

GC-MS, UPLC-MS and UHPLC Profiling of Hippophae rhamnoides Linnaeus Berries Fractions in Correlation to Its Antioxidant, Anti-inflammatory, and Hepatoprotective Effects

Nasira Abbasi¹, Munish Goyal², Rohit Goyal³, Anjana Devi^{4*}

¹Research Scholar, Department of Pharmacy, Career Point University, Hamirpur, HP.

Email: nasiraabbasi4@gmail.com

²Professor, Department of Pharmacy, Aakash Institute of Medical Sciences, Nalagarh.

Email: drmunishgoyal81@gmail.com

³Professor, Department of Pharmacy, Shoolini University, Solan. Email: rohitgoyal@shooliniuniversity.com

^{4*}Associate Professor, Department of Pharmacy, Career Point University, Hamirpur, HP.

Email: anjana.pharmacy@cpuh.edu.in (Corresponding Author)

ABSTRACT

The current investigation used phytochemical evaluation of solvent fractions to assess the hepatoprotective efficacy of Hippophae rhamnoides Linn. berry. Fresh berries were juiced and fractionated using different solvents like ethyl acetate, n-butanol, chloroform and water. Ethyl acetate and chloroform fractions were analysed using GC-MS and further characterisation of Chloroform (HR-Chl), ethyl acetate (HR-EA), and butanol (HR-But) fractions were using UPLC-Q-TOF/MS with the aim of identifying phytoconstituents for bioactive components with hepatoprotective, anti-inflammatory, and antioxidant properties. Here, the metabolic profiling of Hippophae rhamnoides Linn. Fractions of Berry via gas chromatography-mass spectrometry and Ultra Performance Liquid Chromatography was performed to screen and identify the reported bioactive chemical constituents. Total of 72 diverse chemical constituents were identified which belong to various classes, including polar compound, non polar compound flavonoids, fatty acids, terpenoids, carboxylic acids, phenolic acids, quinones, and alkaloid. Among these metabolites flavonoids were found to be the major component of Hippophae rhamnoides Linn. berry fractions. Moreover, Caffeic acid was found in the ethyl acetate and butanol fractions, while quercetin was found in the chloroform fraction, based on the quantitative analysis with UHPLC-DAD.

Keywords: Sea Buckthorn, GC-MS, UHPLC-DAD, UPLC-Q-TOF/MS

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Conflict of interest: None

1. Introduction

Siberian pineapple, sand thorn, sea berry, and sallow thorn are other names for the deciduous shrub or tree known as sea buckthorn (*Hippophae rhamnoides* L.) [45]. Sea buckthorn is a member of the Elaeaceae family of deciduous trees and shrubs and the *Hippophae* L. genus.

Hippophae L. is found in temperate regions of Eurasia and originated in the Hengduan Mountains and East Himalayas region [10]. This plant's fruits, leaves, stems, branches, roots, and thorns have all historically been utilized in medicine, as a nutritional supplement, to preserve soil and moisture, and to provide habitats for wildlife. As a result, sea buckthorn is sometimes referred to as "Wonder Plant," "Golden Bush," or "Gold Mine" [46]. The fruit of sea buckthorn is high in

vitamins and minerals, and the oils from the seeds and fruit meat are used as nutraceutical supplements or in the medical and cosmetic industries. Buckthorn's fruit, leaves, and other parts are rich in bioactive compounds and have been utilized in traditional medicine, particularly in China, Tibet, Mongolia, and Central Asian nations [53]. The current investigation used phytochemical evaluation of solvent fractions to assess the hepatoprotective efficacy of *Hippophae rhamnoides* Linn. berries. Fresh berries were juiced and fractionated using different solvents like ethyl acetate, n-butanol, chloroform and water.

The *Hippophae rhamnoides* Linn. Berries of chloroform fraction revealed identify the compound using GC-MS instrument pyridine, Octadecane, 3-ethyl-5(2-ethylbutyl)-, Octadecane, 9-ethyl-9-heptyl-,

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Tetratetracontane, Tridecane, Nonadecane, Tetradecane, Hexadecanoic acid, methyl ester, Heptadecanoic acid, 16-methyl-,methyl ester and (22R)-6á,11á,21-Trihydroxy-16á,17á-propylmethylenedioxyregna-1,4-diene-3,20-dione which has been reported to have hepatoprotective, anti-inflammatory, and antioxidant properties show in table 2. Then identification of ethyl acetate fraction of *Hippophae rhamnoides* Linn. Berries compound using GC-MS instrument Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Solasodine, Tetratetracontane, Pinostrobin, Pterostilbene, Nonadecane, Tetradecane, Hexadecanoic acid, methyl ester, Heptadecanoic acid, 16-methyl-,methyl ester and Hexanoic acid which has been reported to have hepatoprotective, anti-inflammatory, and antioxidant properties show in table 2.

