Chapter 3

Extraction and characterization of phytoconstituents from *Daruharidra* (*Berberis aristata*) extract.

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3.1 Background-

Natural medications include very little concentrations of active substances. The extraction and isolation procedure, which is labour-intensive and time-consuming, has been the bottleneck for the use of natural products in medication development. There is a dire need to develop efficient and selective technologies for extracting and isolating bioactive natural compounds. Extracts from different parts of the plant, particularly those obtained using aqueous and ethanolic solvents, have shown high levels of phenolic and flavonoid compounds, which are known for their antioxidant and antidiabetic effects. Solvent extraction of dried plant's root and bark with eight different solvents according to polarity was taken into account. Aqueous and ethanolic extractions yielded the highest total phenolic content (TPC), while methanol and ethanol extracts exhibited the highest total flavonoid content (TFC). In contrast, petroleum ether extraction resulted in lower TPC and TFC values. High-resolution liquid chromatography-mass spectrometry (HR-LCMS) was employed to identify phytoconstituents in the extracts. It helped in screening of the ethanolic extract has identified several alkaloids, flavonoids and phenols as major constituents. Among these, compounds such as pirenzepine, platyphylline, Dihydroartemisinin, berbamine, and desmethylnefopam.

3.2 Introduction-

The process of extracting natural products involves several key stages: initial penetration of solvent into the solid matrix, dissolution of solute into the solvent, diffusion of solute out of the solid matrix, and finally, collection of the extracted solutes. Enhancing factors such as diffusivity and solubility throughout these stages facilitates extraction. Efficiency of extraction

is affected by a number of variables, such as the characteristics of the extraction solvent, the size of the raw material particles, the solvent-to-solid ratio, temperature, and duration. A good extraction depends on selecting the appropriate solvent, taking into account variables including specificity, solubility, expenditure, and safety. Solvents with polarity values close to those of the solute, following the principle of "like dissolves like," tend to perform better. Commonly, polar compounds such as alcohols such as ethanol and methanol serve as universal solvents for phytochemical investigation in solvent extraction. *Daruharidra* contains isoquinoline alkaloids, mainly berberine, berbamine and other phytoceuticals like oxyberberine, aromoline, alloxanthin, palmatine, oxyacanthine. [51] *Daruharidra* plant is traditionally accomplished and its root extracts have been used for decades in northeast (Sikkim) India to treat diabetes.[113] Herbal drugs have low risks of side effects; they are in expensive and show valuable results in due course of time as compared to conventional drugs.[114]

3.3 Methodology

3.3.1 Plant material collection and authentication, Chemical utilized-

Dried samples of *Daruharidra* (stem and bark) were procured from a local herb shop in Varanasi, INDIA. The plant material consisted of the bark and stem of *Daruharidra*'s characteristic yellow color. The plant was authenticated from Raw materials herbarium and museum, Delhi (RHMD), authentication number- NIScPR/RHMD/consult/2022/4385-86. All other chemical utilized in the experiments performed were of analytical grade.

3.3.2 Preparation of sample and Sequential Solvent Extraction-

Plant material was washed with normal water, deionized water, and 70% ethanol to remove dust or microorganisms. It was then dried in the sun before grinding to yield fine powder. The sample was ground and made into powder by a mechanical grinder. Sequential extraction was performed (hexane, petroleum ether, chloroform, acetone, isopropanol, ethanol, methanol, and

water) for the extraction of powdered stem and bark of *Daruharidra* in increasing polarity order. [115] *Daruharidra* powder 30g was dissolved in 100 ml of solvents for sequential extraction based on increasing polarity. Hexane being the non-polar was used to start the extraction carried on by petroleum ether, isopropanol, chloroform, acetone, methanol, ethanol, and water at 50°C with shaking at 180rpm for 48hours. Then they were first filtered with a muslin cloth and then passed through Whatman no1 filter paper. The filtrate obtained was evaporated and collected with the use of a rotary vacuum evaporator. [116] Fine powder of extract was obtained. 20mg/ml of this extract was mixed with 10% DMSO for further storage and use.

3.3.3 Phytochemical screening-

Chemo profiling of all the *Daruharidra* solvent extracts to test the presence of the active chemical components present in them was carried out. Standard documented procedures were used to test the presence of alkaloids, flavonoids, phenol, tannin, terpenoids, saponin, protein, and steroids, as mentioned below.[117]

Alkaloid- To 1 ml of extract, add a few drops of Wagner's reagent; the occurrence of reddish-brown precipitate shows the presence of alkaloid.

Flavonoid- To 1 ml of extract, add a few drops of dilute NaOH solution; yellow color indicates a positive test for flavonoid. Color disappears with the addition of dilute acid.

Phenol- Add 10% of ferric chloride solution to 1:2 water diluted extract; dark green color indicated the presence of phenolic compounds.

Terpenoids- Add 2ml of chloroform and 3ml of concentrated sulphuric acid to 5 ml extract. The reddish-brown color at the interface of the solution states a positive test.

Tannin-1 ml of extract diluted with 10 ml water and filtered; to the filtrate, add 1% ferric chloride solution. Appearance of intense green, purple, blue, or black color shows positive for tannins.

Saponin- Dilute 1 ml sample extract with 5 ml water and shake vigorously; the occurrence of froth is positive.

Protein- A few ml of 0.25% w/v ninhydrin reagent was added to 1 ml sample extract and boiled for 2 mins; blue color indicates the presence of amino acids.

Steroids- Add equal amounts of chloroform and concentrated sulfuric acid (5ml) to 1 ml extract. The upper layer in the test tube turns red, and the layer below shows yellow color with greenish tint is positive.

3.3.4 Extractive value determination-

15gm of powdered plant sample was used for extraction with 250ml of different solvents at 50°C, 180rpm for 2 days. The solution thus obtained was filtered, and we used pre-weighed Petri dishes for the filtrate to dry in. Petri dishes were left to dry in a vacuum evaporator and weighed to set out the extractive value in mg.[118] Percentage extractive value was calculated as mentioned-

$$\%\ extractive\ value = \frac{weight\ of\ dried\ extract}{weight\ of\ plant\ sample} * 100$$

3.3.5 Total phenolic content (TPC)-

Measurement of total phenol content in each solvent extract was handed out by the modified method of Slinkard et al. and McDonald et al.[119] A solution was prepared of each powder extract by taking 5 mg extract in 10 ml methanol and mixing it by a vortex. 200µl of each extract was taken in separate test tubes, 600µl of distilled water, and added 200µl of FC reagent (1:1). The reaction requires 10 mins of incubation followed by the addition of 1.5ml saturated sodium carbonate solution (8% w/v). Subsequently, add 1.5ml distilled water to make up the volume. Carry out Dark incubation of 30 mins and then centrifuge the test tubes. Take the

absorbance at 750nm. Total phenolic content is expressed as µg of gallic acid equivalents per ml.

