Phytochemical Characterization & investigation of *Haldina cordifolia* Leaf extract *as potential herbal drug*

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Abstract:

The current learning was intended to analyze about the important phytoconstituents of methanolic fraction of *Haldina cordifolia* leaves extract. Qualitative extraction of Haldina leaves was prepared using Methanolic extract. *H cordifolia* leave extract confirms presence of Flavanoids, Alkaloids, phenols, saponins, anthraquinone, Phytosterols, coumarins and anthocyanins, which was confirmed from the TLC analysis. The FT-IR spectrum confirmed presence of alkyl, methyl, ether, amino, alkene, alcohol, carboxyl and carbonyl groups. UV spectral reports indicate presence of unsaturated groups and hetero atoms such as S, N, and O [8]. The absorption spectrum for *H. cordifolia* extract shows five identifying peaks and confirms presence of organic chromophores and variety of functional groups within the *H. cordifolia* extract. LC-MS analysis data suggests existence of hydroxyl coumarins compounds namely scopoletin , isoscopoletin , umbelliferone and phytosterols like ß-sitosterol,epiglobulol, campesterol and Caryophylene oxide etc., that can be considered for antimicrobial activity and it might largely contribute in action againest multidrug resistant microbes.

Keywords: IR-spectroscopy, LC-MS, Scopoletin, Umbelliferone, ß-sitosterol and Campesterol.

1. Introduction:

Phytochemical drugs now becoming a genuine alternative to the conventional medicine worldwide. In recent times it is affecting public health and also has made an impact on the global trade. India was known to be one of the most abundant sources of medicinal agents amongst ancient culture. Phytomedicines keep on playing significant part in the therapeutic and health care system of big chunk of population. The plants with biologically active molecules are harvested for different parts of the plant, including the roots, leaves, barks, seeds, and flowers [1]. The plants having bio active principles are very helpful in management of number of illness and disorders. They can be utilized to treat a vast range of illness, so the reputation is growing day by day in the world health care system[2]. The ancient and current people have a sound of understanding about the herbal drugs that surrounds them. They usually share their knowledge with the locals, botanist and pharmacognosist across the humankind for several of purposes, with the modern conventional medicines [3]. As a whole maximum population trying to use herbal drugs and in the processes making a habit to utilize lesser modern medicines on their own initiative [6]. In the earth there are a huge number of herbs that are regarded as wild or weeds due to their lack of clear economic earlier now been revealed to be exceptionally valuable in provisions of phytomedicines. The most important sources of latest bioactive principles are now found in lesser known wild plants, unusable shrubs, and negligible used trees [8].

2. Plant Profile: *Haldina cordifolia*

The plants belongs to family Rubiaceae has several vernacular names like yellow teak, haridu, haldu, pasupukadamba and hethega etc. [9] [10]

Figure 1: Leaves of Haldina cordifolia

Table.1-Morphological character: [9][10]

Tree	Deciduous, bulky and blossoming.
Stem	Fluted irregular and buttressed, 15-40m height.
Bark	Brownish to grey, chunky, Sometime off white or silverfish white.
Wood	Normally yellow sometime orange in color .Hard and even.

Leaves	Reverse in nature, diameter ranging from 10-20 cm. Obicular , curtly acuminate. Bottom is cordate where as top is pubescent.
Petiole	4-11 cm long, stout in shape.
Stipules	1.2-1.7 cm length. Quadrilateral/ovate in shape.
Flowers	Very attractive, ball shaped with a perimeter 2-3 cm.Yellow in color with a shadow of pink.
Fruits	These capsules split into two dehiscent cocci.
seed	Narrow and small tailed upwards.

3. Collection of Plant material:

The plant parts (laves) of *H. cordifolia* were collected during autumn season from Lucknow district, Uttar Pradesh and herbarium was made for authentication. It was authenticated at integral university Lucknow Uttar Pradesh, India [12].

4. Preparation of plant material:

After collecting the leaves it was first washed well using tap water and allowed to dry in the shade. When optimum drying was achieved these dried leaves were grinded into course powder. The powdered leaves were then kept in a tight container to prevent exposure from moisture[14].

5. Extraction of Plant material:

The dried powders were then extracted using both methanol and water. Soxhlation method was preferred for extraction with methanol cold maceration was done for aqueous extract. The duration of extraction was 72 hrs. The Extracts were filtered and dried and stored in tight container for further experimentation[16].

6. Results and Discussion:

6.1Qualitative analysis:

6.1.1. Detection of Anthocyanins: To the required amount of sample 2 ml of 2N HCl and ammonia solution was added. Presence of Anthocyanins was confirmed by the appearance of pink-red color which turns into blue-violet.

6.1.2. Detection of Coumarins: To the required amount of sample Few ml of 10% NaOH was added. Presence of coumarins was confirmed by the formation of yellow color [17].

6.2Detection of phenols

6.2.1Ferric Chloride Test: To the Required amount extract freshly prepared 1% ferric chloride was added followed by potassium ferrocyanide. Phenol was confirmed by

Formation of bluish-green color.
Required amount of sample was treated in water. A little amount of ferric sulphate transferred to the mixture. Presence of phenol confirmed by dark-violet color formation.

6.3. Detection of Flavonoids

6.3.1. Alkaline Reagent Test: Required amount of sample was added to few ml of sodium hydroxide solution. Presence of flavonoids confirmed when Formation of intense yellow color, which decolorizes on addition of dilute hydrochloric acid.

