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Phytochemicals screening and chromatographic separation of bio active compound from the roots of *Berberis lycium*

[1] Shakir Ullah, [1] Lubna Begum, [2] Maria Khattak, [2] Robeena Naz, [2] Mohammad Sohil and [2] Mohsin Ihsan

[1] Department of Botany, Govt Post Graduate Collage Timargara, Lower Dir, Pakistan

[2] Abdul Wali Khan University, Department of Botany Garden Campus, Mardan, Pakistan

Abstract
In the present research work the phytochemical and Chromatographic separation of bio active compounds *Berberis lycium* occurred. Phytochemical investigation of the CH_2Cl_2/CH_3OH (1:1) extract ethanol of root shown the presence of terpenoids, flavonoids, saponins, anthraquinones and alkaloids, steroids and tannins was absent. Chromatographic separation of CH_2Cl_2/CH_3OH (1:1) root ethanol extract yielded methyl 9, 10-dihydro-3, 8-hydroxy-6-methyl-9, 10-dioxoanthracene-2-carboxylate, isolated for the first time from the species of *Berberis lycium*. With the help of spectroscopic methods (IR, ^1H NMR, ^{13}C NMR, and DEPT-135 and UV-Vis,) Partial characterization of the isolated compound was done.

Keywords: *Berberis lycium*, Phytochemical Screening, Chromatographic Separation, Bio Active Compound

1. Introduction

Phytochemicals are primary and secondary compounds. In primary constituents Chlorophyll, proteins and common sugars are included and alkaloids, terpenoids and phenolic compounds are secondary compounds (Krishnaiah *et al.*, 2007) [7]. Anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities are Terpenoids exhibit these pharmacological activities (Sen and Mahato, 1997) [9]. Phytochemicals are naturally occurring chemical, biologically active compounds found in plants, which be responsible for health benefits for humans further these recognized to micronutrients and macronutrients (Hasler &Blumberg, 1999) [3]. They protect plants from damage and disease and contribute to the plant's color, flavor and aroma. In common, the plant chemicals that defend plant cells from environmental threats such as stress, drought, pollution, pathogenic attack and UV exposure are called as phytochemicals (Sharmila, 2017) [15]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher *et al.*, 1999) [11]. Medicinal plants represent an amusing source of natural products especially phytochemicals. Natural products include lignans, steroids, sugars, alkaloids, flavonoids, glycosides and terpenoids isolated from animals, microorganisms and plants. In different countries due to their secondary metabolites plants are used medicinally are a source of many powerful and potent drugs (Uniyal *et al.*, 2006, Srivastava *et al.*, 1996) [20, 5]. *Berberis lycium* belonging to family Berberidaceae is a high-value medicinal plant with a known history of uses in folk medicine. It is used traditionally for curing a broad range of human illnesses and diseases in the Indian Himalayan region of Pakistan and India. Its ethno medicinal uses include its use for treatment of jaundice, diabetes, eye infections, fractured bones, internal wounds, diarrhea, rheumatism, stomachache, and its use as a general body tonic (Sood *et al.*, 2013) [17].

2. Material and Method

2.1 Plant material collection and their Identification

The root of *Berberis lycium* was collected in October 2018 from Sheen Ghar Dir Lower Region of Khyber Pakhtun Khwa, Pakistan. With the help of Flora of Pakistan Plant were taxonomically identified and placed in the Herbarium of Department of Botany, Govt Post Graduate Collage Timargara Lower Dir Pakistan.

2.2 Crashing and filtration of the plant

The dried plant was powdered with the help of electric grinder. The powder were kept in air tight plastic bottles for further phytochemical analysis and antibacterial activities. In distinct conical flask 90 ml of ethanol was added to the powdered separately. With the help of aluminum foil Flask were covered and for the shaking purposes retained in shaker for 72 hours. After 72 hours with the help of Whatman filter paper extracts were filtered (Welch *et al.*, 2010) [21]. For chromatographic study the root was dried, powdered to suitable size and made ready for solvent extraction. 400g of the powdered root was soaked sequentially with CH_2Cl_2/CH_3OH (1:1) for 3x24hrs with random shaking. Using rotary evaporator at 40 ^oC to give black crude (50g, 10% yield) the extract was filtered.

