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Original Article

Identification of Bioactive Metabolites in *Bhadrikadi Ghrita*: A Phytochemical and GC-MS Approach to Investigating Analgesic and Anti-inflammatory Properties

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ABSTRACT

Background: Ayurveda healthcare system holds considerable reputation in today's era. Treatises of Ayurveda explained numerous formulations with their potential therapeutic benefits but their global acceptance is poor due to the lack of scientific validity. Bhadrikadi Ghrita is one such formulation indicated for *Marmakshat Ruja*. Thus, there is an obvious need to overcome this lacuna by subjecting these medicines to thorough tests in light of modern research about their pharmacological, pharmacokinetic, and toxicity standards. **Objectives:** The present study employs Gas Chromatography-Mass Spectrometry (GC-MS) analysis to investigate pain-relieving Ayurvedic Ghee, *Bhadrikadi Ghrita*, aiming to establish correlations between its medicinal activity and the biomolecules it contains. **Materials and Methods:** *Bhadrikadi Ghrita* was prepared according to Ayurvedic standards and subjected to GC-MS analysis. The detected compounds were identified by comparing their mass spectra with standard libraries and previous literature. A functional classification of the bioactive compounds was conducted to understand their potential therapeutic implications. **Results and Discussion:** The resulting profile revealed the presence of diverse array of bioactive compounds, including fatty acids, terpenoids, phenolic derivatives, and sterols. Notable compounds included linoleic acid, oleic acid, squalene, and curcumene. These constituents are associated with anti-inflammatory, antioxidant, antimicrobial, and neuroprotective properties. **Conclusion:** The identification of these bioactive compounds supports the therapeutic potentials of *Bhadrikadi Ghrita* as an effective pain-relieving ghee in *Marmakshat Ruja,* warranting further pharmacological and clinical studies.

Keywords: *Bhadrikadi Ghrita*, Bioactive Metabolites, GC-MS, *Marmakshat Ruja*, Phytochemical Analysis, Traditional Medicine.

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INTRODUCTION

Contemporary and alternative medicines, including Ayurveda, have been prevalent for centuries. Despite its 3,000-year history, Ayurveda has not received the recognition it deserves globally, due to insufficient scientific evidence. Challenges such as poor-quality control in Ayurveda formulations can lead to adulteration, contamination, and variability in active compounds, affecting therapeutic outcomes and safety.[1] Therefore, scientific validation and standardization are crucial. *Brihatrayi*[2-5] highlights the use of Ghritapana for pain relief and assisting the recovery from surgical injuries**,** *Bhadrikadi Ghrita* is one such ghee based Polyhedral formulation mentioned in *Sahasrayoga*,[6] and it is indicated in *Marmakshat Ruja* (pain due to Vital Part injury). *Bhadrikādi Ghṛita* is a traditional Ayurvedic medicated ghee formulated with *Bhadrika* (*Aerva lanata*), *Kataka* (*Strychnos potatorum*), *Sharkara* (*Saccharum officinarum*), *Goghrita* (clarified butter), and Goksheera (cow's milk) as its key ingredients.

However, to ensure their efficacy, safety, and consistency in modern healthcare, there is a pressing need for analytical methods that can provide reliable data on their chemical composition and quality.[7] Gas Chromatography-Mass Spectrometry (GCMS) offers a robust standardization and quality control method of Ayurvedic products. It identifies and quantifies a sample's chemical components and ensures the consistency of ingredients across different batches, facilitating the Pharmacological Authentication and relating its bioactive molecules to identified pharmacological actions, These analyses would provide an in-depth understanding of the chemical profile of the formulation, Bridging Traditional Knowledge with Modern Science,[8-11] This method is vital for analyzing complex mixtures, like herbal formulations, where

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multiple active compounds contribute to the overall therapeutic effects.

In the context of *Bhadrikadi Ghrita*, GC-MS analysis of the acetone extract has been essential in revealing the key chemical components that contribute to its analgesic and therapeutic effects. Such detailed chemical profiling links traditional knowledge with modern scientific methodologies, providing a scientific basis for the formulation's therapeutic efficacy. By bridging the gap between ancient Ayurvedic wisdom and contemporary scientific analysis, the application of GC-MS in this study enhances the reliability and standardization of *Bhadrikadi Ghrita*, supporting its clinical and pharmacological applications while ensuring consistency, efficacy, and safety. Overall, this approach underscores the importance of integrating modern analytical techniques like GC-MS into the study and standardization of Ayurvedic formulations, thus ensuring the quality and therapeutic potential of products like *Bhadrikadi Ghrita* in contemporary medical practice. This analysis could provide insights into the potential mechanisms behind the medicinal properties claimed by Ayurveda.

MATERIALS AND METHODS

Bhadrikadi Ghrita was obtained from a GMP-certified KLE Ayurveda pharmacy in Belgaum and was subjected to GC-MS analysis following standard procedures.

Study conduction

The GCMS study was conducted by Amrith Pvt. Lab, Nisargam Pvt. Ltd., Shivamogga. Karnataka, the phytochemical study was conducted by KLE Shri BMK Ayurveda Mahavidyalaya, Belagavi Karnataka.

Instrument

The GC-MS instrument, model QP 2010SE, is widely used for precise qualitative and quantitative analysis in various applications, offering high sensitivity and reliability.

Sample Preparation

Bhadrikadi Ghrita was obtained from GMP certified KLE Ayurveda pharmacy, a trusted supplier of genuine Ayurvedic products. The preparation for analysis involved several steps:

Preparation: The formulation was thoroughly inspected and homogenized to ensure uniformity before extraction.

Acetone Extraction: 10 mL of acetone was used to extract 1 g of the ghrita sample. After any solid residues were eliminated by filtering the extract, a clear solution was obtained for additional analysis.

Analysis: GC-MS Analysis to analyze the filtered acetone extract (1 µL), gas chromatography-mass spectrometry was used.

