



Phytochemical Identification and *in vitro* Radical Scavenging Activities of Medicated Butter Milk “Gauri Takra”: The Ancient Traditional Recipe of Ayurveda

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ABSTRACT

Background: Gauri takra (GT) was found to be an excellent refreshing drink. The current investigation attempts to assess the phytochemical identification and antioxidant properties of Gauri Takra.

Methods: According to the standard procedures, the food recipe GT was examined for phytochemical identification, total flavonoids, total phenolic content, *in vitro* radical scavenging assays and HPTLC analysis.

Result: High-Performance Thin-Layer Chromatography analysis of the methanolic extract of GT has been screened for phytochemical identification. The qualitative test revealed that the methanolic extract of GT contained the presence of quercetin and their Rf value was 0.62 and the 6-gingerol Rf value was detected as 0.68. The phenolic content of GT was 2.69 ± 0.02 mg TAE/gm and a total flavonoid content of 4.35 ± 0.34 mg QAE/gm. Aqueous extract of GT were investigated for their antioxidant properties using DPPH, FRAP and ABTS^{•+} radical scavenging assay. There was a significant dose-dependent inhibition of *in-vitro* radical scavenging assays as demonstrated by the aqueous extract of GT. Thus, our results show that GT was exhibited strongest antioxidant activity.

Key words: Antioxidant, Ayurveda, Gauri takra, HPTLC, Phenolic content, Radical scavenging assays.

INTRODUCTION

In Ayurveda, *Kshemakutuhalam* is a major compendium referred for indigenous dietetics, this book contains numerous therapeutic recipes that can help improve one's immunity and in maintenance of health. In the preview of palatability, easily available ingredients and its therapeutic potential, preparation of GT was selected. Butter milk is popular drink across India especially in Gujarat and Rajasthan (Halder *et al.*, 2021). The worldwide buttermilk production is raised with an average growth rate of about 2-3%. Nowadays various types of pack butter milk are available in the market along with its powder expected to have 5.4% growth in demand from 2025 to 2026 (Señoráns *et al.*, 2025). Making butter from fermented, fat-rich summer milk was traditionally a significant method of preserving the milk's valuable fat content for use in the winter. First, the cream would spontaneously ferment due to contamination by LAB and yeasts from the environment (Seo *et al.*, 2020). When cream is churned, large amounts of buttermilk are released, which is traditionally drunk as a cool, sour summer beverage (Gebreselassie *et al.*, 2016). But from a business perspective, this acidic and fermented product has poor keeping quality because of oxidation, which causes metallic after tastes to appear in a few days (Walstra *et al.*, 2005). The oxidation of phospholipids transferred from the fat globule membrane during churning is thought to be the initial cause of the auto-oxidation of fat in buttermilk (Narvhus *et al.*, 2023).

Unfermented cream is the primary ingredient used to make butter in modern commercial operations, while sweet

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buttermilk is a commodity with higher economic value. Sweet buttermilk can, however, ferment on its own to produce a product similar to “real” buttermilk (Rose *et al.*, 2023).

To enhance the palatability of buttermilk, it is fortified with spices, packed and stored for marketing (Augustyńska-Prejsnar *et al.*, 2020). The final step before packaging in spiced buttermilk involves adding natural or natural extracts of spices, whose level of spiciness varies depending on the region (moderate spiciness, for example) (Ghanshyambhai *et al.*, 2015). When stored at a higher temperature, the

spices used to make buttermilk may lose some of their volatile components (Barukčić *et al.*, 2019).

However, Ayurveda always emphasizes freshly prepared Takra. Hence, Takra fortified with spices was searched in compendium dedicated to traditional Indian recipes. GT was prepared by Ayurveda and nutritional expert of Tilak Maharashtra Vidyapeeth, Pune, by following the verse described in *Kshemkutuhalam*. To ensure the quality, antioxidant and phytochemical properties of the final product, the GT was analyzed for total flavonoids, total phenolic content, *in vitro* radical scavenging assays and HPTLC profile.