2.3 Phytochemical Investigation

Phytochemical screening tests were carried out on the

CH_2Cl_2:CH_3OH (1:1) extract using the following Test.

2.4 Test for alkaloids
For of alkaloids detection, a few drops of Wagner's reagent (Potassium iodine) are add to 2 ml of extracts. The presence of alkaloids was confirmed by the formation of reddish brown precipitate (Ullah *et al.,* 2015).

2.5 Test for tannins
For the tannins detection Ferric chloride was used. The plants extracts was mixed with Ferric chloride ($FeCl_3$) solution. The presence of tannins was showed by Formation of blue green coloration (Mahajan and Badgujar, 2008) [8].

2.6 Test for anthraquinones
With concentrated hydrochloric acid about 0.5g of the ethanol extract was mixed. To it equal $CHCl_3$ solution was added. Few drops of ammonia were added to the mixture and in water bath it was heated. The presence of anthraquinones was showed by the formation of rose-pink color (Rani *et al.,* 2017) [13].

2.7 Test for saponins
In test tube 5 ml of extracts were shaken vigorously. The presence of saponins was indicated by the formation of froth (Shah and Yadav, 2015) [14].

2.8 Test of terpenes
To the 0.25g of ethanol extract 1 mL of concentrated H_2SO_4 was added carefully and mixed with 2 mL of $CHCl_3$ which form a layer. The presence of terpenes was indicated Reddish-brown coloration formation (Ullah *et al.,* 1995).

2.9 Test for flavonoids
For flavonoids detection, with sodium hydroxide (NaOH) solution extracts were treated. The presence of flavonoids was indicate by Red precipitation formation (Kokate *et al.,* 2008) [6]**.**

2.10 Tests for steroids
0.5g extract of sample with the addition of 2ml H_2SO_4 was mixed with 2ml of acetic anhydride. The presence of steroids is indicates by colour change from violet to blue or green (Kokate *et al.,* 2008) [6].

2.11 Isolation and purification of compounds
To silica gel column chromatographic separation (140g silica gel) the CH_2Cl_2: CH_3OH (1:1) crude extract (15g) was subjected and eluted with increasing gradient of ethyl acetate in n-hexane. A total of 28 fractions (each 50 mL) were collected. The constituent profile of each fraction was monitored by TLC and visualized under UV-Vis light (λ max 254 and 366nm). Fraction 1-13 were discarded because their TLC results showed unclear spots. Fractions 14-19 were combined based on their TLC profile and was further purified by column chromatography (eluent; increasing gradient of acetone in chloroform) and fractions 14-19 were combined further purified by PTLC to give compound S2 (40mg).

3. Result
3.1 Preliminary phytochemical screening
Preliminary phytochemical screening of CH_2Cl_2:CH_3OH (1:1) extract shown the presence of anthraquinones, terpenoids, flavonoids, alkaloids and steroids tannins and saponins was absent in the plant extracts (table 1).

Table 1: Qualitative phytochemical investigation of the CH_2Cl_2:CH_3OH (1:1) extract of *Berberis lycium*

S.No	Phytochemicals Name	Present / Absent
1	Alkaloids	+++
2	Anthraquinones	+ +
3	Tannins	_
4	Terpenoids	+ + +
5	Saponins	+
6	Steroids	_ _ _
7	Flavonoids	+ + +
8	Phenols	+ + +
9	Glycosides	+

(+++) presence of Phytochemicals in high amount (---) Absence of plant constituent,

3.2 Spectroscopic Analysis
Compound S2 was isolated as deep red crystal powder with Rf value of 0.37 (7:3) n-hexane/ethyl acetate. The UV spectrum (Appendix-1) (λmax 280 and 300 nm) is comparable to anthraquinone chromophore (Ali. F, 2014).

