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STARTLING IMPACT OF DAIRY WASTE EXTRACT ON PHARMACEUTICALS

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ABSTRACT

Since last decade, there has been much research work done in the potential health benefits of dietary plant polyphenols as antioxidant. Flavonoids and phenolic acids are the most persistent groups of plant phenolics and play a significant role in plants and human health, so it is important to study their potentials as therapeutic agents. Some types of phenolics were expected from diary waste "Ghee residue". As phenolic compounds are also found as secondary metabolites in green plants, these phenolic compounds get accumulated in the milk of herbivores. The present article is to unmask the potentiality of Ghee residue (an uncommercial Dairy waste) in Pharmaceuticals. In addition to its application as a natural source of antioxidants, it also contains a number of Bio-active compounds. An endeavor of extracting and identifying bio-active compounds by GC/MS, uncovered the proximity of some industrial and pharmaceutically applicable bio active compounds like phenol, 2, 5-bis (1, 1-dimethyl ethyl), 17-Octadecynoic acid, Ethaneperoxoic acid,1-cyano-1-[2-(2-phenyl-1,3-dioxalan-2yl)ethyl]pentyl ester, Isopropyl palmitate, (E,Z)-2,13-octadecadien-1-ol, 9, 12-Octadecadienoyl chloride, (Z,Z)-, Eicosanoic acid, Oleic acid and Squalene.

KEYWORDS: Dairy waste "Ghee residue", Poly phenols, Antioxidants, Pharmaceuticals, GC/MS.

INTRODUCTION

Characteristic compounds have filled in as a critical wellspring of medications since ancient times and about portion of the helpful drugs today are derived from natural sources.

A bio-active compound affects a living organism, tissue or cell obtained from plant, animal or artificially delivered. They are not basic to the manageability of a body but rather have an impact on wellbeing. Various bioactive substances have been appeared to go about as an antioxidant.[1]

The investigation of bio-active secondary metabolites, customarily completed for the most part by physicists, has progressively pulled in the consideration of pharmacologists, scholars, botanists, agronomists, and so forth, fortifying helpful work.[2]

Customarily ghee is produced using cow milk or some other milk subsequent to beating coagulated entire milk, separating the butter after aging, and heating it by warming it in container on low fire. On the other hand, it is set up by elucidating cream gathered from crude entire milk at modern scale. Ayurvedic works describe eight sorts of ghee from eight distinctive animal milk; among them ghee produced using cow milk is said to be the predominant.[3] Ghee is basically utilized for cooking and searing and as dressing or garnishes for different nourishments. It is likewise utilized as a part of snacks and desserts.[4]

The hypothesis demonstrated that the ghee residue can be a decent antioxidant. Along these lines, it can be utilized as a characteristic antioxidant for enhancing the time span of usability of nourishment items. It also includes dairy items where utilization of synthetic antioxidants for the most part is not favored on account of their harmful impacts. The nearness of the bioactive compounds containing high therapeutic esteems in the ghee residue has not been researched. The aim of this research is to extract phenolics (the most prominent antioxidant) in higher concentration.[5] Consequently, this investigation is attempted to discover the bioactive compounds exhibited in the ghee residue by utilizing Gas chromatography and Mass spectroscopy (GC-MS) technique.

MATERIALS AND METHODS

Sample

5 g of Ghee residue (GR) was altogether boiled in refined water enthusiastically by keeping the heap of GR inundated in the boiling water for 30 min. The package was evacuated and crushed to deplete out the rest of the water. The filtrate meant as Ghee residue filtrate (GRF) was additionally utilized for extraction of phenolics and other bioactive compounds. It was then treated with ethyl acetate derivation in a 250 ml conical flask. With the assistance of a

separating funnel, the organic layer containing phenolics and other bioactive compounds was isolated from the aqueous layer. The extraction procedure was performed in triplicates.

Preparation of concentrate

The above sample was concentrated utilizing rotary evaporator (cyber Lab. model CR-2000).

GC-MS analysis

The concentrated GRF sample extract of ethyl acetate was again extracted with ethanol to analyze through Gas chromatography –Mass spectrometry for identification of different compounds.It was performed using equipment Scion 436-GC Bruker with column BR-5MS (5%Diphenyl/ 95% Dimethyl poly Siloxane), 30m × 0.25mm ID × 0.25µm df.

