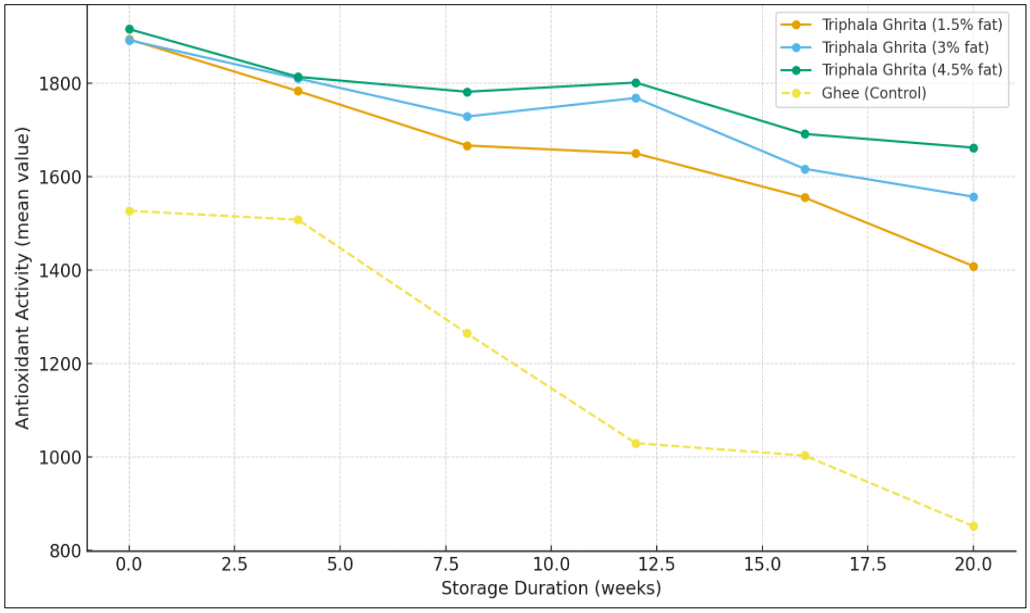

Fig 1: Antioxidant activity of Triphia ghrita during storage

Discussion

Antioxidants such as gallic acid, ellagic acid, tannins, flavonoids and emblicanins from *Triphala* incorporated into *ghrita* act as a supplementary reservoir of radical-scavenging compounds, buffering lipid oxidation and sustaining higher antioxidant activity compared to plain ghee (Pawar *et al.*, 2014) [13]. Phenolic compounds and other plant-derived bioactives are prone to thermal, oxidative, and hydrolytic degradation during storage. Kinetic studies across food systems often follow first-order or pseudo-first-order models, consistently demonstrating progressive losses with increasing time and temperature (de Vilela Silva *et al.*, 2023) [5]. The post hoc analysis in the present study clearly indicates that the interval-by-interval decline in antioxidant activity was steady and significant, which can be attributed to the possible influence of pro-oxidant trace metals or residual water that accelerate degradation reactions. In addition, environmental factors such as storage temperature, oxygen availability, and light exposure are well-documented drivers of lipid and polyphenol instability (Musakhanian *et al.*, 2022) [10]. Losada-Barreiro *et al.*, (2023) [8] suggested that based on polarity of phenolic compounds, amphiphilic or lipophilized phenolics at oil–water interfaces are less exposed to aqueous pro-oxidants show that the local environment (oil volume, interfacial area, emulsifiers) strongly affects antioxidant efficiency and apparent retention.

Conclusion

Triphala Ghrita shows progressive decline in antioxidant activity during storage at room temperature on the other hand *Triphala Ghrita* retained higher antioxidant activity than plain ghee. It was also noted that increasing the fat content in the milk used also positively influence the antioxidant activity. *Triphala* enhances oxidative stability against free radicle oxidation thus providing a potential scope for utilising *Triphala Ghrita* as a functional/therapeutic ghee. It is also validated from the study that role of *Triphala* as a natural antioxidant source, suggesting that *Triphala* could replace synthetic antioxidants in fat-based formulations.

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