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Research Article

GC-MS analysis of bio-active compounds in the methanolic extract of Ghee Residue

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Abstract

Ghee residue is a disuse by-product of ghee manufacturing industry and it is a rich potential source of natural antioxidants. It is used as the ingredient for the preparation of chocolates, cakes and cookies in many industries because of the presence of high protein content in it. The aim of this study is to identify and characterize the vital bioactive compounds from the methanolic extract of ghee residue by Gas chromatography and Mass spectroscopy (GC-MS). The GC-MS analysis of the methanolic extract revealed the presence of 30 bioactive compounds. Among the 30 compounds, the most predominant compounds are fatty acids like hexadecanoic acid, tetradecanoic acid, dodecanoic acid, octadecanoic acid and an amino acid N-glycyl L-threonine. This study forms a basis for the biological characterization and importance of the compounds which could be exploited for future development of drugs.

Keywords: GC-MS analysis; bioactive compounds; ghee residue; antioxidant.

1. Introduction

Ghee is known as *ghrta* [1] (commonly spelled *ghrita*) in Sanskrit. *Ayurveda* has traditionally considered ghee to be the healthiest source of edible fat, with many beneficial properties. According to *Ayurveda*, ghee promotes longevity and protects the body from various diseases [2]. In ancient India, ghee was the preferred cooking oil. It was considered pure and was felt to confer purity to foods cooked with it.

Traditionally ghee is made from cow milk or any other milk after churning curdled whole milk, separating the butter after fermentation, and clarifying it by heating it in pan on low flame. Alternatively, it is prepared by clarifying cream collected from raw whole milk at industrial scale. Ayurvedic classics describe eight kinds of ghee from eight different animal milk; among them ghee made from cow milk is said to be the superior [1]. Ghee is primarily used for cooking and frying and as dressing or toppings for various foods. It is also used in the manufacture of snacks and sweets [3].

Ghee residue is the disuse by-product of ghee manufacturing industry. Not much of the ghee residue is been used and it is considered as an expensive byproduct. Very few research is been carried out for the usefulness of ghee byproduct. The presence of the phenolic compounds in the ghee residue is used for increasing laccase production [4]. Lysine is appeared to be the first limiting amino acid in the ghee residue. If the lysine is supplemented with methionine, the ghee residue is appeared to be a good dietary feed ingredient containing high protein content for poultry [5]. Ghee residue fat (GRF) substituted for hydrogenated vegetable fat (vanaspathi) in preparation of nankatai type cookies and sponge cakes increased the protein content of the cakes and cookies compared with those made with vanaspathi alone [6]. The speculation showed that the ghee residue can be a good source of antioxidants. So, it can be used as a natural source of antioxidants for improving the shelf life of food products including dairy products where use of synthetic antioxidants is generally not preferred because of their toxic

effects. The presence of the bioactive compounds containing high medicinal values in the ghee residue has not been investigated. Hence, this study is undertaken to find out the bioactive compounds present in the ghee residue by using Gas chromatography and Mass spectroscopy (GC-MS) technique.

2. Materials and Methods

2.1 Preparation of ghee residue

Butter was collected from cow and buffalo milk. For making ghee, butter was heated to remove its moisture by evaporation. After heating, it was allowed to stand undisturbed for a while until the black colored residue was started precipitating down. Then, the precipitated residue was filtered out from the ghee by using sieve.

2.2 Preparation of extract

The ghee residue was collected and shade dried. The required quantity of the ghee residue was weighed, packed in Whatmann filter paper and transferred to the thimble. The Soxhlet extraction process was carried out using methanol (99.8% assay) as extraction solvent. The extract was concentrated using rotary evaporator (cyber Lab. model CR-2000).

2.3 GC-MS analysis

GC-MS analysis of the methanol extract of *the ghee residue* was performed using Thermo GC- Trace ultra version 5.0 and Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm films. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed. The injector was operated at 250 °C and the oven temperature was programmed from 70 °C with an increase of 6 °C /min to 260 °C. Mass spectra were taken at 70 eV and the total GC-MS running time was 37.50 min. The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The constituents were identified after comparison with those available in the computer library attached to the GC-MS instrument and the results obtained have been tabulated.

3. Results and Discussion

The present study was undertaken to find out the bioactive compounds present in the methanolic extract of *the ghee residue* by using Gas chromatography and Mass spectroscopy.

