ANALYTICAL PROFILING OF HAWTHORN FRUIT EXTRACTS USING METHANOL AND HEXANE SOLVENT VIA GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

By

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Faculty of Veterinary Medicine

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ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 55204 – Research Project.

In this study, the Hawthorn fruit was extracted using both methanol and hexane solvents, followed by gas chromatography-mass spectrometry (GC-MS) to identify the chemical constituents of the hawthorn fruit. According to the GC-MS chromatography result for the methanol crude extraction of hawthorn fruit, five of the highest compounds were identified, with hexadecanoic acid and octadecyl ester (38.46%) showing the highest percentages. Then, followed by the n-Decanoic acid (20.05%), 5-Hydroxymethylfurfural (14.81%), beta.-D-Glucopyranose, 1,6-anhydro (2.27%), and lastly 9-Octadecenoic acid (Z)-, tetradecyl ester (2.19%). However, five different compounds were identified in the hexane crude extract from the same fruit. Tetracontane was found in the highest concentration (50.59%), followed by 7-Tetradecenal (11.65%), octacosyl trifluoroacetate (8.74%), n-Hexadecanoic acid (4.66%), and octacosane (3.21%). This result provides valuable insights for future research on isolating and applying these compounds for use in veterinary medicine as food additives or pharmaceutical preservatives.

Keywords: Hawthorn fruit, methanol extract, hexane extract, phytochemical compounds

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Dalam kajian ini, buah hawthorn diekstrak menggunakan kedua-dua pelarut metanol dan heksana, diikuti dengan kromatografi gas-spektrometri jisim (GC-MS) untuk mengenal pasti konstituen kimia di dalam buah hawthorn. Berdasarkan keputusan kromatografi GC-MS bagi ekstrak metanol buah hawthorn, lima sebatian dengan peratusan tertinggi telah dikenal pasti, dengan hexadecanoic acid dan octadecyl ester (38.46%) menunjukkan peratusan tertinggi. Seterusnya diikuti oleh n-Decanoic acid (20.05%), 5-Hydroxymethylfurfural (14.81%), beta.-D-Glucopyranose, 1,6-anhydro (2.27%), dan akhir sekali ialah 9-Octadecenoic acid (Z)-, tetradecyl ester (2.19%). Walau bagaimanapun, lima sebatian yang berbeza telah dikenal pasti dalam ekstrak heksana daripada buah yang sama. Tetracontane didapati pada kepekatan tertinggi (50.59%), diikuti oleh 7-Tetradecenal (11.65%), octacosyl trifluoroacetate (8.74%), n-Hexadecanoic acid (4.66%), and octacosane (3.21%). Hasil ini memberikan pandangan yang bernilai untuk penyelidikan masa hadapan mengenai proses pengekstrakan dan penggunaan sebatian-sebatian ini bagi kegunaan dalam perubatan veterinar sebagai aditif makanan atau bahan pengawet farmaseutikal

Kata kunci: Buah hawthorn, metanol ekstrak, heksana ekstrak, sebatian fitokimia

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LIST OF ABBREVIATIONS

GC-MS:	Gas chromatography-mass spectrometry
HS-SPME:	Headspace solid-phase microextraction
PC12 cells:	Pheochromocytoma cells
HepG2:	Hepatocellular carcinoma cells
Nrf2/HO-1:	Nuclear factor erythroid 2-related factor 2/heme oxygenase 1
MLCK:	Myosin light chain kinase
MLC:	Myosin regulatory light chain
ERK1/2:	Extracellular signal-regulated kinase 1/2
NF-κB:	Nuclear factor kappa-light chain enhancer of activated B cells
ROS:	Reactive oxygen species
QHP:	Quaternized hawthorn pectin
MCF-7:	Hormone receptor-positive
MDA-MB-231:	Triple-negative breast cancer
ARPE-19:	Human retinal pigment epithelial
FID:	Flame ionization detection
DPPH:	2,2′-diphenyl-1-picrylhydrazyl

LIST OF SYMBOLS

%:	Percent
g:	Gram
µg:	Microgram
ml:	Milliliter
α:	Alpha
β:	Beta

CHAPTER 1

INTRODUCTION

1.1 Research background

Hawthorn is universal used as a wild fruit for consumption, food, and medicine purposes. The fruits of hawthorn either fresh or dried, are used to make flavour, teas, drinks, and food supplements (Li *et al*., 2022). Hawthorn is well known for their wide range of health benefits consisting of active ingredients such as polyphenolic compounds, flavonoids, lignans, and triterpenoids (Alirezalu *et al*., 2020).

According to Zhang *et al*. (2022), compounds isolated from hawthorn have a wide range of anticancer activities, show positive cardioprotective effect, exert antibacterial and anti-inflammatory activities, and have high antioxidant effects.