3.3.6 Total flavonoid content (TFC)-

Prepared stock quercetin solution 1mg/ml in methanol was taken and serially diluted (0.1- 1mg) to obtain the calibration curve. The solution of each sample was prepared as same as mentioned in TPC and 500 µl of each extract was used for reaction in separate test-tubes. To this 300µl sodium nitrite (0.5M) solution was added, followed by 300µl of aluminum chloride (0.3M) solution after 10 min, 2 ml of sodium hydroxide solution (1M) was added, and volume was made up to10ml with distilled water. Take absorbance at 510nm. T.F.C. is expressed as µg of quercetin equivalent per ml.[120]

3.3.7 Total alkaloid content (TAC) -

The total alkaloid calibration curve was prepared using caffeine solution in accurately measured aliquots 0.2- 1.2ml and transferred into different separatory funnels. Phosphate buffer of pH 4.7 and Bromocresol green (BCG) solution were taken in equal volume added to the funnels. The solution was mixed vigorously containing 2-8 ml of chloroform. Extracts thus obtained were collected in a volumetric flask of 10 ml. dilutions were made to adjust volume 10ml by adding chloroform as required. Absorbance was measured at 470 nm against blank. Similar to the standard curve, the powdered extract of *Daruharidra* 30mg was mixed with 2 N Hydrochloric acid and was then filtered. A part of this filtrate was taken and washed with 10 parts of chloroform in a separatory funnel keeping the pH neutral. This solution was added to Bromocresol Green and phosphate buffer (5+5ml). After vortexing, the mixture, the compound thus obtained was extracted with 2-, 4-, 6-, and 8-ml chloroform by vigorous shaking. The extracted complex was transferred in separate tubes, and the volume was made up to 10 ml by chloroform. TAC was expressed in milligram caffeine equivalent (mg CE).[141]

3.3.8 DPPH Radical scavenging Activity-

Antioxidant properties of *Daruharidra* extract were determined using the DPPH assay. This assay uses the stable radical, 2, 2- diphenyl-1-picrylhydrazyl (DPPH), as a reagent. The free radicals in the extract by donation of a hydrogen atom converts the purple-colored DPPH solution to yellow.[121] The working solution were prepared by adding 5mg of powder extract in methanol and this was subjected to mixing for 2 hours. Sample thus formed was used further. 1 ml each of prepared samples was taken. To this 1 ml DPPH and 2 ml methanol was added. It was kept for incubation in dark for 15 mins. Observe the visible change in color and recorded the absorbance spectrophotometrically at 517nm. [122]50µg/ml Ascorbic acid was used as standard, and methanol was used as blank.

Calculate antioxidant activity using formula –

$$\%inhibition = \frac{(ABcontrol) - (AB\ sample)}{ABcontrol}$$

3.3.9 Antioxidant assay by evaluating Superoxide Dismutase (SOD) activity-

The amount by which the SOD enzyme prevented the photoreduction of nitroblue tetrazolium (NBT) can be exploited to measure the activity of superoxide dismutase. The antioxidant assay was performed by making a reaction mixture containing 100 µL of crude extract (20mg/ml), 10 µM vitamin B2, 10 mM L-methionine, 50 µM NBT, 50 mM sodium carbonate, 50 mM sodium phosphate buffer (pH 7.0), and 0.1 mM EDTA making the final volume to 3.0 mL. Control was taken having no plant extract. The SOD test was performed by shining white light on the reaction mixture for 10 minutes at room temperature. After 15 minutes of incubation; using a spectrophotometer, absorbance at 560 nm was measured. A single unit (U) of SOD activity was defined as the amount of enzyme necessary to block the photochemical degradation of NBT by 50%. A unit of SOD is the quantity of enzyme required to cause a superoxide radical to dismutase by 50%. Each measurement was made in triplicate.[96]

3.3. 10 Investigations of phytoconstituents by Chromatography techniques –

Two different chromatography techniques, such as HR-LCMS (High Resolution- Liquid Chromatography-Mass Spectrometry) and HPLC (High-Performance Liquid Chromatography) were used for detection of various phytochemical compounds present in the extract of *Daruharidra* (stem and bark). As methanolic extract had a highest extractive value, it was used for the detection of hits in HRLCMS. Various phytochemicals in the ethanolic extract of *Berberis aristata* were put through High Resolution-Liquid Chromatography-Mass–Spectrometry (HR-LCMS) provided by SAIF, IIT-Bombay. The Agilent high resolution liquid chromatography and mass spectrometry model- chipcube 6550 with 0.01% mass resolution was used to create the chemical fingerprints of the *Daruharidra* extracts. Mass range was kept at 50-3200 amu having resolution: FWHM 40000, Superior Mass Accuracy (Typically less than 1ppm) High Sensitivity (100:1 Reserpine/Serpine ratio, 1 pg), API Ionization Method 1) Positive and negative ESI 2) Positive and negative APCI. For HR-LCMS, solvent was used was 100 % water and 100% acetonitrile. Phytochemicals of *Berberis aristata* were detected by their mass spectra and mass fragmentation patterns. PubChem was used for the identification of the compound. [123][124]

3.3.11 HPLC study condition-

For HPLC extracts, they were subjected to the Shimadzu HPLC system with a diode array detector (SPD M20A). Use of an ODS C18 column (Luna 5U) with 5µm particle size and 250 × 4.6 mm column dimensions. We used 70% acetonitrile as the mobile phase for an isocratic system at a flow rate of 1 ml/min. Acarbose was chosen as a reference point for different extracts as active ingredient (polyphenols) in *Daruharidra*, and Acarbose may work similarly for alpha-amylase inhibition.[125]

3.3.12 CHNS and FTIR studies-

For knowing chemical constitution and presence of the different functional groups in the dried alcoholic extract samples, CHNS and FTIR were carried out. CHNS analyser facility of SAIF, IIT-Bombay was used. CHNS is a product of Thermo Finnigan, Italy model FLASH EA1112 series that finds percentages of carbon, hydrogen, nitrogen, and sulfur present in organic compounds.[126][127] It works on "Dumas method" in which flash combustion of sample takes place. The product is separated and detected by the chromatographic column and thermal conductivity detector (TCD), respectively. FTIR facility was provided by Central Instrumentation Facility, CIF, IIT-BHU. FTIR Analyzer is the Nicolet iS5 model from THERMO Electron Scientific Instruments LLC. This technique helps in knowing the functional groups as the groups of sample generates a characteristic absorption fingerprint that helps in identifying the chemical compound. Chromatograms were obtained from both and were analysed.[128]

3.4 Results and discussion-

3.4.1 Extraction and phytochemical screening-

Different dried powdered extracts obtained by sequential solvent extraction (hexane, petroleum ether, chloroform, acetone, isopropanol, ethanol, methanol, and water) were subjected to evaluation of percentage extractive value. Methanol had the highest extractive value of 2.35%, whereas hexane had a value of only 0.01%, shown in Table 3.1. All the extracts were then screened for the presence of phytochemicals, as seen in Table 3.2.