The IR spectrum (Appendix-2) revealed broad absorption bands at 3425 cm^{-1}, medium sharp peak at 1600 cm^{-1}, strong sharp peak at 1200 cm^{-1}, weak sharp peak at 2900 cm^{-1}, and 3050 cm^{-1} attributed to the hydroxyl group, C-O ester moiety, SP^3 C-H stretching vibration and SP^2 C-H stretching vibration respectively.

The ^1H NMR analysis of compound S2 was done in DMSO and hence the peak at 2.5 and 3.3 in ^1H NMR and 40 ppm in ^{13}C NMR belongs to the solvent DMSO. The ^1H NMR shows a total of 11 protons (Appendix 3). A peak at 1.2 ppm(s,3H) indicated the presence of methyl protons connected to sp2 quaternary carbon, peak at 3.7 ppm(s,3H) is a proton connected to a methyl part of methyl ester, proton at 14.4 ppm(s,1H) shows a hydroxyl group peri to carbonyl carbon.

There are total of four aromatic protons at 7.53 ppm (s,1H), 7.55 ppm (s,1H), 7.2 ppm (dd,1H, J=8,1.2) and 7.5 ppm (dd,1H, J=7.6,1.6) of which two of them belong to ring A having ABX multiplicity pattern and two of them belongs to ring C. The presence of peak at 14.4 ppm (s, 1H, 1-OH) shows that there is a peri hydroxyl group at C-1 position. The presence of methoxy of ester group at C-7 was evident from the peak 3.7 ppm(s,3H) and the presence of one methyl clearly evident from the peak at 1.2(s, 3H).

From ^{13}C NMR (Appendix-4), the two carbonyl carbon evident 194 ppm (C-9) and 185 ppm (C-10). The difference of more than 5 ppm, between the two peaks suggest that one of the carbonyl (C-9) is peri to the hydroxyl group (C-1). The peak at 171.02 ppm suggest that there is acetyl moiety at C-7 which is supported by the methoxy peak at 51.71 ppm. The chemical shift value of methyl at C-3 position appeared at 1.2 ppm and the upfeild chemical shift value of this methyl may be attributed to the absence of the anisotropic effect through space which is far from the carbonyl carbon. There is aromatic methines between 117

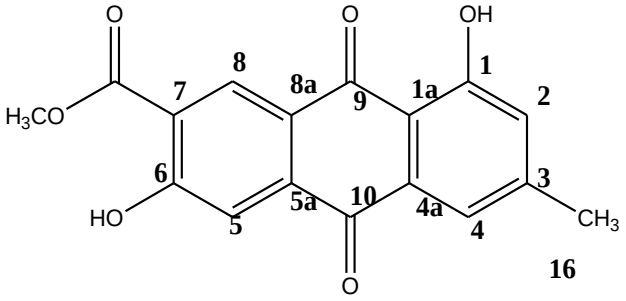
and 141 ppm. The above NMR features are good agreement with anthraquinone skeleton. The ^{13}C NMR revealed a total of 17 carbons that is good agreement with anthraquinone skeleton.

DEPT-135 showed six signals at 134.26, 124.19, 119.03, 117.47 are four aromatic methines and 51.72, 21.23 are methyl connected to methyl ester and aromatic ring respectively.

