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Analysis of Bioactive Phytochemicals in *Aloe perryi* Aqueous Leaf Gel Extract Using FT-IR, GC-MS and HPLC Techniques

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Abstract

This study characterizes the phytochemical composition of the aqueous gel extract from *Aloe perryi* Baker, a medicinal species endemic to Socotra Island, Yemen. The primary objective was to investigate and characterize the functional groups and chemical composition of *Aloe perryi* aqueous extract as compared with quercetin (standard of flavonoids), gallic acid (standard of phenols), aloin (standard of anthraquinones), and ascorbic acid (standard of antioxidants). Aloe leaves gel was blended, extracted with distilled water using a shaker, filtered, and lyophilized. Then the yield of extract (10%) was subjected to FTIR, GC-MS, and HPLC analysis. FTIR analysis confirmed the presence of phenol & alcohols (OH), alkane (C-H), unsaturated ketone (C=C), carbonyl (C=O), Ether (C-O-C), amine (NH), methyl (CH_3) functional groups in the Aloe aqueous extract. GC-MS analysis revealed the presence of alcohols, many alkanes, aldehydes, ketones, aliphatic and aromatic esters, and fatty acids in the Aloe aqueous extract. HPLC emphasized the presence of functional groups by the presence of quercetin (3.59%), gallic acid (5.39%), aloin A (19.27%), and aloin B (29.64%) in Aloe extract, but ascorbic acid was not separated during this method. The three approaches (FTIR, GC-MS, HPLC) were complementary to one another in identification and the simple quantification of compounds in Aloe extract.

Article Info

Keywords:
Aloe perryi, Socotra Island, Aqueous, Gel, FT-IR , HPLC, GC-MS, Quercetin, Gallic acid, Aloin, Ascorbic acid

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1. INTRODUCTION

Aloe, which belongs to the family *Aloaceae* [1, 2], has been used as a folk remedy worldwide [3]. Because aloe thrives in arid environments, its leaves contain a thick gel covering enriched with water and active chemicals [4]. The gel in aloe leaves contains many active constituents, such as minerals, sugars, enzymes, amino acids, anthraquinones, vitamins, phenols, and flavonoids [5]. According to earlier research, aloe has therapeutic qualities, including wound healing and anti-ulcer effects. Furthermore, contemporary pharmacological studies have indicated that aloes have novel therapeutic qualities, including anticancer, antimicrobial, antidiabetic, and hypoglycemic activities [6].

Approximately 33 Aloe species have been found in Yemen, 17 of which are considered endemic. *Aloe perryi*, also known as Perry's Aloe, is endemic to the island of Socotra in Yemen [7].

Fourier-transform infrared (FTIR) spectrophotometry is presumably the most powerful tool for characterizing and identifying functional groups in an unknown plant extract mixture [8].

Owing to the small amount of plant extract required for analysis, GC–MS is an extremely effective, rapid, and accurate analytical method for the detection of a variety of substances, such as alcohols, alkaloids, nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids [9]. Numerous investigations using GC–MS to analyze Aloe extracts have been conducted, and the results were comparable to or near our findings [10–12].

High-performance liquid chromatography (HPLC) is an advanced analytical technique that is employed to separate, identify, and quantify components in complex mixtures. This method operates based on the differential affinities of sample molecules for the mobile and stationary phases within a chromatographic column. HPLC is recognized for its high resolution, accuracy, and efficiency, making it indispensable in various fields, including pharmaceuticals, biotechnology, environmental analysis, and forensic science [13].

Plants endemic to Yemen may have medicinal importance, as evidenced by their use as folk remedies; however, these plants have not been the subject of extensive analytical studies.

Most studies have indicated that the therapeutic properties of Aloe plants are attributed to their antioxidant properties, which are due to the presence of flavonoids, phenolic acids, anthraquinones, and vitamin C. Therefore, this study was designed to investigate the presence of these compounds and perform a simple quantification of their quantities in the aqueous extract of Socotra *Aloe perryi* using FTIR, GC-MS, and HPLC methods, by comparing them to the available standards for these compounds: quercetin, gallic acid, aloin, and ascorbic acid.

2. MATERIALS AND METHODS

Chemicals

Standard aloin with purity ≥97% was purchased from Sigma-Aldrich, Egypt. Standards quercetin, ascorbic acid, and all HPLC analysis solutions were kindly obtained from the Global Modern Pharmacognosy Company. Gallic acid was kindly gifted by Dr. Faten Hameed Thamer, and KBr was obtained from the Chemistry Department at Sana'a University.

Collection of plant materials

Aloe perryi leaves were collected from Socotra Island, Yemen, and immediately transported to Sana'a with permission from the Chairman of the Environment Protection Authority, Yemen. Two specimens of the collected taxa (inflorescence and leaves) were mounted on herbarium sheets and identified by Dr. Hassan Ibrahim, Professor of Plant Taxonomy, Biology Department, Sana'a University, Yemen. Furthermore, the two mounted specimens were preserved in the Herbarium of the Faculty of Science of Sana'a University under herbarium numbers BHSS 1692 and BHSS 1693.

Preparation of gel extraction

In our previous publication [14], we discussed the extraction of Aloe leaves. *Aloe perryi* leaves were first washed

Figure 1. General view of *Aloe perryi* plant in Socotra Island

with running tap water and rinsed with distilled water. The spines around the leaves were removed using a knife, and the thick epidermis was removed to collect the solid mucilaginous gel with a sterilized spoon. The gel was weighed, cut into small pieces, and blended in an electric blender for 3 min [15].