Table 3.1. Extractive value of different solvents

Solvents	Extractive value percentage
Water	2.033± 0.127 %
Methanol	2.356± 0.0498 %
Ethanol	1.086± 0.089 %
Isopropanol	0.753± 0.0205%
Acetone	0.983± 0.250 %
Chloroform	0.838± 0.012%

Pet. Ether	0.053± 0.001%
Hexane	0.010± 0.0009%

Table 3.2. Phytochemical analysis of different extracts

Solvents	Alkaloid	Flavonoid	Phenol	Tannin	Terpenoid	Steroid	Saponin	Sugar	Protein
Control	-	-	-	-	-	-	-	-	-
Water	+	+	+	+	-	-	+++	-	-
Methanol	+++	+++	+	++	+	+	+	+	+
Ethanol	+++	+++	+	+	+	+	-	++	+
Isopropanol	+	+	+	+	+	+	-	+	+
Acetone	++	+	+	+	+	+	-	+	+
Chloroform	++	+	+	+	+	+	-	+	+
Pet.Ether	+	+	+	-	-	-	-	+	+
Hexane	+	+	+	+	-	-	-	-	-

3.4.2 Total phenol and flavonoid content-

All 8 extracts were used to evaluate their total phenolic and flavonoid content. The concentration was then utilized for shortlisting amongst the extracts to be used for alpha-amylase inhibition analysis. The maximum total phenolic content (TPC) of 101.4 and 111.8 mcg of gallic acid equivalent were obtained via ethanolic and aqueous extraction, respectively. Total flavonoid content (TFC) of ethanol and methanol extract was 319.6 mcg of quercetin equivalent and 288.3 mcg, respectively. On the other hand, the lowest TPC of 23.6 and TFC of 8.33 mcg were obtained by petroleum ether extraction. (Table 3.3 & 3.4).

Table 3.3 Total phenolic content per microgram gallic acid.

Solvents	Total phenol (mcg)
Water	101.406 ± 2.424
Methanol	77.326 ± 2.497
Ethanol	111.863 ± 1.956
Isopropanol	89.610 ± 1.57
Acetone	93.026 ± 2.56
Chloroform	39.640 ± 1.47
Pet. Ether	23.636 ± 2.44
Hexane	46.516 ± 1.23

Table 3.4. Total flavonoid content per micro gram quercetin

Solvents	Total flavonoid (mcg)
Water	120.666 ± 4.18
Methanol	319.166 ± 2.65
Ethanol	288.333 ± 4.71
Isopropanol	232 ± 4.08
Acetone	155.833 ± 6.23
Chloroform	116.667 ± 4.24
Pet. Ether	8.33 ± 2.11
Hexane	124.833 ± 5.328

Table 3.5: Total alkaloid concentration per mg CE/100g present in different solvent (polar to nonpolar).

Solvents	Alkaloid (mg CE/100g)
Water	40.073± 0.931
Methanol	17.283± 1.668
Ethanol	55.363± 2.238
Acetone	32.783± 1.831
Chloroform	1.216± 0.091
Isopropanol	29.610± 1.095
Pet. Ether	0.063± 0.043
Hexane	1.416± 0.188

3.4.3 Total alkaloid content (TAC)-

All the sequentially extracted sample extracts were used to evaluate their quantitative alkaloid content. A few drops of Wagner's reagent were mixed with 1 ml of fresh extract; the change of color to reddish-brown precipitate gives affirmation to the presence of alkaloid.[154] Table 3.5 shows solvent extraction with ethanol had the highest TAC of 55.363 mg caffeine equivalent (mgCE/100g). In comparison, petroleum ether yielded the lowest TAC of 0.62 mg.[155][156] Alkaloids' therapeutic effects on blood glucose aetiology are mediated by a range of signalling cascades and pathways, either by blocking or activating a variety of systems, such as enhancing insulin sensitivity and controlling oxidative stress by inhibiting the alpha-glucosidase enzyme. Alkaloids may be utilised to treat and prevent endocrine diseases, including diabetes, as well as serve as catalysts for the development of novel antidiabetic drugs.[157]

3.4.4 Radical scavenging Property-

Antioxidant activity of various solvent extracts of *Daruharidra* was determined and was compared with the standard of ascorbic acid. 5mg of each extract was used to determine DPPH radical scavenging activity. It was observed that methanol extract had the highest 87.32% inhibition activity, and chloroform extract showed the least inhibition of 6.36% in Table no.3.6.

Table 3.6 Antioxidant activity of different extracts by DPPH assay

Solvent	**DPPH inhibition %**
Water	36.94
Methanol	87.32
Ethanol	36.94
Isopropanol	35.03
Acetone	21.27
Chloroform	6.36
Pet. ether	15.92
Hexane	24.84

3.4.5 Antioxidant assay by evaluating superoxide dismutase SOD activity-

The ethanol-based extracts of *Daruharidra* were tested for antioxidant properties. It was found to be 2.82±0.059 units/mg protein as seen in table 3.7. A control reaction was performed without crude plant extract. It is hypothesized that the extract used in this study may have SOD protective actions attributable to their potential concertation of excess copper ions in the SOD complex, which stops the metal from interacting with H_2O_2. As an alternative approach it can be suggested that, the copper-bound radicals that shield the histidine residues in the SOD complex from denaturation may be helped by the *Daruharidra* extract by acting as electron donors.[151] *Daruharidra* has a strong potential as an antioxidant. The herb is a prospective source of natural antioxidants, and based on the findings, it may be commonly used to treat disorders linked to oxidative stress.[158]

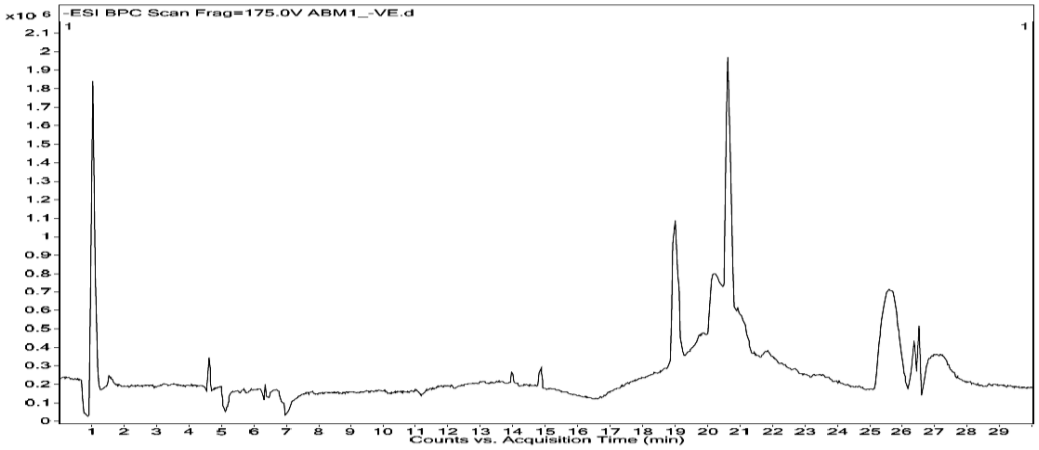
Table 3.7: Antioxidant activity of different extracts v/s control.

