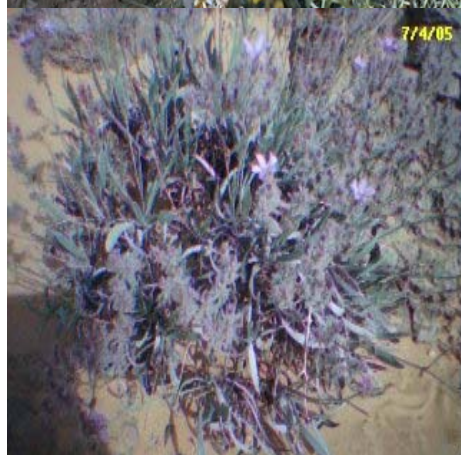
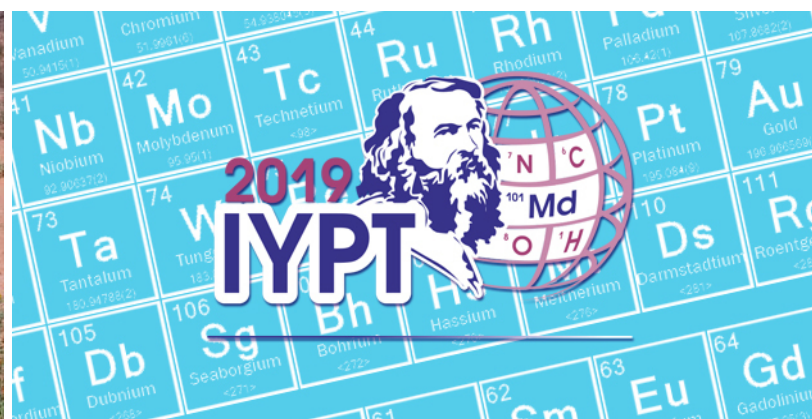


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Peer-reviewed research journal on Phytochemistry & Bioactives Substances

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Chromatography study (HPLC/DAD/MS) of *Haloxylon Scoparium Pomel* extracts

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Abstract. *Haloxylon scoparium* is a medicinal plant, widespread and quite famous in South Algeria, Where it is known as Remth. The investigation and inquiries held as far as its traditional use is concerned has revealed that it is very well known in curing some desases in different regions of the country.

The analytical study applied by high performance liquid chromatography coupled with diode array detection and tandem mass spectrometry from the ethyl acetate and butanol extracts by using Agilent Prep - C₁₈ HPLC column with a gradient elution of acetic acid in water and methanol as the mobile phase. The compounds in the mixture were clearly identified by comparing their HPLC-DAD ultraviolet spectra, and MS data with those of corresponding reference compounds. The obtained results allowed the detection of various flavonoids among which Seventeen flavonoid glycoside.

Key Words: *Haloxylon; Scoparium; Remth; Chromatographiy; ultraviolet spectra; mass spectra Flavonoid glycoside.*

1. INTRODUCTION

The term “flavonoid” is generally used to describe a broad collection of natural products that include a C₆-C₃-C₆ carbon framework, or more specifically a phenylbenzopyran functionality. Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, there are a well-defined group of compounds with established physical and chemical characteristics. This especially counts for their absorption of ultraviolet (UV) radiation, which makes their UV spectra very characteristic and UV spectroscopy a method of choice for their characterization, thus UV spectroscopy is used as a main method for the identification and the quantification of flavonoids for decades[1-2].

There is several published information regarding the isolation and the identification of flavonoids in plant material using different methods, mainly chromatographic and spectroscopic [3-10]. Today, high-performance liquid chromatography (HPLC) is established as the most convenient method which enables separation and identification of flavonoids using various detection systems [11-14].

Therefore, this paper presents qualitative identification of the content of flavonoids present in the aerial parts of *Haloxylon Scoparium* by using of high performance liquid chromatography coupled with diode array detection and tandem mass spectrometry.

2. METHODS

2.1. Plant material

The aerial parts of *H. scoparium* were collected from Ghardaia (Berienne region) in November 2012. The plants were identified by Pr. Abdelmadjid Chehma from Ouargla University. The plant materials were dried under shade and then ground and stored in closed container away from light and moisture.