Table 2: NMR data of compound S2 (400 MHz, DMSO-d_6) δ in ppm

Position of carbon	Compound S2			Reported Literature(Ali,F 2014)	
	δ ^{1}H NMR	**δ ^{13}C NMR**	**DEPT-135**	**δ ^{1}H NMR**	**δ ^{13}C NMR**
1	-	162.08	-	-	162.9
1a	-	133.61	-	-	125.3
2	7.2(dd,8,1.2)	124.19	-	7.29(dd,7.5,1.0)	124.4
3	-	122.62	-	7.68(t,1.9)	119.3
4	7.5(dd,7.6,1.6)	117.47	-	7.70(dd,5.5,2.5)	113.0
4a	-	134.24	-	-	133.6
5	7.53(s)	134.26	-	7.73(s)	136.9
5a	-	137.15	-	-	133.8
6	-	160.44	-	-	159.5
7	-	141.23	-	-	142.9
8	7.55(s)	119.03	-	-	130.8
8a	-	133.26	-	-	125.2
9	-	194	-	-	190.4
10	-	185.30	-	-	182.3
COOCH3	3.7(s)	51.71	-	3.95(s)	52.7
COOCH3	-	171.02	-	-	167.9
CH3	1.2(s)	21.2	-	2.70(s)	20.3
1-OH	14.4(s)	-	-	12.8(s)	-

*From the above spectroscopic data and comparing with literature the structure of compound S2 was proposed to be:



methyl 9,10-dihydro-3,8-dihydroxy-6-methyl-9,10-dioxoanthracene-2-carboxylate

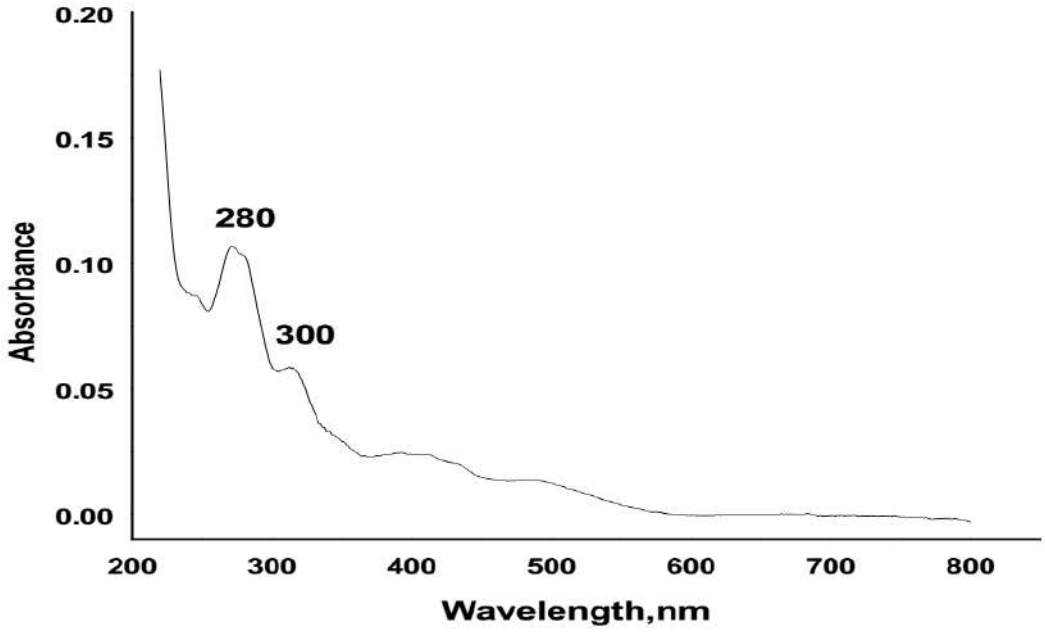


Fig 1: UV spectroscopic data

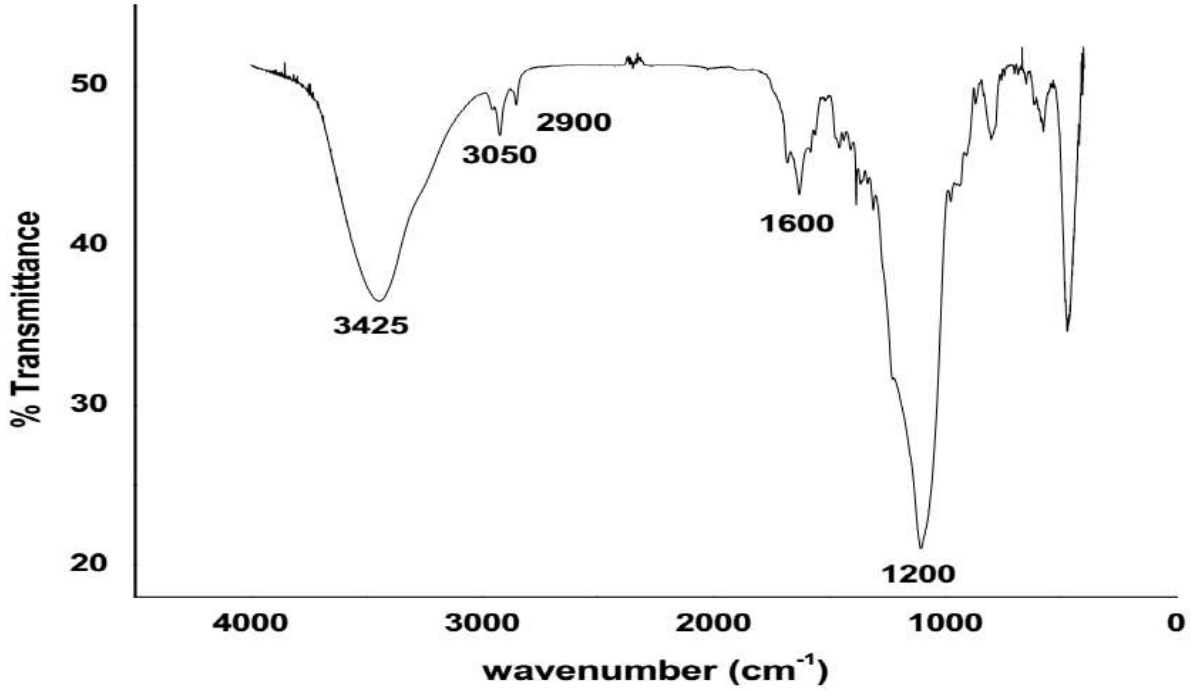


Fig: 2 IR spectroscopic data

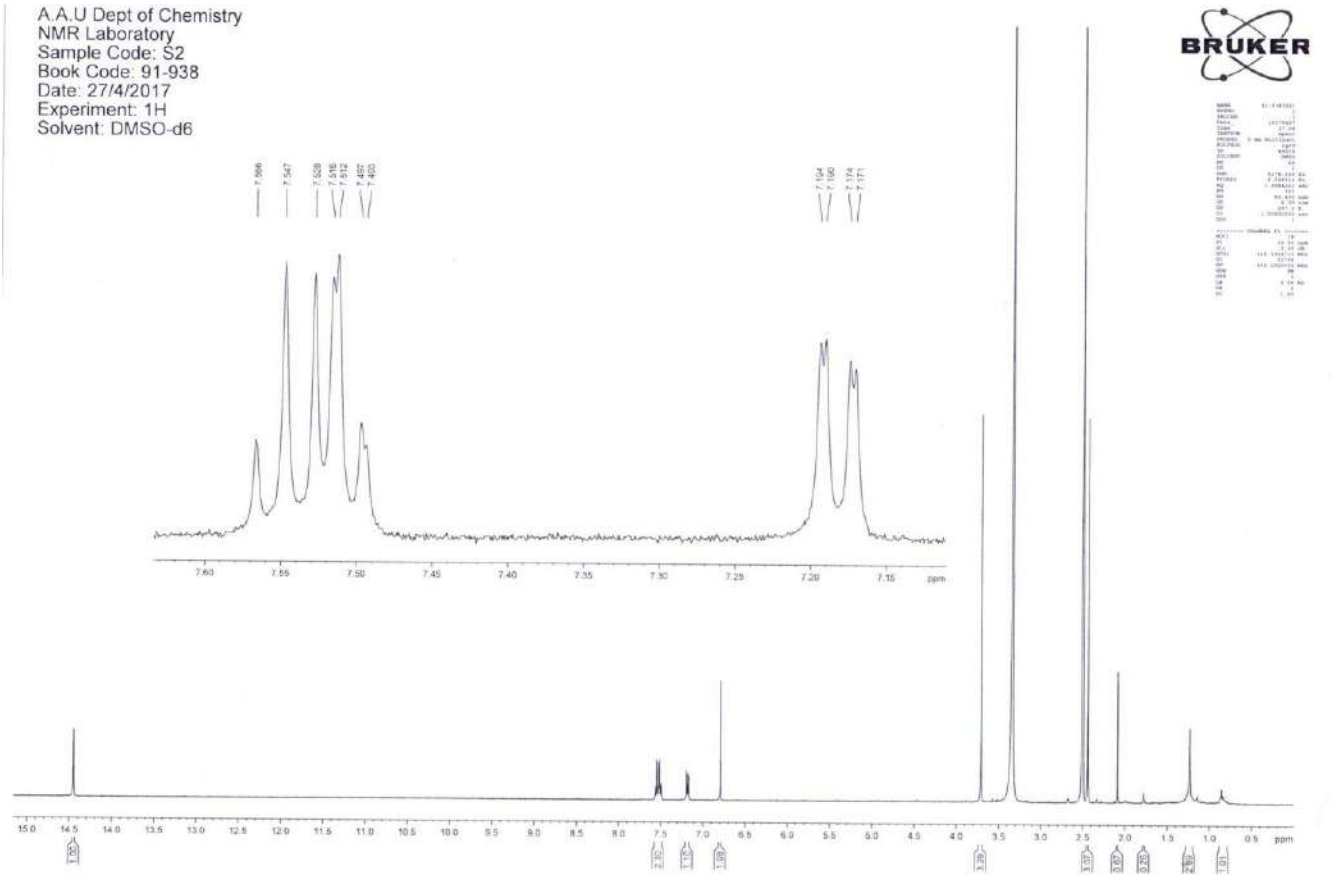


Fig 3: 1H- NMR spectroscopic data

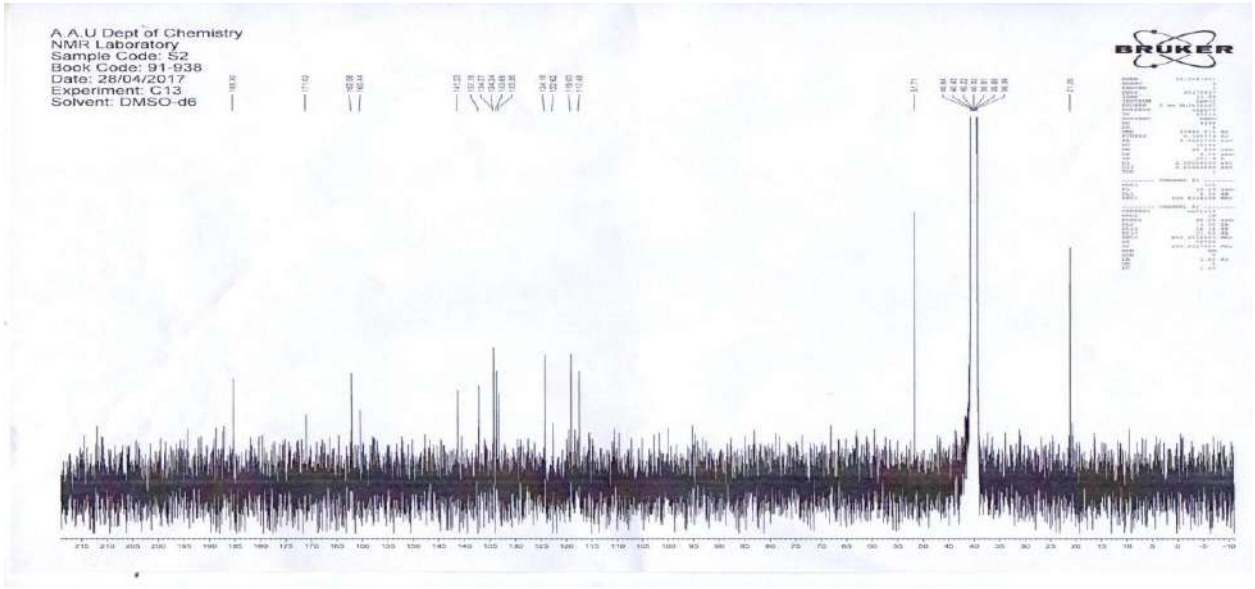


Fig 4: NMR spectroscopic da

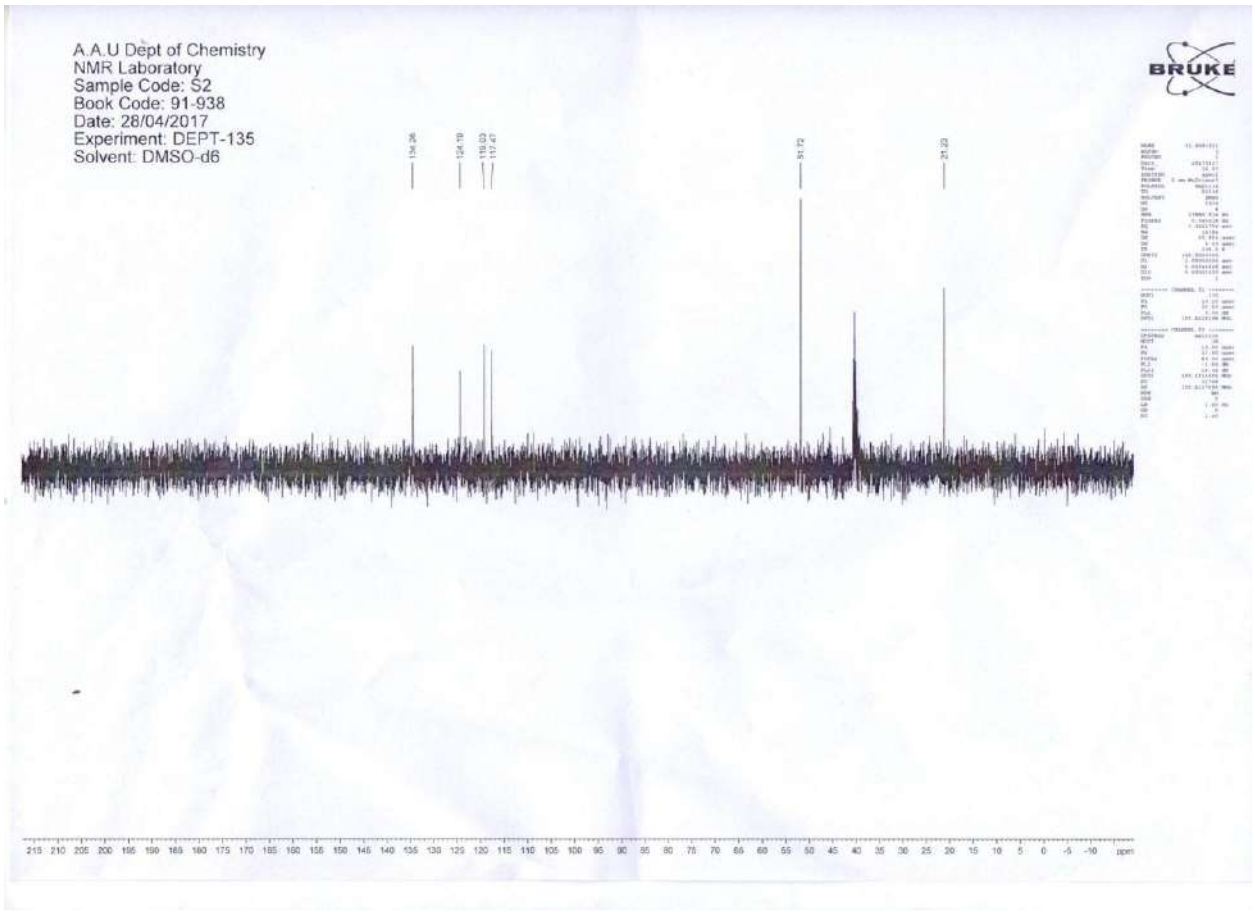


Fig 5: DEPT -135 spectroscopic data

5. Discussion

In the present research work the qualitative phytochemical investigation of ethanolic extracts and Chromatographic investigation occurred of *Berberis lycium.* The bioactive compounds on the medicinal plants employed contain various secondary metabolites such as alkaloids, flavonoids, steroids, phenols, tannins, and glycosides in appreciable quantities. Phytochemical screening tests of the crude CH_2Cl_2/CH_3OH (1:1) and CH_3OH root extract of *Berberis lycium* showed the presence of