Aqueous extract was prepared as follows: 100 g of blended gel was extracted with sterilized distilled water at a 1:10 (w/v) ratio and pH between 4.4 and 6.5 using a shaker provided with a Gallenkamp orbital incubator at 80℃ [16] for 3 h. The obtained extract was filtered using Whatman No.1 filter paper and lyophilized using a freeze dryer (LABCONCO, Freezone 4.5, USA). The dried crude extracts were collected, weighed, packed in dark glass containers, and stored at -20℃ until used. Extraction was carried out in the laboratory of the Biology Department, Faculty of Sciences, Sana'a University.

Calculation of the extract yield percentage (%):

Yield = w2/ w1 X 100

where w2 is the weight of the extracted sample (g) and w1 is the weight of the leaf gel (g) before extraction [17].

Fourier transform infrared (FT-IR)

Spectroscopy was used to determine the functional groups present in *Aloe perryi* leaf gel aqueous extract, as well as standard quercetin, aloin, gallic acid, and ascorbic acid, using the KBr disk method at room temperature. Samples (Aloe extract and standards) were weighed (2.0 mg) and homogenized with 2.0 mg spectral grade potassium bromide (KBr) powder, which was anhydrous. The samples and KBr mixture were pressed using a vacuum hydraulic (Graseby Specac) at 1.2 psi to obtain a clear, transparent disc with a diameter of 13 mm and a thickness of 1 mm. The scanned samples passed through infrared, where their continuing waves were detected by a detector connected to a computer with set values of the tested samples' spectra. The samples were scanned in the absorption area of 4000–4000 cm^{-1} at a resolution of 4 cm^{-1}, cosine apodization, gain 4, and scanning speed of 2 mm/s using a Fourier-transform infrared spectrometer (JASCO FT/IR-410 model) available in the Department of Chemistry Analytic Lab, Sana'a University, Yemen. The obtained results were analyzed using Perkin Elmer Co. software [18]. This spectroscopy was repeated thrice to ensure the reliability and quality of the results.

GC-MS analysis

A 1-μL aliquot of the aqueous aloe extract was injected in splitless mode into a Shimadzu Scientific GC System equipped with a TG-5 MS fused-silica capillary column (30 m × 25.0 mm i.d., 25.0 mm film thickness) for partial component separation. –he MS analysis was performed using a QP-2010 GC mass detector coupled to a single-quadrupole mass spectrometer. The ionization energy for the positive-ion electron-impact (EI) mass spectra was 70 eV, and helium was used as the carrier gas at a flow rate of 1.2 mL/min. The GC oven temperature was programmed as follows:

Heating was initiated at 60℃ (holding for 2min), subsequently increased to 270℃ at a rate of 21℃ /min for 15 min. The total GC run time was 25 min. The injector and detector temperatures were 250 and 200℃, respectively. The quantification of all the identified components was investigated using the percent relative peak area. The relative percentage of each component was calculated by dividing the peak area by the total peak area. Tentative identifications of the compounds were performed based on comparisons with those in the National Institute of Standards and Technology (NIST) and Wiley library mass spectral databases [19, 20].

Plastic materials, such as plastic-coated stir bars, most pipette tips, plastic gloves, some filter holders, plastic vials, and so on, were not used during sample collection, preparation, or storage. Only glass equipment, such as flasks, vials, pipettes, syringes, and so on, that had been rigorously cleaned was used. Only high-performance liquid chromatography (HPLC) or optima grade solvents were used. Glass syringes and stainless-steel needles/dispensers were used. Work was performed in a dedicated, well-ventilated clean room. The injection syringe was rinsed several times with a clean solvent before and after each injection. Manual injections were performed quickly to minimize the time the syringe needle was exposed to laboratory air. A systematic blank was injected to check the entire system, and solvents and glassware were used to monitor background levels and ensure method reliability. The injector head was frequently heated to release any adsorbed contaminants. Only the person working in the laboratory was allowed to enter [21].

Preparation of samples for HPLC analysis

Each standard (10 mg) was accurately weighed and transferred to a 100 mL volumetric flask. The standard was then dissolved in 100 mL methanol (70%) to prepare a standard stock solution of 100 μg/mL. The extract (0.2 g) was dissolved in methanol (70%) and transferred to a 10 mL volumetric flask and diluted to a mark at 20 mg/mL. Standard stocks and the extract were sonicated in an ultrasonic bath (SCT-SONIC-6) for 15 min and filtered through a 0.45 μm nylon syringe filter. Standards were diluted to 25 μg/mL for HPLC analysis and then transferred with the extract to 2 mL glass vials and injected in triplicate [22]. The identification of the compounds was verified by comparing their retention times and UV absorption spectra with those of the standards. Chromatograms of the standards and extract were recorded at the selected wavelength.

Table[1]: Mobile phase gradient for HPLC compounds separation

Time (min)	Solvent A (%)	Solvent B (%)
-	88	12
3	88	12
8	70	30
13	45	55
15	40	60
17	10	90
21	10	90
23	88	12
25	88	12

Optimization of analytical conditions

Chromatographic analysis was performed using a method described previously [23, 24]. Separation of the Aloe extract and standards (aloin, quercetin,, gallic acid, and ascorbic acid) was performed using a Waters Hypersil TM C18 column consisting of 18 carbon chains immobilized to a silica matrix column with a length of 250 mm and an internal diameter of 4.65 mm, filled with Waters' proprietary phase chemically bonded to porous modified silica particles with a diameter of 5 μm (250 × 4.65 mm I.D, 5 μm; Waters, USA). The column was thermostated at 28 ℃. High-performance liquid chromatography (HPLC) was performed using a photodiode-array detector (PDA (Waters 2998) as detector, a binary HPLC pump (Waters 1525, U.S.A.) and an autosampler injector (Waters 2707). Separation was performed using gradient

Table[2]: FT-IR absorption peaks and corresponding functional groups identified in of *A. perryi* leaves gel aqueous extract and the selected standards.