Then further characterisation of Chloroform (HR-Chl), ethyl acetate (HR-EA), and butanol (HR-But) fractions of *Hippophae rhamnoides* Linn. were using Ultra Performance Liquid Chromatography – Quadrupole Time of Flight Mass Spectrometry (UPLC-Q-TOF/MS) with the aim of identifying tentatively phytoconstituents for bioactive components. Twenty bioactive compound is identify in chloroform fraction and reported as Antioxidant, Anti-inflammatory and Hepatoprotective activity show in table 3, Seven bioactive compound is identify in chloroform fraction and reported as Antioxidant, Anti-inflammatory and Hepatoprotective activity show in table 3 and Five bioactive compound is identify in chloroform fraction and reported as Antioxidant, Anti-inflammatory and Hepatoprotective activity show in table 3. These findings suggest that *Hippophae rhamnoides* Linn. fractions contain significant hepatoprotective potential, and that is primarily due to polyphenolic substances with proven anti-inflammatory and antioxidant qualities.

The Ultra High Performance Liquid Chromatography – Diode Array Detector (UHPLC-DAD) based quantitative analysis of *Hippophae rhamnoides* Linn. fractions demonstrated a distinct variation in the distribution of major flavonoids compounds across different solvent systems. Caffeic acid was quantified in both ethyl acetate (HR-EA) and butanol (HR-BUT) fractions, with a comparatively higher concentration observed in the butanol fraction (6.579 ppm) than in the ethyl acetate fraction (5.268 ppm), indicating its moderate polarity and affinity toward polar solvents. Similarly, Quercetin was quantified in the chloroform fraction (2.928ppm). The retention times of the identified compounds ranged between 9.133 and

10.473 minutes, confirming good chromatographic separation and method suitability. Caffeic acid has been reported to antioxidant activity [41]. And Quercetin has been shown to have anti-inflammatory and antioxidant properties.

[53, 31].

2. Materials and Methods

2.1. Materials and chemicals

n-Hexane, Chloroform, Ethyl Acetate, n-Butanol, Methanol (analytical grade), Formic acid, acetonitrile and Test samples: chloroform, ethyl acetate, and n-butanol fractions of *Hippophae rhamnoides* Linn.. All chemicals and solvents used in this study were of commercial or analytical grade.

2.2. Collection and identification of plant

Fresh healthy berries of *Hippophae rhamnoides* Linnaeus collected from Manali Sissu, Lahaul and Spiti, Himachal Pradesh, India, were washed with double distilled water (DDW) and dry in room temperature. The berries of *Hippophae rhamnoides* were wash were crushed and prepare juice for separation and stored in an airtight container at - 4°C until further extraction. *Hippophae rhamnoides* Linn. plant (UHF-Herbarium no. 14191) were authenticated at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan.

2.3 Extraction and fractionation:

The berries of *Hippophae rhamnoides* Linn. were wash were crushed and prepare juice for separation [4]. The berries juice was dis-solved in distilled water and defatted with n-hexane and successive fractionation was done using chloroform, ethyl acetate, and n-butanol and remaining aqueous fraction [33].

2.4 GC-MS analysis:

The column utilized for the measurement of chloroform fraction and ethyl acetate fraction of berries of *Hippophae rhamnoides* Linn. was Thermofisher column-TG-5MS (40m × 0.15m x 0.15 µm). The initial temperature of 70 °C was maintained for 1 min, then ramped up to 270 °C at 7 °C/min and held for 20 min. The injection port temperature was 270 °C, the injection volume was 1 µL and was kept spitless. Solvent delay time was 4 min. The carrier gas was high-purity helium gas (99.99%) at a flow rate of 0.700 mL/min. The column temperature range was adjusted between 70°C to 270 °C. Electron ionization (EI) mode was used with an ionization energy of 70 eV, transmission line temperature of 270 °C, ion source temperature of 250 °C, and scan range of 35–450 m/z. The plots of the mass spectra were generated

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by scanning each spectrum of the total ion flow. The GC-MS runs for 45 minutes in total. The chemical composition was finally determined by software search of the NIST version 2.2 and reviewed relevant literature. The relative amount of each component was computed by comparing each component's average peak area to the overall area. The relative amount of each component was computed by comparing each component's average peak area to the overall area.

2.5 UPLC-Q-TOF/MS Analysis

An ultra-performance liquid chromatography system with a four-element pump, an online degassing machine, an automatic sampler, and a thermostatically controlled column chamber was used for the chromatographic analysis. The separation was carried out on an ACQUITY UPLC H CLASS Series machine, and MassLynx version 4.2 software C18 column (100 × 2.1 mm, 1.7 μm) was used for data acquisition and processing. The mobile phase was composed of solvent A (0.1% Formic acid in LC-MS Grade water) and solvent B (0.1% Formic acid in acetonitrile), and the elution gradient system was optimized on this basis. Elution gradient technology was used for the study, with a constant flow rate of 0.2 mL/min. The injection volume was 5 μL. The gradient proceeded as follows: 0–5.0 min, 5% B; 30.0–35.0 min, 90% B; 36.0–55.0 min, 5% B; 0–5.0 min, 95% A; 30.0–35.0 min, 10% A; 36.0–55.0 min, 95% A. The column and autosampler were kept at 45 and 4 °C, respectively. MS detection was carried out on Synapt-G2-SI MS system. The high collision energy ranged from 15 to 25 eV, whereas the low collision energy was fixed at 4 eV, and the ionization mode was set as ESI⁺ and ESI⁻. The mass was between 50 and 1200 Da. The cone voltage was 40 V, the capillary voltage was 3.22 keV in the negative mode and 2.59 kV in the positive mode. The ion source temperature stayed at 120 °C, while the desolvation temperature was set at 550 °C. Cone gas (N₂) flowed at 50 L/h, whereas dissolution gas (N₂) flowed at 950 L/h.