Solvent	**SOD (units/mg protein)**
Control	1.149±0.062
Ethanol	2.8236±0.059
Methanol	3.441±0.063

3.4.6 Chromatography-

The active phytoconstituents in the ethanolic extract were found using the High Resolution-Liquid Chromatography-Mass Spectrometry (HR-LCMS) approach in the methanolic dried extract as it had the highest extractive value percentage of 2.356± 0.0498 %. The chromatogram obtained was analyzed to have many therapeutic constituents. Numerous medicinal components were discovered in the chromatogram that was subsequently analysed. The chemical makeup of the active extract can be easily investigated using HR-LCMS. Considering the length of their retention, the difference in the database (library), experimental m/z, MS/MS fragments, metabolite class, and proposed compounds, this approach was used to separate and identify the phytoconstituents. Both negative and positive ionisation mode MS data were reported. In our extract, the bulk of the m/z values fell between 187 to 810 as seen in Figure 3.1 and MS zoomed spectrum of shortlisted ligands are shown in Figure 3.2. Among them were Berbamine, platyphylline, dihydroartemensin, ambelline, acetoxykhevorin, oxycodone, and others mentioned in the Table 3.8.[124]

A



B

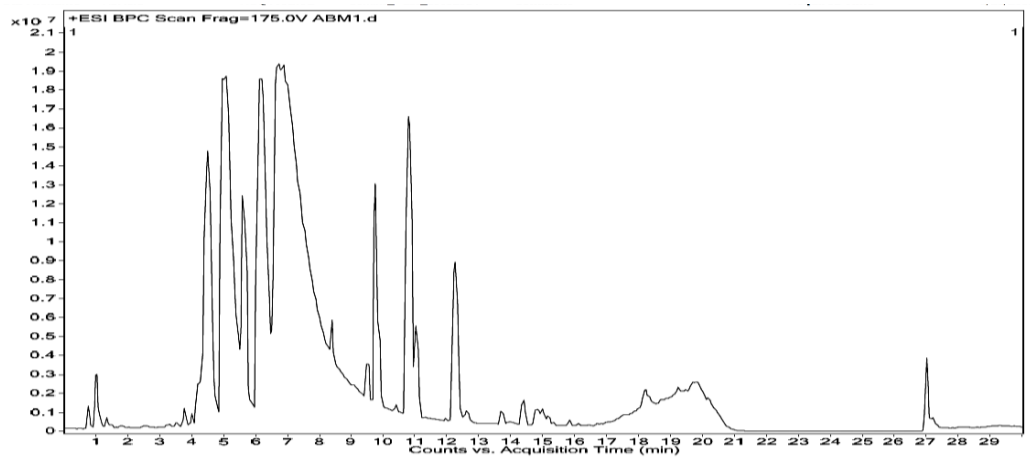
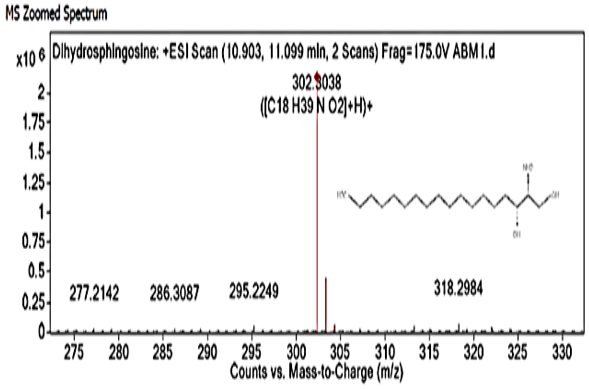
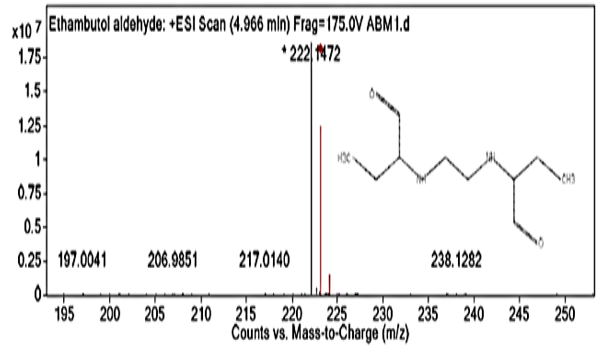


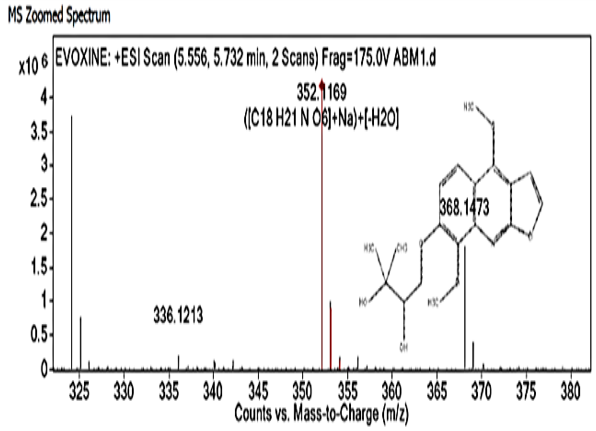
Figure 3.1. HR-LCMS chromatogram of ethanolic extract of *Daruharidra* (*Berberis aristata*) showing (A) Negative and (B) positive electron spray ionization spectra.