MATERIALS AND METHODS

Procurement of food recipe

All the raw materials for GT (Butter Milk) were procured from a local Ayurvedic shop. The food recipe for raw materials was authenticated by Dr. Manoj A. Joshi, M.D. (Ayurveda), Department of Ayurveda and Yoga, Tilak Maharashtra Vidyapeeth, Pune, Maharashtra, India.

Preparation of the recipe

GT was Takra (Buttermilk) fortified with spices, as referred from *Ksemakutuhalam* Fig 1. To prepare GT, 100 g of curd was added into a container, then 200 mL of water was poured and churned well to make buttermilk. Spices such as black pepper powder, dry ginger powder, rock salt, cumin seed powder and orange peel powder were added and mixed thoroughly. In a pan, ghee was heated and asafoetida was fried for a while. The buttermilk mixture was then tempered with the ghee and fried asafoetida and the buttermilk was churned well. This preparation was known as GT. As all the ingredients helped to improve digestion, it mitigated thirst, imparted taste and also provided energy and satiety.

High performance thin layer chromatography (HPTLC)

A precise weight of 4.22 g of GT lyophilized powder was measured in 10 mL of methanol. The mixture was sonicated for 15 minutes at 25°C, then centrifuged at 10,000 rpm for ten minutes and the supernatant was collected in a glass vial for HPTLC analysis. The mobile phase consisted of Toluene: Ethyl acetate: Methanol: Formic acid: Glacial acetic acid (6:8:3:1:1 v/v/v/v/v). A pre-coated silica gel 60F254 TLC plate (Merck) with a consistent 0.2 mm thickness was utilized and 10 µL of GT was applied on different tracks. The plate was then developed up to 80 mm in the specified mobile phase. After the plate had dried, it was observed at 254 nm and 366 nm wavelengths. During the derivatization stage, the plate was heated at 105°C until the color of the bands or spots appeared after dipping it in the Natural Product reagent (NP) and the Anisaldehyde Sulfuric Acid reagent (ASR) (Shinde *et al.*, 2015).

Determination of flavonoid content

The aluminium chloride colorimetric method, according to Ribarova *et al.* (2005), had been employed to ascertain GT

total flavonoid contents. 0.3 ml of 5% NaNO₂ was combined with 1 mL of aqueous extract of GT in a flask containing 4ml of distilled water using a pipette. 0.3ml of 10 percent AlCl₃ had been added after 5 minutes. After standing for an additional six minutes, this mixture was combined with two milliliters of 1M NaOH. Following that, distilled water had been added to reduce the mixture's volume to 10 ml. The reaction mixture's absorbance at 510 nm was measured at multimode reader (Synergy HTX, Biotek, USA).

Determination of phenolic content

The Folin-Ciocalteu method had been used to calculate the total phenolic content for GT recipe (Pérez *et al.*, 2023). One ml of GT aqueous extract and one ml of Folin-Ciocalteu reagent were combined. Following a 5-minute incubation period, 4.0 mL of Na₂CO₃ had been added to the mixture and distilled water had been added to bring the volume to 10 ml. The solution was centrifuged for five minutes at 2000 rpm after being allowed to stand at room temperature in the dark for two hours to measure the absorbance at 760 nm was measured in multimode reader (Synergy HTX, Biotek, USA).

Determination of *in vitro* radical scavenging activity

DPPH assays

The GT extract capacity to scavenge DPPH[•] radicals was assessed through the utilization of the previously documented technique by (Brand-Williams *et al.*, 1995). 100 µL of tris buffer was added to a DPPH solution (0.1mM DPPH in methanol) along with 25 µL of GT and standard ascorbic acid. After that, 125 µL of DPPH solution was thoroughly added as well as allowed to stand for 30 minutes at room temp and the mixture's absorbance at 517 nm was measured in multimode reader (Synergy HTX, Biotek, USA).