In this study, Butter collected from cow and buffalo milk was heated to remove its moisture and made it remain undisturbed until the black colored residue precipitated down. The precipitated residue was filtered out from the ghee. The soxhlet extraction process was done with methanol as a solvent and the extract was concentrated using rotary evaporator. The physical properties of ghee residue were tested and the results were given below.

3.1 Physical properties of ghee residue

Color: Dark brown

Texture: Soft earlier, hard and granular on storage

Odor: Pleasant

Taste: Slightly sweet

Solubility: Soluble in hot concentrated in H_2SO_4 and methanol.

3.2 GC-MS analysis

The methanolic extract of the ghee residue was analysed for the detection of bioactive compounds in it by using GC-MS. The GC-MS profile of methanolic extract of the ghee residue is represented in figure 2 which showed the prominent peaks for the presence of 30 bioactive compounds. The probable bioactive compounds corresponding to the peaks with their retention time (RT), molecular formula, molecular weight (MW), structure and concentration (peak area %) are tabulated in Table 1.

From the analysis, it is revealed that there are 30 probable bioactive compounds present in the ghee residue. Among these, there are 10 aromatic compounds, 16 aliphatic compounds and 4 cyclic compounds. The figure 1 represents the different type of compounds present in the ghee residue.

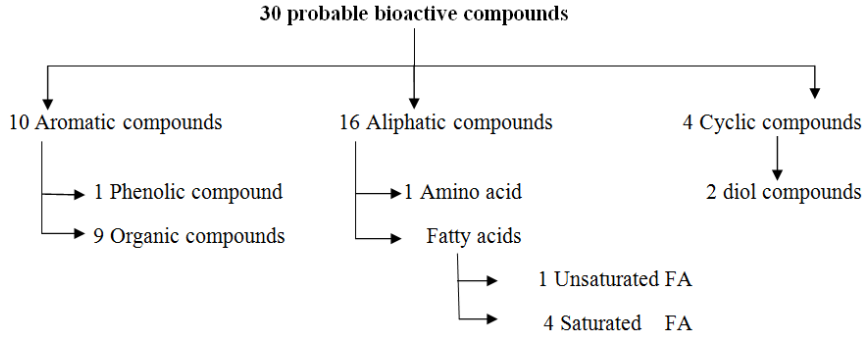


Figure 1: Different types of compounds present in the ghee residue

The phenolic compound is 6-hydroxy-mellein diacetate. The organic acid is methylsuccinic acid. The amino acid present in it is N-glycyl-L-threonine. The unsaturated fatty acid is octadecanoic acid and the saturated fatty acids are dodecanoic acid, tetredecanoic acid, pentadecanoic acid and hexadecanoic acid.

