

GC-MS analysis of anti-mark ointment formulation for skin burns

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Abstract

Wounds generated from burns are fatal and cause marks that last for a long period of time. The formulation of anti marks cream especially for burns would provide hope for a better way of treating scars. The aim of this study is to prepare an ointment using hair ash and oil along with aloe vera followed by the characterization of compounds by using Gas Chromatography and Mass Spectroscopy (GC-MS). The natural compounds used could possibly reduce any side effects on the skin. The GC-MS analysis of the ointment revealed the presence of 30 bioactive compounds. The predominant bioactive compounds were especially fatty acids such as hexadecanoic acid, tetradecanoic acid, dodecanoic acid and octadecanoic acid. The antibacterial, antioxidant, anti-inflammatory, antifungal properties of these bioactive compounds could be effectively used in the treatment of scars.

Keywords: GC-MS analysis, bioactive compounds, hair ash, anti-inflammatory.

1. Introduction

An ointment is a viscous semi-solid preparation used topically on a variety of body surfaces [1]. Burn represents a major health problem, but there is no clear consensus on evidence. Massage therapy could have a positive result on scar pliability and moisturizers and lotions could have an effect on itching of burn scars [2][3]. The mortality from burns have diminished tremendously, but there is no reflect whether the victim could lead a normal life due to the burn scars which are inevitable and other deformities [4].

Since ancient time, local and systemic remedies have been used for burn wound dressing and scar prevention. Antimicrobial creams which are ineffective in deep burns are used for curing traumatic wounds. Separate medical treatments have been designated for burns and burn wounds which are different from other diseases. This is a generalised disorder since most of the body is affected unlike the other diseases. Ancient Egyptians used honey,

Aloe and Tannic acid to heal burns along with thyme, opium and belladonna. [5][6]

The leaves, flowers, stem and root of *Cassia alata* was formulated as ointment for tropical use and it was investigated to have antibacterial activity[7]. So, it could be used as a antibacterial agent in the treatment of common skin conditions.[8] St-John's wort Flowers containing antiseptic properties were used to heal burns[9]

Aloe Vera has been used in traditional medicine as a cathartic and remedy for burns and wounds because of its wound healing capacity with known anti-fungal, anti-microbial, anti-diabetic, and hypoglycemic properties.[10] Aloe Vera as a very good skin healer helps in soothing of skin and moisturizing the skin injuries caused due to burning and it also cures skin itching and swellings due to its bactericidal property. Further it repairs the damaged skin cells that cause aging by acting as an effective detoxifier.[11]

Linseed (*Linum usitatissimum*) which is also known as flaxseed have many properties such as antioxidant, antiviral, antibacterial, antifungal, anti-inflammatory and anti-atherosclerosis along with several therapeutic effects[12]. The flowers of *C. officinalis* were used as medicine in the treatment of inflammation and skin wounds. In spite of having medicinal properties, this is also used in perfumes, pharmaceuticals, cosmetics and foods.

Hair ash and other essentials such as coconut oil, Aloe Vera, Pomegranate peels were known to contain antioxidant properties which are used to cure burns[13][14]. The preparation of an ointment by the combination of hair ash and oil has not been investigated yet. Hence this study is undertaken to poise an ointment for burns and scars with hair ash and oil followed by the characterization of bioactive compounds present in it by using Gas chromatography and Mass spectroscopy (GC-MS).

2. Materials and Methods

2.1 Preparation of Hair ash

Hair was collected from parlours in and around Sathymangalam, Erode. For making hair ash, the hair was burnt under optimal conditions. After burning the hair, ash was sieved by nylon mesh sieve of 0.18mm pore size. The fine particles of the hair ash collected after sieving were used for further process.

2.2 Preparation of essentials

Essentials such as Pomegranate peels and Aloe Vera were taken. Pomegranate peels were shade dried for about 7 days. Then, they were crushed by using a Philips HL 7720 750 watt mixer grinder to make fine particles. Aloe Vera leaves were freshly collected and washed thoroughly using distilled water. The leaves were cut open at the top because of the thinness of the leaves at the top. The spines present were removed by using a sharp knife. The top and the bottom layers were sliced off to get the clear gel present at the centre.