Functional groups	Aloe Aqueous extract	Standard			
		Quercetin ($C_{15}H_{10}O_7$)	Gallic acid ($C_7H_6O_5$)	Aloin ($C_{21}H_{22}O_9$)	Ascorbic acid ($C_6H_8O_6$)
-OH	3435.56	3406.46	3370.9	3389.28	3525.24
-C-H	2960.2 & 2859.92	2924.52	—	2923.56	2855.1
-C=O	1723.09 1685.48 1654.62 1637.27	1655.59	1701.87	1636.3	1673.91
C=C	1617.98	1615.09	1617.98	1603.52	1526.38
N-H	1609.31	—	—	—	—
$-CH_3$	1383.68	—	—	1457.92	—
-OH bending vibrations of phenols	1383.68	1363.43	1339.32	1383.66	1364.39
Ether group (C-O-C)	1269.9	1287.25	—	1291.11	—
C-N	1269.9	—	—	—	—
C–O stretching of esters and phenols	1249.65	—	—	—	1248.68

elution with solvents A and B, namely acetonitrile and water, respectively. The gradient conditions are shown in Table (1). The flow rate was adjusted to 1 mL/min for 5 min at room temperature, and good resolution and the quantitation wavelength (λ max) were observed; thus, the wavelength was set between 200 and 800 nm for the pilot study and then we selected the wavelength 370 nm for the determination of aloin A, aloin B, and quercetin; 272 nm for the determination of gallic acid; and 245 nm for the determination of ascorbic acid (vitamin C) as preferred wavelengths. Optimal separation of the analyses was achieved within 25 min, and the injection volume was 20 μL for standards and Aloe aqueous extract. For quantitative analysis of the compounds, the retention time and peak area were compared with those of the standards. BreezeTM 2 HPLC System software was used during the HPLC analysis. The HPLC analysis was performed by the Global Modern Pharmacognosy Company.

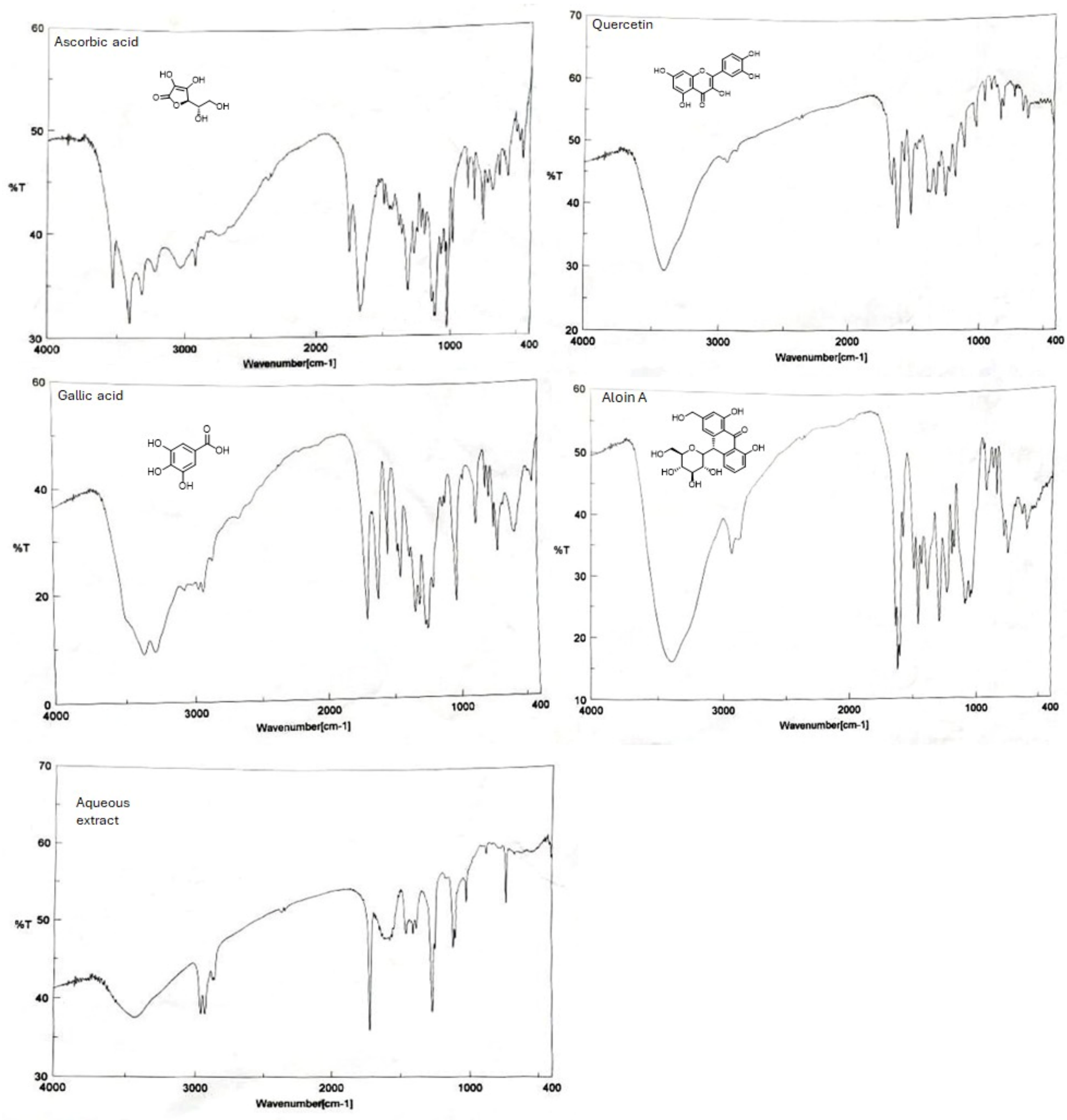
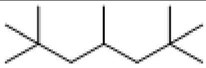
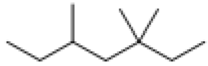
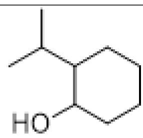
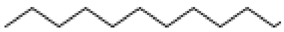
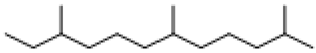
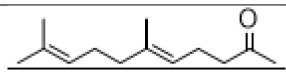
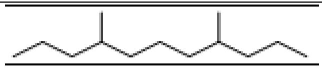
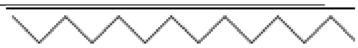
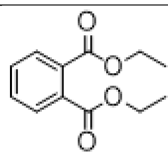
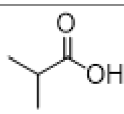
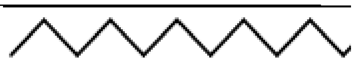
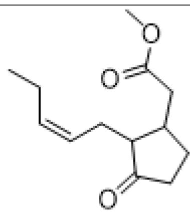
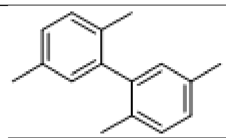
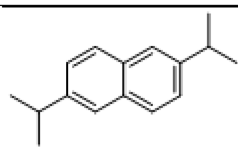
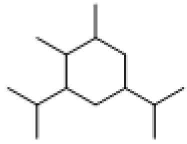
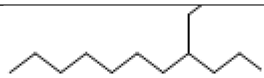
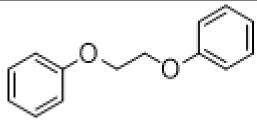
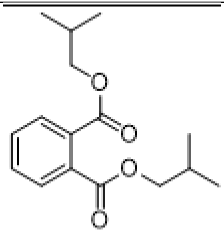
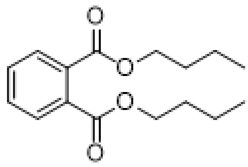
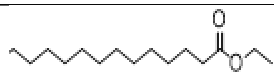