Taleghani *et al*. (2024) revealed that fruit hydro-methanolic extract demonstrated the highest antioxidant and antibacterial activity, followed by the leaf and root extracts. Besides that, various petroleum ether extract shows 28, 42, and 25 chemical compounds belonging to different chemical families, representing 91.13%, 95.71%, and 89.67%, respectively, of the relative area in the fruit, leaf, and root extracts were identified using GC-MS as major components.

Interestingly L. Zhang *et al*. (2020), demonstrated that the extracting solvents markedly influenced the chemical composition and antibacterial activity of hawthorn extract. The results showed that methanol and ethanol extract exhibited high levels of total flavonoids and polyphenol content compared to petroleum ether extracts. Additionally, the methanol extract exhibited the strongest antibacterial activity against the tested bacteria.The total polyphenols content was measured by colorimetric Folin–Ciocalteu method and the total flavonoids content was determined by measuring reducing power, inhibition of peroxidation using linoleic acid system and 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity.

Based on Bazgir and colleagues (2020), the essential and volatile oils were extracted from hawthorn fruit using headspace solid-phase microextraction (HS-SPME) and their chemical compounds were analysed by gas chromatography–mass spectrometry (GC-MS). According to the results obtained, the major fruit essential oil compounds of this plant are α-bisabolol oxide A(19.07%), α-Pinene (15.50%), β-Bisabolene (12.56%), spathulenol (9.23%), β-Farnesene (8.95%), α-Bisabolol(4.72%), caryophyllene oxide (4.46%), trans-Farnesole(3.75%) and dl-limonene (3.47%).

Moreover study by Iwara et al. (2018), demonstrated that highest bioactive compounds of *Vernonia calvoana* leaves using gas chromatography-mass spectrometer after n-hexane extraction were phytol (46.67%), 8,11,14-eicosatrienoic acid (33.40%), octadecanoic acid (11.25%), pentadecane carboxylic acid (10.69%), 9, 12, 15-octadecatrien-1-ol (8.12%) and ethyl palmitate (7.68%). Besides that, distinct compounds were observed after methanol extraction includingoleic acid (33.40%), hexadecanoic acid (12.49%), 2-butanone (14.32%), palmitadehyde (8.36%), 11-octadecenoic acid (5.56%), Z-4-nonadecen-1-ol-acetate (5.36%) and limonene Oxide (4.37%).

Hence, the objective of this study is to perform a comparative quantitative analysis of hawthorn fruit phytochemicals through solvent extraction using methanol and n-hexane, followed by characterization via gas chromatography-mass spectrometry.

1.2 Problem statement

Existing research has demonstrated the presence of beneficial phytochemicals in hawthorn fruit through basic screening such as Folin–Ciocalteu method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, yet the specific chemical compositions of its methanol and hexane extracts remain largely unexplored. To better understand the chemical constituents of these extracts, there is a critical need to move beyond general assays and perform a detailed characterization using gas chromatography-mass spectrometry.

1.3 Research question

1. What are the variations in hawthorn fruit extract composition when using different solvents?

2. What are the five most concentrated phytochemical compounds observed in the hawthorn fruit using hexane and methanol crude extracts?

1.4 Research hypothesis

1. There is a significant difference in the sample extraction of hawthorn fruit using different solvents.

2. The chemical identities of the five most concentrated phytochemicals differ significantly between methanol and hexane hawthorn fruit extracts

1.5 Research objective

1. To determine the types of phytochemical compounds obtained from hawthorn fruit using different solvents

2. To determine the five most concentrated phytochemical compound levels in hawthorn fruit for different solvents

1.6 Significance of study

The data generated from this study will provide a comparative analysis of phytochemical compounds obtained via methanol and hexane extractions of hawthorn fruit. These findings are crucial as they will highlight the critical importance of selecting the appropriate solvent to maximize the yield of desired phytochemical compounds before further study. To date, no study has reported a comparative gas chromatography-mass spectrometer analysis of methanol and hexane extracts of hawthorn fruit. Therefore this study aims to fill this gap by evaluating the phytochemical constituent of hawthorn fruit of different solvent polarity using GC-MS.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of hawthorn

Hawthorn berry (*Crataegus* L.) is a genus of over 1000 species belonging to the subfamily Maloideae in the Rosaceae family, widely distributed in Asia and Europe. Hawthorn fruits contain high numbers of phenolic compounds, and are used as medicinal remedies with a variety of biological activities including antitumor, antispasmodic, cardiotonic, diuretic, hypotensive, anti-atherosclerotic, and anti-inflammation effects (Alirezalu *et al*., 2020). Hawthorn consists of small trees and shrubs and is widely distributed around the world containing a richness of bioactive substances suitable for medicinal and food consumption. Hawthorn is the most valuable species in the genus and is often found on forest margins or in scrub on mountain slopes where it can reach heights of up to 15 m (Zhang *et al*., 2022). Common names for hawthorns may include may blossom, quick thorn, whitethorn, haw hazels, halves, hagthorn, and bread and cheese tree. The colour of the ripe fruit may range from yellow, green, to red and on to dark purple (Özcan *et al*., 2004).

