# Phytochemical and antioxidant studies of *Berberis lycium*.

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**Abstract**: Six compounds have been isolated from methanolic and petroleum ether extracts of *Berberis lycium* (Barberry). Four out of six isolated compounds are reported for the first time from this plant. Purification of different compounds has been accomplished by conventional extraction and chromatographic techniques. The compounds have been structurally characterized by IR, Low Resolution MS, $^{1}$H-NMR and $^{13}$C-NMR spectroscopic techniques. All plant extracts and isolated compounds were assayed for the first time for their antioxidant activity.

**Keywords**: *Berberis lycium*, Daruharidra, berberine, ß-sitosterol, alkaloid.

## INTRODUCTION

The plant was described under the name of Ambaribis by *Al-Biruni.* He also mentioned its Persian name as Zirkash. Its common name is Kashmal. However, it is called Ishkeen and Sumbal in the area of its collection. (Said *et al,* 1996). *Berberis lycium* was described in 1837 by John Forbes Royle. *Berberis lycium* commonly known as Barberry belongs to the genus Berberis of family Berberidacae. It sustains its leaves in all seasons. It produces flowers from May to June. Its flowers are bisexual and pollinated by insects. The plant is widely used for medicinal purposes. A common recipe is to boil sliced pieces of root and its bark in water. The water extract is strained and further boiled until a semi solid mass, called “Rasaut” is obtained. The extract of roots is used for the treatment of urinary tract infections, enlargement of spleen, gastric and duodenal ulcer and liver disorders. The product is mixed with butter and alum to be used as an external application for the eyelids in acute conjunctivitis. Similar ointment containing camphor is used against acne, pimples and other skin infections (Chiang *et al.,* 1977; Chauhan *et al*., 1990). The main biological activities are attributed to its principal constituent, berberine (an alkaloid). The compound is bitter in taste and imparts the Rasaut unpleasant taste. To make it palatable, Rasaut is sometime mixed with sugar and maize like meal. This cooked recipe is called “Halva”. The “Halva” thus formed, is used regularly as a remedy by rheumatic patients for a longer period of time (Hassan *et al.,* 2007). The local inhabitants also use the dried mass of the root bark in powder form after mixing with molten animal fat as bandage for bone fractures. In Indochina, the fruit is given as a cure for renal disorder. The fruit juice is used for gums and teeth ailments. Decoction of fruit is used in typhoid and common cold (Shah, *et al.,* 2006). Stems of the plant are used for the stomach pain, diarrhea, jaundice and in the inhibition of melanohialdehyde (MDA) (Ahmad *et al*., 2009). The bark of the plant shows wound healing activity (Asif *et al*., 2007). The plant leaves are used as tea substitute. The fruits are in the form of berries and are used as fresh or in dried form. The plant on the whole is used by the local inhabitants for the treatment of swollen and sore eyes, broken bones, internal injuries, ulcer, juindice and rheumatism (Khan *et al*., 2010).

Previous investigations on this plant showed isolation of different types of compounds including alkaloids and steroids. Some biologically important isolated compounds are berberine, palmatine (Kinghorn *et al.,* 1993), Berbamine (Khan *et al.,*1969), Aromoline, Oxyacanithine, Umbellatine (Hamidulla *et al.,* 2003), ß-sitosterole (Ali *et al.,* 1996), Punjabine, Balochistanamine, oxyberberine (Barry *et al.,* 1982), berberinechloroform and palmatine chloroform (Miana., 1973 ). Berberine and its analogues represent a structural class of organic cations, isolated from numerous plants of Genera Berberis, Mahonia and Coptis. They have been shown to exert a broad spectrum of antimicrobial, anticarcinogenic and antimutagenic activity (Chopra., 1958; Chiang *et al.,* 1977). In Pakistan *Berberis lycium* is also of great importance as a house hold remedy for the treatment of various diseases.