2.3 Preparation of cream base

Purely refined coconut oil and raw Shea butter (Pioneer unrefined raw Shea butter) were procured. The ingredients were mixed together and heated for about 15 minutes until the cream base was obtained. All the ingredients were added to the cream base and whipped for about 10 minutes to obtain the cream.

2.4 Gas Chromatography Mass Spectrometry analysis (GC-MS)

The sample was then subjected to gas chromatography and mass spectrometry (GC-MS). GC-MS analysis of the sample 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 37.49 min. The identification of components present in the sample was done based on Willey and NIST libraries as well as comparison of their retention indices. The relative percentage 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.

3. Results and Discussion

The present study was undertaken to investigate the presence of bioactive compounds present in the cream produced by the combination of hair ash and coconut oil using Gas Chromatography and Mass Spectrometry (GC-MS).

In this study, Hair was collected and made into hair ash by burning. Essentials like Aloe Vera gel and pomegranate peel powder were prepared. The preparation of cream base was done by heating purely refined coconut oil and Shea butter together. Then, both the essentials were added to the cream base and mixed together and the cream was obtained by whipping. The physical properties of the cream were tested and the results were given below.

3.1 Physical properties of cream

Colour : Light brown
 Odour : Pleasant
 Texture : Oily
 Homogeneity : Smooth

3.2 GC-MS analysis

The sample was analysed for the detection of bioactive compounds in it by using Gas Chromatography and Mass Spectrometry (GC-MS). The Gas Chromatography and Mass Spectrometry (GC-MS) profile of the sample is represented in figure 1 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 of the 30 probable bioactive compounds present in the sample 10 are aromatic compounds, 16 are aliphatic compounds and 4 are cyclic compounds. These compounds are represented in the table 1.

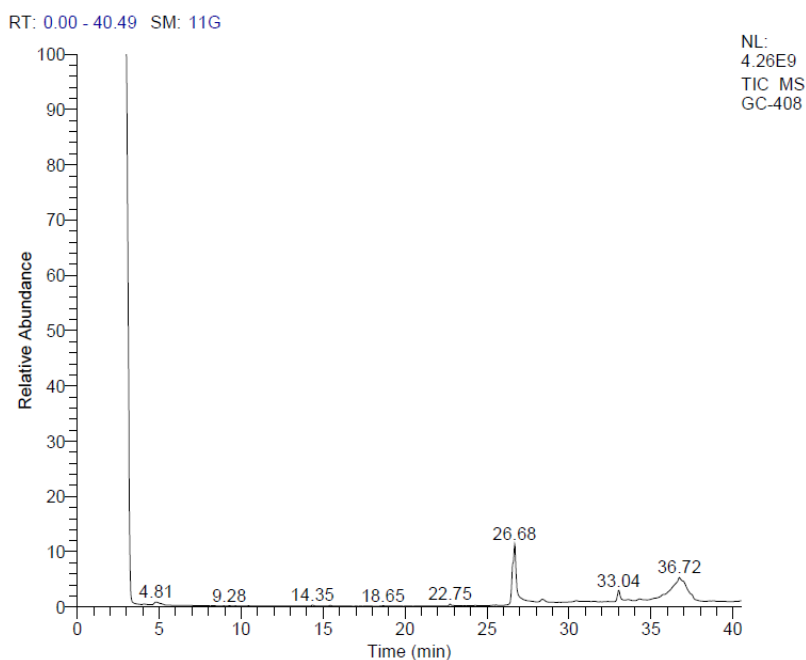
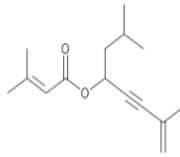
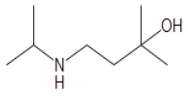
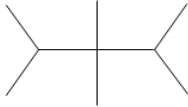
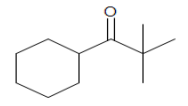
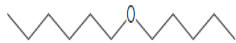
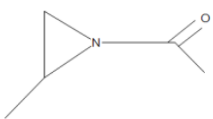