Phytochemical screening qualitative analysis

The phytochemical investigation revealed the presence of various bioactive constituents, including sterols, triterpenoids, fatty acid, alkaloids, flavonoids, tannins, polysaccharides, sugars, starch, and saponins. These compounds were identified through the analysis of multiple solvent extracts, following a standardized methodology, ensuring a comprehensive profile of the active components.

Chromatography and Mass Spectrometry Method

A sample weighing 1 g was extracted using 10 mL of acetone, and 1 µL of the resulting extract was injected into the analytical system. The injection was executed in split mode at a temperature of 280°C, with a split ratio of 10:1. Flow control was established using linear velocity, with a pressure setting of 24.2 kPa, the overall flow rate is 19.5 mL/min., and a column flow rate of 1.50 mL/min. The linear velocity was consistently maintained at 45.1 cm/sec, accompanied by a purge flow of 3.0 mL/min. The temperature program for the oven commenced at 80°C, held for 2 min, then increased to 280°C at a rate of 10°C/min, where it was maintained for 10 min before rising to 330°C at a rate of 20°C/min, holding for an additional 5 min. The temperatures for the ion source and interface were set at 200°C and 300°C, respectively. The solvent cut time was established at 1.40 min, and the detector gain mode was adjusted relative to the tuning result at 0.95 kV. The scan range for m/z values extended from 35.00 to 500.00, with a scan speed of 1666 and an event time of 0.30 sec. Data acquisition commenced at 2.00 min and concluded at 33.00 min. This methodology, including the pressure settings, facilitates accurate compound detection, establishing a reliable framework for GC-MS analysis.

RESULTS

The phytochemical analysis of *Bhadrika* and *Nirmali Beej* (Seed) revealed different compositions based on the extraction method. *Bhadrika* water extract contained carbohydrates, reducing sugars, flavonoids, alkaloids, cardiac glycosides, and saponin glycosides, whereas its alcohol extract showed only carbohydrates. In contrast, *Nirmali Beej's* water extract had carbohydrates, reducing sugars, and saponin glycosides, while its alcohol extract also contained only carbohydrates. These results emphasize the significance of using appropriate extraction techniques to identify active compounds effectively. The *Bhadrikadi Ghrita* sample, procured from KLE GMP Ayurveda Pharmacy, underwent comprehensive testing to ensure quality and safety. The organoleptic analysis confirmed a lemon-yellow color, rich characteristic odor, and sweet taste.

Physicochemical evaluations showed a loss on drying of 0.196%, refractive index of 1.476, specific gravity of 0.942, acid value of 3.449, iodine value of 17.206, and saponification value of 157.77: using. Tests using microbiology verified that Pathogens such

as *P. aeruginosa, E. coli, S. aureus*, and *S. albany* were tested, with bacterial and fungal counts well within permissible limits, confirming compliance with standard specifications. *Bhadrikadi Ghrita* contains a variety of bioactive compounds that contribute to its therapeutic potential. Anti-inflammatory and pain-relieving effects are attributed to compounds like glyceraldehyde, cyclohexene, Caryophyllene oxide, and Tumerone, which also exhibit neuroprotective properties beneficial for managing neurodegenerative disorders.

Antimicrobial and antioxidant activities are enhanced by 2-decenal, Caryophyllene oxide, and Tumerone, while Hexanoic acid esters and 2-pentadecanone provide significant antibacterial benefits. Asarone further demonstrates anti-anxiety, anti-depressant, and anti-cancer properties, along with neuroprotection. Additionally, pharmacologically active compounds such as squalene, octane, and Hexanoic acid derivatives show promise for combating inflammation, oxidative stress, and microbial infections, making the formulation valuable in addressing chronic diseases and supporting neurological health. Figure 1 presents the Gas Chromatography-Mass Spectroscopy (GC-MS) chromatogram of the acetone-extracted *Bhadrikadi Ghrita*, showing distinct peaks corresponding to various bioactive metabolites. The retention times, peak areas, and identified compounds from the acetone extract are detailed in Table 1. The analysis revealed the presence of key bioactive compounds such as Nootkatone ($C_{15}H_{22}O$), Cyclohexene-1-carboxylic acid, 1,2-bis(2-ethyl-1-oxobutoxy)-, ethyl ester ($C_{21}H_{34}O_6$), Asarone ($C_{12}H_{16}O_3$), and Caryophyllene ($C_{15}H_{24}O$) (Figure 2). Additionally, Curlone (C15H22O), 2H-Pyran-2-one ($C_{14}H_{26}O_2$), E, E-2,13-Octadecadien-1-ol ($C_{18}H_{34}O$), and Hexanoic acid, 4-hexadecyl ester ($C_{22}H_{44}O_2$) were identified in the acetone extract (Figure 3). These bioactive metabolites exhibit analgesic, anti-inflammatory, antimicrobial, and antioxidant activities, supporting the traditional therapeutic use of *Bhadrikadi Ghrita*. The pharmacological properties of these identified compounds are summarized in Table 2.