FRAP assays

The FRAP assay method was used to calculate the reduction of GT extract (Oyaizu, 1986). 1.5 mL of potassium ferric cyanide (1%), 1.5 mL of phosphate buffer (0.2 M, pH = 6.6), along with 0.5 mL of GT and standard BHT had been mixed. The mixture had spent 20 minutes being incubated at 50°C. After adding 1.5 mL of 10 per cent TCA to the mixture, the mixture had been centrifuged for 10

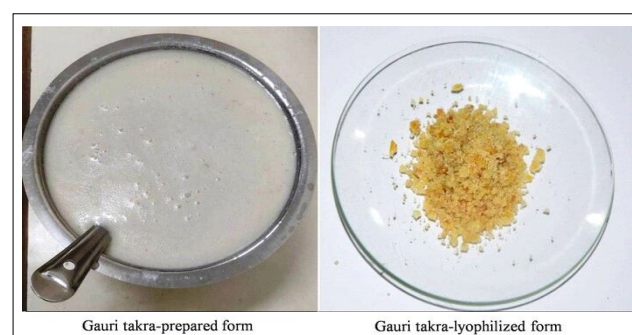


Fig 1: Final product of lyophilized powder of gauri takra.

minutes at 6000 rpm. 1.5 mL of the solution's upper layer, 1.5 mL of distilled water and 0.3 mL of FeCl_3 (0.1%) were mixed. In the presence of GT and BHT to study the $\text{Fe}^{3+}/\text{Fe}^{2+}$ transformation and absorbance values were measured at 700 nm in a multimode reader (Synergy HTX, Biotek, USA).

ABTS^{•+} assays

The decolorization assay method was developed by (Wolfenden and Wilson, 1982) GT extract with antioxidant method of reducing the ABTS^{•+} radical. A volume of 10 μL of GT and standard ascorbic acid had been added to 990 μL of ABTS^{•+} free radical solution, as well as the mixture had been left in the dark at room temp for 60 minutes the absorbance was measured at 734 nm in a multimode reader (Synergy HTX, Biotek, USA).

Statistical analysis

Graph pad Prism version-5 (California, United States) for Windows was used to conduct statistical analysis. For each group, the values have been presented as mean $\pm$ SD for 3 replicates, with $p < 0.05$ being deemed significant.

RESULTS AND DISCUSSION

HPTLC phytochemical identification

The HPTLC analysis of GT revealed the presence of quercetin and 6-gingerol as illustrated in the Table 1. After scanning at UV 254 nm and 366 nm, the chromatograms Fig 2 to 3 were obtained and peak tables were demonstrated. The Rf values of the methanolic extract of

GT have been screened for phytochemical identification. In HPTLC, the Rf value helps identify and confirm marker compounds by comparing the distance a compound travels with that of known standards (quercetin and 6-gingerol). A matching Rf value and similar fluorescence color indicate the presence of the target compounds. It also helps assess purity and ensures quality control through consistent fingerprinting patterns in GT. Based on HPTLC qualitative examination, the GT methanolic extract contained the presence of quercetin with a Rf value of 0.62 and 6-gingerol with a Rf value of 0.68.

Analysis of total phenolic content and total flavonoid content

The total phenolic content of GT had been estimated using the Folin-Ciocalteu method. The tannic acid standard calibration curve's linear regression equation had been used to determine GT total phenolic content. The mg of tannic acid equivalent per gram dry weight of food recipe extract had been used to express the total phenolic content. The phenolic content of Gauri takra is 2.69 ± 0.02 mg TAE/gm. The aluminium chloride method had been used to estimate the total flavonoid content of GT. Gauri takra had a total flavonoid content of 4.35 ± 0.34 mg QAE/gm.

DPPH assay

Fig 4 illustrates how the concentrations range of ascorbic acid 2.5 to 25 $\mu\text{g}/\text{mL}$ and 10 to 100 mg/mL of GT aqueous extract inhibited the formation of DPPH radicals. The purple color of the solution decreased in proportion to the concentration, indicating the antioxidant activity of the extracts, when compared to standard ascorbic acid, the DPPH radical scavenging activity of the GT extract was significantly lower. A concentration v/s percentage DPPH inhibition linear regression analysis had been performed. Ascorbic acid and GT regression coefficients, $R^2 = 0.99$ and 0.959, respectively, indicate that the activity of DPPH radical scavenging was concentration-dependent. The regression

Table 1: HPTLC analysis of gauri takra in comparison with standards 6-gingerol and quercetin.