2.6 Construction of UNIFI Theoretical Library on chemical constituents of *Hippophae rhamnoides*

A list of the substances referenced in the HR literature was compiled using a variety of online sources, including SciFinder, PubMed, PubChem, and Reaxys. Up until March 2026, published material was searched using the terms "*Hippophae rhamnoides* Linn." Combining UPLC-Q-TOF/MS data with the UNIFI information management platform and its integrated Traditional Medicine Library helps simplify the process of identifying chemical structures

in complicated natural goods. Ultimately, Compounds' structures from *Hippophae rhamnoides* Linn. species were gathered and stored in a sdf file as a theoretical library. For quick matching screening with the theoretical library data of *Hippophae rhamnoides* Linn. compounds, the QA-TE MS data was transferred into the UNIFI platform.

2.7 UHPLC-DAD Quantitative Analysis of Main Components

2.7.1 Development of UHPLC-DAD Analytical Method

Quantitative analysis of major phenolic compounds in different fractions of *Hippophae rhamnoides* Linn. was performed using UHPLC-DAD system. The fractions including chloroform fraction, ethyl acetate fraction and butanol fraction of berries of *Hippophae rhamnoides* Linn. [4, 33]. were analyzed for caffeic acid, ellagic acid, rutin, and quercetin. Chromatographic separation was carried out using an UHPLC-DAD system was employed for the analysis. The instrument used for this analysis was the Ultimate 3000 UHPLC (Thermo Fisher Scientific, Milan, Italy). Water soluble phenols were determined using 0.1 mg of fraction dissolved in 1 ml methanol then sonicate for 45 minutes at 45°C. After sonication solution was filtered with a 0.5 μm syringe filter in UHPLC vials.

Flavonoids compounds were analyzed using a UHPLC system equipped with a diode array detector (DAD). Chromatographic separation was carried out on a Merck Lichrospher RP-18 column (100 × 2.1 mm, 2 μm particle size) maintained at 25°C. The mobile phase consisted of solvent A (water adjusted to pH 3.5 with orthophosphoric acid) and solvent C (acetonitrile) [7]. The gradient elution procedure was: 5 min, 15% C to 30% C, 10 min, 30% C to 50% C. Each specimen took 20 minutes to analyze, including a 4-minute column equilibration time. The flow rate and column temperature were kept at 0.8 mL/min and 25 °C, respectively. In order to detect phenolic acids and flavonoids, chromatograms were examined at 280 nm, 260 nm, 325 nm, and 350 nm using the DAD integrated into the UHPLC system. The scanning range wavelengths were 190-400 nm. ICH criteria were followed in the validation of the developed UHPLC technique [7] to ensure fulfillment of current regulatory standards.

3. Results

3.1. Percentage Yield of fractions

The *Hippophae rhamnoides* Linn. fractions percentage yield of n-Hexane 22.5, chloroform

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26.5%, ethyl acetate 14%, butanol 11% and aqueous 26%.

3.2. Gas chromatography–mass spectrometry (GC-MS) analysis of plant fraction

3.2.1 GC–MS analysis of chloroform fraction of *Hippophae rhamnoides* Linn.

GC–MS analysis of chloroform fraction revealed the presence of twelve bioactive compounds as reported in Table 6 and Fig. 1. The compounds with a higher peak area were Pyridine (0.21%), octadecane, 3-ethyl-5(2-ethylbutyl)- (0.65), Octadecane, 9-ethyl-9-heptyl- (0.33%), Tetratetracontane (0.33%), Tridecane (0.34%), Nonadecane (0.24%), Tetradecane (0.24%), Hexadecane (0.24%), Hexadecanoic acid, methyl ester (1.31%), Methyl stearate (0.74%), Heptadecanoic acid, 16-methyl-,methyl ester (0.74%), (22R)-6á,11á,21-Trihydroxy-16á,17á-propylmethylenedioxypregna-1,4-diene-3,20-dione (0.24%).

3.2.2 GC–MS analysis of ethyl acetate fraction of *Hippophae rhamnoides* Linn.

GC–MS analysis of ethyl acetate fraction revealed the presence of twelve bioactive compounds as reported in Table 7 and Fig. 2. The compounds with a higher peak area were Octadecane, 3-ethyl-5(2-ethylbutyl)- (0.40%), Solasodine (0.22%), Tetratetracontane (0.19%), Pinostrobin (0.84%), Pterostilbene (0.84%), Tetradecane (0.15%), Nonadecane (0.15%), Hexadecane (0.15%), Hexanoic acid (90.29%), Hexadecanoic acid, methyl ester (0.52%).

In the present study, various phytochemical components were observed when the chloroform and ethyl acetate fractions of *Hippophae rhamnoides* Linn. were subjected to GC-MS analysis. Analysis of peak area, retention time, and molecular formula confirmed the presence of these phytochemicals. Figures 1 and 2 explain the results of the present study.

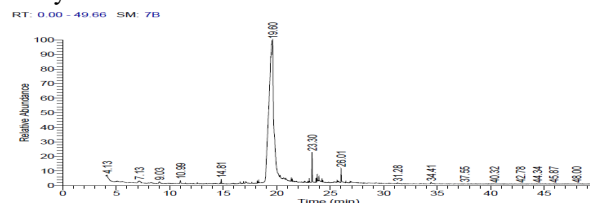


Figure 1: GC-MS chromatogram of Chloroform fraction of berries of *Hippophae rhamnoides* Linn.