DISCUSSION

Bhadrikadi Ghrita is a traditional medicinal formulation used for relieving pain, yet its medicinal benefits lack scientific validation. This study may be the first to report its potential role in pain management. The compounds discussed in this study include Glyceraldehyde, an intermediate in carbohydrate metabolism. Its phosphorylated form, glyceraldehyde-3-phosphate (G3P), is crucial in glycolysis and the Calvin cycle, supporting energy production, redox balance, and biosynthesis.[12] 4-Hydroxy-4-methyl-2-pentanone, a hydroxy ketone, may act as an antioxidant by donating hydrogen atoms to neutralize free radicals. Its potential antibacterial activity could arise from interactions with bacterial membranes or proteins, showing significant action against both Gram-positive and Gram-negative bacteria, similar to other hydroxyl-containing compounds,[13-15] 2-Decenal, an unsaturated aldehyde, exhibits antimicrobial potential due to its reactive nature, which disrupts microbial proteins and cell walls, thereby inhibiting bacterial and fungal growth.[16-18]

Decenal exhibits antioxidant properties, as evidenced by its performance in DPPH free radical scavenging and β-carotene bleaching assays.[19] Similarly, cuparene, a sesquiterpene found in essential oils, interacts with microbial membranes through lipophilic mechanisms and has demonstrated activity against bacteria like *Bacillus subtilis* and *Staphylococcus aureus*, Cuparene reduces inflammation by suppressing the NF-κB signaling pathway.[20-22] While direct evidence on nitroisobutylglycerol antimicrobial effects is limited, nitro derivatives are generally reactive and may possess such properties. Its role as a precursor in energetic material synthesis suggests possible microbial

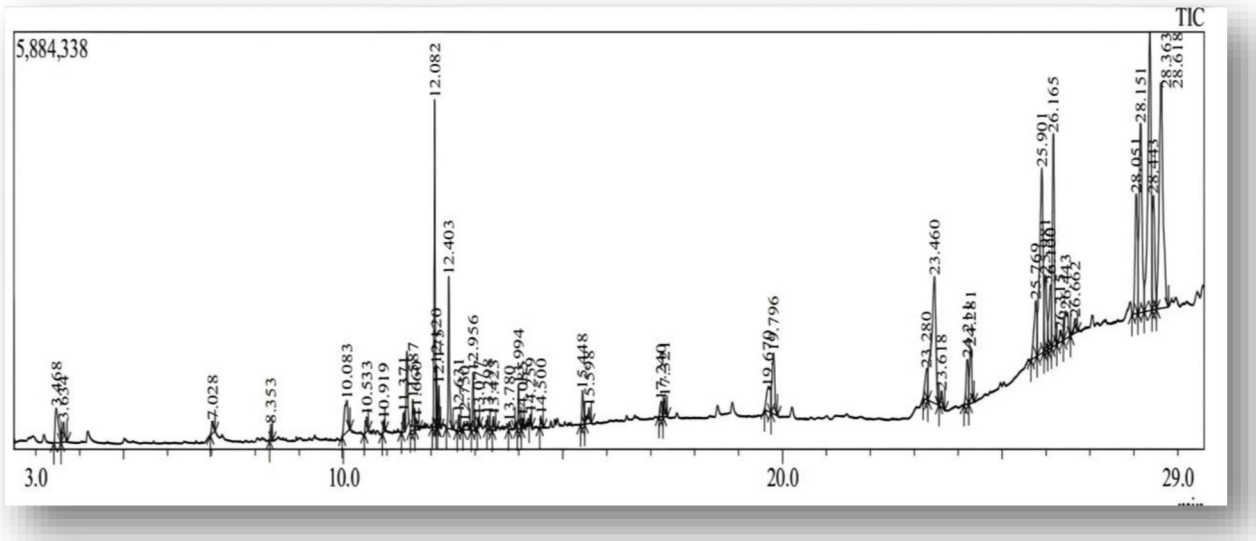


Figure 1: Gas chromatography-mass spectroscopy chromatogram of acetone extract of bhadrikadi Ghrita.

Table 1: Phytocomponents detected in the acetone-extracted *Bhadrikadi Ghrita* using GC-MS.

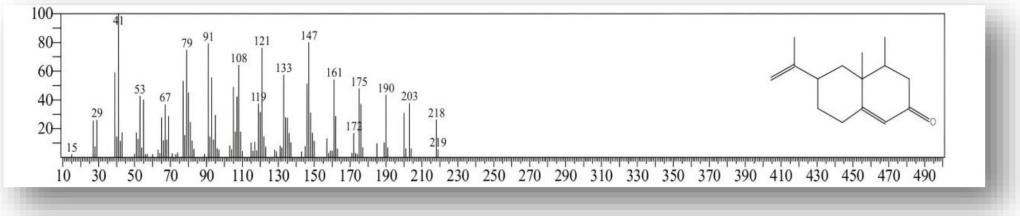
Sl. No.	Retention time	Start	End	Area	Area %	Height	Height %	Factor	Name
1	3.468	3.41	3.575	2530751	1.57	484034	1.15	5.23	Glyceraldehyde
2	3.634	3.575	3.71	1021906	0.63	280981	0.67	3.64	2-Pentanone, 4-hydroxy-4-methyl-
3	7.028	6.975	7.08	515252	0.32	168982	0.4	3.05	Glyceraldehyde
4	8.353	8.325	8.39	331544	0.21	226448	0.54	1.46	2-Decenal, (E)-
5	10.083	9.975	10.15	2477449	1.53	444405	1.05	5.57	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
6	10.533	10.48	10.57	539077	0.33	204479	0.48	2.64	Benzene, 1-(1,5-dim ethyl-4-hexenyl)-4-methyl-
7	10.919	10.89	10.95	266266	0.16	166520	0.39	1.6	Cyclohexene, 3-(1,5-dim ethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-
8	11.371	11.325	11.405	531480	0.33	259727	0.62	2.05	2-Methyl-6-(p-tolyl) hept-2-en-4-ol
9	11.587	11.53	11.63	1046872	0.65	430145	1.02	2.43	Asarone
10	11.66	11.63	11.72	417225	0.26	159475	0.38	2.62	Caryophyllene oxide
11	12.082	12.03	12.11	7721870	4.78	4612013	10.92	1.67	AR-Turmerone
12	12.12	12.11	12.15	1191022	0.74	873070	2.07	1.36	Tumerone
13	12.175	12.15	12.215	930635	0.58	593340	1.41	1.57	2-Pentadecanone
14	12.403	12.35	12.495	4071298	2.52	2139934	5.07	1.9	Curlone
15	12.631	12.6	12.675	415563	0.26	228316	0.54	1.82	(3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2H-3a,7-methanoazulen-2-one
16	12.75	12.725	12.88	527295	0.33	95182	0.23	5.54	(6R,7R)-Bisabolone
17	12.956	12.915	13.005	1529558	0.95	811656	1.92	1.88	(E)-Atlantone
18	13.077	13.05	13.1	212692	0.13	146778	0.35	1.45	Heptadecanal
19	13.298	13.275	13.34	312052	0.19	185480	0.44	1.68	Perilla alcohol tiglate
20	13.423	13.39	13.47	390264	0.24	190309	0.45	2.05	Nootkatone
21	13.78	13.76	13.915	237806	0.15	94896	0.22	2.51	6-(5-Hydroxy-4-methylidenecyclohex-2-en-1-yl)-2-methylhept-2-en-4-one
22	13.994	13.96	14.055	1121584	0.69	605869	1.43	1.85	2H-Pyran-2-one, tetrahydro-6-nonyl-
23	14.085	14.055	14.115	247442	0.15	148062	0.35	1.67	Turmeronol A
24	14.259	14.235	14.285	161033	0.1	133437	0.32	1.21	Hexadecanoic acid, ethyl ester
25	14.5	14.47	14.55	347564	0.22	156270	0.37	2.22	E,E-2,13-Octadecadien-1-ol
26	15.448	15.405	15.5	1154896	0.72	476895	1.13	2.42	2H-Pyran-2-one, tetrahydro-6-undecyl-
27	15.598	15.5	15.645	856520	0.53	219641	0.52	3.9	1,2-Dicaprin
28	17.24	17.185	17.28	715250	0.44	213485	0.51	3.35	Hexanoic acid, 5-tetradecyl ester
29	17.321	17.28	17.375	700539	0.43	264891	0.63	2.64	Lauric acid, 2-methylbutyl ester

