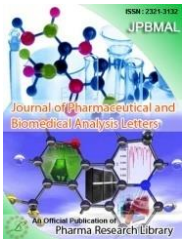

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Screening the efficacy of compounds from Ghee to control cancer through GC-MS Analysis

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A b s t r a c t

Ghee, a clarified form of butter, holds a revered status in Ayurveda as the healthiest source of edible fat, known for its diverse therapeutic and nutritional benefits. Traditionally integrated into the daily Indian diet and religious rituals, ghee is considered essential for promoting longevity, enhancing memory, and supporting the nervous system. Its unique ability to act as a vehicle for transporting herbal constituents to deeper tissues further underscores its medicinal value. Ayurvedic texts suggest that ghee improves digestion, absorption, and assimilation, while also lubricating the nervous tissue and enhancing bodily flexibility. However, from a modern scientific perspective, caution is warranted regarding excessive consumption. Ghee is rich in saturated fatty acids (65%), monounsaturated fatty acids (32%), and polyunsaturated fatty acids (3%), which, in high intake, may elevate cholesterol levels and increase the risk of cardiovascular diseases. Additionally, the role of lipid peroxidation and free radical damage in chronic diseases and aging is well-documented, linking excessive fat intake to pathological conditions such as ischemia, inflammation, stroke, and carcinogenesis. Thus, while ghee offers numerous health benefits as per Ayurvedic tradition, its consumption should be balanced and moderated in the context of modern health guidelines.

Keywords: Ghee, Ayurveda, Edible fat, Longevity, Nervous system, Digestion, Lipid peroxidation, Free radicals, Cardiovascular disease, Saturated fatty acids, Cholesterol, Traditional medicine.

A r t i c l e I n f o

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C o n t e n t s

1. Introduction

Dairy activities have long been an integral part of India's rural economy, serving as both a livelihood source and a cornerstone of the nation's nutritional framework. India ranks as the world's leading producer and consumer of dairy products, and the sector continues to show immense potential. According to the *India Dairy Products Market Forecast & Opportunities, 2017*, the dairy industry has significantly expanded within the food processing sector. With the adoption of hygienic and standardized practices, it holds promising opportunities for aligning with global quality standards. In recent decades, India's dairy market has experienced rapid growth, surpassing the global average

rate of expansion [1]. Among the wide range of dairy products, ghee stands out as the most valuable due to its nutritional richness, high market price, and distinct flavor. The origins of ghee, or *Ghrita*, trace back to ancient India around 1500 BC [2], with similar products reportedly used in the Middle East during the same era [3]. Known for its economic, nutritional, and sensory appeal, ghee continues to be a staple in Indian households. Its production can vary based on whether fermentation of milk to curd is carried out; alternatively, cream may be directly separated from milk and clarified through heat. "Desi ghee" refers specifically to the product derived from fermented milk, where the curd is churned into butter and then clarified to isolate the fat [4].

Various manufacturing techniques exist depending on the raw material (milk, cream, or butter), process stages, and desired final product (semi- or fully clarified ghee). Ghee is cherished not only for the pleasant taste it imparts to food but also for its reputed health benefits. It remains a preferred cooking fat across Indian households, with numerous established brands such as Amul, Patanjali, Nestle Every day, Gowardhan, and Britannia dominating the market. Nonetheless, while ghee contributes positively to health in moderate quantities, overconsumption may pose health risks due to its high cholesterol and saturated fat content [5].