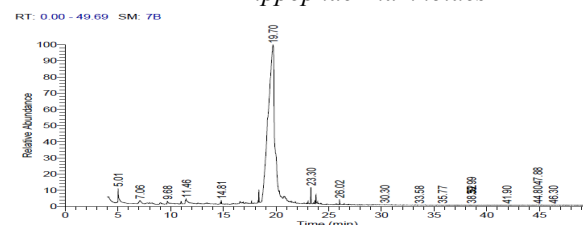
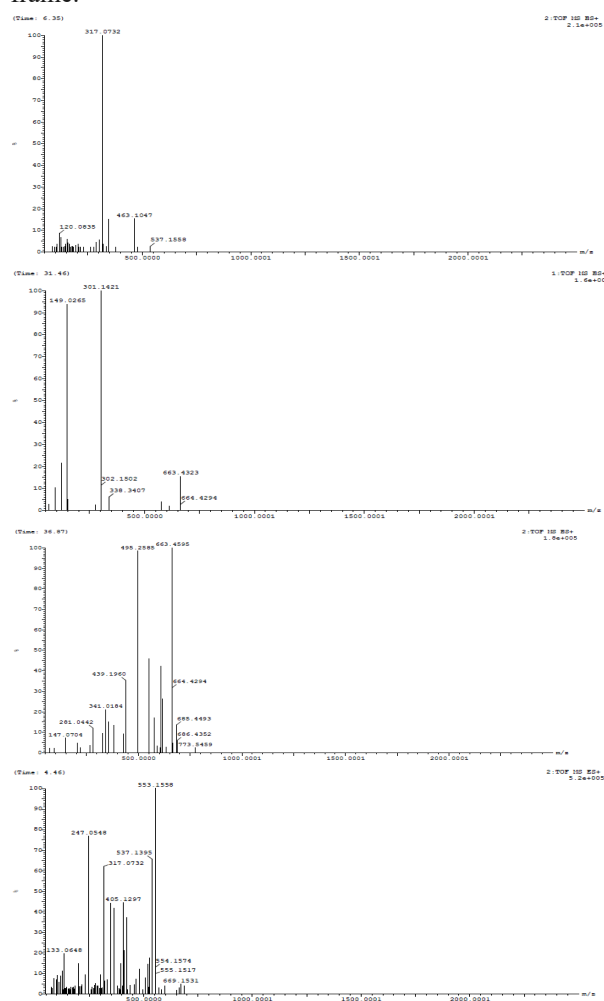


Figure 2: GC-MS chromatogram of Ethyl Acetate fraction of berries of *Hippophae rhamnoides* Linn.

3.3 Identification of bioactive compounds of *Hippophae rhamnoides* Linn. fraction by UPLC-Q-TOF/MS

The of *Hippophae rhamnoides* Linn.fractions solution was detected using UPLC-Q-TOF/MS technology under chromatographic and mass spectrometry conditions. The rapid, efficient and validated UPLC-Q-TOF/MS analytical method was established for the identification of the main chemical components in *Hippophae rhamnoides* Linn.fractions. the identification of the main bioactive components in chloroform, ethyl acetate and butanol fractions as reported in Table 3. The base peak ion chloroform, ethyl acetate and butanol fractions chromatograms (Figure 3) provide the metabolomic analysis, also known as the analytical fingerprint for plant identification and authentication, a fairly integrated frame.



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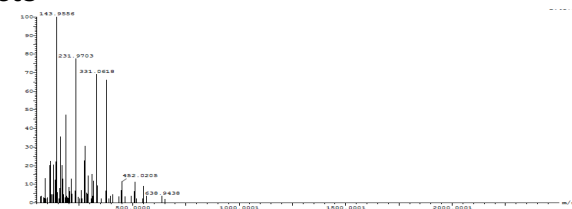
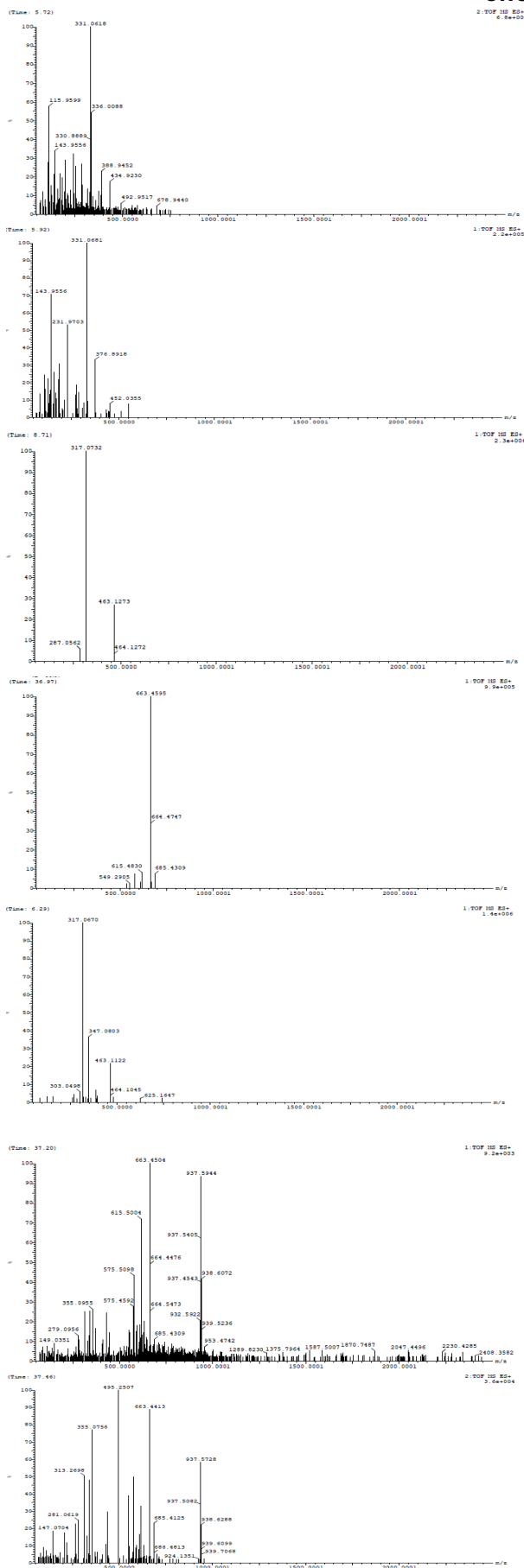