2.2. Preparation of plant extracts

The extracts were prepared by soaking 200 g of the plant powder in a solution of EtOH/H₂O [70/30] for 24H. The procedure was repeated three times and the filtrates were combined before being evaporated under reduced pressure. The resulting extracts were diluted with distilled water and left for a whole night. The filtrates were then subjected to extraction by various solvents with increasing polarity (petroleum ether, dichloromethane, ethyl acetate, and butanol). The organic phases were separated and evaporated.

2.3. HPLC analysis

Reversed-phase high-performance liquid chromatography has proved to be the method of choice for the separation of a variety of flavonoids in different samples. The phenolic nature of these compounds requires the use of acidic mobile phases for satisfactory separation and peak shapes, whereas the detection is usually carried out with photodiode array detectors which are also very helpful for their identification of the characteristic absorption spectra of the flavonoids. In the last decade, mass spectrometers connected to HPLC systems introduced a greater selectivity and sensitivity in flavonoid analysis [3, 15-20]. The conditions applied in this study are

Device type: Agilent Prep - C18 scalar PN 440905 – 902, SN: USAWSO1038, LN: PR045203.

Chromatographic column: Colonne Agilent: (4.6*250mm, 5M).

Stationary phase: Slicagel Griffey (C-18).

Mobile phase: water / methanol / Acetic acid.

Injection volume: 10 Ml.

Scanning range: 200-600 nm.

3. RESULTS AND DISCUSSION

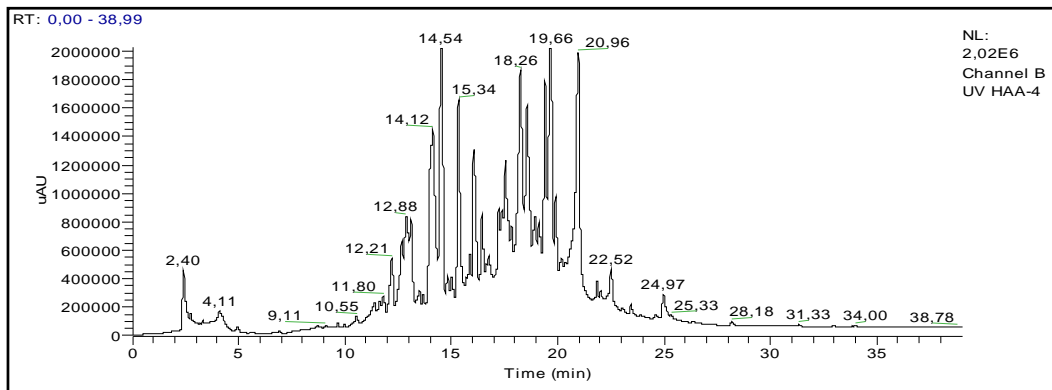


Figure 1. Chromatogramme of Ethyl acetat extract of *Haloxylon scoparium*.

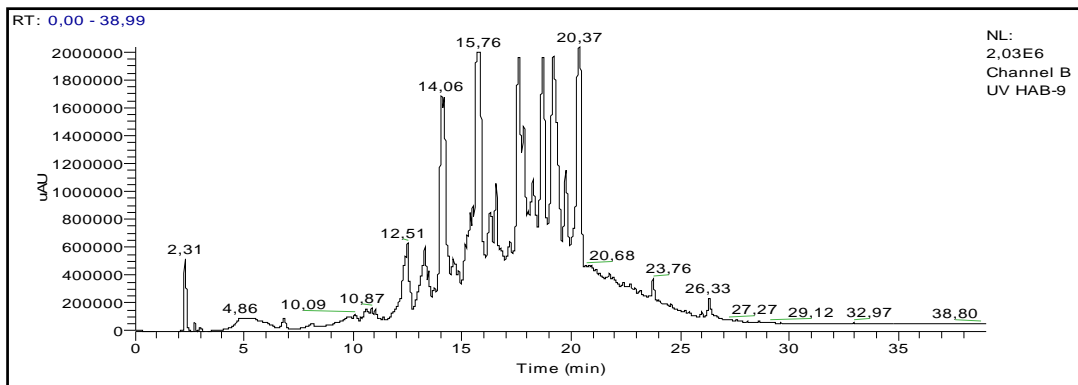
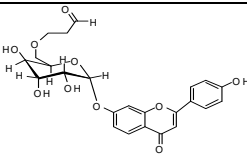
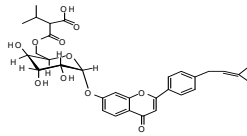


Figure 2. Chromatogramme of Butanol extract of *Haloxylon scoparium*.