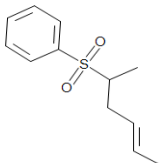

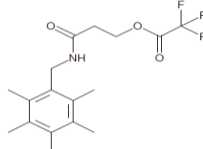
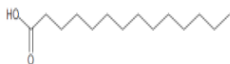
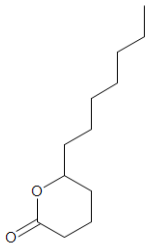

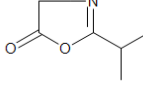

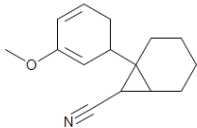

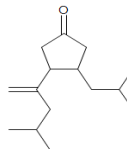
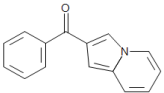
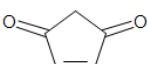
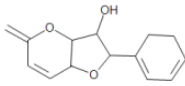

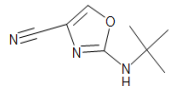
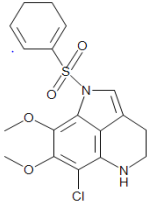
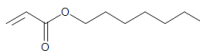
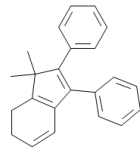
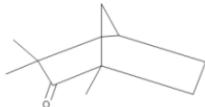
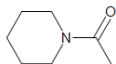
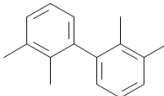
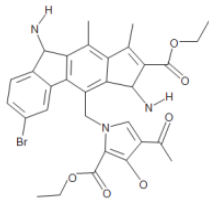


Figure 1: GC -MS Profile of the Sample

Table 1: The presence of bioactive compounds present in the cream using GC-MS analysis

S. No	Retention Time (Min)	Compound Name	Molecular Formula	Molecular Weight	Area	Structure	Reference
1.	3.06	3-Methyl-2-butenic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	$C_{15}H_{22}O_2$	234	9.45		[15]
2.	4.08	4-Isopropylamino-2-methylbutan-2-ol	$C_8H_{19}NO$	145	0.37		[16]
3.	4.81	Pentane, 2,3,3,4-tetramethyl- (CAS)	C_9H_{20}	128	3.75		
4.	10.46	1-Propanone, 1-(3-cyclohexen-1-yl)-2,2-dimethyl- (CAS)	$C_{11}H_{18}O$	166	0.15		
5.	11.31	Ether, hexyl pentyl (CAS)	$C_{11}H_{24}O$	172	0.18		[17][18]
6.	13.35	Aziridine, 1-acetyl-2-methyl-	C_5H_9NO	99	0.16		[19]
7.	14.35	Dodecanoic acid (CAS)	$C_{12}H_{24}O_2$	200	0.57		[20]

8.	14.84	Benzene, [(1-methyl-3-pentenyl)sulfonyl]-, (E)- (CAS)	$C_{12}H_{16}O_2S$	224	0.17		
9.	15.45	Heptadecane (CAS)	$C_{17}H_{36}$	240	0.43		[21]
10.	17.73	3-Oxo-[(pentamethylbenzyl)oxy]propyl - Trifluoroacetate	$C_{17}H_{22}F_3NO_3$	345	0.13		
11.	18.65	Tetradecanoic acid (CAS)	$C_{14}H_{28}O_2$	228	0.30		[22][23]
12	20.08	2H-Pyran-2-one, 6-heptyltetrahydro-	$C_{12}H_{22}O_2$	198	0.14		[24][25]
13.	22.75	Hexadecanoic acid (CAS)	$C_{16}H_{32}O_2$	256	0.95		[26] [22]
14.	24.28	2-Isopropyl-4H-oxazol-5-one	$C_6H_9NO_2$	127	0.15		[27]
15.	25.56	Octadecanoic acid, methyl ester (CAS)	$C_{19}H_{38}O_2$	298	0.52		[22][28]
16.	26.68	3-(p-tolyl)-1-oxo-2,3,4,5,6,7-hexahydro-1H-isindole	$C_{15}H_{17}NO$	227	43.25		[29]
17.	28.37	Docosane(CAS)	$C_{22}H_{46}$	310	2.41		[30]
18.	30.43	Cis-4-Isobutyl-3-(1-isobutylvinyl)cyclopentanone	$C_{15}H_{26}O$	222	0.94		[31]
19.	31.08	2-Indolizinyphenylmethanone	$C_{15}H_{11}NO$	221	0.16		[32]
20.	31.61	2-cyclopentene-1,4-dione	$C_5H_4O_2$	96	0.23		[33]