2.2 Antioxidant effects in hawthorn

The hawthorn extract and its polyphenols demonstrated antioxidant properties in cellular and animal models. The complete hawthorn extracts markedly elevated cell viability and augmented the activities of superoxide dismutase, catalase, and glutathione peroxidase, while reducing lactate dehydrogenase release, reactive oxygen species levels, and hydrogen peroxide-induced malondialdehyde content in rat pheochromocytoma cells (PC12 cells) (Wang *et al.*,2022). The application of whole hawthorn fruit extract enhanced the antioxidant capacity of human hepatocellular carcinoma cells (HepG2) and mitigated oleic acid-induced hyperlipidemia by activating the nuclear factor erythroid 2-related factor 2/heme oxygenase 1 (Nrf2/HO-1) signaling pathway (Feng *et al.*, 2022).

2.3 Anti-inflammatory potential in hawthorn

The flavonoids were found to inhibit the synthesis of inflammatory cytokines, such as IL-6, IL-8, and IL-1β in human colorectal adenocarcinoma cells, potentially by downregulating the 4 phosphorylation of myosin light chain kinase (MLCK)/myosin regulatory light chain (MLC) and obstructing the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and nuclear factor kappa-light chain enhancer of activated B cells (NF-κB) signaling pathways (F. Liu *et al.*, 2020).

2.4 Antibacterial effects in hawthorn

Furthermore, hawthorn also exhibited effective inhibitory action against both Gram-positive and Gram-negative bacteria, with its antibacterial mechanism involving the induction of oxidative stress through the production of reactive oxygen species (ROS) (Golabiazar *et al.*, 2021). Likewise, it enhanced the resistance of *Exopalaemon carinicauda* to *Vibrio parahaemolyticus*, as indicated by improved immune responses and growth in the shrimp (Cao *et al.*, 2022). Furthermore, it demonstrated antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli*, with a minimum inhibitory concentration of 0.11 μg/mL (Ebrahimzadeh *et al.*, 2020). Additionally, it was noted that the quaternized hawthorn pectin (QHP) derivatives displayed a certain inhibitory effect on *E. coli* and *S. aureus* (Qin *et al*., 2022).

2.5 Anti-cancer function in hawthorn

Methanolic extracts from the whole hawthorn fruit, which are high in polyphenols, were shown to be cytotoxic to human breast cancer cells, specifically hormone receptor-positive (MCF-7) and triple-negative breast cancer (MDA-MB-231) cell lines. These extracts effectively inhibited the proliferation of tumor cells and caused cell cycle arrest at the G1/S transition, likely due to the modulation of the Wnt signaling pathway (Kombiyil & Sivasithamparam, 2022). Furthermore, polyphenols were observed to inhibit apoptosis via the AMPK/SIRT1/NF κB pathway and diminish the activation of the miR-34a/SIRT1/p53 pathways in human retinal pigment epithelial cells (ARPE-19 cells) (Liu *et al*., 2021).

2.6 Gas chromatography-mass spectrometer

According to Iordache et al. (2008), the study of plant-based organic compounds on numerous extraction techniques and analytical techniques developed for the study of plant active compounds include spectrophotometry, high performance liquid chromatography, capillary electrophoresis, gas chromatography (GC) with flame ionization detection (FID), and gas chromatography–mass spectrometry (GC–MS). The same paper also mentioned that GC–MS is a perfect technique for both qualitative and quantitative analysis of volatile and semivolative compounds because it combines the best separation method (GC) with the best identification method (MS).

It was supported by Smriti et al. (2023), they stated that gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas chromatography and mass spectrometry, facilitating the identification of various compounds in a sample. The methodology includes the separation of chemical mixtures using the GC component and the subsequent identification of individual components at a molecular level through the MS component. Moreover,the applications of GC-MS are extensive, including pharmaceutical drug detection, fire investigation, environmental analysis, explosives detection, flavor and fragrance analysis in food and beverages, forensics, biological and pesticide determination, geochemical research, petrochemical analysis, clinical toxicology, and identification of unknown samples.

2.7 Methanol and hexane solvents

According to Daniel (2013), the study demonstrated that nonpolar extract such as n-hexane, dichloromethane and ethyl acetate were less active in trapping antioxidant activity of hawthorn fruit using DPPH radical compared to the polar extracts such as acetone, ethanol, and methanol at 100 mg/L. Similarly in study by L. Zhang and colleagues (2020) observed total polyphenols content using colorimetric Folin–Ciocalteu method and flavonoids content using DPPH.