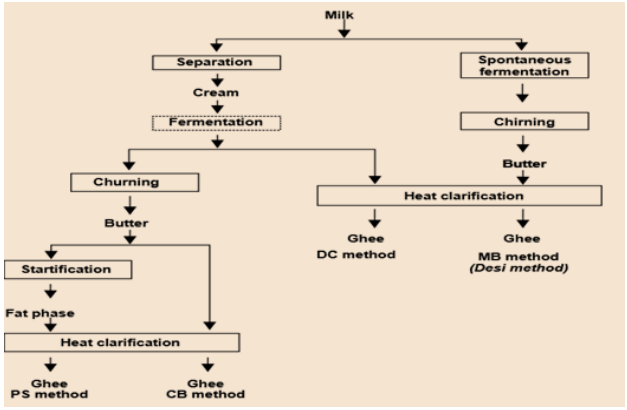

Figure.1. Flow diagram illustrating four methods of ghee manufacture: milk butter (MB) (desi); cream butter (CB); direct cream (DC); pre-stratification (PS).

Physico-Chemical Properties of Ghee

Milk fat is among the most complex natural lipids, and ghee is a refined form of this fat, commonly referred to as clarified butter or anhydrous milk fat. Its primary composition includes mixed glycerides, accounting for approximately 98% of its content. In addition to glycerides, ghee contains minor constituents such as free fatty acids, phospholipids, sterols, sterol esters, fat-soluble vitamins, carbonyl compounds, hydrocarbons, and carotenoids particularly present in cow-derived milk fat. Trace amounts of charred casein, calcium, phosphorus, and iron are also found. The moisture content in ghee is minimal, around 0.3%, contributing to its long shelf life [6]. Ghee is also known to contain conjugated linoleic acids (CLA), which have been reported to possess anti-cancer properties [7]. One of ghee's most distinguishing attributes is the rich flavor it imparts to food, which significantly enhances product acceptability. This characteristic flavor is primarily influenced by factors such as fermentation of cream or butter and the specific heat treatments applied during production. Key compounds responsible for its flavor include carbonyls, lactones, and free fatty acids [8].

The low moisture content in ghee makes it a highly stable fat, especially suitable for use in tropical climates where butter and cream may spoil quickly. Its shelf stability is further supported by its potential antioxidant properties, which help prevent lipid oxidation. Additionally, the presence of phospholipids (approximately 400 mg/kg), low acidity, and natural antioxidants are believed to contribute to its microbial resistance and extended shelf life [9]. These properties limit bacterial growth, making ghee safer and more durable than many other dairy fats.

Ghee is nutritionally beneficial for individuals across all age groups, serving as an excellent carrier for fat-soluble vitamins such as A, D, E, and K, along with essential fatty acids like linolenic and arachidonic acids. However, a concern with ghee consumption is its cholesterol content, which ranges from 0.2% to 0.4%. High intake can significantly contribute to dietary cholesterol, leading to increased health awareness among consumers and impacting the market demand for cholesterol-rich foods [10].

Recognizing the importance of ghee in the Indian diet, the Government of India has implemented quality and safety standards under the FSSAI (2011) and Agmark (1981) regulations to ensure product authenticity. Nevertheless, these standards are limited in their ability to detect adulteration, especially in identifying the type and concentration of added substances. This limitation arises due to the natural variability in the physico-chemical properties of milk fat, influenced by factors such as animal breed, diet, and management practices.

Gas chromatography-mass spectrometry (GC-MS) is a synergistic analytical method employed for the identification and quantification of compounds within a sample [11]. This technique holds a pivotal role in the analysis of phytochemicals and in conducting chemotaxonomic studies, particularly in medicinal plants containing biologically active constituents [12].

2. Methodology

Clarified ghee was obtained from the milk of Malnad Gidda cattle, a breed recognized by the National Bureau of Animal Genetic Resources (NBAGR), India.

Chemicals:

All the chemicals and reagents used for the research were of analytical grade.