Track no	Name of standards	
	6 Gingerol	Quercetin
12	Rf- 0.68	Rf- 0.62
	Detected	Detected
	Rf- 0.68	Rf- 0.63

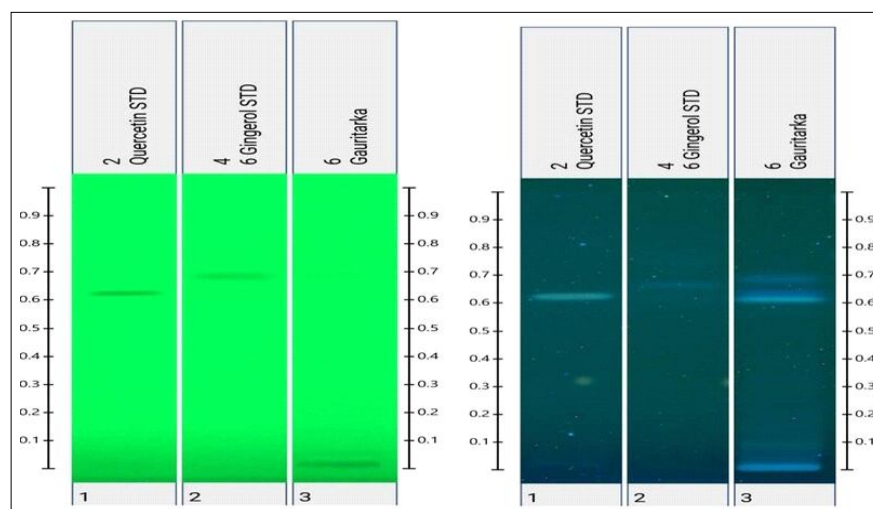


Fig 2: HPTLC development image of quercetin, 6-gingerol and gauri takra at 254 nm and 366 nm.

curve yielded the IC_{50} values for GT and ascorbic acid, which were 43.24 mg/mL and 14.47 μ g/mL, respectively.

FRAP assay

The concentration range of 3.9 to 1000 μ g/mL of Standard BHT (Butylated hydroxyl toluene) and AQ extract of GT had been in the concentration range of 3.9 to 1000 μ g/mL and showed ferric-reducing activity. A concentration versus absorbance graph comparing BHT and extract of GT was

plotted and depicted in Fig 5. An increase in the green color absorbance measured at 700 nm indicates that there had been a significant concentration-related decrease of ferricyanide to ferrocyanide in the aqueous extract of GT, which is in the concentration range of 3.9-1000 μ g/mL. The decrease of ferricyanide to ferrocyanide indicates a reduction reaction, showing the antioxidant potential (reducing power) of the GT extract. Standard antioxidant-BHT had been also found to have a similar effect in the