6.3.2. Lead acetate Test: Required amount of sample was added to two drops of lead acetate solution. Presence of flavonoids confirmed by the yellow color precipitate formation [20].

6.4. Detection of Phytosterols

6.4.1. Salkowski's test: Required amount of sample was dissolved in few drops of chloroform .Conc. Sulphuric acid was added carefully on the wall of the test tube. Presence of phytosterols was confirmed when reddish brown color observed at the interface.

6.4.2. Liebermann Burchard's test: Required amount of sample was treated with chloroform shaken well and filtered. The resulted filtrate was exposed to 3ml ethanoic anhydride, after boiling and cooling, Sulphuric acid was mixed into it. Phyto-sterols was confirmed when brown ring was formed [22].

6.5.Detection of Fatty acids: Required amount Extract was treated with 5 ml of ether and was allowed for evaporation on a filter paper, after drying of the filter paper presence of fatty acid was confirmed by appearance of transparency on filter paper.

6.6. Test for Tannins:

6.6.1. FeCl3 test.: Required amount of sample was dissolved in water and filtered; 10% $FeCl_3$ solution was added to it. Presence of tannin was established when there is a change in color to bluish black[23].

6.6.2. $Pb(CH_3COO)_2$ Test: Required amount of sample was dissolved in water and dilute $Pb(CH_3COO)_2$ was added. Presence of tannin was established through the appearance of yellow color.

6.5.3. Pot. Dichromate Test. Required amount Extract was dissolved in water followed by addition potassium dichromate solution. Presence of tannin was established by the formation orange to yellow ppt.

6.7. Test for Saponins:

6.7.1.Foam Test: Required amount of Extract was diluted with 20ml of water and was mixed thoroughly for 15 minutes. Presence of saponin was confirmed by the formation "honey comb" froth.

6.8. Detection of alkaloids: The extracts were made soluble in HCl and were made clear by filtration.

6.8.1. Mayer's Test: Required amount sample exposed to Mayer's reagent.Presence of alkaloid was confirmed by the yellow precipitate formation.

6.8.2. Wagner's Test: Required amount of extract treated with Wagner's reagent .Presence of alkaloid confirmed by the brown/reddish Precipitate formation.

6.8.3. Dragendroffs Test: Required amount of sample was treated with Dragendroffs reagent. Presence of alkaloids confirmed by red precipitate Formation.

6.8.4. Hager's Test: Required amount of extract was mixed with Hager's reagent. Presence of alkaloid was confirmed by the formation of yellow precipitate.

6.9. Detection of Anthraquinone

6.9.1. Borntrager's test: Required amount of extract was shaken vigorously with 10 ml of benzene and filtered. 5 ml of 10% ammonia solution were added to the mixture. After shaking for a moment formation of pink, violet or red color confirms the presence of Anthraquinone.

6.9.2. Modified Borntrager's Test: Required amount of sample was treated with Ferric Chloride solution and warmed in boiling water. After cooling it was extracted with same volume of benzene. The benzene layer was separated and mixed with ammonia solution. Presence of Anthraquinone is confirmed by the Formation of rose-pink color.

6.10. Detection of carbohydrates:

6.10.1Fehling's Test: required amount of sample were treated with dilute HCl and treated with an alkali andheated with Fehling's solutions A and B. existence of reducing sugar was confirmed development of reddish brown color.

6.11. Test for Terpenoids: Required amount of sample was taken in a test tube which was added with 2 ml of ethanoic anhydride and concentrated sulphuric acid. Absence of terpenoid due to lack of Formation of blue, green rings.

Table.2-Qualitative tests for Haldina cordifolia:

Bioactive components	Aqueous extract	Methanol extract
Anthocyanins	+	+
Coumarin	+	+
Phenol	+	+
Flavanoid	+	+
Phytosterol	+	+
Fatty acid	+	+
Tannin	-	+
Saponin	+	+
alkaloids	+	+
Anthaquinone	+	+
Sugar	-	+
Terpenoid	-	-

7. Thin layer chromatography:

Commercial TLC sheet were used for the analysis was cut to required size and base line was drawn above 1cm from one end. Mobile phase was selected by testing out the samples in various solvents. A very little volume of methanolic extract was applied in the plate by a thin capillary tube on the base line. Plates are placed inside the TLC chamber where the spotted sides were kept down in to the chamber For chromatogram development. The chromatographic plates were held inside the chamber until the mobile phase travelled up to ¾th of the distance of the plate. The plate is removed and allowed to dry, and then the spots were observed under UV lights where lighted spots are observed. Rf values obtained for the extracts ranges from 0.4 to 0.8, So the extract may contain compounds like Flavonoids, phenols, saponin, Anthraquinone, Phytosterols, coumarins and Alkaloids. Mobile phases used and Rf values are given in the table no-3[24].