21.	32.36	(+)-Altholactone	$C_{13}H_{12}O_4$	232	0.13		[34]
22.	33.04	1-Tricosene	$C_{23}H_{46}$	322	7.00		[35]
23.	33.61	2-([t-butyl]amino)oxazole-4-carbonitrile	$C_8H_{11}N_3O$	165	0.68		[36][37]
24.	34.28	6-chloro-1,3,4,5-tetrahydro-7,8-dimethoxy-1-(phenylsulfonyl)pyrrolo[4,3,2-de]quinoline	$C_{18}H_{17}ClN_2O_4S$	392	1.11		[38][39][40][41]
25.	35.18	Acrylic acid heptyl ester	$C_{10}H_{18}O_2$	170	0.15		[42][43]
26.	35.71	1,1-dimethyl-2,3-diphenylindene	$C_{23}H_{20}$	296	0.46		[44]
27.	36.72	Fenchone	$C_{10}H_{16}O$	152	24.57		[45]
28.	37.50	Piperidine,1-acetyl-(cas)	$C_7H_{13}NO$	127	0.52		[46]
29.	38.82	2,2',3,3'-Tetramethylbiphenyl	$C_{16}H_{18}$	210	0.68		[47]
30.	39.39	8-Bromo-10-[(1,3'-acetyl-4'methyl-5'-ethoxycarbonyl)pyrrolymethyl]pyrrolo[3,2-b]carbazole	$C_{30}H_{30}BrN_3O_5$	591	0.29		[48][49]

4. Conclusion

From the table, the identified bioactive compounds like Tetradecanoic acid, Octadecanoic acid, and Hexadecanoic acid possess antibacterial activity[26][22]. The compound Altholactone exhibits antimicrobial activity against some bacteria and fungus [34]. The surface protection against invasive organisms functioning as an antistaphylococcal agent is provided by Hexadecanoic acid[26]. Tetradecanoic acid finds its application in the treatment of corneal vascularisation by reducing the linear incursion of new blood cells[23] where as Octadecanoic

acid acts as a strong anti-inflammatory and antioxidising agent[28]. Butenoic acid is used for the treatment of anti-apoptotic drugs and molecular imaging including the treatment for blotting clotting [15]. Oxazole has a wide range of pharmaceutical properties including other properties such as anticancer, antimicrobial, antitumor, anti-inflammatory and antiviral[50]. Oxazolones are generally known to increase the secretion of IgE antibodies and also induce changes in the affected area of skin which is characterized by influx of inflammatory cells in dermis and epidermis [27].

Dodecanoic acid commonly called Lauric acid is known to exhibit antimicrobial property[20]. Docosane is a compound which exhibits good antimicrobial effects[30]. Fenchone is extensively used to inculcate aroma to the cream formulations and is also used in perfumery.[45]

Hence the various bioactive compounds present in the formulated cream justifies that it could be used to treat scars created due to skin burns. This serves as an alternative for other chemical treatment of scars which may produce side effects. Therefore adoption of this method could be a boost to the pharmaceutical industries as the raw materials used are cheaper and produces efficient results. However the time taken for complete healing has to be analysed for proper administration. Further work on this study is to carry out animal testing for commercialisation.

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