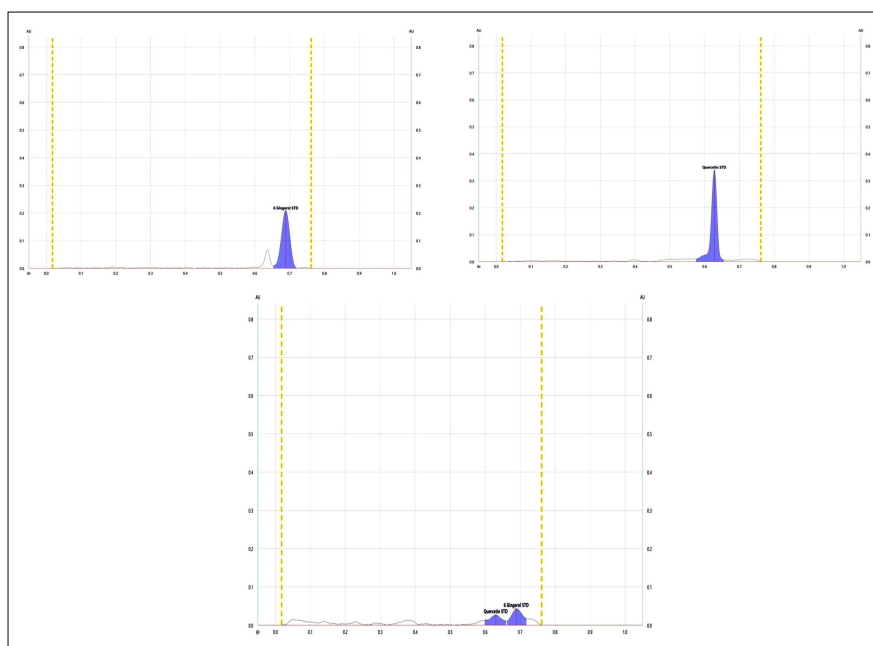


Fig 3: HPTLC densitometry of standards of 6-gingerol, quercetin and gauri takra.

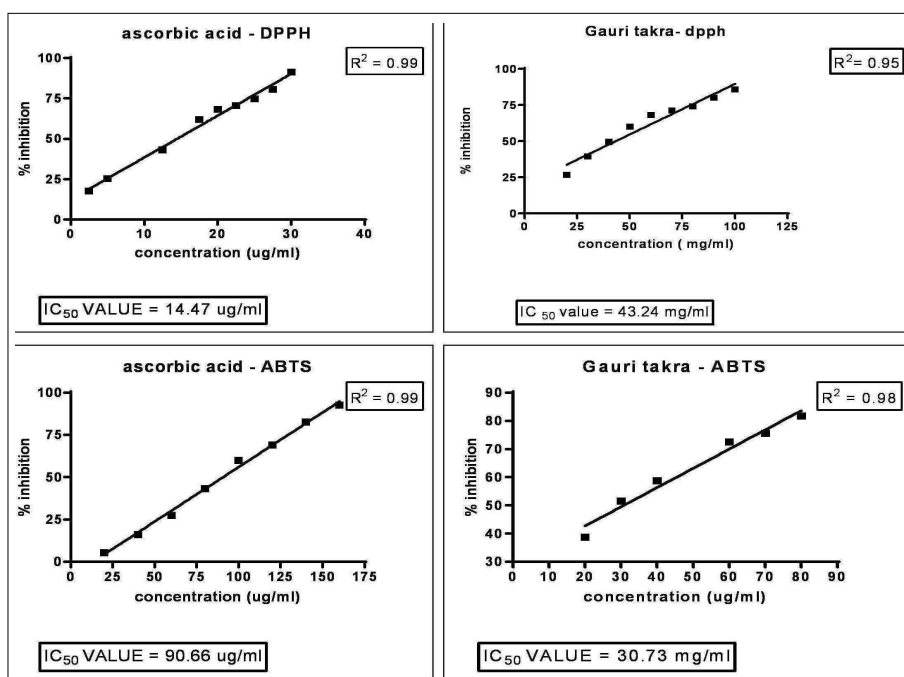


Fig 4: Changes in the levels of DPPH and ABTS+• radical scavenging activity of different concentrations aqueous extract of gauri takra.

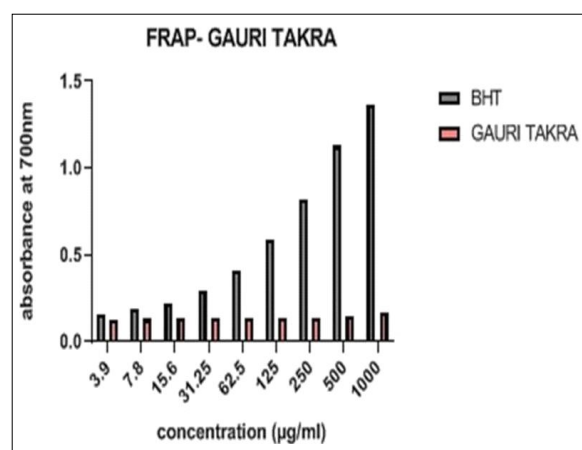


Fig 5: Changes in the levels of FRAP-radical scavenging activity of different concentrations aqueous extract of gauri takra.

concentration range of 3.9 to 1000 µg/mL. AQ extract of GT showed significantly decreased radical scavenging activity when compared with standard BHT.