Analysis of the extract of the aerial parts of *Haloxylon scoparium* by HPLC-DAD-MS showed the presence of various compounds flavonoids (Table 1, Table 2). The compounds were identified by comparison of UV spectra of the peaks and MS spectra with the corresponding standards and fragmentation pathways reported in the previous literature.

Table 1. Flavonoids compounds likely to be present in the ethyl acetate extract for the ethanol and water extraction method of the plant *Haloxylon scoparium*.

t_R (s)	Spectra UV	Spectra MS	Chemical formula	Scientific name
13.48	237,280,310	191.11,208.04,238.00(B) 322.92,390.98,474.87(M)		3-(((3R,4R,5S,6R)-3,4,5-trihydroxy-6-(2-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yloxy)tetrahydro-2H-pyran-2-yl)methoxy)propanal
14.58	254,319	203.99,219.98,288.01(B) 309.88,392.73,532.69 596.73(M)		3-methyl-2-(((3R,4R,5S,6R)-3,4,5-trihydroxy-6-(2-(4-(3-methylbut-2-enyl)phenyl)-4-oxo-4H-chromen-7-yloxy)tetrahydro-2H-pyran-2-yl)methoxy)carbonyl)butanoic acid

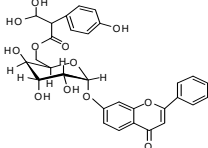
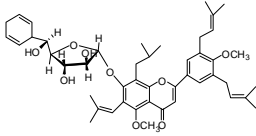
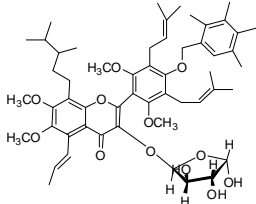
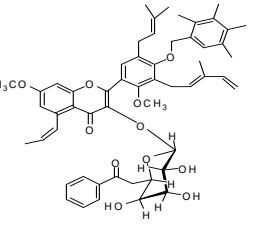
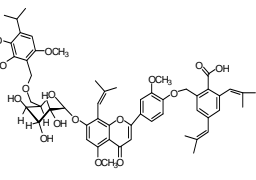
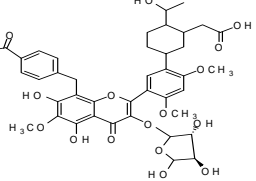
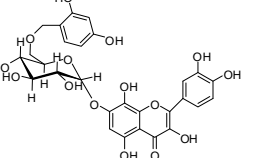
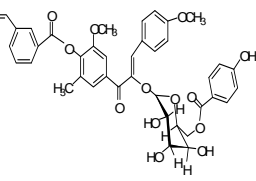
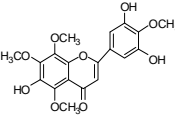
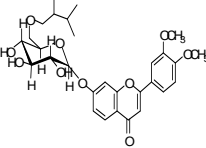
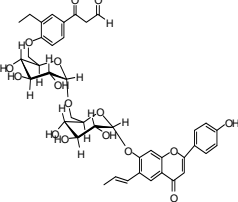
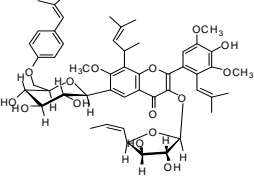
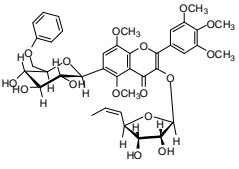
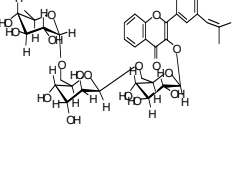
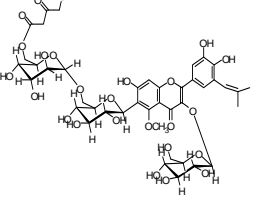
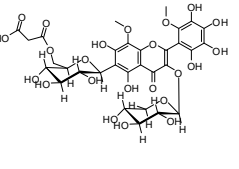
15,87	239,289,322	180.85,222.00(B),280.91 372.83,436.65,543.73 582.53(M)		((3R,4R,5S,6R)-3,4,5-trihydroxy-6-(4-oxo-2-phenyl-4H-chromen-7-yloxy)tetrahydro-2H-pyran-2-yl)methyl 3,3-dihydroxy-2-(4-hydroxyphenyl)propanoate