Table.3: TLC analysis report

Sl.No	Mobile Phase composition	Rf value
1	Toluene, Ethyl acetate(8:2)	0.61
2	Toluene ,Ethyl acetate ,Glacial acetic acid(5:4:1)	0.73
3	Petrolium ether,Chloroform(6.5:3.5)	0.48
4	Ethyl acetate + Methanol(1:1)	0.74
5	Hexane+Dichloro methane(1:1.5)	0.59
6	Ethyl acetate + Methanol(2:1)	0.80

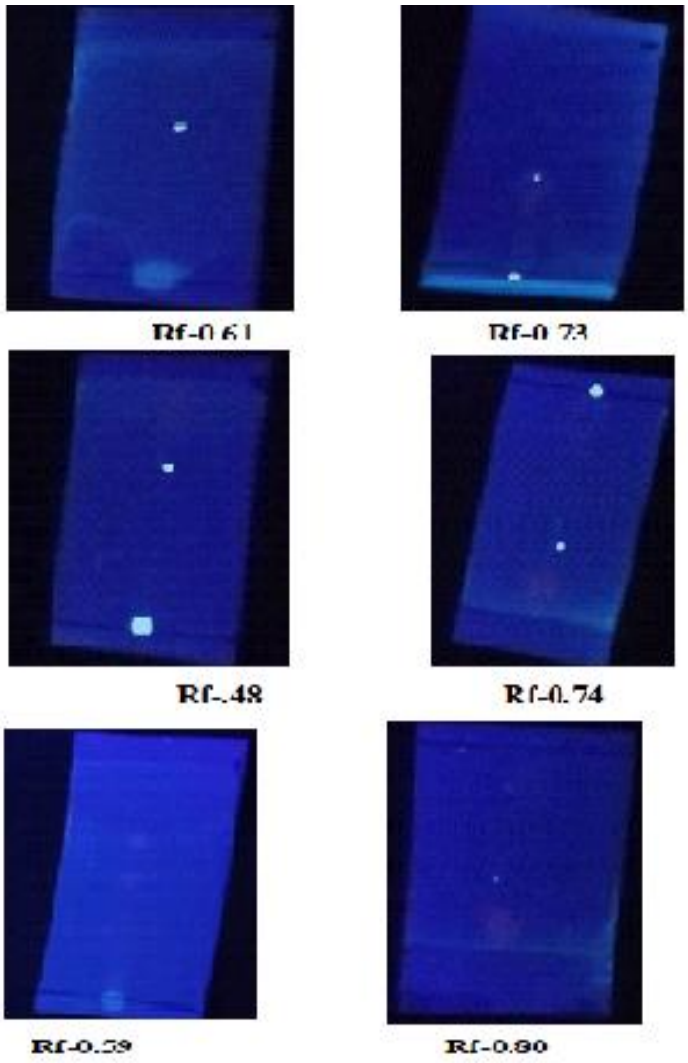


Figure 2 TLC plates with Rf values

8. FT-IR analysis

The powder leaves was mixed with potassium bromide by a mortar and pestle, and converted into a thin pellet. Infrared spectra were recorded as Potassium bromide and sample pellets on an IR21E28SEP19 transmission, between 4000 − 400 cm −1. The study of aqueous and methanolic extract of *H. cordifolia gave* the following characteristic absorption peaks are revealed in the tables. It is also given in fig.8 and 9 From the FT-IR spectral data, C=O stretching (1722.64 cm^{-1}), C-H stretching (2919.27 cm^{-1}), C=C bending (827.21 cm^{-1}), O-H Stretching(2651.97 cm^{-1}), C-CHO skeletal(1380.56 cm^{-1}), N-H Stretching (3397.42 cm^{-1}), S=O Stretching (1056.13 cm^{-1}), CH3 symmetrical bending(1452.49 cm^{-1}), N=C=S stretching(2113.17cm^{-1}), and C-X strong stretching(593.19cm^{-1}) were identified [26].

Table 4: IR Spectroscopic data (Methanol extract)

S.No	Group Stretching	Frequency (cm^{-1})
1	N-H Stretching	3397.42
2	C-H Stretching (carbonyl)	2919.27
3	O-H Stretching	2651.97
4	C=O stretching (carbonyl)	1722.64
5	CH3 Symmetrical bending	1452.49
6	C-CHO Skeletal	1380.56
7	C-O strong Stretching	1222.22
8	S=O Stretching	1056.13
9	C=C bending	827.21
10	-C-H Stretching	768.42
11	C-X strong stretching	593.19

Table-5: IR Spectroscopic data of (aqueous extract)

S.No	Group Stretching	Frequency (cm^{-1})
1	N-H Stretching	3393.53
2	C-H Stretching (carbonyl)	2926.19
3	N=C=S Stretching	2113.17
4	C=C stretching (carbonyl)	1619.58
5	C-H medium bending	1384.65
6	C-O strong Stretching	1271.33
7	S=O Stretching	1071.31
8	C=C strong bending	886.31
9	C=C bending	718.16
10	C-X Stretching	609.50