terpenoids, flavonoids, saponins, anthraquinones and alkaloids tannins and steroids was absent in the plant. Silica gel column chromatographic separation of the CH_2Cl_2/CH_3OH (1:1) extract proposed to be, methyl 9, 10-hydro-3, 8-dihydroxy-6-methyl-9, 10-dioxoanthracene-2-carboxylate, reported for the first time from the species of *Berberis lycium*. The structure of the compound was determined by ^{1}H-NMR, ^{13}C-NMR, IR and UV spectroscopic analysis. The plant extracts were also

revealed to contain saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000) [16]. The saponins are used in hyperglycemia, antioxidant, anticancer, anti as inflammatory activity, hypercholesterolemia and weight loss. Saponins act as antimicrobial activity and extremely cold blooded animals, but toxicity to mammals is low (Verma *et al.*, 2013) [4]. Alkaloids have the antispasmodic and antibacterial and analgesic potential (Malik *et al.*, 2017) [10] properties. Alkaloids have been used as both antibacterial and antidiabetic properties and useful for such activities. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared (Akinyeye *et al.*, 2014) [1]. Glycosides are known to lower the blood pressure. Tannins are also known antimicrobial agent. Tannins (commonly referred to as tannic acid) are water soluble polyphenols that are present in many plant foods. Tannins are water soluble plant polyphenols that precipitate proteins. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Ullah *et al.*, 2018) [18, 19]. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al.*, 1998) [2].

6. Conclusion

This work is one of the few attempts to analyze the phytochemical constituents of the polar extracts from the roots of *Berberis lycium* from Lower Dir. Phytochemical investigation of the crude CH_2Cl_2/CH_3OH (1:1) and CH_3OH root extract of *Berberis lycium* showed the presence of terpenoids, flavonoids, saponins, anthraquinones, alkaloids tannins and steroids was absent. Silica gel column chromatographic isolation of the CH_2Cl_2/CH_3OH (1:1) extract proposed to be, methyl 9, 10-hydro-3, 8-dihydroxy-6-methyl-9, 10-dioxoanthracene-2-carboxylate, reported for the first time from the species of *Berberis lycium*. The structure of the compound was determined by ^{1}H-NMR, ^{13}C-NMR, IR and UV spectroscopic analysis.

7. Recommendation

Since this plant has alkaloid, flavonoid, terpenoids and anthraquinones as assured from the results of phytochemical test, it is possible to isolate additional compounds from this plant. Especially if reverse phase chromatography is available it is possible to isolate more compounds from methanol extract. *In vitro* bioassay and structural activity relationship (SAR) has to be done on the crude as well as isolated compounds on various strains so as to isolate lead compounds.

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