Figure 2. FTIR spectra of the selected standards and aqueous extract of Aloe leaves gel.

Table[3]: Major bioactive compounds identified in the *A. perryi* leaves gel aqueous extract using GCMS.

Peak no.	RT (min)	Peak area (%)	Molecular formula	Name	Chemical Structure
1	5.875	9.92	$C_{12}H_{26}$	2,2,4,6,6-pentamethylheptane	
2	6.157	2.07	$C_{10}H_{22}$	3,3,5-trimethyl-Heptane	
3	7.003	2.16	$C_{11}H_{24}$	Undecane	
4	7.828	0.89	$C_9H_{18}O$	2-isopropylcyclohexan-1-ol	
5	9.622	0.67	$C_{14}H_{30}$	Tetradecane	
6	9.757	1.34	$C_{15}H_{32}$	2,6,10-trimethyl-dodecane	
7	9.935	3.12	$C_{15}H_{32}$	Pentadecane	
8	10.205	0.41	$C_{14}H_{24}O$	6,10-dimethyl, 5,9-Undecadien-2-one	
9	10.366	0.28	$C_7H_{14}O$	(E)-hept-2-enal (Heptenal)	
10	10.498	0.54	$C_{13}H_{28}$	4,8-dimethyl-undecane	
11	10.789	2.11	$C_{17}H_{36}$	Heptadecane	
12	11.245	3.88	$C_{12}H_{14}O_4$	Diethyl Phthalate	
13	11.483	1.23	$C_4H_8O_2$	2-methyl propanoic acid	
14	11.594	3.36	$C_{16}H_{34}$	Hexadecane	
15	11.766	2.35	$C_{13}H_{22}O_3$	methyl (Z)-2-(3-oxo-2-(pent-2-en-1-yl)cyclopentyl)acetate (Dihydro methyl jasmonate)	
16	11.933	1.54	$C_{16}H_{18}$	2,2',5,5'-tetramethyl 1,1'-Biphenyl	
17	12.143	0.73	$C_{16}H_{20}$	2,6-diisopropylnaphthalene	
18	12.357	3.22	$C_{18}H_{38}$	Octadecane	

19	12.525	4.84	$C_{14}H_{28}$	1,5-Diisopropyl-2,3-dimethylcyclohexane	
20	12.823	0.82	$C_{22}H_{46}$	8-heptyl pentadecane	
21	12.88	1.86	$C_{14}H_{14}O_2$	1,1'-[1,2-ethanediylbis(oxy)]bis-Benzene	
22	13.081	3.75	$C_{20}H_{42}$	Eicosane	
23	13.183	3.53	$C_{17}H_{34}O_2$	Isopropyl myristate	
24	13.309	3.77	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	
25	13.828	6.36	$C_{17}H_{34}O_2$	Methyl palmitate	
26	13.931	2.89	$C_{16}H_{22}O_4$	Dibutyl phthalate	
27	14.279	3.14	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester	
28	15.359	1.06	$C_{20}H_{38}O_2$	Ethyl Oleate	
29	15.522	1	$C_{19}H_{38}O_2$	Heptadecanoic acid, ethyl ester	

Table[4]: Biological activities of compounds from *Aloe perryi* leaves gel aqueous extract.