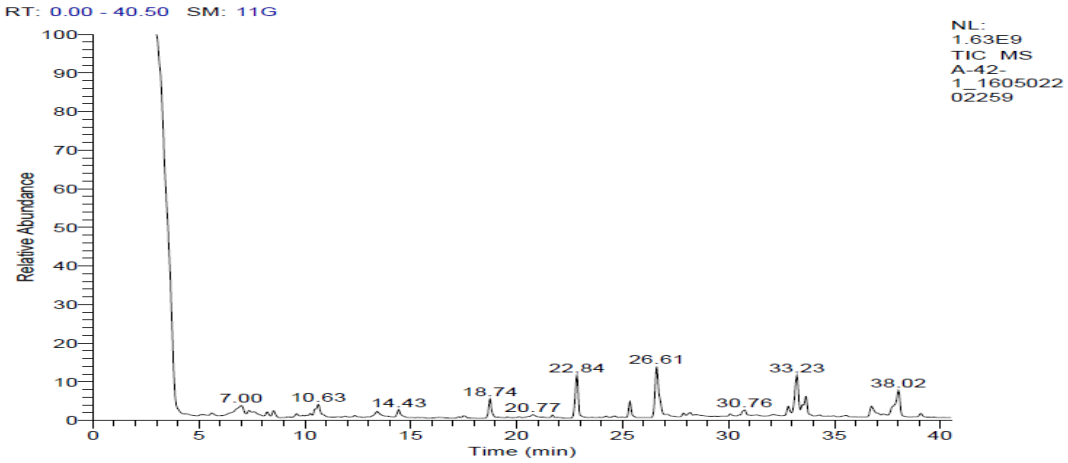


Figure 2: GC-MS Profile of Methanol extract of Ghee residue

Table 1: Bioactive components identified in the methanol extract of Ghee residue

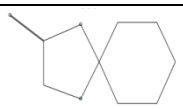
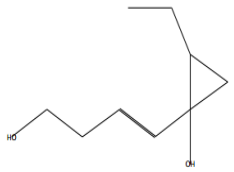
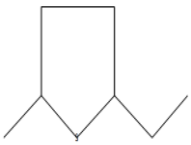
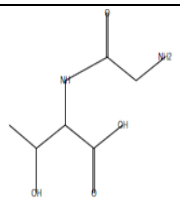
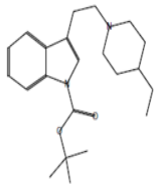
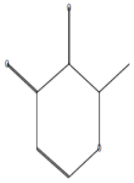
S. No	Retention Time	Compound name	Molecular Formula	Molecular weight	Area	Structure	Reference
1	3.21	1,4 Dioxaspiro(4.5)decane-2-one	$C_8H_{12}O_3$	156	24.10		[7,8]
2	5.11	(E)-2-Ethyl-1-(4-hydroxybut-1-enyl)cyclopropanol	$C_9H_{16}O_2$	156	0.49		[9,10]
3	5.60	2-Ethyl-5-methylthiophene	$C_7H_{10}S$	126	0.70		[11]
4	7	N-glycyl- L-Threonine	$C_6H_{12}N_2O_4$	176	2.74		[12]
5	7.37	4-Ethyl-1-[2-[1-(tert-Butoxycarbonyl)indol-3-yl]ethyl]pyrrilidine	$C_{22}H_{32}N_2O_2$	356	1.63		
6	8.22	Maltol	$C_6H_6O_3$	126	0.81		[13-15]

Table 1 continue.....

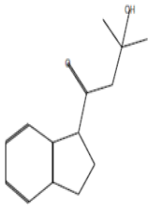
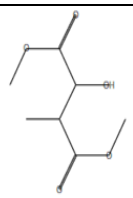
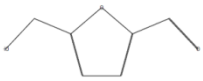
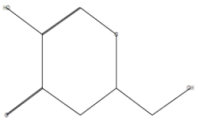
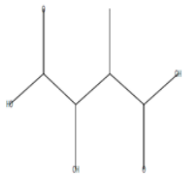
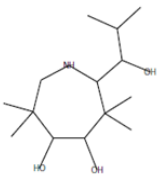
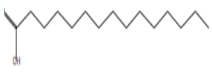
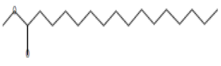
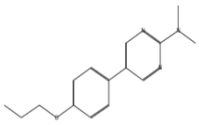
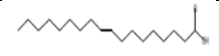
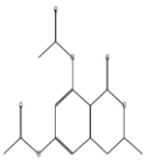
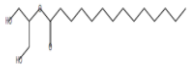
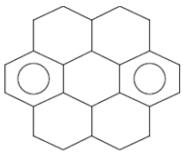
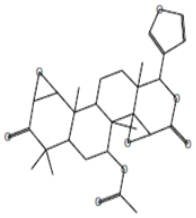
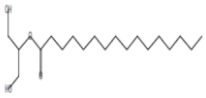
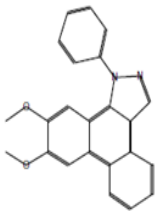
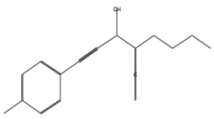
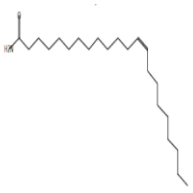
7	8.52	1-(2',3'-Dihydro-1H-inden-3'-yl)-3-hydroxy-3-methylbutan-1-one	$C_{14}H_{18}O_2$	218	1.25		[16,17]
8	9.60	Dimethyl ester of (2S,3R)-2-Hydroxy-3-methylsuccinic acid	$C_7H_{12}O_5$	176	0.65		[18]
9	10.63	5 Hydroxymethylfurfural	$C_6H_6O_3$	126	3.30		[19-21]
10	12.36	2 - hydroxymethyl - 5 - hydroxy - 2,3 - dihydro - (4H) - pyran - 4 - one	$C_6H_8O_4$	144	0.40		[16]
11	13.42	2-Hydroxy-3-methylsuccinic acid	$C_5H_8O_5$	148	1.62		[18]
12	14.43	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.62		[22-24]
13	17.51	Octadecane	$C_{18}H_{38}$	254	0.80		[25,26]
14	18.74	cis-N-2-(1-Hydroxy-2-methylpropyl)-3,3,6,6-tetrame thyl-1-azacycloheptane-4,5diol	$C_{14}H_{29}NO_3$	259	3.50		[27,28]
15	20.77	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.92		[29,30]
16	21.69	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.54		[31-34]
17	22.84	2-(Dimethyl amino)-5-(4-propyloxyphenyl)pyrimidin e	$C_{15}H_{19}N_3O$	257	8.28		[35-37]
18	25.35	Docosane (CAS)	$C_{22}H_{46}$	310	2.68		[38]
19	26.61	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	12.46		[34]
20	27.87	Monomethyl monobutyl "capped" tetraethylene glycol	$C_{13}H_{28}O_5$	264	0.58		[39]