17,56	245, 320	180.96,280.88,351.81 384.57,390.81,449.86 527.72(B),546.55,618.46 681.95,754.28(M)		7-((2R,3R,4R,5S)-5-(cyclohexa-2,5-dienyl(hydroxy) methyl)-3,4-dihydroxytetrahydrofuran-2-yloxy)-8-isobutyl-5-methoxy-2-(4-methoxy-3,5-bis(3-methylbut-2-enyl)phenyl)-6-(2-methylprop-1-enyl)-4H-chromen-4-one
18,26	254, 291,342	180.27,299.92,363.85 402.68,430.55,590.64 650.41,756.56,778.37(B) 890.50,908.44,914.64(M)		2-(2,6-dimethoxy-3,5-bis(3-methylbut-2-enyl)-4-(2,3,4,5-tetramethylbenzyloxy)phenyl)-8-(3,4-dimethylpentyl)-6,7-dimethoxy-5-((E)-prop-1-enyl)-3-((2R,3R,4R)-3,4,5-trihydroxytetrahydrofuran-2-yloxy)-4H-chromen-4-one.
18,55	256, 290, 353	181.05,207.93,280.96 354.83,408.59,546.51 610.68,632.69(B),695.99 762.31,768.55,898.35(M)		7-methoxy-2-(2-methoxy-5-(3-methylbut-2-enyl)-3-((E)-3-methylpenta-2,4-dienyl)-4-(2,3,4,5-tetramethylbenzyloxy)phenyl)-5-((Z)-prop-1-enyl)-3-((3R,5R,6S)-3,4,5-trihydroxy-6-(2-oxo-2-phenylethyl)tetrahydro-2H-pyran-2-yloxy)-4H-chromen-4-one.
19,34	241, 285,318	212.92,280.97,314.03 335.89(B),384.64,488.49 546.40,646.69,696.20 777.62,874.61,994.03(M)		2-((2-methoxy-4-(5-methoxy-8-(2-methylprop-1-enyl)-4-oxo-7-((2R,3R,5R,6R)-3,4,5-trihydroxy-6-((4-isopropyl-2,3,6-trimethoxybenzyloxy)methyl)tetrahydro-2H-pyran-2-yloxy)-4H-chromen-2-yl)phenoxy)methyl)-4,6-bis(2-methylprop-1-enyl)benzoic acid.
19, 67	255, 353	181.06,280.92,412.72 500.66,578.21,624.65 646.74(B),696.44 776.43(M)		2-(5-(5-(8-(4-formylbenzyl)-5,7-dihydroxy-6-methoxy-4-oxo-3-((2R)-3,4,5-trihydroxytetrahydrofuran-2-yloxy)-4H-chromen-2-yl)-2,4-dimethoxyphenyl)-2-(1-hydroxyethyl) cyclohexyl) acetic acid
20, 96	255, 373	181.02,280.93,302.89(B) 361.99,472.75,518.40 578.55,602.61(M)		7-((2R,3S,4R,5S)-6-((2,4-dihydroxybenzyloxy)methyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yloxy)-2-(3,4-dihydroxyphenyl)-3,5,8-trihydroxy-4H-chromen-4-one
22, 52	237, 369	180.98,212.91,280.95(B) 316.92,390.92,488.39 546.40,650.53,754.31(M)		2-methoxy-4-((Z)-3-(4-methoxyphenyl)-2-((2R,3R,5R,6R)-3,4,5-trihydroxy-6-((4-hydroxybenzyloxy)methyl)tetrahydro-2H-pyran-2-yloxy)acryloyl)-6-methylphenyl 3-(2-methylprop-1-enyl)benzoate