Antioxidants are substances which scavenge or eliminate free radicals from living bodies. The main purpose of the antioxidants is to slow down or eliminate the oxidation processes. The ultimate end of oxidation processes is to produce free radicals. These free radicals produced inside the living bodies are the main source of cancer cells. Antioxidants terminate oxidation processes by removing free radical intermediates by being oxidized themselves. The antioxidants are basically the reducing agents. (Sies., 1997). It was thought that antioxidants are useful for better health but the large clinical trials did not detect any benefit. Afterwards it was suggested that the excessive use of antioxidants may be harmful. The antioxidants can

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be used industrially as well. Antioxidants are used as food preservatives, in cosmetics and preventing the degradation of rubber and gasoline (Bjelakovic *et al*., 2007).

The chemical structure of all the antioxidants contains hydroxyl groups which actually take part in the oxidation reduction process.

Antioxidants are classified as naturally occurring antioxidants and synthetic antioxidants. Naturally occurring antioxidants are some minerals, vitamins and phytochemicals. Minerals containing cupper, iron, zinc, maganese and selenium metals are useful antioxidants. The vitamins are vitamin B, vitamin C and vitamin E. Phytochemicals are mostly flavonoids (Hamid *et al*., 2010). Synthetic antioxidants are mostly phenolic compounds that perform the function of scavenging and capturing free radicals. These include butylated hydroxyl anisole (BHA), butylated hydroxytoulene (BHT), propyl gallate and metal chelating agent (EDTA), tertiary butyl hydroquinone (TBHQ) and nordihydro guratic acid (NDGA)( Hurrell., 2003).

The chemical structure of all the antioxidants contains hydroxyl groups which were helpful for oxidation reduction processes.

Keeping in view the therapeutic effect of *Berberis lycium* the roots of the plants were subjected for phytochemical studies. The isolated compounds after identification showed that they contain hydroxyl groups. Due to the presence of hydroxyl groups in the compounds including different extracts were subjected to antioxidant activities. The earlier research showed only the antioxidant studies of plant extracts. The antioxidant activities of the fruit of *Berberis lycium* was studied voltametrically (Safeer., 2012). There were no reports of antioxidant activities of root extracts of this plant. The details of the structural effect on free radical scavenging was tried to study in this report. The main focus of the research was to find whether hydroxyl groups present on the isolated compounds were able to scavenge free radicals or not.

## MATERIALS AND METHODS

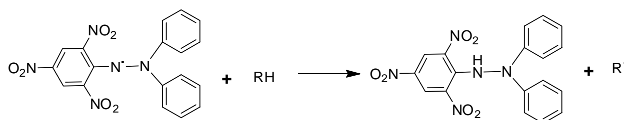
Roots of *Berberis lycium* were collected from Tehsil Rawalakot, District Poonch (Azad Jammu and Kashmir) Pakistan. The plant was identified by Botanical section of University of Agriculture, Rawalakot (AJK) Pakistan.

### *Isolation*

The roots were thoroughly washed with water to remove dust and other contaminants. The clean roots were dried under shade at room temperature for two months. The roots were assumed completely dried, as there was no significant change in weight with the time. They were cut into small pieces and then pulverized into fine powder. 2kg of powdered material were extracted three to four times with 8 liters of Pet ether for fifteen days. After every extraction, plant material was removed from solvent by decantation. The extract was evaporated to obtain 0.21g of dry residue. Plant material, after ether extraction, was again dried and further extracted with methanol two to three times for a month with total 13 liters of the solvent. After that it was filtered. Methanol extract was evaporated similarly under reduced pressure to yield 350ml of dark brown syrup of methanol extract. Methanol extract was mixed with 750ml of 5%hydrochloric acid. Supernatant along with precipitates, formed on the acid addition, were kept in refrigerator overnight and then filtered. These precipitates after drying, weighed 10.2 g representing compound (berberine).The filtrate (1025ml) was adjusted to pH 8-10 by adding 30% ammonia. The precipitates formed under basic conditions were allowed to settle down in the form of salt and filtered. The filtrate was extracted with 10 liters of chloroform. Chloroform extracts were evaporated on rotary evaporator to yield 10g of chloroform residue. The pH of aqueous layer was adjusted at 7 by the addition of HCl. This neutral aqueous layer was extracted with 6 liters of n-Butanol. n-Butanol extracts were evaporated on rotary evaporator to yield 12.1g of dry residue. The pet ether extract was evaporated under reduced pressure to yield 0.21g of dry extract. This dry extract was than subjected to fractionation by column chromatography. Elution was made with Pet ether / Ethyl acetate (6:4). It afforded one pure compound (ß-sitosterol) and other impure fractions were collected and again subjected to fractionation by passing through a column. Elution was made with Pet ether / ethyl acetate (8.5:1.5). Another pure compound was obtained which is (4, 4-dimethylhexadeca-3-ol). 10 g of dry chloroform extract was dissolved in $CHCl_3$, adsorbed on silica and was subjected to column chromatography over silica gel eluting with n-hexane/chloroform in increasing polarity to get Compound (butyl-3-hydroxypropyl phthalate) and (3-(4'-(6-methyl butyl) phenyl) Propan-1-ol). The remaining impure fractions were again collected and subjected to column chromatography using chloroform/methanol in increasing polarities to get compound (3-(4'-(6-methyl butyl) phenyl) Propan-1-ol).