Sl. No.	Retention time	Start	End	Area	Area %	Height	Height %	Factor	Name
30	19.67	19.59	19.74	2294057	1.42	329060	0.78	6.97	Myristin, 1,3-diaceto-2-
31	19.796	19.74	19.88	3543025	2.19	855284	2.03	4.14	Butyric acid, 3-pentadecyl ester
32	23.28	23.2	23.31	1877750	1.16	436005	1.03	4.31	Hexanoic acid, 4-hexadecyl ester
33	23.46	23.31	23.555	12374342	7.66	1785569	4.23	6.93	Octane, 1,1'-oxybis-
34	23.618	23.565	23.695	762165	0.47	227122	0.54	3.36	Butyric acid, 4-pentadecyl ester
35	24.211	24.13	24.24	2245229	1.39	627127	1.49	3.58	Squalene
36	24.281	24.24	24.36	2744197	1.7	787483	1.86	3.48	Hexadecanoic acid, 2-(acetyloxy)-1-[(acetyloxy) methyl]ethyl ester
37	25.769	25.655	25.795	3571258	2.21	792532	1.88	4.51	Glycerol tricaprylate
38	25.901	25.795	25.955	14386237	8.91	2608644	6.18	5.51	2-Cyclohexene-1-carboxylic acid, 1,2-bis(2-ethyl-1-oxobutoxy)-, ethyl ester
39	25.981	25.955	26.04	3892768	2.41	1074378	2.54	3.62	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester
40	26.1	26.04	26.12	3317400	2.05	886011	2.1	3.74	Glycidyl palmitate
41	26.165	26.12	26.245	9365389	5.8	2998793	7.1	3.12	Tributyrin
42	26.315	26.245	26.39	643883	0.4	154343	0.37	4.17	Hexanoic acid, 4-hexadecyl ester
43	26.443	26.39	26.555	2205528	1.37	392278	0.93	5.62	9-Octadecenoic acid (Z)-, 2-(acetyloxy)-1-[(acetyloxy) methyl]ethyl ester
44	26.662	26.555	26.71	697919	0.43	206122	0.49	3.39	Octadecanoic acid, 2,3-bis(acetyloxy)propyl ester
45	28.051	27.96	28.09	6309170	3.91	1682521	3.98	3.75	Laurin, 2-capri-1,3-di-
46	28.151	28.09	28.235	12942465	8.01	2666128	6.31	4.85	V 3-(Octanoyloxy) propane-1,2-diyl bis(decanoate)
47	28.363	28.235	28.41	20023485	12.4	3926239	9.3	5.1	2-Ethylbutyric acid, 3-methylpent-2-yl ester
48	28.443	28.41	28.51	6117438	3.79	1609449	3.81	3.8	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
49	28.618	28.51	28.785	19670472	12.18	3169250	7.5	6.21	Cholesterol

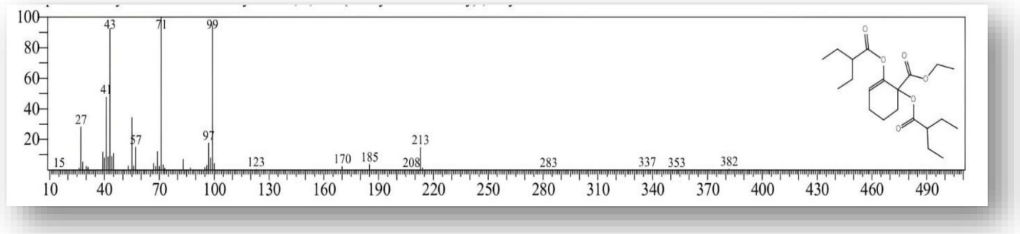
disruption, though further empirical studies are needed.[23] α-Humulene, a sesquiterpene, exhibits anti-inflammatory properties by inhibiting pro-inflammatory mediators and cytokines. It modulates the NF-κB pathway, a key regulator of inflammation. Alpha-humulene is particularly effective against histamine-induced edema and inhibits TNF-α and IL-1β production, while (-)-trans-caryophyllene mainly reduces TNF-α. Both suppress PGE_2 production, iNOS, and COX-2 expression. Their effects are comparable to dexamethasone.[24-26] 3-(1,5-dimethyl-4-hexen)-Methyl-6-(p-tolyl) hept-2-en-4-ol, with its phenyl, alcohol, and alkene groups, shows potential for interacting with inflammatory pathways like NF-κB and COX enzymes.[27,28]