Table 2. Flavonoids compounds likely to be present in the butanol extract for the ethanol and water extraction method of the plant *Haloxylon scoparium*.

t_R (s)	Spectra UV	Spectra MS	Chemical formula	Scientific name
15.34	244,290, 318	114.04, 174.11,204.03 208.07,222.07,287.94 (B) 322.86,336.84,390.52 (M)		2-(3,5-dihydroxy-4-methoxyphenyl)-6-hydroxy-5,7,8-trimethoxy-4H-chromen-4-one
16.34	241,272, 381	143.97(B),186.99,219.94 261.03,280.88,302.92 343.82,372.89,375.16 488.27,505.09,527.61 543.32(M)		2-(3,4-dimethoxyphenyl)-7-((2S,3S,4R,5S)-6-((2,3-dimethylbutoxy)methyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yloxy)-4H-chromen-4-one
18.21	255, 332	157.98,186.99,206.00 251.95,278.89(B) 280.97,306.30,403.65 410.66,415.80,540.49 546.48,624.64,754.60 770.52,792.60(M)		3-(3ethyl-4-(((3S,4R,5S,6S)-3,4,5-trihydroxy-6-(((3S,4R,5S,6R)-3,4,5-trihydroxy-6-(2-(4-hydroxyphenyl)-4-oxo-6-((E)-prop-1-enyl)-4H-chromen-7-yloxy)tetrahydro-2H-pyran-2-yl)methoxy)tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-3-oxopropanal
18,63	255, 290, 353	143.95,181.00,216.97 280.52,354.83,360.78 429.74,486.79,610.48 743.17,756.52(B),778.40 831.95,908.60,914.50 (M)		3-((2S,3R,5R)-3,4-dihydroxy-5-((Z)-prop-1-enyl)tetrahydrofuran-2-yloxy)-2-(4-hydroxy-3,5-dimethoxy-2-(2-methylprop-1-enyl)phenyl)-7-methoxy-8-(4-methylpent-3-en-2-yl)-6-((2S,3S,4S,5S)-3,4,5-trihydroxy-6-((4-(2-methylprop-1-enyl)phenoxy)methyl)tetrahydro-2H-pyran-2-yl)-4H-chromen-4-one
19,18	256, 290, 353	145.93,199.04,236.03 280.40,363.83,368.93 464.65,486.83,546.18 610.44(B),632.63,723.40 762.27,768.42(M)		3-((2S,3R,4R,5R)-3,4-dihydroxy-5-((Z)-prop-1-enyl)tetrahydrofuran-2-yloxy)-5,8-dimethoxy-6-((2S,3S,4S,5S)-3,4,5-trihydroxy-6-(phenoxy)methyl)tetrahydro-2H-pyran-2-yl)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one
19, 66	254, 336	106.01,144.11,201.01 270.87,289.00(B),377.60 429.95,489.21,546.49 632.51,716.87,756.55 778.33(M)		2-(4-hydroxy-3-(2-methylprop-1-enyl)phenyl)-3-((3S,4S,5S,6S)-3,4,5-trihydroxy-6-(((2R,4R,5R,6R)-3,4,5-trihydroxy-6-(((2S,3S,4S,5R,6R)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)methyl)tetrahydro-2H-pyran-2-yloxy)methyl)tetrahydro-2H-pyran-2-yloxy)-4H-chromen-4-one
19,73	254, 350	170.92,180.96,201.04 280.78,321.94,401.71 478.87,517.78,646.54 726.59,756.55,778.38 (B) 786.73,908.46,955.84 (M)		((2R,3R,4R,6R)-6-(((2R,3R,4R,5S,6S)-6-(2-(3,4-dihydroxy-5-(2-methylprop-1-enyl)phenyl)-7-hydroxy-5-methoxy-4-oxo-3-((2R,3S,4S,5R,6R)-3,4,5-trihydroxy-6-((hydroxymethyl)tetrahydro-2H-pyran-2-yloxy)-4H-chromen-6-yl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)methoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)methyl 4-hydroxy-3-oxobutanoate
20, 37	256, 342,369, 382	216.78,280.82,316.76 348.16,416.12,478.65 500.46,594.82,624.66 (B) 646.60,692.43,776.39 (M)		3-(((3S,4S,5S,6S)-6-(5,7-dihydroxy-8-methoxy-4-oxo-2-(2,3,4,5-tetrahydroxy-6-methoxyphenyl)-3-((2S,3S,4R,5R)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yloxy)-4H-chromen-6-yl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)methoxy)-3-oxopropanoic acid