Helium gas was utilized as a carrier gas at a consistent stream rate of 1 ml/min, and an infusion volume of 2µl (split ratio of 10:1) was applied. The injector worked at 280 °C and the oven temperature was modified from 110 °C hold for 3.50 min up to 200°C with an expansion of 10 °C /min then 5°C/min to 280 °C. Mass spectra were taken in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min. and the aggregate GC-MS running time was 40.50 min.[6]

RESULTS AND DISCUSSION

The present investigation was attempted to extract higher concentrations of phenolics[5] and discover the bioactive compound present in the ethyl acetate concentrated extract of the ghee residue later extracted with ethanol by utilizing Gas chromatography and Mass spectroscopy.

The GC-MS profile of ethyl acetate extract of the ghee residue is represented in figure.1 which showed the prominent peaks for the presence of 19 bioactive compounds.

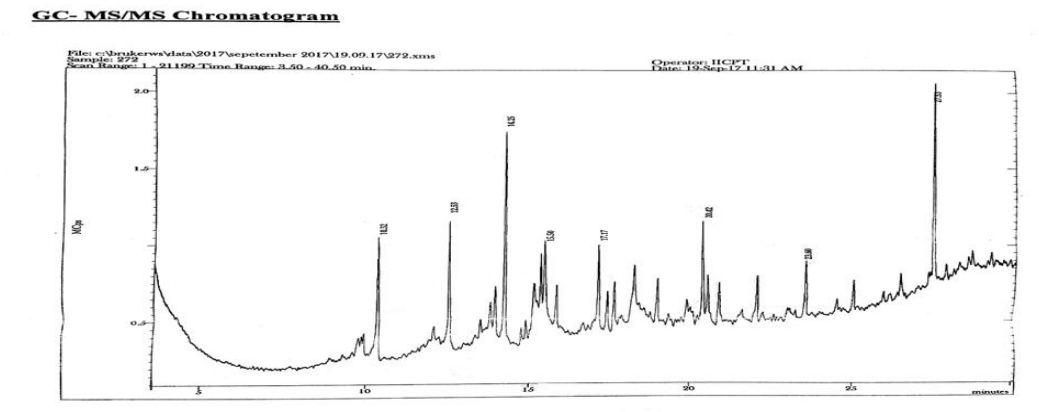
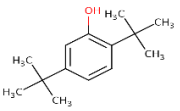
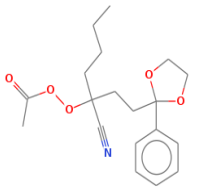


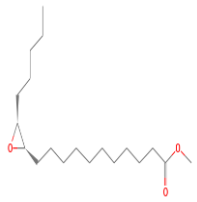
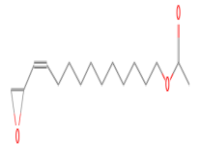
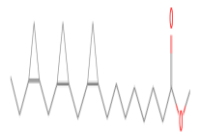
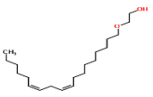
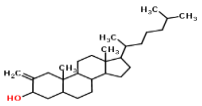
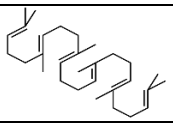
Fig.1

GC/MS Chromatogram of Ethyl acetate extract of GRF.

The 19 distinguished bioactive compounds from GRF extract were tabulated in Table.1 with their respective structures, molecular weight, retention time (RT) and their percentage of peak area. The RT of identified bioactive compounds was tabulated in increasing order. Phenol, 2,5-bis(1,1-dimethyl ethyl)- is with minimum RT of 9.91 and Squalene with maximum of 27.53.

Table 1: Identified Bioactive compounds in ethyl acetate extract of Ghee residue by GC-MS.

S.N0	Name of the Compound	Molecular Formula	Molecular Wt.	Structure	RT	Peak Area %
1	Phenol, 2,5-bis (1,1-dimethyl ethyl)-	$C_{14}H_{22}O$	206		9.91	1.48
2	1-Heptadecyne	$C_{17}H_{32}$	236		10.32	7.45
3	1-Octadecyne	$C_{18}H_{34}$	250		12.53	5.85
4	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280		13.96	4.73
5	1-Hexadecyne	$C_{16}H_{30}$	222		14.25	11.19
6	Oleic acid	$C_{18}H_{34}O_2$	282		15.17	4.73
7	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxalan-2yl)ethyl]pentyl ester	$C_{19}H_{25}NO_5$	347		15.37	6.68
8	Isopropyl palmitate	$C_{19}H_{38}O_2$	298		15.50	5.94
9	13-Heptadecyne-1-ol	$C_{17}H_{32}O$	252		15.88	2.20
10	Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268		17.17	4.56