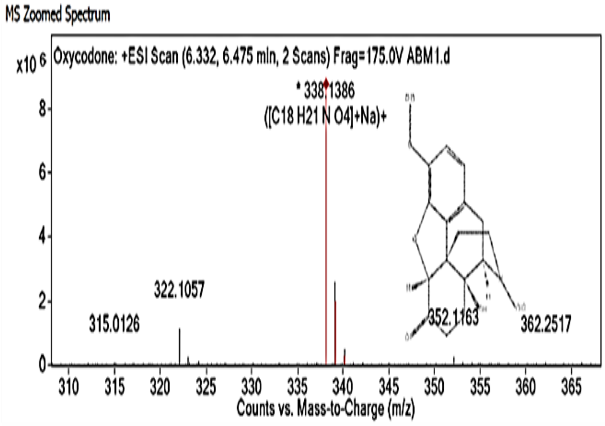
Dihydrosphingosine



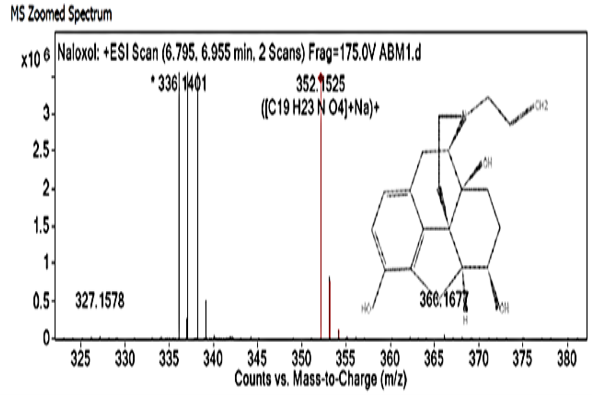
Ethambutol



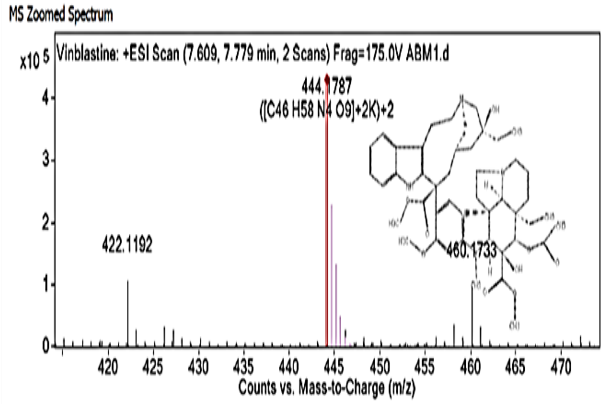
Evoxine



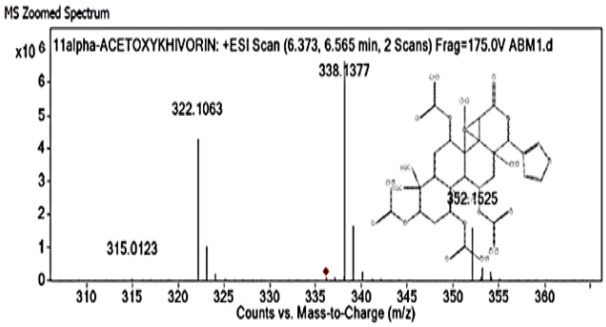
Oxycodone



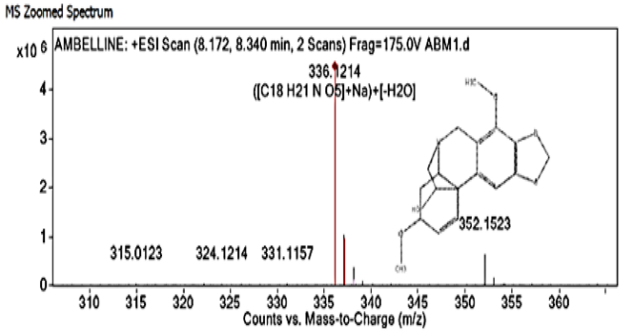
Naloxol



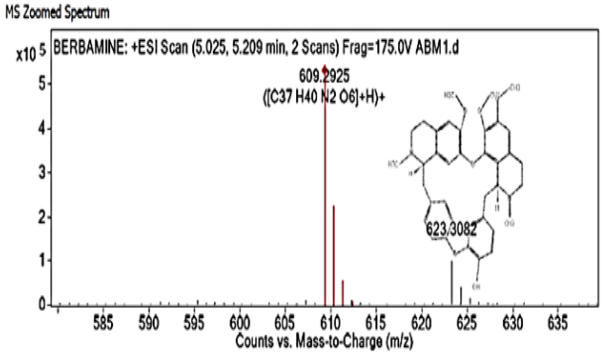
Vinblastine



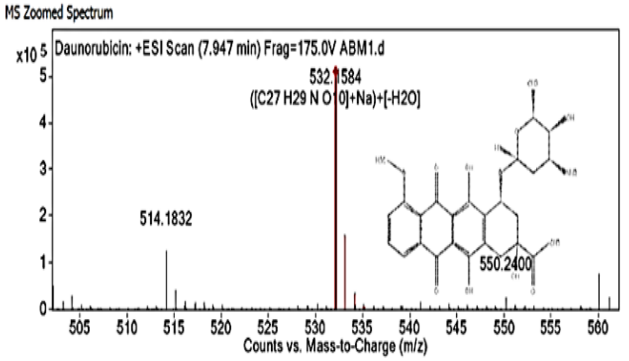
Acetoxykhivorin



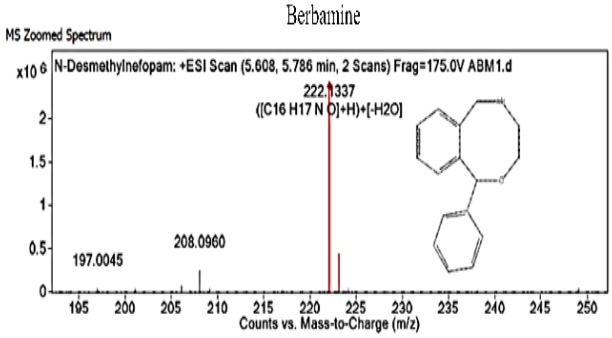
Ambelline



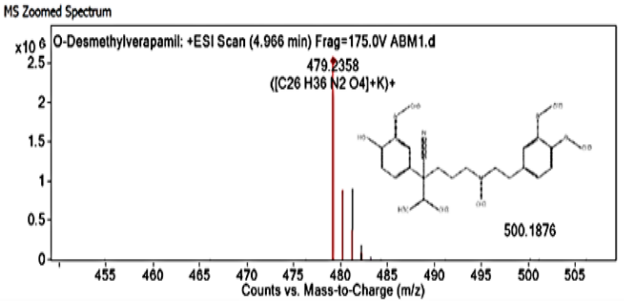
Berbamine



Daunorubicin



N-desmethylnefopam



O-desmethylverapamil

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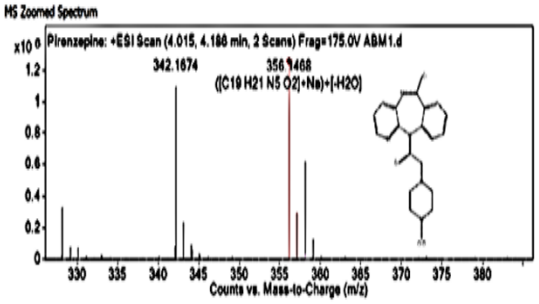
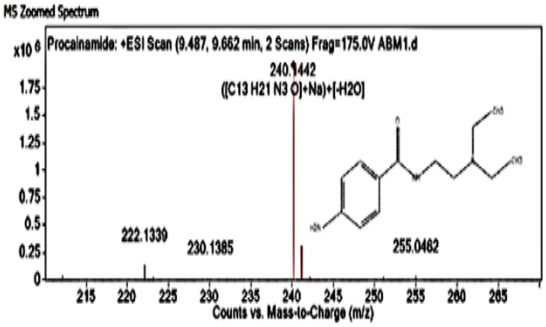
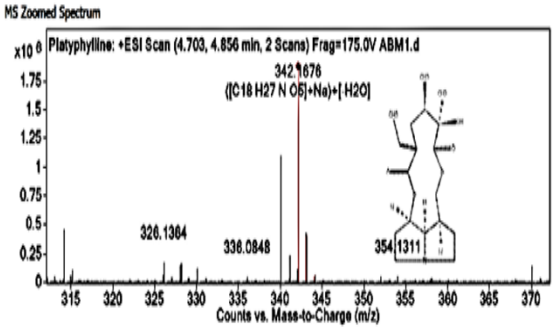