The information from the UV spectrum, it may be possible to identify the compound subclass or perhaps even the compound itself. UV spectral data of 175 flavonoids in several solvents can be found, for example, in a book by Mabry et al, These are typically to be found at 270 and 330 to 365 nm for flavones and flavonols, at 290 nm for flavanones, at 236 or 260 nm for isoflavones, at 340 to 360 nm for chalcones, at 280 nm for dihydrochalcones, at 502 or 520 nm for anthocyanins, and at 210 or 280 nm for catechins.

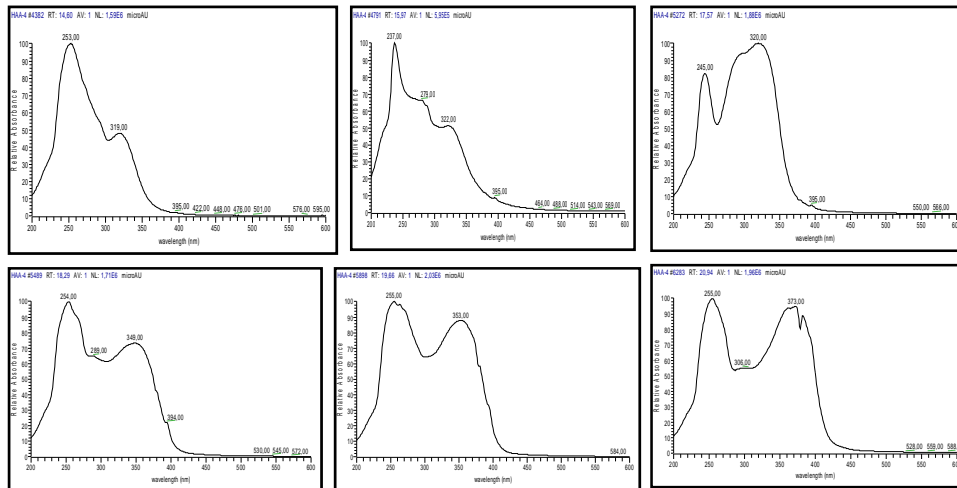


Figure 3. Spectra UV of some compounds of Ethyl acetate extract.

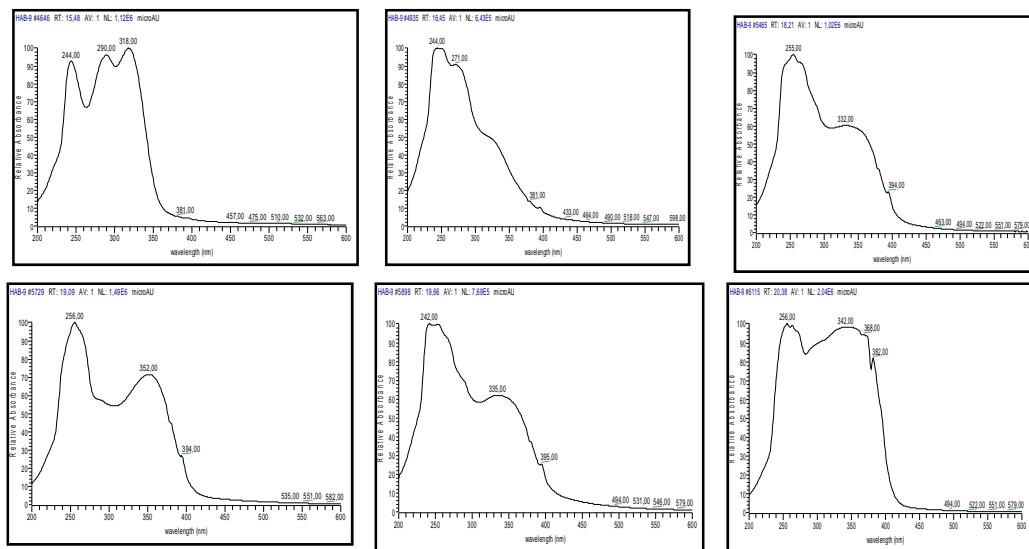


Figure 4. Spectra UV of some compounds of Butanolic extract.