Abubakar and Haque (2020),claimed that n-hexane is the least polar which is 0.009 polarity compared to methanol which is at 0.762 polarity. In general, polar solvents such as methanol, ethanol, and water are used in extraction of polar compounds while nonpolar solvents such as hexane and dichloromethane are used in extraction of nonpolar compounds.

CHAPTER 3

METHODOLOGY

3.1 Study design

The study was conducted as an analytical cross-sectional study design with laboratory work to prepare hawthorn fruit crude methanol and hexane extracts.

3.2 Sample collection and preparation

A thousand grams of dried hawthorn fruits (Figure 1) were purchased from a supermarket located in Kota Bharu, Kelantan. Then, hawthorn fruits were ground into powder form using a high-speed multifunction comminutor (SEAAN). The ground samples were stored at room temperature in a sealed plastic bag containing silica gel desiccant.

Figure 1: Dried hawthorn fruit

3.3 Sample extraction

Approximately 1000 g of ground hawthorn fruit was soaked in 1000 ml of hexane l00% for 24 hours. After that, the sample was passed through Whatman No. 1 filter paper to obtain a particle free extract for 1 hour. Then, the extract was concentrated under reduced pressure using a rotary evaporator to obtain a paste-like consistency extract for 1 hour. The same procedure was applied using methanol 100%. The end product from both extractions were designated as the hexane crude extract and the methanol crude extract (Figure 2 and 3).

Figure 2: Hawthorn methanol crude extract

Figure 3: Hawthorn hexane crude extract

3.4 Compound profiling

Compound profiling was conducted using gas chromatography-mass spectrometry (GC-MS) at the Institute of Marine Biotechnology, University Malaysia Terengganu. Prior to delivery, samples were stored in a closed airtight container during transportation. The results were obtained 2 weeks following submission.

CHAPTER 4

RESULT

4.1 Compound profiling of methanol and hexane crude extract for hawthorn fruit

Table 1 showed a list of compounds from hawthorn methanol extract after profiling using gas chromatography-mass spectrometry (GC-MS) method. GC-MS analysis reveals that 50 compounds in methanol extract of hawthorn fruit.

Table 1: List of compounds in methanol extract using GC-MS

PEAK#	NAME	AREA%	HEIGHT%
1	Glycerin	0.17	1.05
2	Trifluoroguanidine	0.46	2.06
3	Methyl formate	0.12	0.85
4	4-(Methylthio)-1-butene	0.08	0.39
5	Propanenitrile, 3,3-dimethoxy	0.31	1.41
6	4H-Pyran-4-one	0.06	0.39
7	Furfural	1.76	13.44
8	Methyl isovalerate	0.22	1.09
9	2,5-Furandione, dihydro-3-methylene	0.42	1.67
10	2(1H)-Pyridinone, 3-hydroxy	0.69	4.84
11	1,2,5,6-Tetrahydropyridin-2-one, 5-methyl	0.38	2.44
12	Acetamide, N-methyl-N-(2-propynyl)	0.47	2.32
13	2,5-Furandione, dihydro-3-methylene	1.74	5.19
14	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.48	3.12
15	3-Aminopyrazine 1-oxide	0.48	3.03
16	2(1H)-Pyridinone, 3-hydroxy	0.18	1.40
17	Dimethyl dl-malate	0.82	1.90
18	Propanamide, 3-(1-piperazinyl)	0.48	0.93
19	Bicyclo[2.2.1]heptane-2-carboxamide	0.13	0.30
20	2(1H)-Pyridinone, 5-methyl	0.25	0.71

21	Glutaric acid, dec-2-yl 3-methyl-5-methoxypentyl	0.14	0.36
22	Fumaric acid, 5-methoxy-3-methylpentyl	0.43	0.79
23	Glutaric acid, isohexyl 5-methoxy-3-methylpentyl	0.16	0.35
24	5-Hydroxymethylfurfural	7.44	4.80
25	5-Hydroxymethylfurfural	0.71	0.94
26	5-Hydroxymethylfurfural	0.88	1.27
27	2-Undecene, 4,5-dimethyl	0.49	0.73
28	5-Hydroxymethylfurfural	0.94	1.10
29	5-Hydroxymethylfurfural	4.84	3.37
30	2-Chlorobenzoic acid, undec-2-enyl ester	1.13	1.57
31	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	0.59	0.87
32	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	0.32	0.62
33	Isosorbide	0.51	0.47
34	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	0.20	0.60
35	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	0.31	0.60
36	2-Isopropyloxan-4-ol	0.73	0.87
37	.beta.-D-Glucopyranose, 1,6-anhydro	2.27	1.01
38	2-Hydroxypropane-1,2,3-tricarboxylic acid	1.94	1.35
39	n-Decanoic acid	20.05	3.64
40	n-Hexadecanoic acid	0.75	0.79
41	Furo[3,4-b]furan-2,6(3H,4H)-dione	0.56	0.53
42	Hexadecanoic acid, methyl ester	0.72	0.48
43	n-Hexadecanoic acid	0.57	0.65
44	9,12-Octadecadienoic acid	0.92	0.74
45	9,12,15-Octadecatrienoic acid, methyl ester	0.71	0.49
46	8,11,14-Eicosatrienoic acid	0.57	0.69
47	9,12-Octadecadienoic acid	0.52	0.39
48	Heptacosane	0.26	0.44
49	9-Octadecenoic acid (Z)-, tetradecyl ester	2.19	1.12
50	Hexadecanoic acid, octadecyl ester	38.46	19.83
		100.00	100.00