Caryophyllene oxide, a bioactive terpenoid, possesses notable anti-inflammatory and antioxidant properties. It modulates inflammation through the CB2 receptor and NF-κB pathway while mitigating oxidative stress by scavenging free radicals and influencing ferritinophagy and ferroptosis.[29-31] Asarone exhibits promising anti-inflammatory and energizing properties. It relieves Spain and prevents tissue damage by suppressing

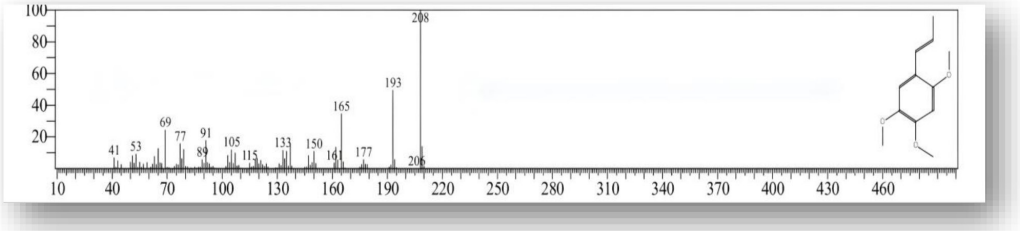
proinflammatory cytokines and reducing oxidative stress.[32] It demonstrated potent anti-inflammatory effects in LPS-induced paw edema models, significantly reducing swelling by over 60% within 2-4 hr of treatment. It also decreased leukocyte infiltration at multiple time points and suppressed the expression of iNOS and the production of TNF-α following LPS stimulation. These effects suggest that α-asarone exerts its anti-inflammatory action through modulation of key inflammatory mediators.[33] Tumerone, a bioactive compound in turmeric essential oil, possesses anti-inflammatory, analgesic, and immunomodulatory properties. It inhibits COX-2, iNOS, and pro-inflammatory cytokines like TNF-α and IL-1β,[34,35] Ar-turmerone suppressed Hedgehog signaling in HaCaT cells by downregulating the expression of Shh, Gli1, and SMO. However, the addition of recombinant human Shh (rhShh) counteracted ar-turmerone's influence on cell growth, programmed cell death, and TNF-α-induced expression of inflammatory cytokines.[36] 2-Pentadecanone exhibits antibacterial activity against *Staphylococcus aureus* and promotes wound healing by enhancing collagen deposition and epithelial cell migration, making it a potential agent for managing infected



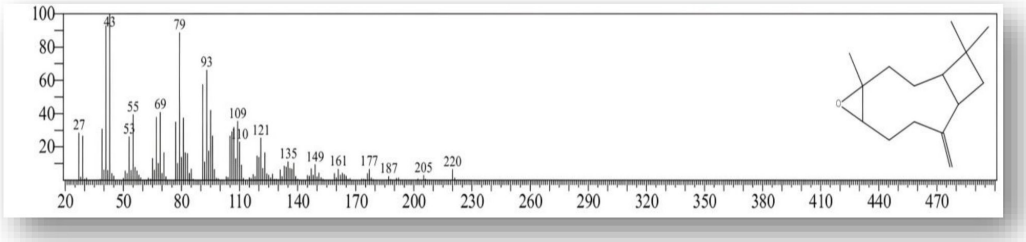
Nootkatone ($C_{15}H_{22}O$)



2-Cyclohexene-1-carboxylic acid, ethyl ester($C_{21}H_{34}O_6$)



Asarone ($C_{12}H_{16}O_3$)



Caryophyllene oxide($C_{15}H_{24}O$)

Figure 2: Retention time of compound Nootkatone ($C_{15}H_{22}O$),Cyclohexene-1-carboxylic acid, 1,2-bis(2-ethyl-1-oxobutoxy)-, ethyl ester ($C_{21}H_{34}O_6$), Asarone ($C_{12}H_{16}O_3$), and Caryophyllene ($C_{15}H_{24}O$).

Table 2: Pharmacological Activity of compounds found in *Bhadrikadi Ghrita*.