RT (min)	Name of compound	M. Wt.	Match score (%)	CAS number	Functional classification	Biological activity
11.24	Diethyl Phthalate	222.24	80% (WILEY7)	84-66-2	Ester (phthalates)	Antimicrobial [25]
13.30	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278.34	81% (WILEY7)	84-69-5	Ester (phthalates)	Anticancer [26]; Induced apoptosis [27]; Antimicrobial [28]
13.08	Eicosane	282.55	86% (WILEY7)	112-95-8	Hydrocarbon (an alkane)	Antitumor [29]
13.82	Methyl palmitate	270.46	88% (WILEY7)	112-39-0	Ester (Fatty acid)	Antioxidant, Anticancer, Antimicrobial [30]
14.27	Hexadecanoic acid, ethyl ester	284.48	91% (NIST27)	628-97-7	Ester (Fatty acid)	Antioxidant, Anti-inflammatory, Anticancer [31, 32]; Antimicrobial [33–35]

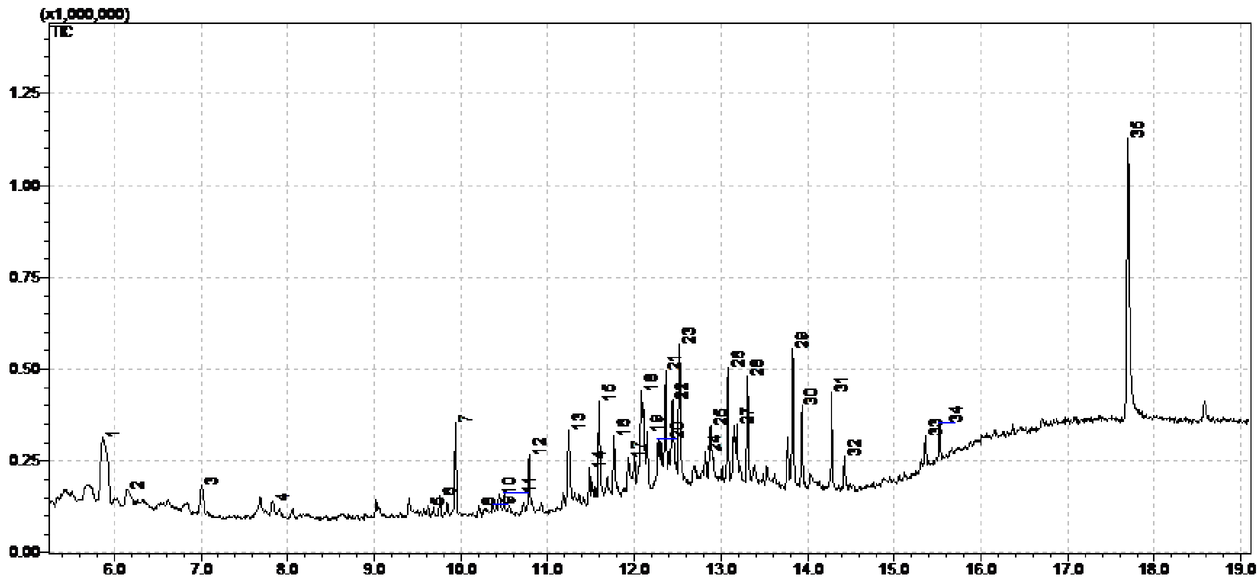


Figure 3. GC–MS chromatogram of aqueous gel extract of *A. perryi* leaves.

3. RESULTS

The yield of gel extraction of aloe leaves with distilled water was 10 %.

The infrared spectra of *A. perryi* aqueous gel extract and standard compounds (quercetin, gallic acid, aloin and ascorbic acid) were analyzed over a wavelength range of 4000 to 400 cm^{-1} (Table (2) & Fig.(2)). Fourier transform infrared spectrophotometer (FTIR) represented the first step in the identification of compounds in Aloe extract, while GC-MS and HPLC were the second and third emphasize steps. FTIR revealed the presence of OH phenol and alcohol group in Aloe extract at the peaks 3435.56 & 1383.68 cm^{-1}, compared to standards, which was later identified by HPLC and GC-MS as quercetin (3.59 % and 2-isopropylcyclohexan-1-ol (0.89%), respectively.The group C-H stretch was found in Aloe extract at the peaks 2960.2 and 2859.92 cm^{-1} which may correspond to the alkanes 2,2,4,6,6-pentamethylheptane(9.92%),3,3,5-trimethyl-Heptane(2.07%), Undecane(2.16 %),Tetradecane (0.67%),2,6,10-trimethyl-dodecane (1.34%), Pentadecane (3.12%),4,8-dimethyl-undecane(0.54%),Heptadecane (2.11%),Hexadecane(3.36%),Octadecane (3.22%),1,5-Diisopropyl-2,3-dimethylcyclohexane(4.84%),8-heptyl pentadecane (0.82%), Eicosane (3.75%) that identified by GCMS. The peaks 1723.09, 1685.48, 1654.62 and 1637.27 cm^{-1} in Aloe extract may correspond to C=O stretching of the aliphatic esters Isopropyl myristate (3.53%), Methyl palmitate (6.36%), Hexadecanoic acid, ethyl ester (3.14%),Ethyl Oleate (1.06%), Heptadecanoic acid, ethyl ester (1%) and the aromatic esters Diethyl Phthalate (3.88%), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (3.77%), Dibutyl phthalate (2.89%),1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (15.71%), and fatty acid 2-methyl propanoic acid (1.23%) which identified by GCMS. Carbonyl group found in Aloe extract may also indicate the presence of aldehyde (E)-hept-2-enal (Heptenal) (0.28%) that identified by GC-MS. Unsaturated ketone (C=C) group was found in Aloe extract at the peak 1617.98 cm^{-1} may refer to 6,10-dimethyl, 5,9-Undecadien-2-one (0.41%) which identified by GCMS. Ether group (C-O-C) was showed in Aloe extract at the peak 1269.9 cm-1whic may referred to (benzene,1,1'-[2-ethanediylbis(oxy)]bis) (1.86%) that identified by GC-MS. Hydroxyl