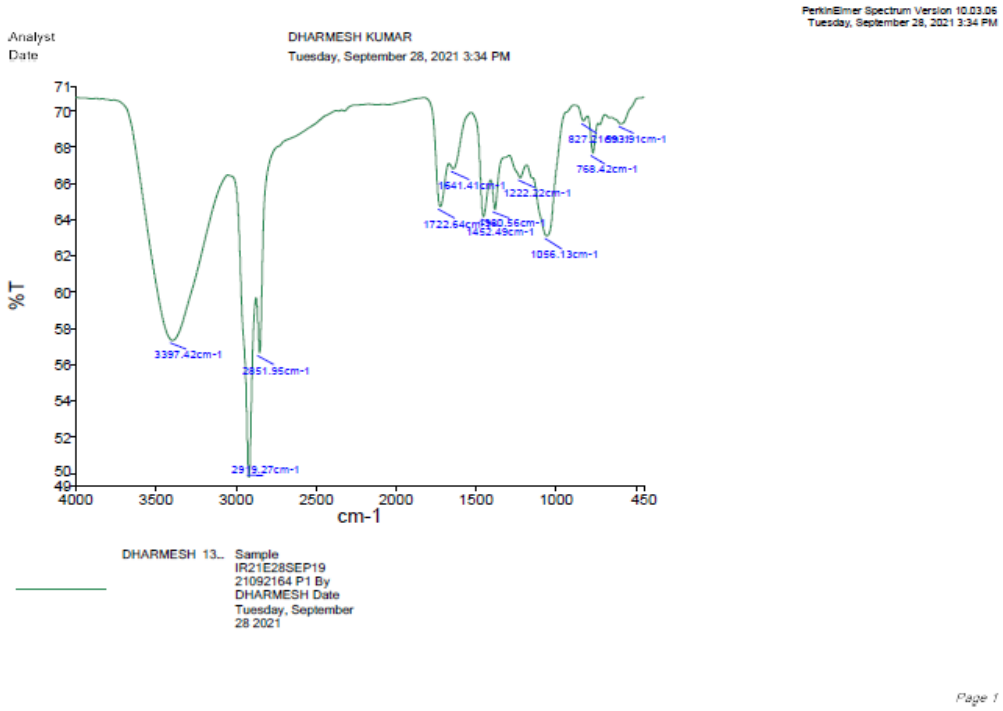


Figure 3-FTIR Images of methanolic extract of H.cordifolia

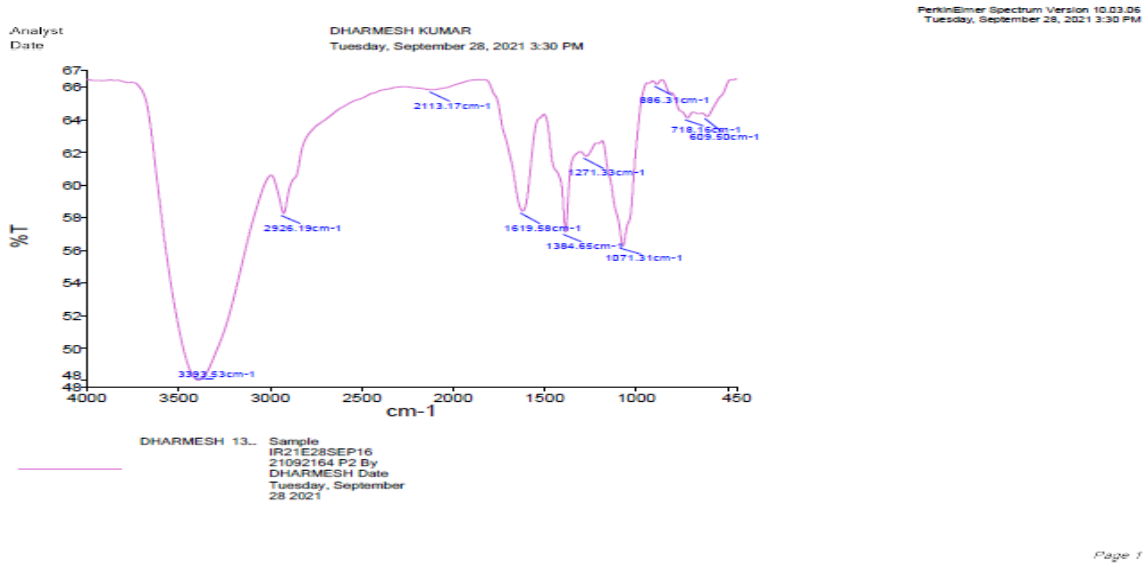


Figure 4-FTIR Images of aqueous extract of H.cordifolia

9. UV Analysis

The UV analysis preformed for identification of phytoconstituents present in methanolic and aqueous extract of *H. cordifolia*. The UV spectra were performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromospheres and