Compound	Pharmacological Activity	Chemical Formula
Glyceraldehyde	Intermediate in carbohydrate metabolism.	$C_3H_6O_3$
2-Pentanone 4-hydroxy-4-methyl	Antioxidant and antibacterial activity.	$C_6H_{12}O_2$
2-Decenal	Antimicrobial activity.	$C_{10}H_{18}O$
1,3-Propanediol2-(hydroxymethyl)/ nitroisobutylglycerol	Oxytocin-induced, antioxidant, anti-staphylococcal activities.	$C_4H_9NO_5$
Benzene 1-(1,5-dimethyl-4-hexenyl)/ cuparene	Anti-microbial	$C_{15}H_{22}$
Cyclohexene/ Humulene (α-humulene or alpha-humulene)	Anti-inflammatory	$C_{15}H_{24}$
3-(1,5-dimethyl-4-hexen 2-Methyl-6-(p-tolyl) hept-2-en-4-ol/ Nootkatone	Anti-inflammatory, antagonistic effect.	$C_{15}H_{22}O$
Asarone	Anti-cancer, anti-inflammatory, anti-Hyperlipidemic, anti-thrombotic, peripheral and central anti-nociceptive, anti-depressant, anti-anxiety, anti-Alzheimer's, anti-Parkinson's, anti-epileptic, and radio-protective properties.	$C_{12}H_{16}O_3$
Caryophyllene oxide-Turmerone/	Antioxidant and anti-inflammatory activities.	$C_{15}H_{24}O$
Tumerone	Anti-inflammatory, analgesic, immunomodulatory, neuroprotective, Hepato-protective, nephron-protective, chemo-preventive, anticancer, Anti-proliferative, antimicrobial, antifungal, antiviral, carminative, diuretic, anti-arthritic, antidiabetic.	$C_{15}H_{22}O$
2-Pentadecanone	antibacterial activities	$C_{15}H_{30}O$
Curlone	Antioxidant, Anti-inflammatory	$C_{15}H_{22}O$
(3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4	Anti-inflammatory	$C_{15}H_{22}O$
(6R,7R)-Bisabolone/6-Methyl-2-(4-methylcyclohex-3-en-1- yl)hepta-1,5-dien-4-o	Anti-inflammatory, Antimicrobial Antioxidant.	$C_{15}H_{24}O$
(E)-Atlantone	Antibacterial, antifungal activities.	$C_{15}H_{22}O$
Heptadecanal	Antioxidant, anti-fungal, surfactant.	$C_{17}H_{34}O$
Perilla alcohol tiglate	Not known	$C_{15}H_{22}O_2$
Nootkatone	Anti-inflammatory.	$C_{15}H_{22}O$
6-(5-Hydroxy-4-methylidenecyclohex-2-e n-1-yl)-2-methylhept-2-en-4-one/Valerenic acid	Anti-Neuroinflammatory Neuroprotective - Effect.	$C_{15}H_{22}O_2$
2H-Pyran-2-one, tetrahydro-6-nonyl	11B-HSD-Inhibitor,17-betahydroxysteroidDehydro-genaseinhibitor, 5-hete-inhibitor, 8-HETE-Inhibitor, arylhydrocarbon-hydroxylase-inhibitor.	$C_{14}H_{26}O_2$
Turmeronol A	anti-inflammatory	$C_{15}H_{20}O_2$
Hexadecanoic acid, ethyl ester/Stearic acid/ Octadecanoic acid	5-α-reductase inhibitor, hypocholesterolemic, suppository, cosmetic, lubricant, surfactant and softening agent, perfumery, propecic, flavor.	$C_{18}H_{36}O_2$
E,E-2,13-Octadecadien-1-ol	Not known	$C_{18}H_{34}O$
2H-Pyran-2-one, tetrahydro-6-undecy	Not known	$C_{16}H_{30}O_2$

Compound	Pharmacological Activity	Chemical Formula
1,2-Dicaprin	A synthetic diacylglycerol, is the substrate for human pancreatic lipase. It activates protein kinase C in platelets and enhances anterior pituitary hormone secretion *in vitro*.	$C_{23}H_{44}O_5$
Hexanoic acid, 5-tetradecyl ester	anti-inflammatory and anti-proliferative properties.	$C_{20}H_{40}O_2$
Lauric acid, 2-methylbutyl ester	It falls under the category of flavoring agents. A mixture of 2-methyl-1-butyl laurate and 3-methyl-1-butyl laurate is used in cosmetic, dermatological, or pharmaceutical compositions with antimicrobial properties.	$C_{17}H_{34}O_2$
Myristin, 1,3-diaceto-2-	Not known	$C_{21}H_{38}O_6$
Butyric acid, 3-pentadecyl ester	Not known	$C_{19}H_{38}O_2$
Hexanoic acid, 4-hexadecyl ester	anti-inflammatory and anti-proliferative properties.	$C_{22}H_{44}O_2$
Octane, 1,1'-oxybis-	It inhibits the cancer cell proliferation without affecting the normal cell and program cell death apoptosis in addition to others compounds in the oils.	$C_{16}H_{34}O$
Butyric acid, 4-pentadecyl ester	Not known	$C_{19}H_{38}O_2$
Squalene	Immunostimulant, cancer preventing, Hepatoprotective activities, antihistaminic, sedative action, Hypolipidemic effects, hypoglycemic, potential antiplatelet components, Antinociceptive, antitumor, antioxidant, anti-inflammatory, and anti-atherosclerotic properties *in vivo* and *in vitro*.	$C_{30}H_{50}$
Hexadecanoic acid, 2-(acetyloxy)-1-[	Not known	$C_{23}H_{42}O_6$
Glycerol tricaprylate	Not known	$C_{27}H_{50}O_6$
2-Cyclohexene-1-carboxylic acid, 1,2-	Not known	$C_{21}H_{34}O_6$
Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	Not known	$C_{23}H_{42}O_6$
Glycidyl palmitate	Not known	$C_{19}H_{36}O_3$
Tributyrin	Antibiotic anti-inflammatory	$C_{15}H_{26}O_6$
Hexanoic acid, 4-hexadecyl ester	Anticancer and antibacterial activities.	$C_{22}H_{44}O_2$
9-Octadecenoic acid (Z)-, 2-(acetyloxy	Not known	$C_{25}H_{44}O_6$
Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	Perfumery properties, surfactant and softening agent, lubricant, cosmetic, suppository, hypocholesterolemic, and 5-α-reductase inhibitor.	$C_{25}H_{46}O_6$
Laurin, 2-capri-1,3-di-	Not known	$C_{37}H_{70}O_6$
3-(Octanoyloxy)propane-1,2-diyl bis	Not known	$C_{31}H_{58}O_6$
2-Ethylbutyric acid, 3-methylpent-2-y	Not known	$C_{12}H_{24}O_2$
V 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	Not known	$C_{16}H_{30}O_4$
Cholesterol	Anti-inflammatory, antioxidant	

wounds,[37,38] 2-Pentadecanone exhibits notable anticancer potential by inducing cytotoxic effects, altering cell morphology, and disrupting cellular integrity in HeLa cells, as reported by Kumar *et al*. It also promotes cell cycle arrest and apoptosis in A549 lung cancer cells, effectively inhibiting cell proliferation.