Mass spectral data provide structural information on flavonoids and are used to determine molecular masses and to establish the distribution of substituents between the A- and B-rings. A careful study of fragmentation patterns can also be of particular value in the determination of the nature and site of attachment of the sugars in O- and C-glycosides. For flavonoid aglycones and glycosides with a limited number of sugars units.

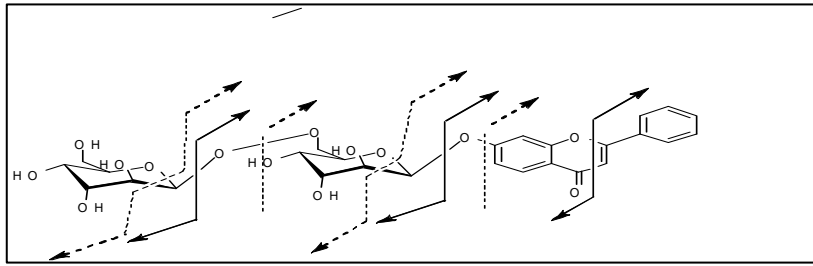


Figure 5. Possible fragmentation of glycoside flavones

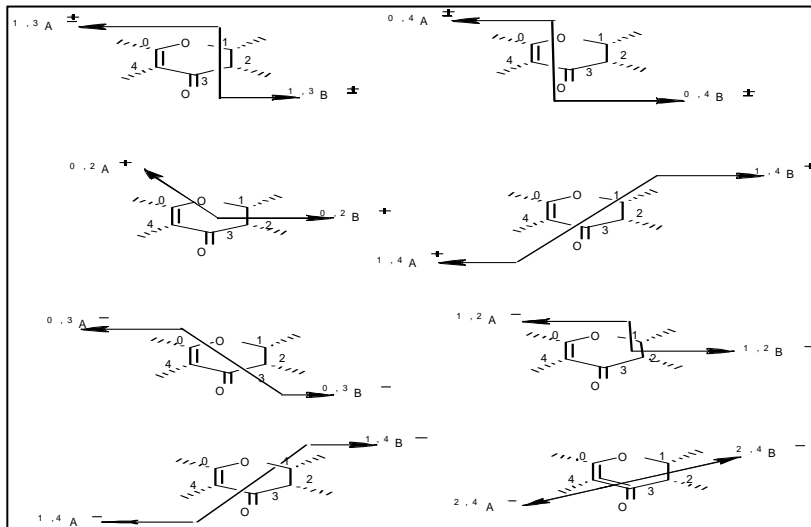


Figure 6. Possible fragmentation of the C ring of flavones

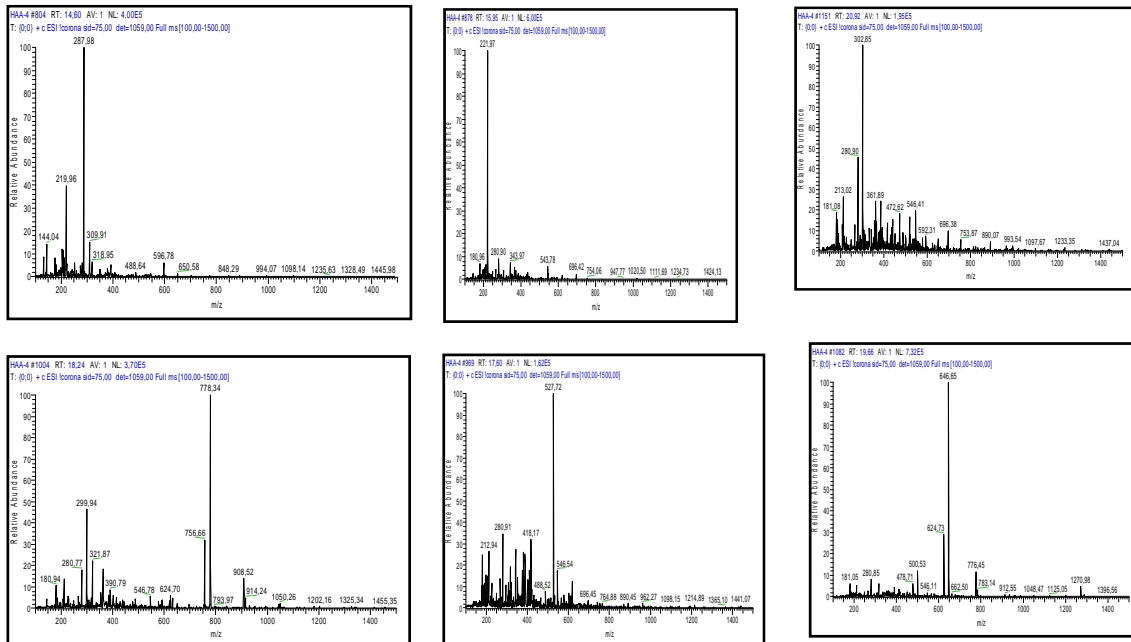