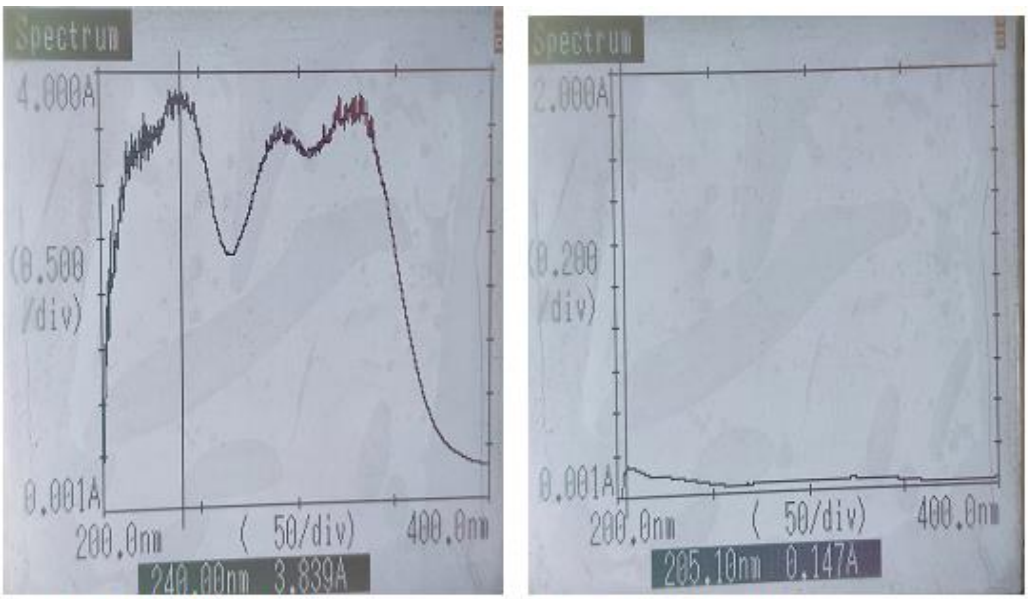
aromatic rings. The qualitative UV profile of alcoholic extract of *H. cordifolia* was taken at the wavelength of 200-400nm. The profile showed the peaks at 240, 284, and 294, 313and 332 nm with the absorption 3.911, 3.373, 3.505, 3.511 and 3.797 respectively. Figure-5 shows the absorption spectrum of *H. cordifolia* methanolic extract. Absorption bands observed pertaining to *H. cordifolia* plant extract are displayed in Table 6. The qualitative UV profile of Aqueous extract of *H. cordifolia* was taken at the wavelength of 200-400nm. The profile showed the peaks at 205.17nm with the absorption 0.147 respectively.Fig-5 shows the absorption spectrum of *H. cordifolia* Aqueous extract. The absorption bands are shown in Table-7. In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear sign of the existence of unsaturated groups and heteroatom's such as S, N, and O [8]. The spectrum for *H. cordifolia* extract shows two peaks at positions 332 nm, and 294 nm. This confirms the presence of organic chromospheres within the *H. cordifolia* extract. The experimental data of UV-VIS spectroscopic study in these two different extracts of H. *cordifolia* is given in Table 6 and 7 respectively [25].

Table-6: Calibration of methanolic extracts

λ (nm)	Absorbance
332.30	3.797
313.40	3.511
294.40	3.505
284.00	3.373
240.20	3.911

Table-7: Calibration of Aqueous extract

λ (nm)	Absorbance
205.10	0.147



λ_{max} of Methanolic extract λ_{max} of aqueous extract

Figure 5- UV calibration curve of Haldina cordifolia extracts

10. Liquid Chromatography-Mass Spectroscopy (LC-MS) analysis:

LC-MS analysis of methanol extracts of *H.cordifolia* leaves had detected twelve peaks with the retention time 1.93, 2.17, 2.49, 7.19, 10.48, 12.34, 13.50, 14.35, 22.59, 25.31and 27.24 minutes (Figure 12). Then each peak was fragmented, resulting 5 fragmentation spectrum with candidates mass (m/z) 192.5, 192.04, 162.14 and 416. (Figure 7).

The LC-MS spectrums interpretation was performed using a spectrum database for organic compounds in SDBS application. The results of spectrum interpretation on methanol extracts of *H.cordifolia* leaves, indicating that there are substance of scopoletin, isoscopoletin, umbelliferone and Beta-sitosterol with the retention time of 1.93, 7.19,2.17 and 13.51minutes respectively[27]. These results were confirmed by every fragmentation pattern as figure-7.