Table 2 presents a list of compounds identified in the hawthorn fruit hexane crude extract following Gas Chromatography–Mass Spectrometry (GC-MS) profilin

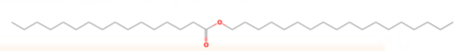
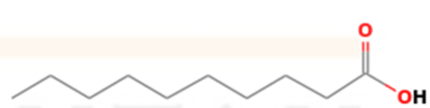
Table 2: List of compounds in hawthorn hexane extract using GC-MS

PEAK#	NAME	AREA%	HEIGHT%
1	Toluene	0.05	0.22
2	Cyclopropane, 2-chloro-1,1,3-trimethyl-	0.08	0.42
3	2-Butyn-1-ol	0.06	0.21
4	1-Pentanol, 3-methyl-	0.07	0.32
5	Tetrachloroethylene	0.09	0.32
6	2-Propenoic acid, 2-methyl-, ethenyl ester	0.04	0.13
7	Ethane, 1,1,2,2-tetrachloro-	0.41	1.36
8	Benzene, 1-ethyl-3-methyl-	0.09	0.21
9	Mesitylene	0.03	0.12
10	Ethane, pentachloro-	0.10	0.31
11	Benzene, 1-ethyl-2-methyl-	0.03	0.13
12	Mesitylene	0.35	0.95
13	Benzene, 1,2,3-trimethyl-	0.16	0.36
14	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	0.06	0.14
15	Benzaldehyde, 2-hydroxy-	0.09	0.18
16	Ethane, hexachloro-	0.04	0.12
17	Benzene, 4-ethyl-1,2-dimethyl-	0.06	0.12
18	1,3-Cyclopentadiene	0.13	0.22
19	Benzene, 1,2,4,5-tetramethyl-	0.20	0.33
20	1,3-Cyclopentadiene	0.08	0.12
21	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	0.76	0.62
22	Pentadecanoic acid, 14-methyl-, methyl ester	0.28	0.27
23	n-Hexadecanoic acid	4.66	3.32
24	2-Methyltetracosane	0.53	0.52
25	9-Octadecenoic acid-, methyl ester	0.33	0.47
26	Methyl stearate	0.11	0.12
27	7-Tetradecenal	11.65	3.94
28	Tetracosane	0.20	0.26
29	Eicosanoic acid	0.53	0.31
30	Hexanedioic acid, bis(2-ethylhexyl) ester	1.88	2.49
31	Tetratetracontane	0.57	0.75
32	Di-n-octyl phthalate	0.15	0.20
33	Eicosanoic acid	0.15	0.13
34	Pentacosane	0.23	0.24
35	Hexacosane	2.20	3.09
36	Heptacosane	0.45	0.47
37	Squalene	2.30	2.85
38	Tetracontane	29.92	32.92
39	Didodecyl phthalate	1.23	0.64

40	Didecyl phthalate	1.57	0.64
41	.delta.-Tocopherol	1.94	0.92
42	Octacosane	2.94	1.39
43	Acetic acid, 6,8,9-trimethyl-4-pentyl-3-oxabicyclo	0.85	0.40
44	Octacosanal	0.89	0.46
45	Tetracontane	20.67	23.49
46	Octacosyl trifluoroacetate	8.74	11.32
47	1-(3-Methylbutyl)-2,4,6-trimethylbenzene	0.71	0.30
48	Vitamin E	0.82	0.55
49	Octacosane	0.27	0.32
50	Hexacosanal	0.27	0.33
		100.00	100.00

Based on Table 3, the percentage composition of five dominant phytochemical compounds of hawthorn fruit extract from methanol solvent is shown as below In the methanol extract, hexadecanoic acid, octadecyl ester is the most abundant compound constituting 38.46% of the extract, followed by n-Decanoic acid (20.05%), 5-Hydroxymethylfurfural (14.81%), .beta.-D-Glucopyranose, 1,6-anhydro (2.27%), and 9-Octadecenoic acid (Z)-, tetradecyl ester (2.19%).

Table 3: List of most concentrated phytochemical compounds with percentage in hawthorn methanol crude extracts.