11	Oxiraneundecanoic acid,3-pentyl-,methyl ester, trans-	$C_{19}H_{36}O_3$	312		17.68	4.82
12	Eicosanoic acid	$C_{20}H_{40}O_2$	312		18.29	5.73
13	9, 12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298		19.02	2.67
14	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268		20.42	6.89
15	Cyclopropaneoctanoic acid, 2-{[2[(2ethylcyclopropyl)methyl] cyclopropyl]methyl]-methyl ester	$C_{22}H_{38}O_2$	334		20.57	4.86
16	Z E-2,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266		22.10	1.59
17	Ethanol, 2-(9,12-Octadecadienyloxy)-, (Z,Z)-	$C_{20}H_{38}O_2$	310		23.60	3.36
18	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	400		25.08	1.77
19	Squalene	$C_{30}H_{50}$	410		27.53	13.50

19 Bioactive compounds in Ghee residue extract with their respective molecular formulae, molecular weight, structures, peak areas and retention time.

The known biological activities and pharmaceutical applications of some of the essential bioactive compounds from the above table (Table 1) were discussed in Table 2.

Table. 2: Sources and Biological applications of some of the most important bioactive compounds identified in Ghee residue extract.

S.No	Bioactive compound	Source	Biological activity	References
1.	Phenol, 2, 5-bis(1,1-dimethyl ethyl)-	*Calotropis gigantean Michelia nilagirica*	Intermediates, Oxidizing/reducing agents, Process regulators, Processing aids, antibacterial and anti-inflammatory, a-glucosidase inhibition antioxidant, UV stabilizers and anticancer properties,	[7], [8], [9], [10], [11]
2.	17-Octadecynoic acid	-	improves contractile response to angiotensin Ⅱ by releasing vasoconstrictor prostaglandins	[12], [13]
3.	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxalan-2yl)ethyl]pentyl ester	*Aerva lanata*,	Anti-microbial, anti-inflammatory and insecticidal properties.	[14], [15], [16]
4.	Isopropyl palmitate	*Psidium salutare* fruits and boiled buckwheat flour	emollient, moisturizer, skin conditioning, thickening agent, and anti-static agent	[17], [18], [19]
5.	(E,Z)-2,13-octadecadien-1-ol	*Moringa oleifera*	Promising sources of novel antibiotic prototypes.	[20]
6.	Eicosanoic acid	*Arachis hypogea*from, inside the human body, particularly in the muscles, liver, and brain	Photographic materials and lubricants, muscle inflammation properties, bodybuilding supplement, treatment for depression in the form of fish oils, treating arthritis	[21], [22], [23], [24], [25], [26], [27]
7.	z-[13, 14-epoxy]tetradec-11-en-1-ol acetate	*Vernonia amygdalina*	Antioxidant, Haemolytic activity.	[28]
8.	Oleic acid	primer pheromone in Honeybees,	solvent for pharmaceutical drug, lubricant and a plasticizer, vehicle for intramuscular drug delivery, responsible for the hypotensive, increase high-density lipoprotein (HDL) cholesterol, excipient in pharmaceuticals, and it is used as an emulsifying or solubilizing agent	[29], [30], [31], [32]
9.	9, 12-Octadecadienoyl chloride, (Z,Z)-	*Wrightia tinctoria*	Phytopharmaceutical importance, Antisecretory Antispermigenic, Antitonsilitic, Antitubercular, Choleretic, and Contraceptive.	[33], [34]
10.	Squalene	shark liver oil, Amaranth seed, rice bran, wheat germ, and olives, Natural found as biochemical intermediate in plants, animals and humans	Precursor for synthesis of all plant and animal sterols, including cholesterol and steroid hormones in the human body, In plants, it is the precursor to stigmasterol. In certain fungi, it is the precursor to ergosterol, Derivatives are used as a skin moisturizer in cosmetics, Antitumor, Cancer preventive, Antibacterial, Antioxidant, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide, Use as an adjuvant in vaccines	[35], [36], [37], [38], [39], [40]

The most important bioactive compounds found in plant extracts with references, also found in Ghee residue.

CONCLUSION

With reference to the above discussion, most of the pharmaceutically applicable bioactive compounds were extracted from plant sources. All these bioactive compounds were together found in ethyl acetate concentrates of ghee residue. The fact might be the source of ghee residue (milk) which is obtained from a herbivore.

In contrast to the available literature and survey reports obtained from dairy industries, every dairy industrialist is looking forward for maximum marketable value to the ghee residue. Hence with the help of this research work I would like to expose the applications of dairy waste “ghee residue” in pharmaceuticals.

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