Figure 3: UPLC-Q-TOF/MS chromatogram of chloroform fraction, ethyl acetate fraction and Butanol fraction of berries of *Hippophae rhamnoides* Linn.

3.4 Quantitative Analysis by UHPLC-DAD

The developed UHPLC method was subsequently applied to quantify 2 bioactive compounds in *Hippophae rhamnoides* fraction shows in Table 4 and (Fig. 4) the quantification results for *Hippophae rhamnoides* Linn. fractions. The quantified two flavonoids compounds in *Hippophae rhamnoides* Linn. fraction.



Figure 4: UHPLC-DAD chromatogram of *Hippophae rhamnoides* Linn. fraction showing major compounds such as Caffeic acid and Quercetin with their respective retention times.

4. Discussion

4.1 Fractionation Yield

4.2 Gas chromatography–mass spectrometry (GC-MS)

4.2.1 GC–MS analysis of chloroform fraction

The *Hippophae rhamnoides* Linn. of chloroform fraction revealed presence of the compound **pyridine** which has been reported to have anti-inflammatory activities [26]. Studies have also shown that **Octadecane, 3-ethyl-5(2-ethylbutyl)-** has Antibacterial, antioxidant antidiabetic and antitumor activity reported [25]. The compound **Octadecane, 9-ethyl-9-heptyl-** has been reported to have antimicrobial activity and antioxidant activity [27]. Earlier studies also reported the Antioxidant, Hypoglycemic activity of **Tetratetracontane** [3]. **Tridecane** has been reported to have Antioxidant and Antimicrobial activity [14]. **Nonadecane** has been reported Antioxidant activity [28]. **Tetradecane**

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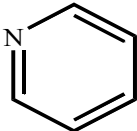
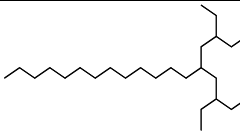
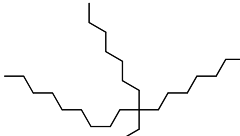
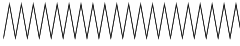
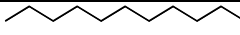
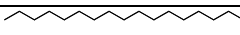
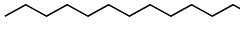
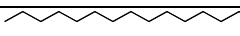
reported Antioxidant activity and preservative [28]. Hexadecane compound has been reported Antioxidant, and Antimicrobial activity [28]. **Hexadecanoic acid, methyl ester** compound has been reported Hepatoprotective Activity & Anti-inflammatory activity [22]. **Heptadecanoic acid, 16-methyl-,methyl ester** compound has been reported Antioxidant activity [40]. And **(22R)-6 α ,11 α ,21-Trihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-3,20-dione** compound also reported as Anti-inflammatory activity [11].

4.2.2 GC-MS analysis of ethyl acetate fraction

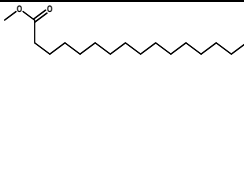
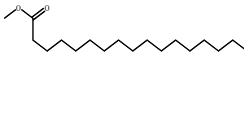
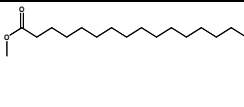
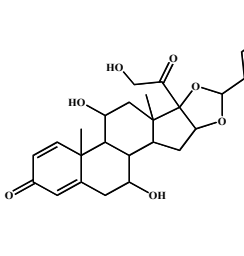
The *Hippophae rhamnoides* Linn. of ethyl acetate fraction revealed presence of the compound **Octadecane, 3-ethyl-5-(2-ethylbutyl)-** which has been reported to have Antibacterial, antioxidant antidiabetic and antitumor activities [25]. Studies have also shown that **Solasodine** has Anti-inflammatory,

Antimicrobial and Hepatoprotective Activity reported [38]. The compound **Tetratetracontane** has been reported to have Antioxidant and Hypoglycemic activity [3]. Earlier studies also reported the Anti-inflammatory activity, Antioxidant and Analgesic activity of **Pinostrobin** [19,44]. **Pterostilbene** has been reported to have Antioxidant activity [1]. **Nonadecane** has been reported Antioxidant activity [28]. **Tetradecane** reported Antioxidant activity and preservative [28]. **Hexadecane** compound has been reported Antioxidant, and Antimicrobial activity [28]. Hepatoprotective and anti-inflammatory properties have been discovered for hexadecanoic acid methyl ester molecule [22]. And **Hexanoic acid** compound also reported as Antioxidant activity [23].