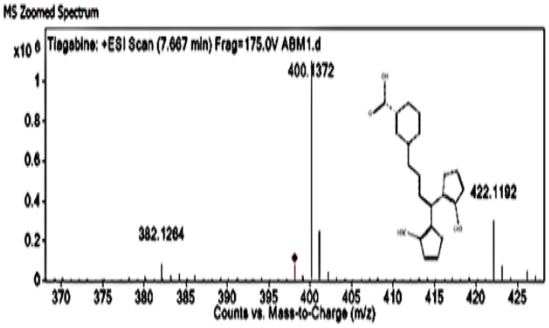
Pirenzepine



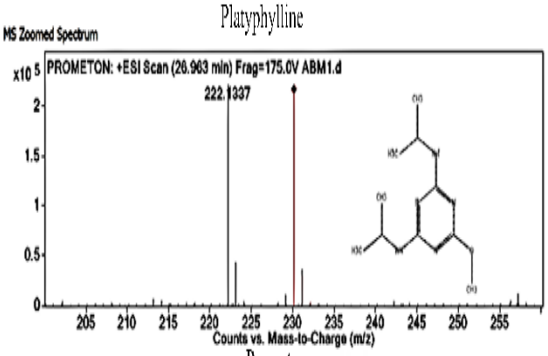
Procainamide



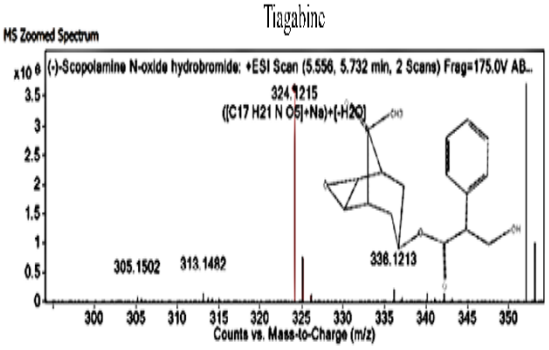
Platyphylline



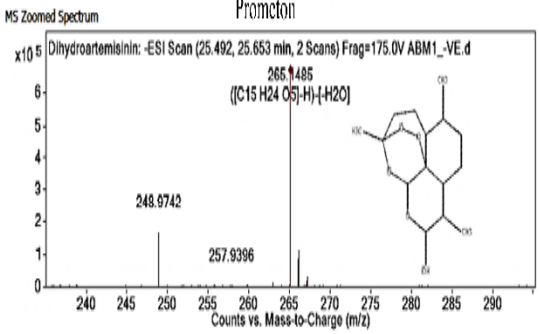
Tiagabine



Prometon



Scopolamine



Dihydroartemisinin

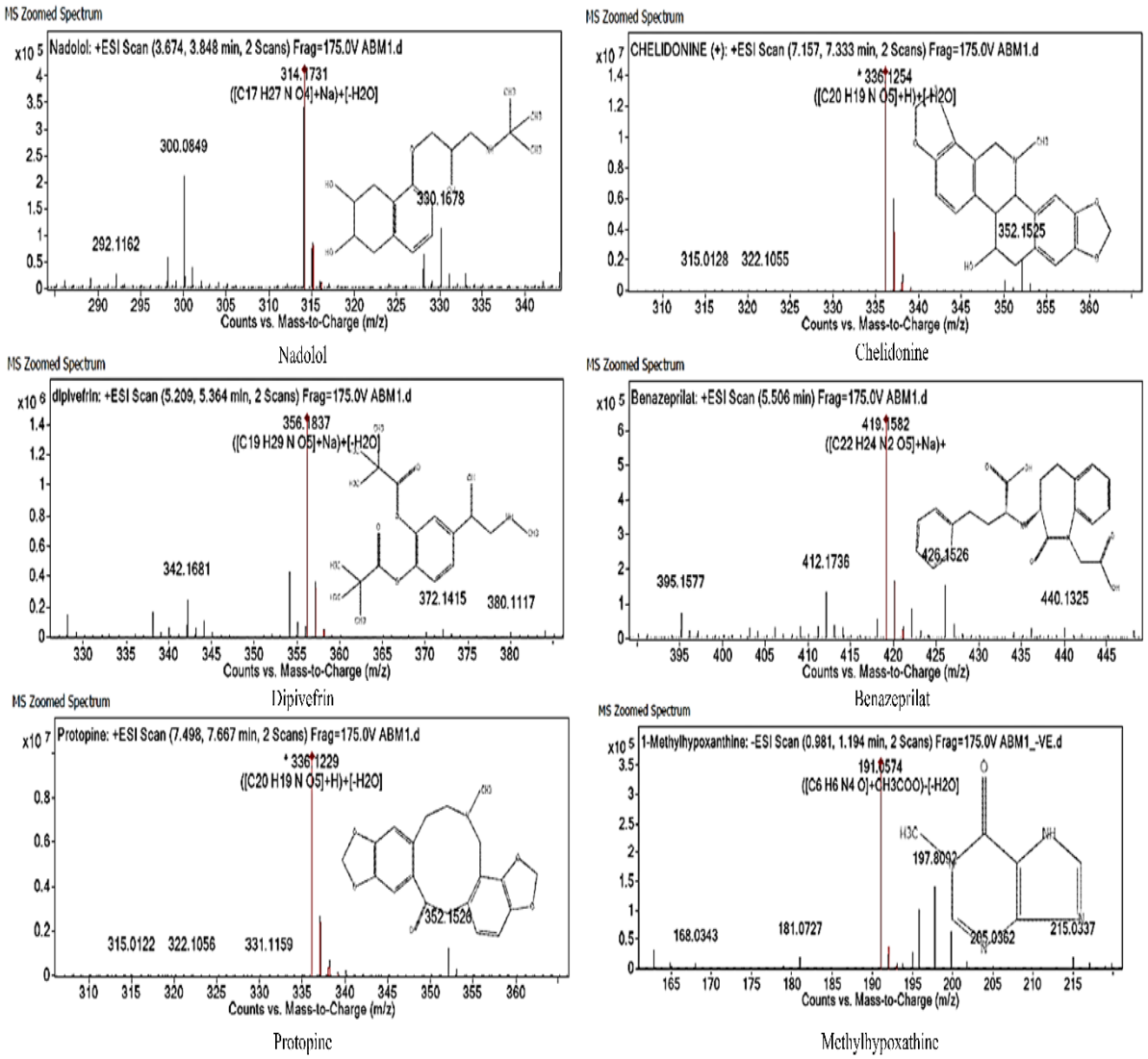


Figure 3.2 Mass spectra of phytochemicals of B. aristata to be used for docking with alpha amylase and alpha glucosidase. They mainly comprised of alkaloids and flavonoids.