The presence of phenol and carbonyl aromatic C=O groups in Aloe extract may also indicate the presence of aloin A and B and gallic acid in Aloe extract. HPLC emphasized the presence of aloin A(19.27%), aloin

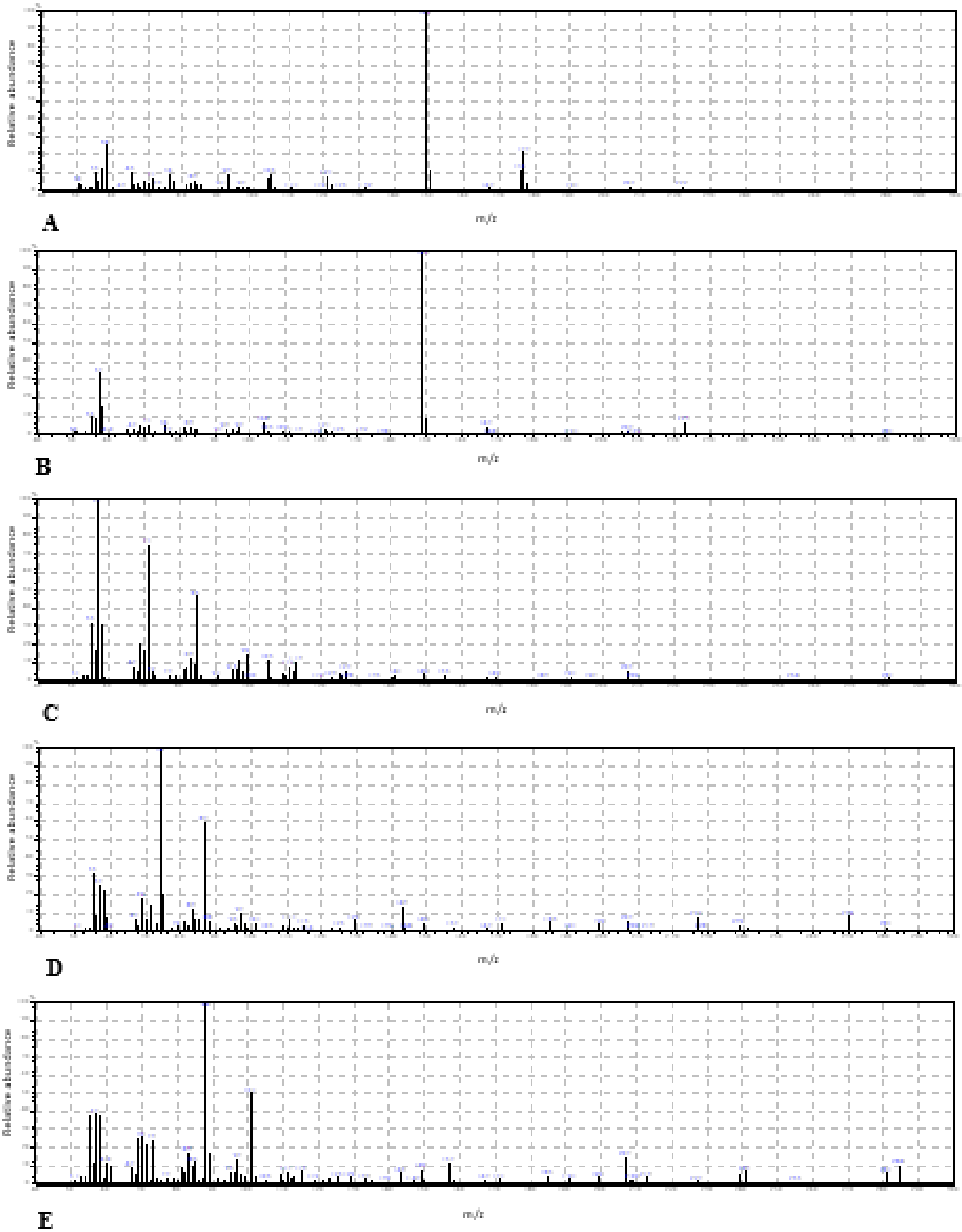


Figure 4. Mass spectra of A, diethyl phthalate; B, 1, 2-benzenedicarboxylic acid, bis(2-methylpropyl) ester; C, eicosane; D, methyl palmitate and hexadecanoic acid, ethyl ester, in *Aloe perryi* extract.

B(29.64%), and gallic acid (2.25%) in Aloe extract. However, ascorbic acid could not be separated using this method.

GC-MS analysis of *Aloe perryi* aqueous gel extract recorded 30 compounds (Table (3) and Figure (3)). 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, exhibited the highest peak area (15.71%), whereas heptenal exhibited the lowest peak area (0.28%). Some compounds identified using GC-MS have been reported to have biological activities, such as antimicrobial,

Table[5]: Identified compounds in *A. perryi* leaves gel aqueous extract using HPLC and standards

Peak	Wavelength (nm)	RT (min)	Area (μV*sec)	Area %	Height (μV)
Aloin A	370	10.77	428689	42.27	72221
Aloin B		11.11	585442	57.73	98395
Extract		10.74 A 11.09 B	456478 A 701982 B	19.27 A 29.64 B	76835 A 84333 B
Quercetin		12.41	2414519	98.18	177016
Extract		12.40	84985	3.59	14622
Gallic acid	272	2.37	2231734	99.40	317807
Extract		2.25	3987	5.39	1652
Ascorbic acid	245	1.78	———-	———-	———-
Extract		2.70	33887	0.66	5096