### *Spectral Techniques*

Melting points were recorded using a digital Gallenkamp (SANYO) model MPD.BM 3.5 apparatus. IR spectra were recorded on AT-FT-IR Spectrophotometer (Omnic) using KBr pellets.$^1$H- and $^{13}$C-NMR spectra were recorded on Bruker spectrometers (AM-300). Chemical shifts have been reported in parts per million (ppm) relative to $SiMe_4$.The coupling constants are reported in Hz. Mass spectra was acquired (EI, 70eV) recorded on Mass Spectrometer Mat 312 system. The purity of isolated compounds was checked on pre-coated TLC plates and by their sharp melting points.




***Antioxidant Activity of plants extracts and isolated compounds***

Stable DPPH (2, 2 Diphenyl, 1-picrylhadrazylhydrate) radical has been used as a reactive compound to measure antioxidant activity When DPPH reacts with an antioxidant the reaction proceeds with change in color (from deep violet to light yellow), which can be measured at 518nm. The extracts and isolated compounds were tested in the DPPH free radical scavenging test by modified method outlined in litrature (Bandoniene *et al.,* 2002).Solutions of four extracts (pet ether, Methanol, chloroform, and n-Butanol) and isolated fractions of Pet ether ,chloroform and methanol were prepared in methanol and assayed for antioxidant activity. 20mg of dry extract and isolated fractions were mixed with 20ml of methanol in each case. The solution of DPPH (100µM) was also prepared in methanol. 2.5ml of DPPH solution was mixed with 0.5ml of extract solution. The mixture was kept in dark for one minute and then decrease in absorbance at 518nm was monitored after every one minute for fifteen minutes. Solution containing 2.5ml DPPH and 0.5ml methanol was used as a blank. The solution of Gallic acid (10µM) was used as a positive control. Radical scavenging activity was calculated by following formula.

***% Inhibition = $((A_B-A_E)/A_B)*100$***

Where $A_B$=Absorption of blank sample (t=0)

$A_E$=Absorption of test extract (t=15min)

The pet ether extract was sparingly soluble in methanol. In this case the extract was dissolved in methanol and only soluble fractions collected after filtration were subjected to the assay for antioxidant activity.

## RESULTS

***Spectral Characterization of Isolated Compounds***

***Berberine***: Yellow needle like crystals. Melting Point=194-196°C. FT-IR (KBr pellet) in $cm^{-1}$: 1210 (C-O), 1370 ($CH_3$), 1462 ($CH_2$).$^1$H-NMR (MeOD, 300MHz), δ (ppm). 9.75 (s, 1H, CH), 7.62 (s, 2 H, $CH_2$), 6.95 (s, 1H, CH), 3.92 (m, 2H, $CH_2$), 4.8 (m, 2H, $CH_2$), 3.31 (s, 3H, $CH_3$), 8.10 (d, J=9.1Hz, 1H, CH), 9.1 (d, J=9.1Hz, 1H, CH) and 6.94 (s, 1H,CH). $^{13}$C-NMR (MeOD, 300MHz), δ (ppm): 152.1 (C), 152.0 (C), 146.4 (C), 145.7 (C), 139.6 (C), 135.1 (C), 131.8 (C), 131.8 (C), 128.5 (C), 124.5 (CH methine), 123.3 (CH methine), 121.8 (CH methine), 121.5 (CH methine), 106.5 (CH methine), 103.6 ($CH_2$ 2a), 62.5 ($CH_3$ 9a), 57.6 ($CH_3$ 10a), 57.1 ($CH_2$ c-6a), 28.1($CH_2$ c-5).