Figure 7. Spectra MS of some compounds of Ethyl acetate extract.

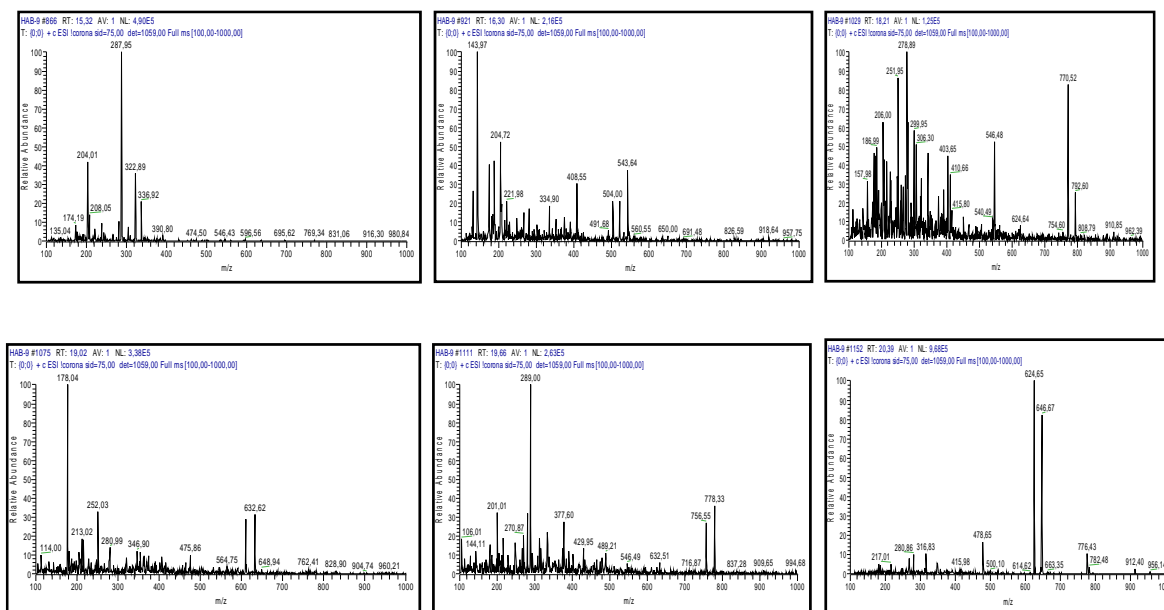


Figure 8. Spectra MS of some compounds of Butanolic extract.

4. CONCLUSIONS

HPLC coupled with diode array and mass spectrometric detection provides an efficient method of rapid identification of flavonoids in a mixture. This technique now finds widespread application. By this means 17 flavonoid glycoside in the extract of the aerial parts of *H. scoparium*, the compounds were identified by comparison of UV spectra of the peaks and MS spectra with the corresponding standards and fragmentation pathways reported in the previous literature.

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