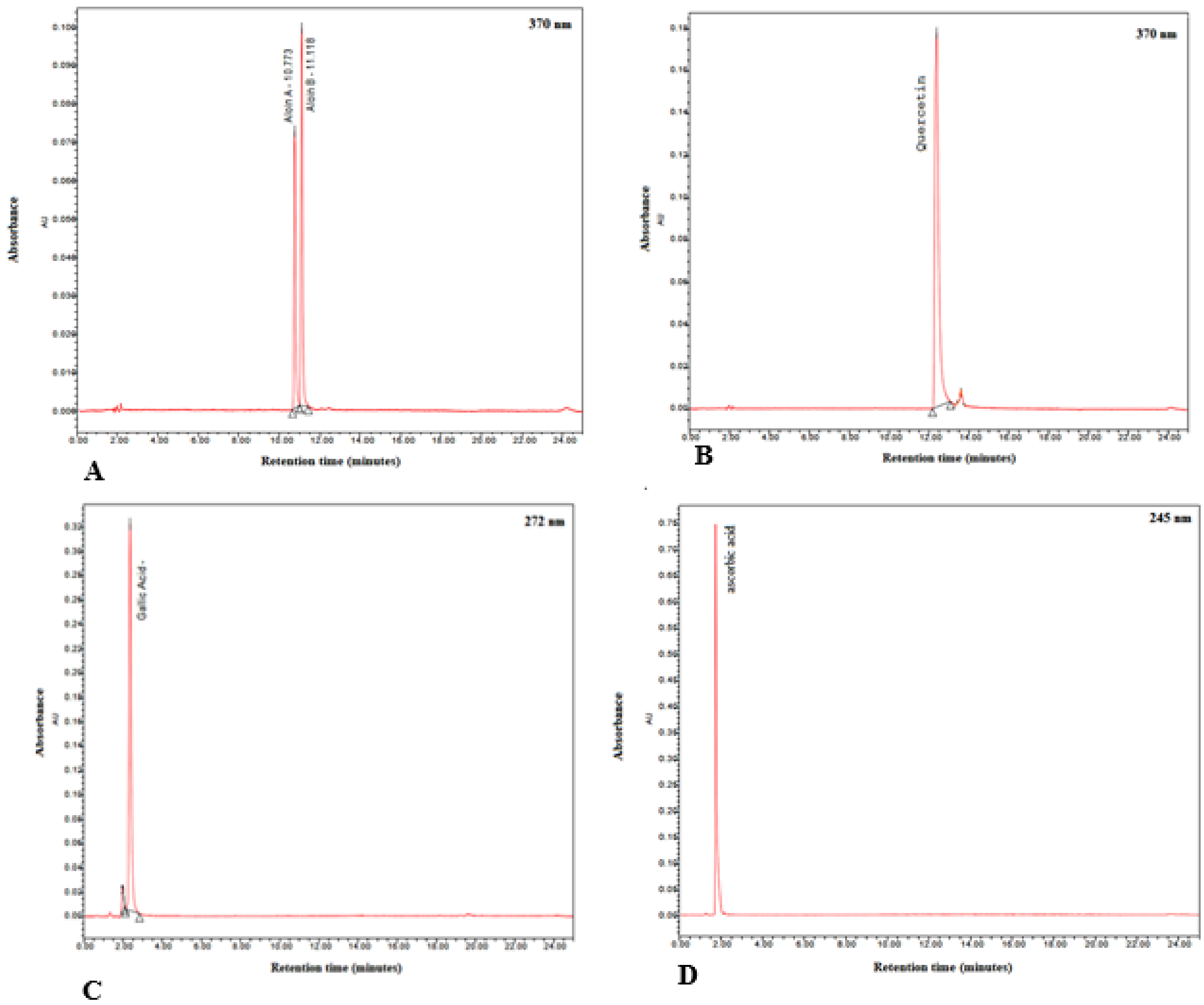


Figure 5. HPLC chromatograms of the selected standards.

anticancer, pro-apoptotic, and anti-inflammatory activities. Table (4) lists the compound names, retention times, nature, molecular formula, and biological activities.

Discussion

Because Aloe glycosides are usually dissolved in water, this study utilized water as the extraction solvent. Although glycosides of flavonoids have increased water solubility, their water solubility is limited [36]. The