ABTS^{•+} assay

Fig 4 illustrates the concentration-dependent reduction in the blue color of the solution, which indicates that the aqueous extract of GT in the range of 10 to 90 µg/mL scavenged the formation of the ABTS^{•+} radical. The concentration versus percentage of inhibition was analyzed using linear regression. Ascorbic acid and GT had regression coefficients of $R^2=0.99$ and 0.98 , correspondingly, indicating that ABTS^{•+} scavenging had been concentration-dependent. The regression curve yielded the IC_{50} values for GT and ascorbic acid, which had been 30.73 µg/mL and 90.66 µg/mL, respectively.

Fermented foods are rich in nutrients, phytochemicals, bioactive compounds and probiotic bacteria, among other health-promoting attributes. These functional foods contain *Lactococcus lactis* which has anti-inflammatory and immunomodulatory properties (Saleena *et al.*, 2023). In colitis models, milk consumption can lessen intestinal tissue damage and shield newborns from necrotizing enterocolitis. Inflammation of the oral mucosa and peri-implantitis may be reduced with milk, but the exact mechanism is unclear (Panahipour *et al.*, 2019). According to the study, mice who consumed milk polar lipids had greater mucus-producing goblet cells in the colon, thus strengthening the colon barrier (Cani *et al.*, 2012). Lactic acid bacteria ferment to produce bioactive peptides with properties including opioid, antithrombotic, hypotensive, immunomodulatory, mineral binding, as well as antibacterial (Korhonen and Pihlanto, 2003). The nutritious properties of buttermilk include milk salts, vitamins and proteins, along with nutritional ingredients (Myagmardorj *et al.*, 2018). There are now buttermilk variations in the market, but because they contain added sugars, flavors and preservatives, they cannot be categorized as health drinks. It is therefore highly

recommended to prepare functional buttermilk with natural ingredients and nutrition-imparting ingredients such as ginger powder, asafoetida, cumin powder, black pepper and orange peel powder to reduce the unpleasant flavor and enhance nutrition and bioavailability (Sharma *et al.*, 2021). The present study identified total flavonoids and total phenolic content, *in vitro* radical scavenging assays and HPTLC profile. Using qualitative HPTLC analysis, the phytochemical screening investigations revealed quercetin and 6-gingerol as two active ingredients in the food recipe. In addition, the screening of phenolic content of GT was 2.69 ± 0.02 mg TAE/gm and flavonoid content of 4.35 ± 0.34 mg QAE/gm was estimated. These phytochemicals can be found in food recipes and nutrients. Studies have shown that flavonoids and many other phenolic components are potent antioxidants, anti-inflammatory, immune system boosters, anti-cancer, anti-bacteria and an intriguing candidate for use in pharmaceutical and medical applications (Sun and Shahrajabian, 2023). Our research findings showed that different concentrations of GT aqueous extract exhibited the best inhibition against *in vitro* tests for DPPH, FRAP and ABTS^{•+} radical scavenging. The presence of flavonoids and phenolic contents, quercetin and 6-gingerol, which donates hydrogen an electron to hydroxyl radicals, stabilizing them and resulting in a relatively stable radical, can be attributed to the DPPH, FRAP and ABTS^{•+} assay of the aqueous extract of GT. Thus, the antioxidant qualities are caused by the free hydroxyl group on the aromatic ring. During the food preparation process, GT recipe was having mixture of phenolic and flavonoid content; it contributed to the highest nutritional value.

CONCLUSION

The conclusion of the experiments demonstrated that GT recipe had a strong antioxidant potential when it came to scavenging free radicals produced by various *in vitro* tests. It was detected by the presence of phenolic and flavonoid content. The prepared food recipe is having the highest nutritional value and it is beneficial for humankind. It may be recommended for the patients with a deficiency of pro-biotic and electrolytes.

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Disclaimers

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Informed consent

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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