Table 3.8: HR-LCMS based chemo profiling details of ethanolic extract of *Berberis aristata*.

S no.	Compound label	m/z	Formula	Retention time	Mass	Adduct	Hits
1	Acetoxykhivorin	336.120	C_{34} H_{44} O_{12}	6.487	644.27	(M+2Na)+ 2[-H_2O]	1
2	Ambelline	336.121	C_{18} H_{21} N O_5	5.951	331.142	(M+Na)+[H_2O]	10
3	Berbamine	609.292	C_{37} H_{40} N_2 O_6	5.139	608.285	(M+H)+	3
4	Desmethylnefopam	222.133	C_{16} H_{17} N O	5.709	239.136	(M+H)+[-H_2O]	1
5	Desmethylverapamil	479.235	C_{26} H_{36} N_2 O_4	4.979	440.27	(M+K)+	10
6	Dihydroartemisinin	265.148	C_{15} H_{24} O_5	25.584	284.166	(M-H)-[-H_2O]	7
7	Dihydrosphingosine	302.303	C_{18} H_{39} N O_2	11.027	301.296	(M+H)+	1
8	Ethambutol aldehyde	223.138	C_{10} H_{20} N_2 O_2	4.979	200.148	(M+Na)+	2
9	Evoxine	352.116	C_{18} H_{21} N O_6	5.659	347.138	(M+Na)+	10
10	Oxycodone	338.138	C_{18} H_{21} N O_4	6.416	315.149	(M+Na)+	10
11	Naloxol	352.152	C_{19} H_{23} N O_4	7.595	329.163	M+Na)+	10
12	Vinblastine	444.178	C_{46} H_{58} N_4 O_9	7.716	810.431	(M+2K)+2	1
13	Pirenzepine	356.146	C_{19} H_{21} N_5 O_2	4.115	351.168	(M+Na)+ [-H_2O]	10
14	Platyphylline	342.167	C_{18} H_{27} N O_5	4.792	337.189	(M+Na)+ [-H_2O]	10
15	Procainamide	240.144	C_{13} H_{21} N_3 O	9.587	235.166	(M+Na)+ [-H_2O]	2
16	Prometon	230.139	C_{10} H_{19} N_5 O	26.98	225.16	(M+Na)+ [-H_2O]	1
17	Scopolamine	324.121	C_{17} H_{21} N O_5	5.656	319.142	(M+Na)+ [-H_2O]	10
18	Tiagabine	398.121	C_{20} H_{25} N O_2 S_2	7.691	375.132	(M+Na)+	10
19	Nadolol	314.173	C_{17} H_{27} N O_4	3.774	309.194	(M+Na)+ [-H_2O]	10
20	Chelidonine	354.131	C_{20} H_{19} N O_5	5.192	353.125	(M+H)+	10
21	Dipivefrine	356.183	C_{19} H_{29} N O_5	5.299	351.205	(M+Na)+ [-H_2O]	-
22	Benazepril	419.158	C_{22} H_{24} N_2 O_5	5.518	396.169	(M+Na)+	10
23	Protopine	336.122	C_{20} H_{19} N O_5	7.594	353.126	(M+H)+[-H_2O]	10
24	Methylhypoxanthine	191.057	C_6 H_6 N_4 O	1.099	150.054	(M+CH_3CO O)-[-H2O]	10

HPLC was used to compare the potential of extracts to the known alpha-amylase inhibitory drug Acarbose. The chromatograms thus obtained predicted the peaks at similar retention times of 19 -20 mins @280nm with different intensities. This result suggests that Acarbose and these

extracts may have a similar compound that acts as an enzyme inhibitor. Acarbose contains acarviosin, inositol linked to a 4-amino-4,6-dideoxy-D-glucopyranose that acts as an enzyme inhibitor. Similar compounds may be present in the alcoholic extracts of *Daruharidra*, as seen in Figure 3.3. [135].