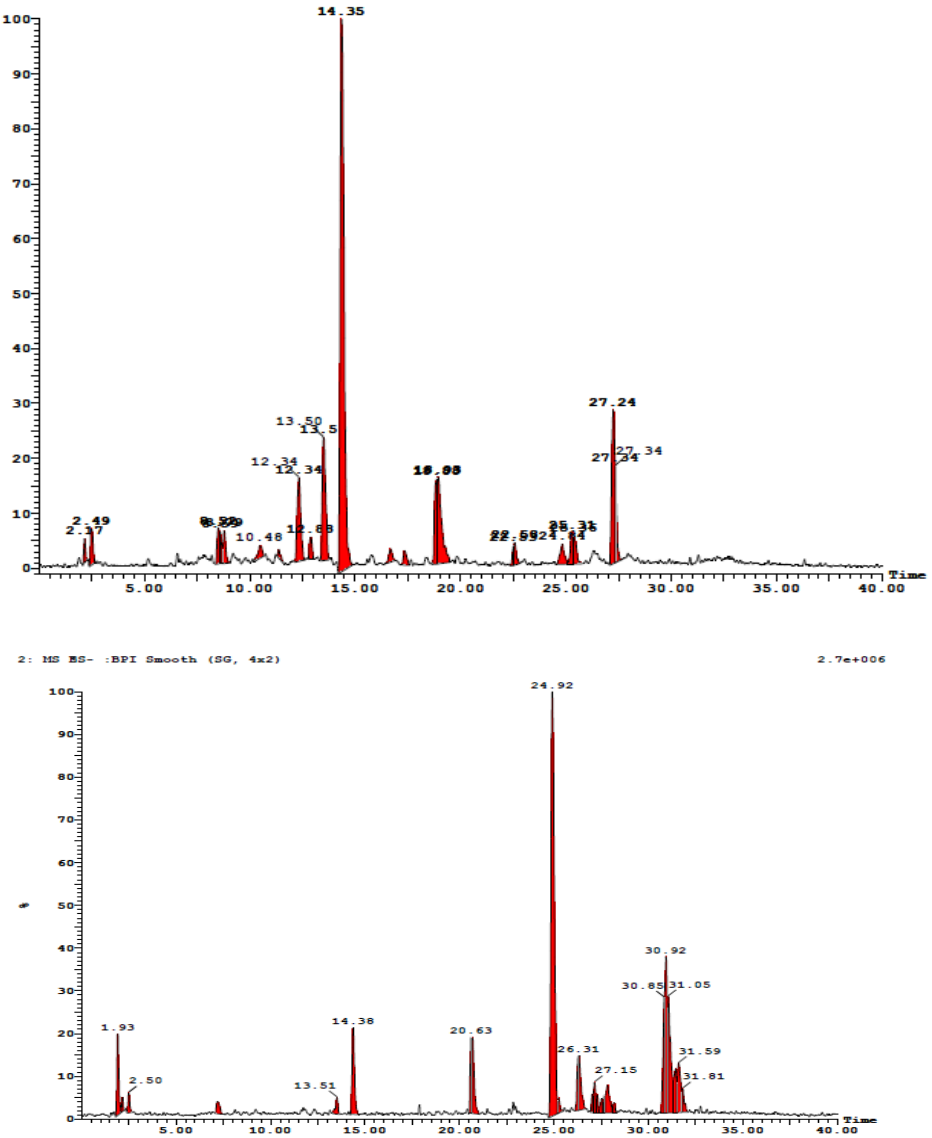
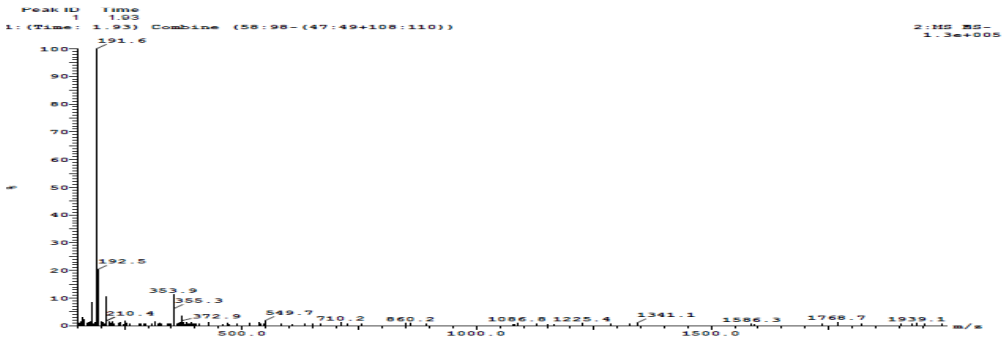
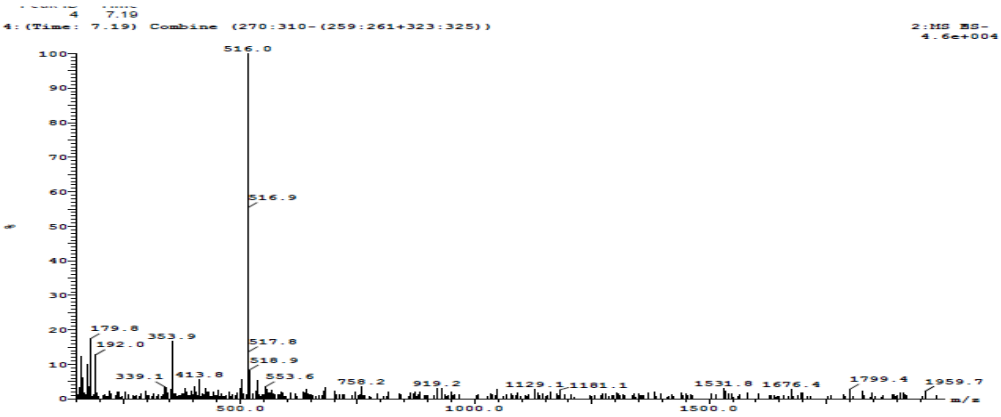


Figure 6-LCMS chromatogram of methanolic extract of Haldina cordifolia




4: (Time: 7.19) Combine (270:310-(259:261+323:325))
2:MS BS-
4.6e+004
516.0
516.9
179.8
192.0
353.9
517.8
518.9
339.1
413.8
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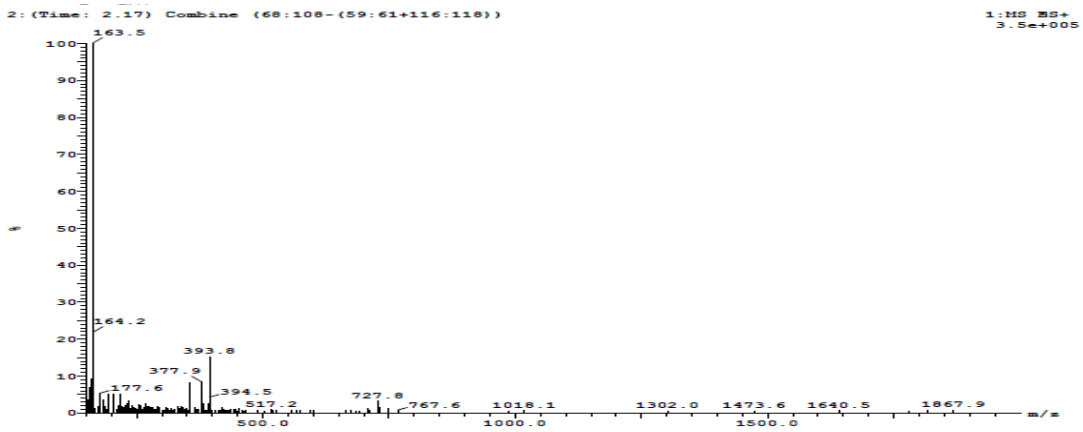
919.2
1129.1
1181.1
1531.8
1676.4
1799.4
1959.7
m/z