NO	NAME	PERCENTAGE (%)	CHEMICAL STRUCTURE & MOLECULAR FORMULA	COMMON NAME
1.	Hexadecanoic acid, octadecyl ester	38.46	 ($C_{34}H_{68}O_2$)	Stearyl palmitate
2.	n-Decanoic acid	20.05	 ($C_{10}H_{20}O_2$)	Capric acid

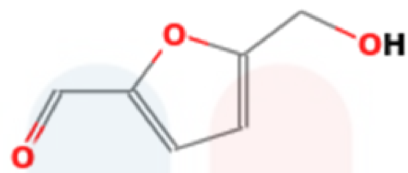
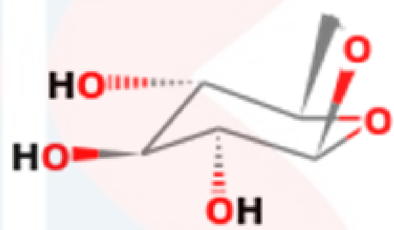
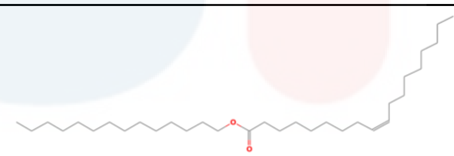
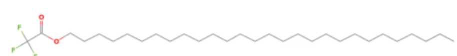
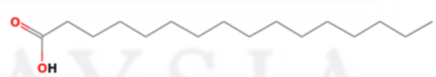
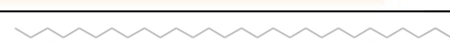
3.	5-Hydroxymethylfurfural	14.81	 ($C_6H_6O_3$)	-
4.	.beta.-D-Glucopyranose, 1,6-anhydro	2.27	 ($C_6H_{10}O_5$)	Levoglucosan
5.	9-Octadecenoic acid (Z)-, tetradecyl ester	2.19	 ($C_{32}H_{62}O_2$)	Myristyl oleate

Table 4 showed the percentage composition of five dominant chemical compounds of hawthorn fruit extract from hexane solvent combined with GC-MS. In hexane extract, tetracontane is the most plentiful compound comprising 50.59% of the extract, followed by 7-Tetradecenal (11.65%), octacosyl trifluoroacetate (8.74%), n-Hexadecanoic acid (4.66%), and octacosane (3.21%).

Table 4: List of most concentrated phytochemical compounds with percentage in hawthorn hexane crude extracts

NO	NAME	PERCENTAGE (%)	CHEMICAL STRUCTURE & MOLECULAR FORMULA	COMMON NAME
1.	Tetracontane	50.59	($C_{40}H_{82}$)	-
2.	7-Tetradecenal, (Z)-	11.65	 ($C_{14}H_{26}O$)	-
3.	Octacosyl trifluoroacetate	8.74	 ($C_{30}H_{57}F_3O_2$)	-
4.	n-Hexadecanoic acid	4.66	 ($C_{16}H_{32}O_2$)	Palmitic acid
5.	Octacosane	3.21	 ($C_{28}H_{58}$)	-

The results from this study strongly suggest that the phytochemical profiles of hawthorn fruit extracts obtained using different solvents differ significantly in both composition (identity of compounds) and concentration (percentage of compounds).

CHAPTER 5

DISCUSSION

The observed differences in phytochemical compounds between hawthorn fruit methanol and hexane crude extracts are influenced by polarity. Methanol, a polar solvent, preferentially extract polar and semi-polar compounds such as fatty acids (n-Decanoic acid), esterified lipids (Hexadecanoic acid, octadecyl ester, and 9-Octadecenoic acid (Z)-, tetradecyl ester), sugars (.beta.-D-Glucopyranose, 1,6-anhydro), and furan derivatives (5-Hydroxymethylfurfural). On other hand, hexane, a nonpolar solvent, predominantly result in lipid soluble compounds rich in long-chain hydrocarbons and wax-associated constituents such as Tetracontane, 7-Tetradecenal, (Z)-, Octacosyl trifluoroacetate, n-Hexadecanoic acid, and Octacosane. These results demonstrate that solvent polarity is a critical determinant in the selective extraction of phytochemicals, as characterized by gas chromatography-mass spectrometry (GC-MS).

The major compounds isolated from methanol extracts were hexadecanoic acid, octadecyl ester, n-Decanoic acid, 5-Hydroxymethylfurfural, .beta.-D-Glucopyranose, 1,6-anhydro, 9-Octadecenoic acid (Z)-, tetradecyl ester, while from hexane extracts were Tetracontane, 7-Tetradecenal, (Z)-, Octacosyl trifluoroacetate, n-Hexadecanoic acid, and Octacosane. Previous studies have demonstrated that these compounds possess a wide range of biological activities, as summarized in Table 5.