***β-sitosterol***: White needle like crystals. Melting Point=194-196°C. FT-IR (KBr pellet) in $cm^{-1}$: 1600, 1430 (C=C), 1370 ($CH_3$), 1465 ($CH_2$), 3040 (OH). $^1$H-NMR (MeOD, 300MHz), δ (ppm): 0.87 (t, J=6.9Hz, 3H, $CH_3$), 0.86 (s, 3H, $CH_3$), 0.91 (d, J=6.2Hz, 3H, $CH_3$), 0.98 (m, $CH_2$), 1.24 (m, $CH_2$), 1.42 (t, J=6.4Hz, 2H, $CH_2$), 1.63 (m, $CH_2$), 1.81 (d, J=3.2Hz, 3H, $CH_3$), 2.01 (m, $CH_2$), 2.26 (m, $CH_2$), 5.32 (t, J=6.9Hz, 1H, CH). $^{13}$C-NMR (MeOD, 300MHz), δ (ppm): 12.1 ($CH_3$, C-29), 18.0 ($CH_3$, C-19), 19.0 ($CH_3$, C-21), 19.6 ($CH_3$, C-26), 20.0 ($CH_3$, C-27), 21.1 ($CH_3$, C-18), 26.15 ($CH_2$, C-23), 28.24 ($CH_2$, C-11), 28.9 ($CH_2$, C-16), 29.4 (CH, C-24), 31.67 (CH, C-24), 31.92 ($CH_2$, C-2), 36.16 (C, C-10), 37.88 (C $H_2$, C-1), 39.8 (CH, C-24), 42.3 (C, C-13), 45.88 ($CH_2$, C-4), 50.17 (CH, C-9) , 56.1 (CH, C-14), 56.8 (CH, C-24), 71.85 (CH, C-3), 121.73 (CH, C-6), 130.86 (C, C-10).

***4,4-dimethylhexadeca-3-ol***: White sticky mass. Melting Point=194-196°C. FT-IR (KBr pellet) in $cm^{-1}$: 1370 ($CH_3$), 1465 ($CH_2$), 3,040 (OH). $^1$H-NMR (MeOD, 300MHz), δ (ppm): 0.332 (s, 3H, $CH_3$), 0.87 (t, J=7.3Hz, 3H, $CH_3$), 2.20-1.67 (m, 3H, $CH_3$), 2.27 (t, J=5.3Hz, 2H, $CH_2$) , 2.42 (m, 2H, $CH_2$) , 4.2 (t, J=5.1Hz, 3H, $CH_3$). $^{13}$C-NMR (MeOD, 300MHz), δ (ppm): 14.09 ($CH_3$, C-16), 19.80 ($CH_3$, C-1), 21.03 ($CH_3$, C-17, C-18), 30.64 ($CH_2$, C-2), 31.4-21.0 ($CH_2$, C-5-15), 37.2 (C, C-4), 77.2 (CH, C-3).

***Butyl-3-hydroxypropyl-phthalate***: Yellow oil. Melting Point=194-196°C. FT-IR (KBr pellet) in $cm^{-1}$: 1213(C-O), 1368 ($CH_3$), 1452 ($CH_2$), 1732 (C=O), 3021(C-H, aromatic), 3,180 (OH). $^1$H-NMR (MeOD, 300MHz), δ (ppm): 0.89(s, 3H, $CH_3$), 1.30(m, 2H, $CH_2$), 1.4(m, 2H, $CH_2$), 1.68(t, J=6.3Hz, 2H, $CH_2$), 4.19(d, J=6.8Hz, 2H, $CH_2$),7.53(d,J=5.1Hz,1H, $CH_1$),7.70(d, J=5.3Hz,1H, CH).