2: (Time: 2.17) Combine (68:108-(59:61+116:118))
1:MS BS+
3.5e+005
163.5
164.2
393.8
377.9
177.6
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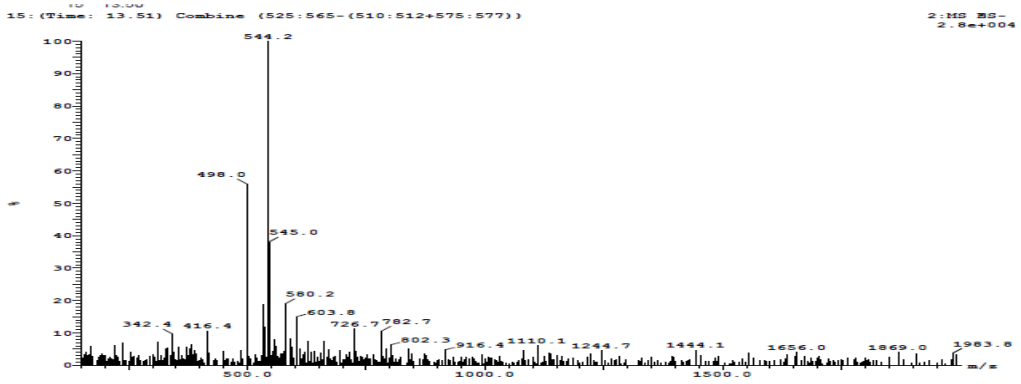
727.8
767.6
1018.1
1302.0
1473.6
1640.5
1867.9
m/z


15: (Time: 13.51) Combine (525:565-(510:512+575:577))
2:MS BS-
2.8e+004
544.2
498.0
545.0
560.2
603.8
342.4
416.4
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1444.1
1656.0
1869.0
1983.6
m/z