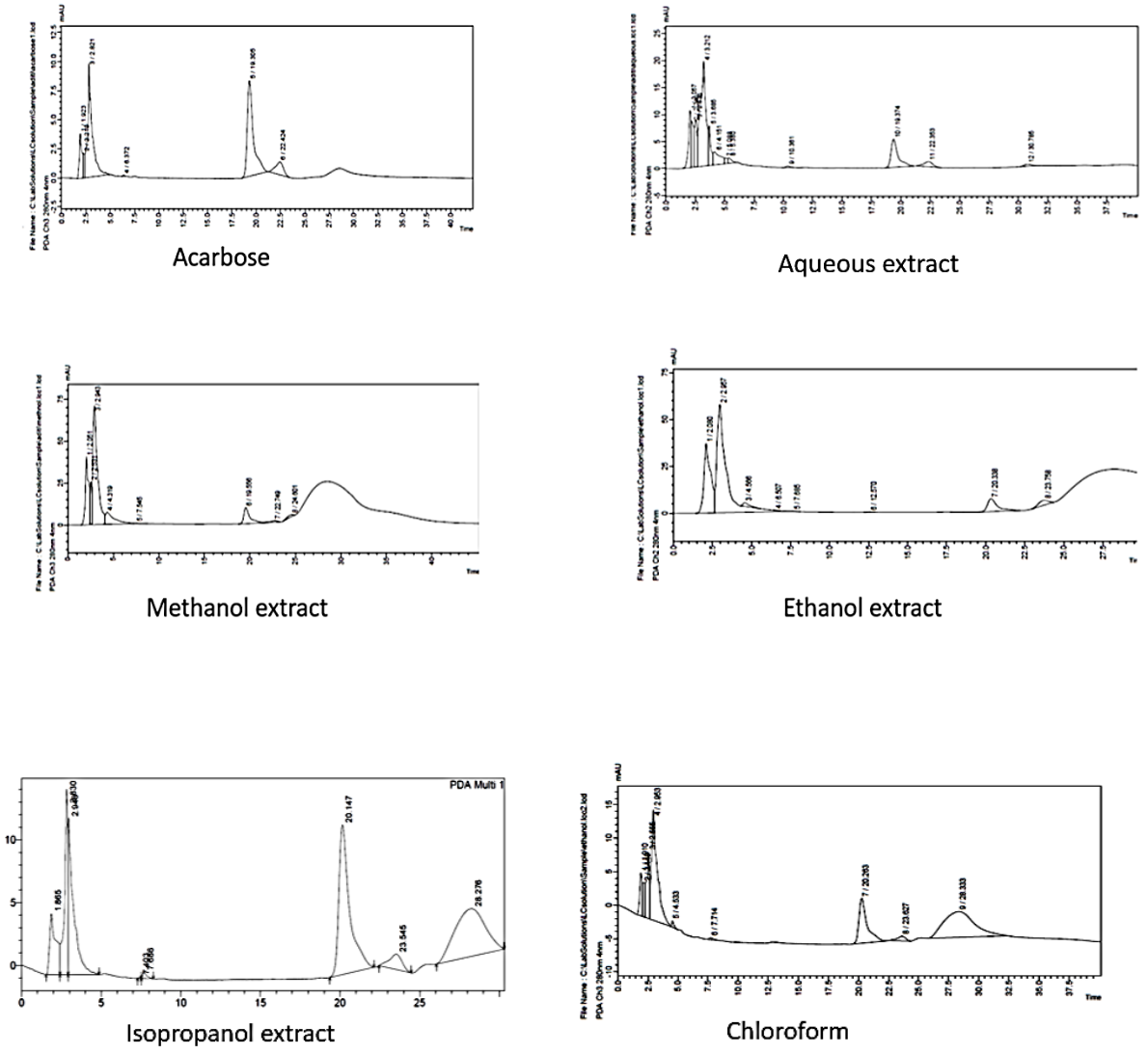


Figure. 3.3 HPLC chromatogram of *B aristata* bark extract in polar and nonpolar solvents.

CHNS and FTIR- Carbon, Hydrogen, and Nitrogen elements in the methanolic extract were evaluated with CHNS analysis. Carbon content was highest with 49.363% and nitrogen and hydrogen being 1.570% and 7.363%, respectively. Functional group analysis showed that methanolic extract had 617.10- halo compound, 1509- nitro compound, 1361-

sulfonamide,3381 primary aliphatic amines, 1717- C-O unsaturated ester. 2000-1650 aromatic compound. 1250- 1350 aromatic amine and aromatic ester are present, which marks the presence of phenols, flavonoid, and alkaloid content in abundance (Figure 3.4 and Figure3.5.)

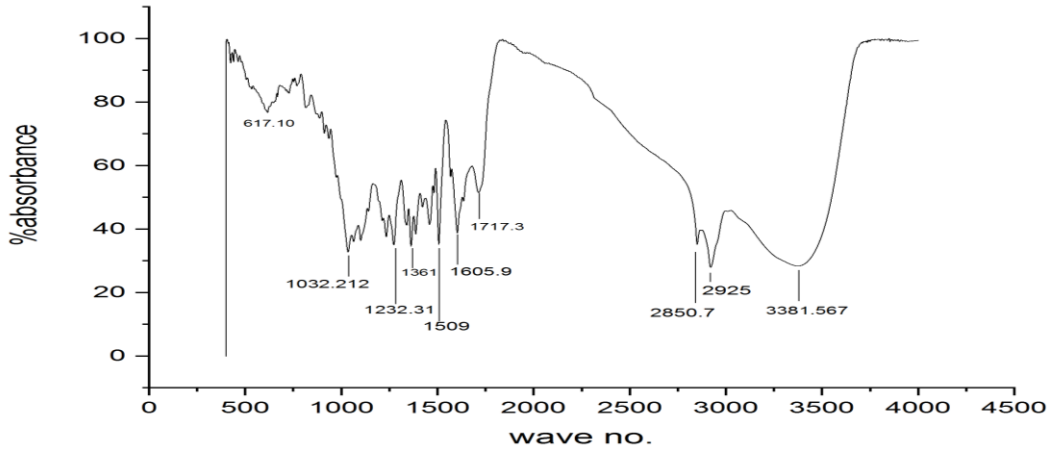
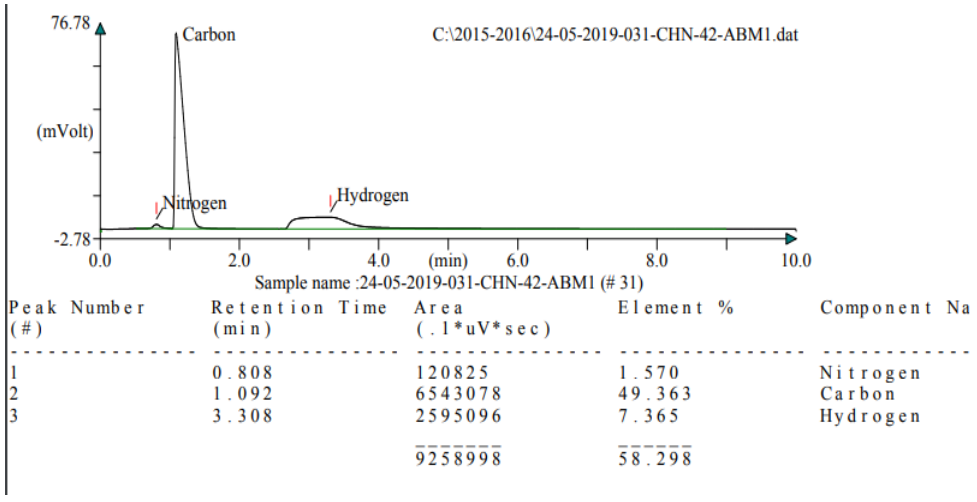


Figure 3.4. FTIR spectra of methanolic extract show peaks of different functional groups.



Peak Number (#)	Retention Time (min)	Area (.1*uV*sec)	Element %	Component Na
1	0.808	120825	1.570	Nitrogen
2	1.092	6543078	49.363	Carbon
3	3.308	2595096	7.365	Hydrogen
		9258998	58.298	

Figure. 3.5 CHNS chromatogram shows element percentage obtained for nitrogen, carbon, and hydrogen as 1.57, 49.363, 7.365, respectively.

3.5 Conclusion-

Approximately 25% of prescription drugs come from plant extracts in today's world. The extraction and phytochemical screening processes indicated considerable differences in extractive values and phytoconstituent content across the various solvent extracts of *Daruharidra*. Methanol proved to be the most effective solvent, giving the highest extractive value and significant amounts of phenols, flavonoids, and alkaloids. Notably, both ethanol and methanol extracts showed excellent antioxidant activities, with the methanol extract having the greatest DPPH radical scavenging activity. Furthermore, ethanol-based extracts demonstrated antioxidant activity via superoxide dismutase (SOD), indicating that they can help prevent oxidative stress-related diseases.

The ethanolic extract's chromatographic study revealed a number of medicinal phytoconstituents, identifying *Daruharidra* as a possible natural antioxidant and enzyme inhibitor. The methanolic extract included a variety of functional groups consistent with phenolic, flavonoid, and alkaloid chemicals, as revealed by CHNS and FTIR studies. These findings highlight the pharmacological significance of *Daruharidra* and its prospective use in the development of therapeutic medicines for oxidative stress-related diseases.