These findings suggest that 2-pentadecanone may interfere with key cellular processes, making it a promising candidate for anticancer therapy.[39] Curlone, a component of turmeric essential oil, may have anti-inflammatory properties similar to curcumin by inhibiting pro-inflammatory cytokines and COX-2, suggesting potential therapeutic benefits.[40] Curlone, comprising 12.48% of turmeric essential oil, contributes to the oil's potent antioxidant and anti-inflammatory effects. Alongside other components, curlone helps reduce free radicals, inhibit inflammation, and alleviate pain, supporting the overall therapeutic benefits of turmeric oil observed in both *in vitro* and *in vivo* studies.[41]

(6R,7R)-Bisabolone exhibits anti-inflammatory properties by suppressing pro-inflammatory cytokines and COX enzymes, similar to β-sitosterol,[42] While direct antimicrobial evidence is limited, sesquiterpenes, Its antioxidant activity is supported by its ability to scavenge free radicals and enhance antioxidant enzymes like SOD and CAT,[43] it demonstrate protective roles against pathogens.[44-46] E)-Atlantone, a sesquiterpene in essential oils, likely exhibits antibacterial and antifungal properties by disrupting microbial membranes and enzyme functions.,[47-49] The antioxidant potential of heptadecanal is theoretically linked to its ability to neutralize free radicals through electron or hydrogen donation,[50,51]

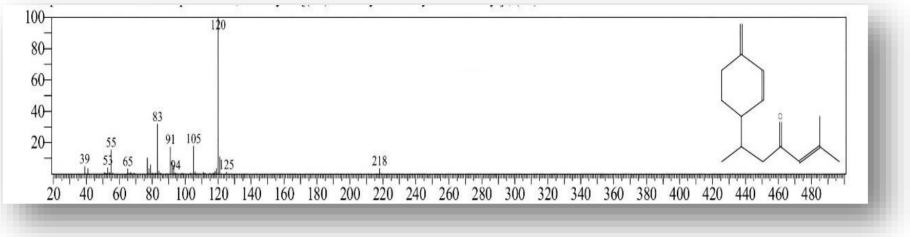
A histamine receptor antagonist showed inhibition similar to the two key interaction regions in the COX-2 binding site, suggesting that NTK-mediated anti-edematogenic effects may involve both COX-2 inhibition and histamine suppression which play a role in pain controx,[52] The compound n-Heptadecanol-1 (HEP), extracted from the MES of *C. sinensis*, was docked with the active sites of Dihydropteroate Synthase (DHPS) from *E. coli* and *S. aureus* using AutoDock Vina to evaluate its potential therapeutic binding interactions.[53] A series of valerenic acid-based compounds was created and assessed for their anti-inflammatory potential through IL-8 and TNF-α assays. Among them, six analogues exhibited moderate activity in inhibiting IL-8, with IC_{50} values between 2.8 and 8.3 μM.[54] Valerenic acid elevates GABA levels, a neurotransmitter that diminishes neuronal excitability, fostering relaxation. Furthermore, it possesses anti-inflammatory properties by inhibiting NF-KappaB activity, a crucial mediator of inflammation.[55] Turmeronols significantly reduced LPS-induced increases in interleukin-1β, interleukin-6, and tumor necrosis factor-α at both the gene and protein levels.

They also blocked the movement of NF-κB into the nucleus, inhibitor pyrrolidine dithiocarbamate, but unlike curcumin. By inhibiting NF-κB activation, turmeronols suppress macrophage activation and inflammatory mediator production, suggesting their potential in preventing chronic inflammatory diseases.[56-58] 2H-Pyran-2-one, tetrahydro-6-nonyl, inhibits several enzymes involved in inflammation and pain, such as 11B-HSD, 17-beta hydroxyl steroid dehydrogenase, and 5-HETE. Blocking these enzymes may help reduce inflammation and oxidative stress, which contribute to pain in conditions like arthritis.[59] Stearic acid has mild antibacterial properties but is not classified as an antibiotic. It showed activity against *Staphylococcus aureus* and *Escherichia coli* in *Excoecaria agallocha*.[60] 1,2-Didecanoylglycerol (DiC10), also known as 1,2-dicaprin, is a synthetic diacylglycerol that serves as a substrate for human pancreatic lipase. It activates protein kinase C in platelets and enhances anterior pituitary hormone secretion *in vitro*.[61]

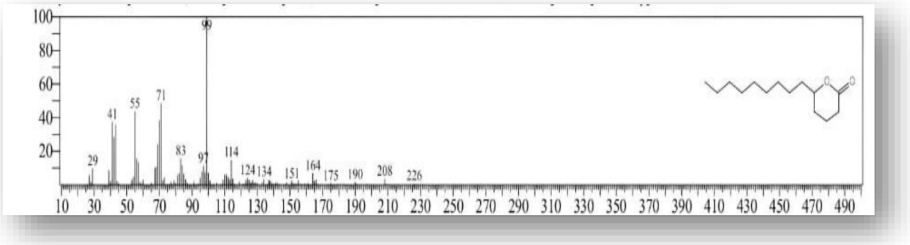
Lauric acid esters, such as erythorbyl laurate, exhibit strong bactericidal activity against pathogens like *Staphylococcus aureus* and Listeria monocytogenes, particularly in acidic conditions,[62,63] Octane, 1,1'-oxybis- It inhibits the cancer cell proliferation without affecting the normal cell and program cell death apoptosis in addition to others compounds in the oils[64] Tributyrin (TB) is a compound that slowly releases butyric acid, which has strong anti-inflammatory properties and helps promote gut health. It effectively reduces the production of LPS-induced inflammatory cytokines and chemokines in adipose tissue samples without showing toxicity at tested concentrations. RNA-Seq analysis identified IL-36γ as highly upregulated by LPS and significantly downregulated when sodium butyrate was added to LPS-stimulated human fat samples. These findings were confirmed at the protein level by IL-36γ ELISA assays.[65] Squalene, a natural compound with antioxidant and anti-inflammatory effects, shows promise for managing pain.