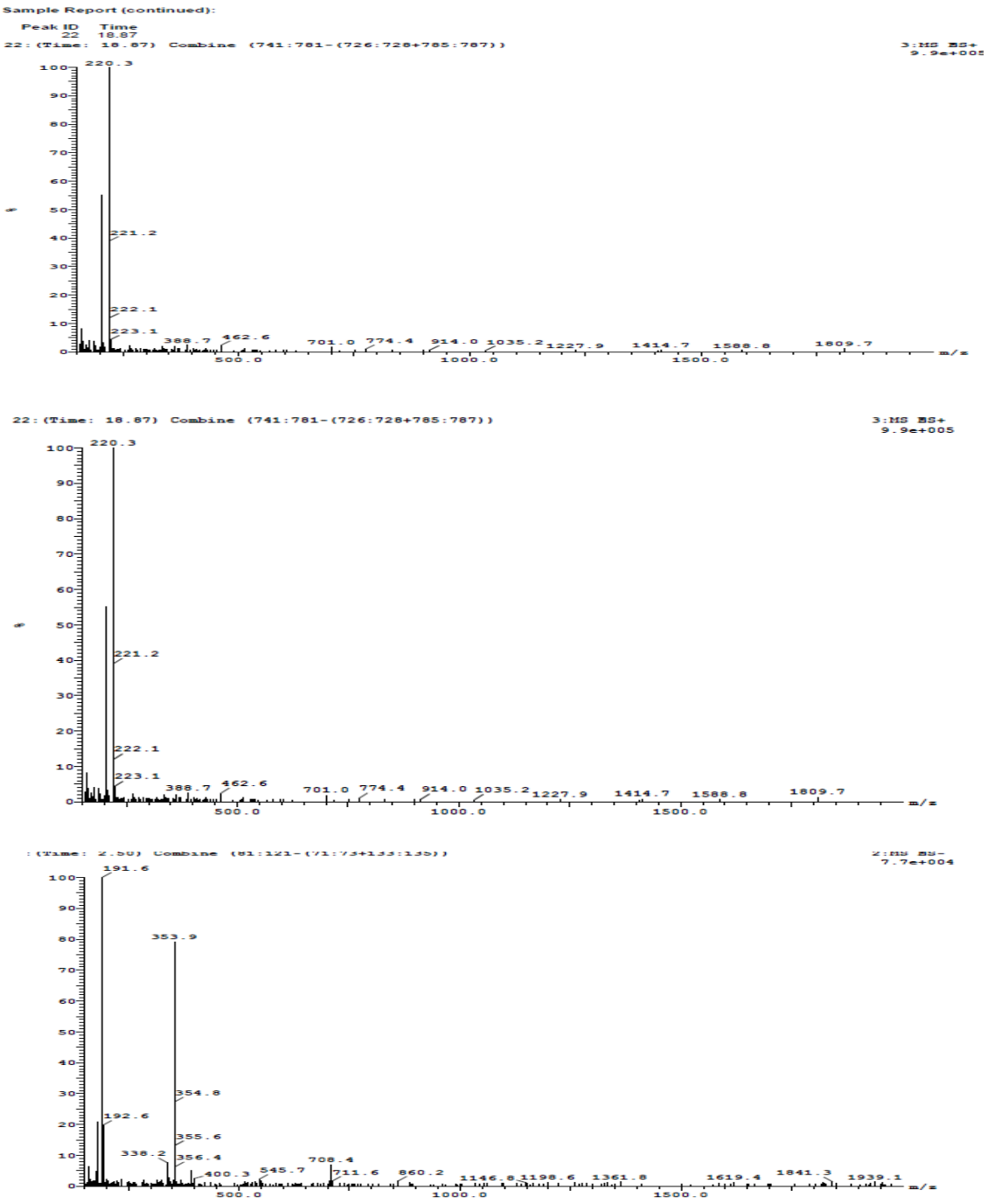

Figure 7- LCMS profiles of methanolic extract of Haldina cordifolia

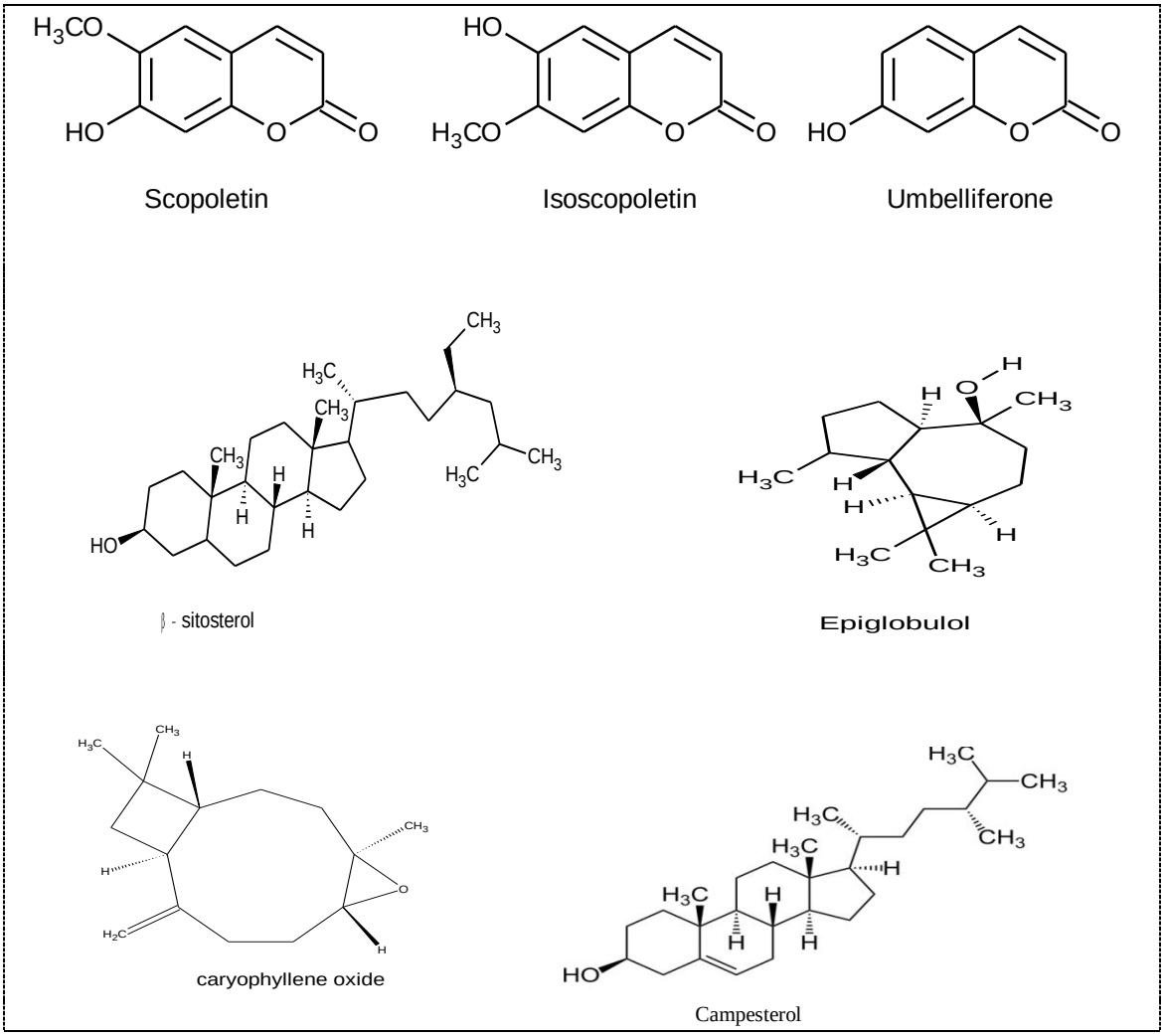

Figure 8-Different established isolates of Haldina cordifolia

11. Conclusion

Phytochemical analysis of Plant parts is important to establish the biological activity, consequently our study focus on the phytochemical characterization and examination of Extract of *Haldina cordifolia* leaves. These parameters are necessary to establish the quality, potential, and safety of the active metabolite. The outcome of our study suggests that Haldina cordifolia leaves contain scopoletin, isoscopoletin, umbelliferone and ß-sitosterol, epiglobulol, Campesterol and caryophylene oxide as phyto-constituents which play a vital role in controlling and treatment for various diseases. The preliminary Qualitative analysis reveals that *Haldina cordifolia* leaves are rich in coumarin, flavonoids, alkaloids, Anthraquinone, Tannin, saponin, fatty acids, phytosterols, sugars and Anthocyanins which provides larger scope for further investigation about it. From the analysis it was observed there are a number of biologically active compounds are present which may be considered for antimicrobial

and antibacterial actions. It can be concluded that methanol extracts of *H. cordifolia* leaves had great potential as biomedicine for antimicrobial diseases.

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