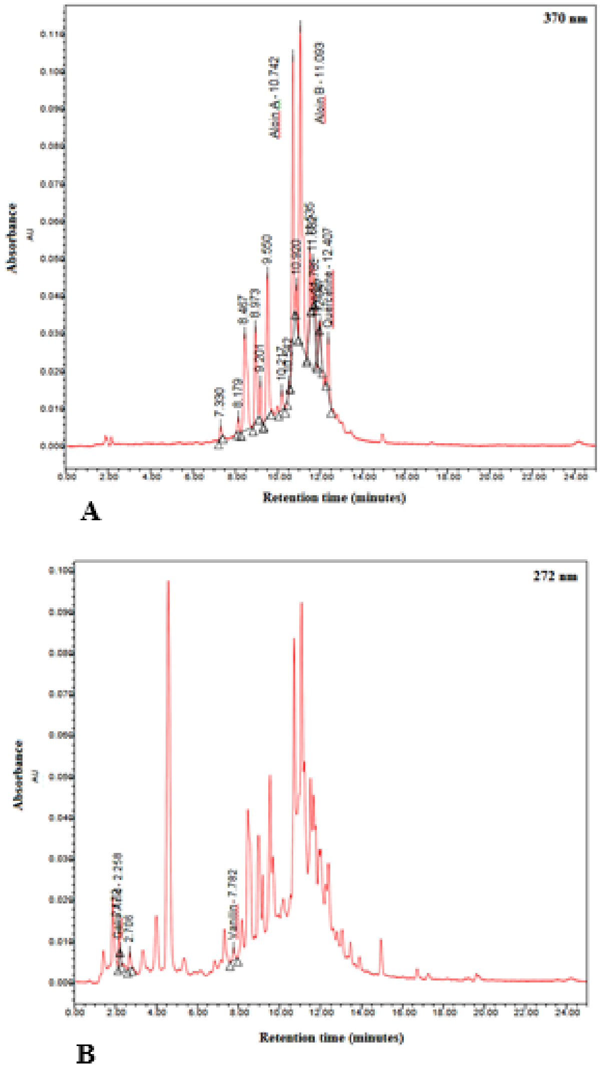


Figure 6. HPLC chromatograms of *A. perryi* leaves gel aqueous extract containing aloin A, B and quercetin (A), gallic acid (B).

chemical structure of flavonoids also has a significant impact on their stability. A higher number of hydroxyl groups in flavonoid compounds results in a reduction in compound stability. [37] stated that the location of the hydroxyl group also affects compound stability. For example, flavonoids with the same number of hydroxyl groups, such as kaempferol (flavonol) with hydroxyl groups in A, B, and C rings, are more prone to degradation than luteolin (flavonone) with hydroxyl groups in A and C rings only. Quercetin, which had –OH phenol groups similar to those of the standard, was also detected in the Aloe extract using HPLC. HPLC results indicated the presence of flavonoids and phenolic acids, which agrees with the results of [38], who reported that the methanol extract of *A. vera* gel contained phenolic acids. Gallic acid (3, 4, 5-trihydroxybenzoic acid) is a naturally occurring polyphenolic compound. It occurs in plants in the form of free acids, esters, catechin derivatives, and hydrolyzable tannins [39],[40]. Based on the results, the amount of Aloin B in Aloe extract and standard was higher than that of Aloin A. A previous study stated that plants contain anthraquinones, phenolic acids, flavonoids, and vitamins [41].

FTIR analysis of functional groups in the aqueous Aloe extract revealed the presence of phenolic, alkane, carbonyl, C=C stretching aliphatic and aromatic, amino, nitro, methyl, ether, and amine groups. This finding is consistent with that of [42], who recorded most of these functional groups in the *Aloe vera rind extract,* but inconsistent with that of [37], who stated that *Aloe rubroviolacea* gel extract did not contain carbonyl and C=C stretching groups. This may be due to variations in plant species, season, environment, and other factors.

The peak at 1609 cm^{-1} in the Aloe extract may correspond to the amine group, which is in agreement with [43], who recorded an amine group at a peak similar to that in our result.

GC-MS result agrees with that of [38] who recorded the presence of hexadecenoic acid, ethyl ester, ethyl oleate, and 1,2 benzenedi-carboxylic acid, bis(2-methylpropyl)ester in the Aloe vera ethanolic extract. GC-MS results are similar to those of [39], who detected isopropyl myristate, 1, 2-benzenedicarboxylic acid, bis (2-methylpropyl) ester, hexadecane, and eicosane in *Aloe vera* ethyl acetate gel extract. These results are supported by [11], who reported the presence of phenolic compounds, anthraquinone glycosides, and esters in A. vera gel and extract.

The results of this study are consistent with those of [44] and [45], who detected aloin A and B in *Aloe vera* and other types of Aloe. Moreover, these results are consistent with those of [34], who detected quercetin and gallic acid in nine Aloe species. HPLC method had some limitations for ascorbic acid, and no recorded data were available for ascorbic acid at a wavelength of 245 nm.T his is likely due to the instability of the compound and

its rapid degradation under HPLC method parameters. Ascorbic acid is highly sensitive to environmental factors and certain analytical conditions. These results are consistent with those of [46], who found that ascorbic acid could not be separated because a special method was required.
Although some studies have suggested that phthalate derivatives are toxic and are identified as plasticizers, there is evidence to suggest that phthalate derivatives have a broad spectrum of bioactive properties. Diethyl phthalate obtained from plants was found to inhibit the growth of the MDA-MB-231 cancer cell line [47]. Previous studies have reported the antimicrobial properties of dibutyl phthalate [48] and diisooctyl phthalate [40], [49], [50].

4. CONCLUSION

Aloe perryi aqueous extract should be investigated for industrial applications; therefore, FTIR, GC-MS, and HPLC methods were selected. FTIR analysis revealed the presence of phenol and alcohols (OH), alkane (C-H), unsaturated ketone (C=C), carbonyl (C=O), ether (C-O-C), amine (NH), and methyl (CH3) functional groups in the Aloe aqueous extract. GC-MS analysis exhibited the presence of various volatile and semi-volatile components, including fatty acids, alkanes, aldehydes, ketones, aromatic and aliphatic hydrocarbons, alcohols, and various esters. HPLC analysis identified and quantified anthraquinones (aloin A and aloin B), phenolic acid (Gallic acid), and flavonoid (quercetin), whereas ascorbic acid could not be separated using this method. In summary, the combined use of FTIR, GC-MS, and HPLC provides a powerful approach for the qualitative and quantitative analysis of Aloe extract because they work together to provide a comprehensive analysis of complex samples. Further studies, including the use of other methods such as quantitative nuclear magnetic resonance (qNMR) spectroscopy and mass spectrometry imaging (MSI), are warranted to quantify the compounds in the Aloe extract.

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