Recent studies highlight its ability to reduce inflammation and oxidative stress, which are key causes of chronic pain in conditions like arthritis and neuropathic pain. Squalene also helps regulate metabolic processes, which may reduce pain linked to metabolic disorders. Its effectiveness in both humans and animals suggests it could be useful for pain relief.[66] Hexanoic acid, 4-hexadecyl ester (Hexadecyl hexanoate) exhibits anti-inflammatory properties, potentially reducing inflammation by inhibiting inflammatory mediators and enzymes. This makes it a promising candidate for natural anti-inflammatory treatments It reduces inflammation in the intestines by blocking pathways that produce inflammatory chemical,[67] 2-Caprin-3-Laurin, a triacylglycerol composed of decanoic (capric) and dodecanoic (lauric) acids, exhibits antimicrobial properties due to its ability to disrupt microbial cell membranes,[68] and Fatty acids have antibacterial and antifungal effects, Long-chain fatty acids (LCFAs), such as n-3, n-6, n-7, and n-9 types, are known for their anti-inflammatory and antibacterial properties. For instance, n-3 fatty acids like EPA and DHA help produce eicosanoids that reduce inflammation.[69]

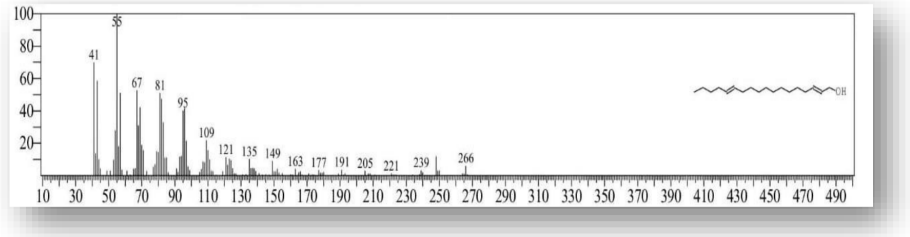
Cholesterol carried by High-Density Lipoprotein (HDL) exhibits anti-inflammatory properties. Functional HDL reduces inflammation by inhibiting the expression of endothelial adhesion molecules and pro-inflammatory cytokines such as TNF-α and IL-1β. It promotes cholesterol efflux from macrophages, reducing foam cell formation and atherosclerosis. Additionally, HDL supports endothelial health by stimulating nitric oxide production and preventing oxidative stress. During acute inflammatory responses, HDL often retains or even enhances its anti-inflammatory and antioxidant functions, contributing to protective physiological changes. While chronic inflammation can challenge HDL's functionality, understanding these mechanisms opens opportunities to restore or optimize its beneficial properties., and may even become pro-inflammatory, contributing to disease progression.[70] Thus, *Bhadrikadi Ghrita* contains numerous bioactive constituents with proven anti-inflammatory, analgesic, antioxidant, and antimicrobial properties. These phytocompounds act through mechanisms such



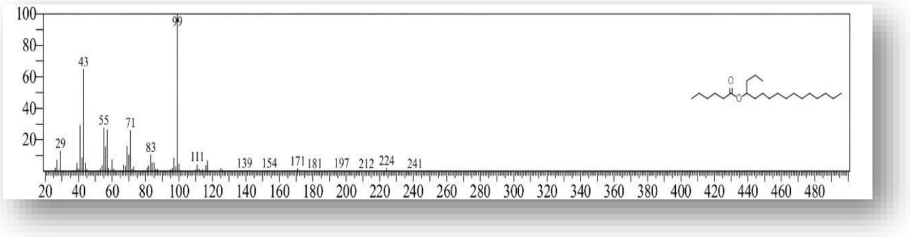
Curlone($C_{15}H_{22}O$)



2H-Pyran-2-one, tetrahydro-6-nonyl-($C_{14}H_{26}O_2$)



E,E-2,13-Octadecadien-1-ol ($C_{18}H_{34}O$)



Hexanoic acid, 4-hexadecyl ester($C_{22}H_{44}O_2$)

Figure 3: Retention time of compound, Curlone ($C_{15}H_{22}O$), 2H-Pyran-2-one ($C_{14}H_{26}O_2$), E,E-2,13-Octadecadien-1-ol ($C_{18}H_{34}O$), and Hexanoic acid, 4-hexadecyl ester ($C_{22}H_{44}O_2$).

as NF-κB inhibition, cytokine suppression, COX-2 modulation, and oxidative stress reduction, supporting their potential role in pain management and inflammatory disorders.

CONCLUSION

The phytochemical and GCMS analysis of *Bhadrikadi Ghrita* highlights the presence of various bioactive compounds, including alkaloids, flavonoids, carbohydrates, and cardiac glycosides. the compounds discussed in this study, including Turmerone, Nootkatone, Caryophyllene Oxide, Asarone, and others, show significant potential in managing inflammation-related pain through their anti-inflammatory, analgesic, and energizing properties. These compounds, by targeting specific pathways involved in pain and inflammation, offer promising alternatives to traditional pain treatments. Furthermore, natural compounds like Squalene, Tributyrin, and *Bhadrikadi Ghrita* support both pain relief and the promotion of gut health, enhancing their therapeutic potential.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RT: Retention Time; **GC-MS:** Gas Chromatography combined with Mass Spectrometry.

SUMMARY

Bhadrikadi Ghrita has been traditionally used as a natural treatment for various health problems. With advances in science, these treatments are now developed safely and effectively. This study looked at the ingredients in *Bhadrikadi Ghrita* using tests like phytochemical screening and GC-MS analysis. The results showed that *Bhadrikadi Ghrita* contains compounds with anti-inflammatory, pain-relieving (analgesic effects). These findings suggest that *Bhadrikadi Ghrita* can be a helpful natural treatment for inflammation, pain, and infections in humans.

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