Table 1: Phytochemical reported compounds identified in the Chloroform fraction of berries of *Hippophae rhamnoides* Linn. by GC-MS

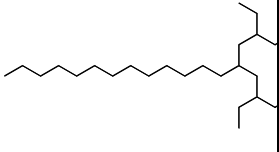
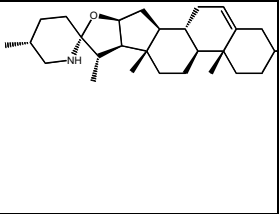
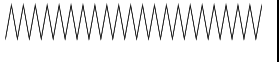
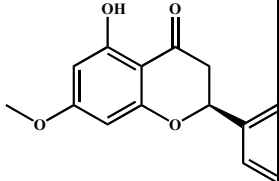
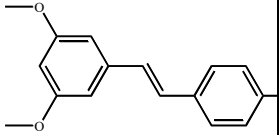
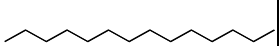
S. No.	RT	Compound Name	Area %	Molecular Weight (g/mol)	Molecular Formula	Molecular Structure	Bioactivity	Ref.
1.	4.13	Pyridine	0.21	79.1	C ₅ H ₅ N		Anti-inflammatory activity	[26]
2.	7.13	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.65	366.7	C ₂₆ H ₅₄		Antibacterial, antioxidant antidiabetic and antitumor	[25]
3.	9.03	Octadecane, 9-ethyl-9-heptyl-	0.33	380.7	C ₂₇ H ₅₆		antimicrobial activity and antioxidant	[27]
4.	9.03	Tetratetracontane	0.33	619.2	C ₄₄ H ₉₀		Antioxidant, Hypoglycemic	[3]
5.	10.99	Tridecane	0.34	184.36	C ₁₃ H ₂₈		Antioxidant, Antimicrobial	[14]
6.	14.81	Nonadecane	0.24	268.5	C ₁₉ H ₄₀		Antioxidant	[28]
7.	14.81	Tetradecane	0.24	198.39	C ₁₄ H ₃₀		Antioxidant, preservative	[28]
8.	14.81	Hexadecane	0.24	226.44	C ₁₆ H ₃₄		Antioxidant, Antimicrobial	[28]

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9.	23.30	Hexadecanoic acid, methyl ester	1.31	270.5	C ₁₇ H ₃₄ O ₂		Hepatoprotective Activity & Anti-inflammatory activity	[22]
10.	26.01	Methyl stearate	0.74	298.5	C ₁₉ H ₃₈ O ₂		Antioxidant	[40]
11.	26.01	Heptadecanoic acid, 16-methyl-,methyl ester	0.74	298.5	C ₁₉ H ₃₈ O ₂		Antioxidant	[40]
12.	34.41	(22R)-6á,11á,21-Trihydroxy-16á,17á-propylmethylenedioxy pregna-1,4-diene-3,20-dione	0.24	446	C ₂₅ H ₃₄ O ₇		Anti-inflammatory activity	[11]

identified in the Ethyl Acetate fraction of berries of

Table 2: Phytochemical reported compounds *Hippophae rhamnoides* Linn. by GC-MS

S. No.	RT	Compound Name	Area %	Molecular Weight (g/mol)	Molecular Formula	Molecular Structure	Bioactivity	Ref.
1.	7.11	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.40	366.7	C ₂₆ H ₅₄		Antibacterial, antioxidant, antidiabetic and antitumor	[25]
2.	7.77	Solasodine	0.22	413.6	C ₂₇ H ₄₃ N ₂ O ₂		Anti-inflammatory, Antimicrobial and Hepatoprotective Activity	[38]
3.	10.99	Tetratetracontane	0.19	619.2	C ₄₄ H ₉₀		Antioxidant, Hypoglycemic	[3]
4.	11.46	Pinostrobin	0.84	270.28	C ₁₆ H ₁₄ O ₄		Anti-inflammatory activity, Antioxidant and Analgesic	[19,44]
5.	11.46	Pterostilbene	0.84	256.3	C ₁₆ H ₁₆ O ₃		Antioxidant	[1]
6.	14.81	Tetradecane	0.15	198.39	C ₁₄ H ₃₀		Antioxidant, preservative	[28]

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7.	14.8 1	Nonadecane	0.15	268.5	C ₁₉ H ₄₀		Antioxidant	[28]
8.	14.8 1	Hexadecane	0.15	226.44	C ₁₆ H ₃₄		Antioxidant, antimicrobial	[28]
9.	19.7 0	Hexanoic acid	90.2 9	116.16	C ₆ H ₁₂ O ₂		Antioxidant	[23]
10	23.3 0	Hexadecanoic acid, methyl ester	0.52	270.5	C ₁₇ H ₃₄ O ₂		Hepatoprotective Activity & Anti-inflammatory	[22]