Table 5: Presence of concentrated compounds in other sources with the biological activities

COMPOUND	ACTIVITIES	OTHER SOURCES
Methanol crude extracts		
1) Hexadecanoic acid, octadecyl ester	• Anticancer • Emollient	• *Althea officinalis* (Ravi *et al*., 2023)
2) n-Decanoic acid	• Antimicrobial • Antioxidant	• Coconut oil • Palm kernel oil • Dairy products • Sesame oil (Jadhav *et al*., 2017)
3) 5-Hydroxymethylfurfural	• Antioxidant • Anti-myocardial ischemia	• Jabara fruit • *Cornus officinalis* (Cao *et al*., 2013) (Uchida *et al*., 2020)
4) .beta.-D-Glucopyranose, 1,6-anhydro	• Antitumor • Antibacterial	• Soybean hull (Giri *et al*., 2017)
5) 9-Octadecenoic acid (Z)-, tetradecyl ester	• Antidiabetic agent • Emollient • Excipient	• *Peronema canescens* (Rahardhian *et al*., 2025)
COMPOUND	**ACTIVITIES**	**OTHER SOURCES**
Hexane crude extracts		
1) Tetracontane	• Emollient • Excipient	• *Grangea maderaspatana* (Jyoti *et al*., 2025)
2) 7-Tetradecenal, (Z)-	• Antimicrobial	• *Vinca rosea* (P. Sharma *et al*., 2025)
3) Octacosyl trifluoroacetate	• Antioxidant	• *Halymenia durvillei* (Chumphoochai *et al*., 2025)
4) n-Hexadecanoic acid	• Antibacterial • Anticancer • Anti-inflammatory	• *A. arborescens* • *Excoecaria agallocha* (Moglad *et al*., 2024) (Purushothaman *et al*., 2025)
5) Octacosane	• Antimicrobial • Antibiofilm • Anti-inflammatory	• Mantidis ootheca • *Marantodes pumilum* (Okechukwu, 2020) (Vanessa *et al*., 2024)

5.1 Functional biological of chemical compounds extracted in hawthorn

As shown in table 3, hexadecanoic acid, octadecyl ester was identified as the highest compound in methanol crude extract with concentration of 38.46%. This compound is also known as stearyl palmitate. According to Ravi *et al*. (2023), the study demonstrated that stearyl palmitate, present in *Althea officinalis*, was a potential anti-breast cancer agent which possessed the ability to inhibit multiple targets of proteins. In a previous study by Fiume *et al*. (2012), it stated that this compound has cosmetic functions such as binders, emulsion, stabilizer, and hair conditioner.

n-Decanoic acid was identified as the second highest compound in methanol crude extract as shown in table 3, with 20.05%. This compound is also known as caprid acid. Jadhav *et al*. (2017) reported that capric acid, which is normally found in coconut and palm kernel oils, has been shown in the infection of human pathogenic yeast *Candida albicans*. The study demonstrated that capric acid can inhibit the filamentous growth, adhesion, and biofilm formation by *C. albicans*. Another study was done by Zhao and colleagues (2021), that reported capric acid has antioxidant functions. The study also demonstrated that capric acid improved intestinal morphology and intestinal barrier functions resulting in the improvement of gut health and growth performance of the host.

As shown in table 3, 5-hydroxymethylfurfural was identified to become 3 highest compounds with 14.81%. 5-hydroxymethylfurfural also was identified in jabara fruit and it is an active component in fermented jabara fruit (Uchida *et al*., 2020). The same study also demonstrated that this compound has been shown to inhibit mast cell granulation by reducing degranulation and cytokine production. According to Cao and colleagues (2013), 5-hydroxymethylfurfural was identified in

Cornus officinalis, which significantly inhibits high glucose-induced oxidative stress. The study demonstrated that this compound prevents cardiovascular and diabetes mellitus diseases.

2.27% of .beta.-D-Glucopyranose, 1,6-anhydro was identified in this study as shown in table 3. This compound is also known as levoglucosan. Itabaiana *et al*. (2020) highlighted that levoglucosan is an anhydrous sugar formed as a major product during pyrolysis of cellulose. The same study also demonstrated that this compound can be converted to different high added- value compounds such as levoglucosenone, and 5-hydroxymethylfurfural directly or through a glucose intermediate via chemical, catalytic and biochemical processes. According to Delbart *et a*l. (2021), the study demonstrated that levoglucosenone has antitumor potential. The previous study by Giri *et al*. (2017) showed that obtained levoglucosenone from bio-oils produced by soybean hull pyrolysis has antibacterial activity against *Salmonella typhimurium* and other bacterial species.