Gas Chromatography–Mass Spectrometry

GC–MS analyses were performed using an Agilent 6890N gas chromatograph coupled with a 5973N mass selective detector (Agilent Technologies, Palo Alto, CA). A HP-5MS

capillary column (30 m × 250 µm i.d., film thickness 0.25 µm) was employed for chromatographic separation. A 1 µL aliquot of the sample was injected into the instrument under splitless mode. The oven temperature program was initiated at 50°C (held for 2 minutes), followed by a ramp to 380°C at a rate of 10°C/min, and held at this final temperature for 10 minutes. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The inlet and GC–MS interface temperatures were maintained at 250°C and 280°C, respectively, while the quadrupole analyzer temperature was set at 150°C. A solvent delay of 2 minutes was applied. For the mass spectrometry conditions, electron ionization (EI) mode was utilized with an EI source temperature of 230°C and an electron energy of 70 eV. The mass spectra were recorded at a scan rate of 1 scan/sec over an m/z range of 29–600. Additionally, chemical ionization (CI) experiments were conducted using methane as the reagent gas. For CI, the source temperature was maintained at 150°C, the electron energy was set at 140 eV, and the scan range was m/z 60–600.

Identification of Compounds:

The identification of components was accomplished by relying on their retention indices, and the interpretation of the mass spectrum was carried out utilizing the database of the National Institute of Standards and Technology (NIST). This extensive database encompasses over 62,000 patterns of known compounds. To elucidate the composition of the unknown components within the obtained ghee fraction, their spectra were systematically compared with the standard mass spectra of known components archived in the NIST library (NISTII). This comparative analysis facilitated the accurate identification of the constituents present in the sample.

Fig.2: Malnad Gidda

3. Results and Discussion

Fig.3: peak representing Cobalt

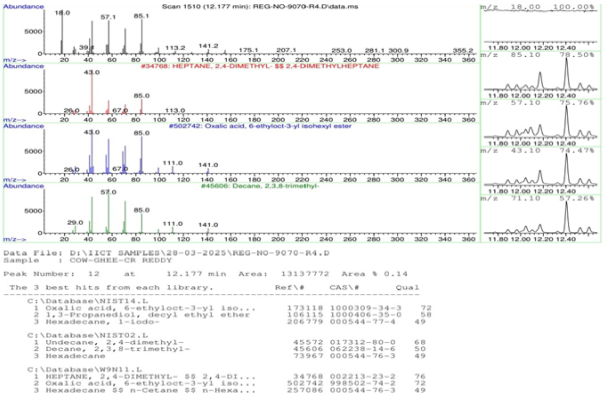

Fig.4: peak representing oxalic acid

Gas chromatography-mass spectroscopy profiling of ghee obtained from Malnad gidda: Multiple compounds have been identified through GC-MS, out of which some of the compounds present in ghee possess anti-cancer and anti-diabetic properties such as oxalic acid, 6-ethyloct-3yl iso, octacosane – iodo, docosane, tetradecanoic acid, indolizine-3-methyl and cobalt etc. as shown in fig 2 & 3.

4. Conclusion

Cobalt, a transition metal with versatile oxidation states and coordination chemistry, has emerged as a promising scaffold for the development of novel anticancer agents. Its complexes demonstrate unique biological interactions, attributed primarily to their redox properties, ability to generate reactive oxygen species (ROS), and capacity to bind with DNA and key intracellular targets. In silico studies including molecular docking, density functional theory (DFT), molecular dynamics simulations, and ADMET profiling have significantly advanced our understanding of the mechanisms by which cobalt-based compounds exert cytotoxic effects. Cobalt complexes exhibit strong binding affinities with cancer-related biomolecules such as DNA, topoisomerases, and kinases, disrupting essential cellular processes like replication, transcription, and signal transduction. Their ability to selectively induce oxidative stress in cancer cells, while minimizing damage to normal cells, positions them as an advantageous alternative to traditional platinum-based therapies. Additionally, in silico models predict favourable pharmacokinetics and safety profiles for several cobalt complexes, supporting their potential as lead candidates for further development. Overall, cobalt-based compounds, supported by computational insights, hold significant promise in the ongoing search for effective and selective anticancer agents. Continued integration of in silico techniques with *in vitro* and *in vivo* studies will be crucial to harness their full therapeutic potential and pave the way for next-generation metal-based chemotherapeutics.

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