4.3 Identification of bioactive compounds of Hippophae rhamnoides fraction by UPLC-Q-TOF/MS

The of *Hippophae rhamnoides* Linn. fractions solution was detected using UPLC-Q-TOF/MS technology under chromatographic and mass spectrometry conditions. Twenty bioactive compound is identify in chloroform fraction and reported as Antioxidant, Anti-inflammatory and Hepatoprotective activity show in table 3, Seven bioactive compound is

identify in ethyl acetate fraction and reported as Antioxidant, Anti-inflammatory and Hepatoprotective activity show in table 3 and Five bioactive compound is identify in butanol fraction and reported as Antioxidant, Anti-inflammatory and Hepatoprotective activity show in table 3.

Table 3: Tentatively identified Reported Bioactive compounds of Chloroform fraction, ethyl acetate fraction and butanol fraction of berries of *Hippophae rhamnoides* Linn. by UPLC-Q-TOF/MS

N o.	Component Name	Observed RT (Min)	Formula	Observed neutral mass (Da)	m/z	Mass Error (mDa)	Adducts	H.R. Fractions	Bioactivity	Reference
1.	Corosolic acid	36.87	C ₃₀ H ₄₈ O ₄	472.2650	495.2585	3.5	M+N a	HR-Chl	Anti-inflammatory	[39]
2.	23-hydroxy Betulinic acid	36.87	C ₃₀ H ₄₈ O ₄	472.2650	495.2585	3.5	M+N a	HR-Chl	Anti-inflammatory and Hepatoprotective activity	[36]
3.	30-noroleanane tri terpenoids	36.87	C ₃₀ H ₄₈ O ₄	472.2650	495.2585	3.5	M+N a	HR-Chl	Anti-inflammatory	[49]
4.	Quercetin	31.46	C ₁₅ H ₁₀ O ₇	302.1328	301.1421	2	M-H	HR-Chl	Antioxidant and anti-inflammatory activities	[51,31]
5.	6β-Hydroxy-27-noroleanolic acid (Eanolic acid)	36.87	C ₃₀ H ₄₈ O ₄	472.2650	495.2585	3.5	M+N a	HR-Chl	Antioxidant, anti-inflammatory and	[47]

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									hepatoprotective activities	
6.	Isoscopoletin derivative (3-aryl coumarin)	31.46	C ₁₆ H ₁₂ O ₆	300.1314	301.1421	3.4	M+H	HR-Chl	Antioxidant & anti-inflammatory activities	[52]
7.	Physcion	31.46	C ₁₆ H ₁₂ O ₆	300.1156	301.1421	0.5	M+N H ₃	HR-Chl	Antioxidant, anti-inflammatory and hepatoprotective activities	[37]
8.	Diosmetin	31.46	C ₁₆ H ₁₂ O ₆	300.0641	301.1421	-6.8	M+H	HR-Chl	Antioxidant & Anti-inflammatory	[34]
9.	α-Tocopherone succinate	36.87	C ₃₃ H ₅₄ O ₅	530.2714	495.2585	1	M+H- 2H ₂ O	HR-Chl	Antioxidant & anti-inflammatory activities	[42,13]
10.	Isorhamnetin	6.37	C ₁₆ H ₁₂ O ₇	316.0651	317.0732	0.8	M+H	HR-Chl	Antioxidant, anti-inflammatory and hepatoprotective activities	[32, 20]
11.	Hexylcyclopropyl acetic acid	31.46	C ₁₁ H ₂₀ O ₂	184.0401	149.0265	0.3	M+H- 2H ₂ O	HR-Chl	Not reported	
12.	Pyrogallol	31.46	C ₆ H ₆ O ₃	126.0367	149.0265	-0.2	M+N a	HR-Chl	Antioxidant & anti-inflammatory activities	[2]
13.	3-O-methylgallic acid	31.46	C ₈ H ₈ O ₄	184.0401	149.0265	0.3	M+H- 2H ₂ O	HR-Chl	Antioxidant activities	[16]
14.	Hydroxymethyl furfural (HMF)	31.46	C ₆ H ₆ O ₃	126.0367	149.0265	0.2	M+N a	HR-Chl	Antioxidant & anti-inflammatory activities	[15]
15.	Phloroglucinol	31.46	C ₆ H ₆ O ₃	126.0367	149.0265	-0.2	M+N a	HR-Chl	Antioxidant & anti-inflammatory activities	[29]
16.	4-O-Methylgallic acid	31.46	C ₈ H ₈ O ₅	184.0401	149.0265	0.3	M+H- 2H ₂ O	HR-Chl	anti-inflammatory activities	[35]
17.	Isorhamnetin 3-O-rhamnosides	6.37	C ₂₂ H ₂₂ O ₁₁	462.1049	463.1122	0	M+H	HR-Chl	Antioxidant, anti-inflammatory and	[48,8]