Table 1 continue.....

21	28.18	6-hydroxy-mellein - diacetate	$C_{14}H_{14}O_6$	278	1.73		[40,41]
22	30.08	Tetradecanoic acid	$C_{17}H_{34}O_4$	302	0.40		[24-34]
23	30.76	Coronene	$C_{24}H_{24}$	312	1.57		[42]
24	32.14	Epoxygedunin	$C_{28}H_{34}O_8$	498	0.43		
25	32.82	Hexadecanoic acid	$C_{19}H_{38}O_4$	330	1.64		[32-33]
26	33.23	Dotriacontane (CAS)	$C_{32}H_{66}$	450	9.39		[43,44]
27	33.65	9,10-Dimethoxy-1-phenylphenanthro[9,10d]pyrazole	$C_{23}H_{18}N_2O_2$	354	4.67		[37-47]
28	36.75	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester(CAS)	$C_{21}H_{40}O_4$	356	2.90		[34,26]
29	38.02	(S)-(+)-1-(4'-Methylphenyl)-4-(n-butyl)hexa-4,5-dien -1-yn-3-o	$C_{17}H_{20}O$	240	7.49		
30	39.09	13-Docosenamide	$C_{22}H_{43}NO$	337	0.72		[48]

4. Conclusion

From the above (Table 1) identified bioactive compounds, Tetradecanoic acid, octadecanoic acid and hexadecanoic acid possess antibacterial activity [34,32]. Hexadecanoic acid gives surface protection against potentially invasive organisms functioning as an antistaphylococcal agent [32]. Tetradecanoic acid reduces the linear incursion of new blood cells into the central cornea finding application in treatment of corneal vascularisation [49] whereas Octadecanoic acid is a strong

antioxidant and antinflammatory agent [50,51]. Dodecanoic acid acts as a repellent to treat tick-born disease by protecting against tick bites [23]. Pyrazole holds potent antioxidant anti-inflammatory, antiallegry, anticancer and antimicrobial properties [47,37]. Pyrazole enhances the activity of ca^{2+}/calmodulin dependent protein kinase II (CaMKII) in reduced cognitive function [52] and also forms motifs forming a pharmacophore for active biological molecules [47]. Octadecane found in the ghee residue (table 1) is known to have antiproliferative activity [25]. Hydroxymethylfurfural is considered to be an important intermediate in the biorefinery because of its rich chemistry and potential availability from carbohydrates such as fructose, glucose, sucrose, cellulose and inulin [19]. Maltol functions as a preventive agent against diabetic complications [13] and it can be used against oxidative damage in the brain [14]. Methylsuccinic acid acts an effective inhibitor for carboxypeptidase A [18]. Docosane owns antimicrobial activity against both gram positive and gram negative bacteria [38]. Docosenamide in its biosurfactant owes to its anticancerous and antiviral activities [48]. Diacetate molecule is known to have both antipyretic and analgesic properties [40]. L-Threonine regulates the embryonic stem cells self-renewal and related signaling pathways [12].

Hence, the presence of various bioactive compounds in the *ghee residue* justifies the use of this in treating various ailments. Hence isolating and characterizing these compounds from the ghee byproduct will give a boost to the milk industry. Therefore, it is recommended as a source of pharmaceutical importance and can be used as target for many known and unknown ailments. Future work on this study is to isolate and characterize the useful bioactive compounds for treating various diseases.

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