Only 2.19% of 9-Octadecenoic acid (Z)-, tetradecyl ester was obtained in methanol crude extract using GC-MS. This compound also known as myristyl oleate has been used as an excipient in topical drug delivery systems due to its emolliency and ability to form emulsions. It stated that myristyl oleate can improve spreadability and potentially enhance skin penetration. Excipients are inactive compounds that improve the delivery of active pharmaceutical ingredients effectively (Cavalcanti *et al*., 2013). According to Rahardhian *et al*. (2025), mystryl oleate has been found in *Peronema canescens* and may contribute to α-amylase inhibitory activity. Inhibition of enzyme α-amylase is considered a strategy for the treatment of carbohydrate disorders such as diabetes and obesity (Sales *et al*., 2012).

Tetracontane was identified as the compound with the highest percentage in the hexane crude extract (50.59%), as shown in Table 4. It was found in higher concentration at the peaks 38 (29.92%) and 45 (20.67%). According to a study by Jyoti *et al*. (2025), tetracontane was identified with concentration at 45.20% in *Grangea maderaspatana.* The previous study done by Jyoti *et al*. (2025) also demonstrated tetracontane as a potential excipient in drug formulations and it has emollient properties to create protective barriers on the skin.

An observed concentration of 11.65% for (Z)-Tetradec-7-enal was calculated based on the peak area, despite the compound exhibiting a low peak height of only 3.94%. This ratio (high area, low height) indicates a short and wide peak in the chromatogram, confirming the presence of a high abundance of this compound but with less efficient separation. The primary consequence of this broad peak is reduced resolution, making it difficult to fully separate this compound from nearby analytes and potentially leading to the co-elution and missed identification of trace components. A study by Ribeiro and colleagues (2022) suggests that (Z)-Tetradec-7-enal may possess antimicrobial properties. Additionally, it has also been shown to be produced by some plants as a defense mechanism against herbivores (Xiong *et al*., 2022). According to P. Sharma *et al*. (2025), the study identified 19.61% of (Z)-Tetradec-7-enal in *Vinca rosea* extracts.

Octacosyl trifluoroacetate showed the third highest compound identified in hexane crude extract with concentration of 8.74%, as shown in table 4. This compound has been identified in ethanol fraction of *Halymenia durvillei* extracts with concentration of 5.03% and possess antioxidant properties and structurally related to octacosanol (Chumphoochai *et al*., 2025). According Pereira *et al*. (2024), the study demonstrated that octacosanol has antidiabetic, anticholesterolemic, and antihypertensive potential.

Besides that, n-hexadecanoic acid was identified in hexane crude extract with 4.66% as shown in table 4. Based on previous studies, n-hexadecanoic has been shown to have an anti-inflammatory effect (F. Liu *et al*., 2020, Zhang *et al*., 2022). Palmitic acid or n-hexadecanoic acid was previously identified with antibacterial and anticancer properties in the petroleum ether extract of *A. arborescens* (Moglad *et al*., 2024). Moreover, Purushothaman *et al*. (2025) highlighted that n-hexadecanoic acid from *Excoecaria agallocha* exhibited strong anti-inflammatory effects through the inhibition of COX-1 and COX-2.

Lastly, only 3.21% of octacosane was found in the hawthorn fruit after GC-MS analysis. Recently, octacosane was identified in Mantidis ootheca extracts, exhibiting antimicrobial and antibiofilm properties against *Pseudomonas aeruginosa* (Vanessa *et al*.,2024). 11.72% of octacosane was identified in purified leaf extract of *Marantodes pumilum* and the study demonstrated it exhibited anti-inflammatory activity (Okechukwu, 2020).

CHAPTER 6

CONCLUSION

This study successfully demonstrated that the selection of the extraction solvent significantly influences the resultant phytochemical profile of Hawthorn fruit, as characterized by Gas Chromatography–Mass Spectrometry (GC-MS). The analysis confirmed distinct chemical compositions based on solvent polarity. The methanol crude extract, a polar solvent, yielded five major concentrated compounds, including Hexadecanoic acid, octadecyl ester (38.46%), n-Decanoic acid (20.05%), 5-Hydroxymethylfurfural (14.81%), beta.-D-Glucopyranose, 1,6-anhydro (2.27%), and 9-Octadecenoic acid (Z)-, tetradecyl ester (2.19%). However, the hexane crude extract, a non-polar solvent, isolated a qualitatively different set of major compounds, such as Tetracontane (50.59%), 7-Tetradecenal, (Z)- (11.65%), Octacosyl trifluoroacetate (8.74%), n-Hexadecanoic acid (4.66%), and Octacosane (3.21%). The pronounced differences in compound identification highlight the selectivity of solvents for specific bioactive molecules. This result provides valuable insights for guiding future research toward the targeted isolation, purification and applying of these compounds. The identified phytochemicals hold significant promise for further application and development in veterinary medicine, specifically exploring their potential as functional food properties or for their desirable pharmaceutical effects.

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