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									hepatoprotective activities	
18	Isorhamnetin	6.37	C ₁₆ H ₁₂ O ₇	316.0651	317.0732	0.8	M+H	HR-Chl	Antioxidant and anti-inflammatory Activity	[18]
19	Nepetin	6.37	C ₁₆ H ₁₂ O ₇	316.0651	317.0732	0.8	M+H	HR-Chl	Antioxidant & anti-inflammatory	[9]
20	6-methoxykaempferol	6.37	C ₁₆ H ₁₂ O ₇	316.0651	317.0732	0.8	M+H	HR-Chl	Antioxidant and Hepatoprotective activity	[30]
21	Isorhamnetin-3-O-rutinoside	36.97	C ₂₈ H ₃₂ O ₁₆	624.4963	663.4595	-0.5	M+K	HR-EA	Antioxidant activity	[48]
22	Isorhamnetin-7-O-neohesperidoside	36.97	C ₂₈ H ₃₂ O ₁₆	624.4963	663.4595	-0.5	M+K	HR-EA	Antioxidant activity	[6]
23	Quercetin 3-(6"-ferulylglucoside)	36.97	C ₃₁ H ₂₈ O ₁₅	640.4697	663.4595	-0.2	M+N a	HR-EA	Antioxidant activity	[43]
24	Diosmetin-5-O-gentiobioside	36.97	C ₂₈ H ₃₂ O ₁₆	624.4963	663.4595	-0.5	M+K	HR-EA	Not reported	
25	Ellagic Acid	5.92	C ₁₄ H ₆ O ₈	302.1494	301.1421	0	M-H	HR-EA	Antioxidant, anti-inflammatory Activity and Hepatoprotective activity	[21]
26	6,7,4'-Trimethoxy-8-hydroxyflavone	5.92	C ₁₈ H ₁₈ O ₆	330.0608	331.0681	0	M+H	HR-EA	Antioxidant and anti-inflammatory Activity	[17]
27	Caffeic Acid	5.92	C ₉ H ₈ O ₄	208.9805	231.9703	-0.2	M+N a	HR-EA	Antioxidant activity	[41]
28	Caffeic Acid	5.86	C ₉ H ₈ O ₄	208.9805	231.9703	-0.2	M+N a	HR-But	Anti-oxidant activity	[41]
29	Rhamnetin-3-O-rhamnoside	6.29	C ₂₂ H ₂₂ O ₁₁	462.1045	463.1122	0.4	M+H	HR-But	heapatoprotective activity	[12]
30	3,5-Dihydroxy-4',7-dimethoxyflavanonol	37.46	C ₁₇ H ₁₆ O ₇	332.0858	355.0756	-0.2	M+N a	HR-But	Anti-oxidant	[24]
31	Isorhamnetin 3-O-robinoside	37.20	C ₂₈ H ₃₂ O ₁₆	624.4862	663.4504	0.5	M+K	HR-But	Anti-oxidant &	[5]

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									Anti-inflammatory	
32	Isorhamnetin-3-O-neohesperidoside	37.20	C ₂₈ H ₃₂ O ₁₆	624.4872	663.4504	-0.5	M+K	HR-But	Anti-oxidant & Anti-inflammatory	[50]

4.4 Quantitative Analysis.

The UHPLC-based quantitative analysis of *Hippophae rhamnoides* Linn. fractions demonstrated a distinct variation in the distribution of major flavonoids compounds across different solvent systems. Caffeic acid was quantified in both ethyl acetate (HR-EA) and butanol (HR-BUT) fractions, with a comparatively higher concentration observed in the butanol fraction (6.579 ppm) than in the ethyl acetate fraction (5.268 ppm), indicating its moderate polarity and affinity toward polar solvents. Similarly, Quercetin was quantified in the chloroform fraction (2.928ppm). The retention times of the identified compounds ranged between 9.133 and 10.473 minutes, confirming good chromatographic separation and method suitability. Caffeic acid has been reported to antioxidant activity [41]. And Quercetin has been reported Anti-oxidant & Anti-inflammatory activity [51,31].

Table 4: Quantification of flavonoids in the Hippophae rhamnoides Linn. fraction by UHPLC-DAD

Hippophae rhamnoides fraction	Compound Name	Regression Equation	Retention Time (Min.)	Area (mAU*)	Height (mm)	Area (PPM)	Relative Area %
HR-EA	Caffeic Acid	Y=0.3547x+0.2829	9.133	2.152	7.448	5.268	18.82
HR-BUT	Caffeic Acid	Y=0.3547x+0.2829	9.177	2.617	14.857	6.579	3.63
HR-Chl	Quercetin	Y=1.6308x+1.7628	15.613	6.538	29.254	2.928	1.25

5. Conclusions

This work deals with the identification and

characterization of metabolites in chloroform ethyl acetate and butanol fraction from *Hippophae rhamnoides* Linn. berries. In this study, 22 compounds were identified by GC-MS and a total of 32 chemical constituents of *Hippophae rhamnoides* Linn. fractions were identified by UPLC-Q-TOF/MS technology. The UHPLC-DAD based quantitative analysis of *Hippophae rhamnoides* Linn. fractions. Caffeic acid was found in the ethyl acetate and butanol fractions, while Quercetin was found in the chloroform fraction. The identified of chemical constituents of *Hippophae rhamnoides* Linn. fractions was reported as hepatoprotective, anti-inflammatory and antioxidant activity. And the identified chemical constituent of *Hippophae rhamnoides* Linn. may provide a basis for further study on the pharmacological basis and resource utilization of *Hippophae rhamnoides* Linn.

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