***3-(4'-(6-methyl-butyl)-phenyl)propan-1-ol***: It was isolated as white amorphous solid. Melting Point=194-196°C. FT-IR (KBr pellet) in $cm^{-1}$: 1359 ($CH_3$), 1450 ($CH_2$), 3010 (OH). $^1$H-NMR (MeOD, 300MHz), δ (ppm): 0.91 (d, J=7.2Hz, 3H, $CH_3$), 1.33 (m, 1H, CH), 1.45 (t, J=7.2Hz, 2H, $CH_2$), 2.17 (t, J=8.4Hz, 2H, $CH_2$), 3.31 (m, 2H, $CH_2$), 4.21 (t, J=4.8Hz, 1H, CH), 7.60 (d, J=2.4Hz, 1H, CH), 7.70 (d, J=3.3Hz, 1H, CH). $^{13}$C-NMR (MeOD, 300MHz), δ (ppm):11.4 ($CH_3$, C-8), 14.4 ($CH_3$, C-7), 24.03 ($CH_2$, C-5), 24.9 ($CH_2$, C-4), 30.15 ($CH_2$, C-3), 31.63 ($CH_2$, C-2), 40.20 (CH, C-6), 48.16 (CH, C-1'), 69.12 (CH, C-1), 129.8 (C, C-4'), 132.4 (CH, C-3', C-5'), 133.4 (CH, C-2', C-6').

***4-methyl, 7-hydroxy coumarin*** : It was isolated as white crystals. Melting Point=186-191°C. FT-IR (KBr pellet) in $cm^{-1}$: 1600, 1435 (C=C), 1370 ($CH_3$), 1465 ($CH_2$), 1738 (C=O), 3020 (=C-H), 3,260 (OH). $^1$H-NMR (MeOD, 300MHz), δ (ppm): 3.39 (s, 3H, $CH_3$), 3.68 (s, 1H, CH), 6.06 (s, H, CH), 6.66 (d, J=6.8Hz, 1H, CH), 7.56 (d, J=8.2Hz, 1H, CH). $^{13}$C-NMR (MeOD, 300MHz), δ (ppm): 18.64 ($CH_3$, C-8), 103.47 (CH, C-8), 112 (C, C-4a), 113.8 (CH, C-3), 114.3 (CH, C-6), 127 (CH, C-5), 155 (C, C-8a), 156 (C, C-4), 162 (C, C-7), 163 (C, C-2).

***Antioxidant Activity of Berberis lycium extracts***

The antioxidant activities of petroleum ether, methanol, chloroform and n-Butanol extracts were studied by means of DPPH free radical discoloration method. The Gallic acid was used as a standard for positive control and DPPH as radical scavenger.

***Antioxidant Activity of Isolated compounds.***

The antioxidant activities of berberine, β-sitosterol, 4,4 dimethyl hexadeca 3-ol, butyl -3-hydroxypropyl phthalate and 3-[4-(6-methyl butyl) phenyl] propan-1-ol were also subjected for antioxidant assay. The Gallic acid was used as a standard for positive control and DPPH as radical scavenger.

## DISCUSSION

***Berberine***

Mass (low resolution) spectrum of the compound afforded molecular ion peak at m/z 337.1 corresponding to molecular formula $C_{20}H_{18}NO_4$ (Calculated=337.12). This compound was identified as berberine (Ali *et al*., 1996). It is an alkaloid.

***ß-sitosterol***

Mass (low resolution) spectrum of the compound afforded molecular ion peak at m/z 414.2 that confirmed the molecular formula $C_{20}H_{18}NO_4$ (Calculated=414.2). This spectral data verified that data reported in literature (Lee *et al.,* 2008) of compound ß-sitosterol.

***4,4-dimethylhexadeca-3-ol***

Mass (low resolution) spectrum of the compound showed a peak at 270 that confirmed the molecular formula $C_{18}H_{32}NO$ (Calculated=270.2922). On the basis of above mentioned structural data compound was identified as 4, 4-dimethylhexadeca-3-ol.

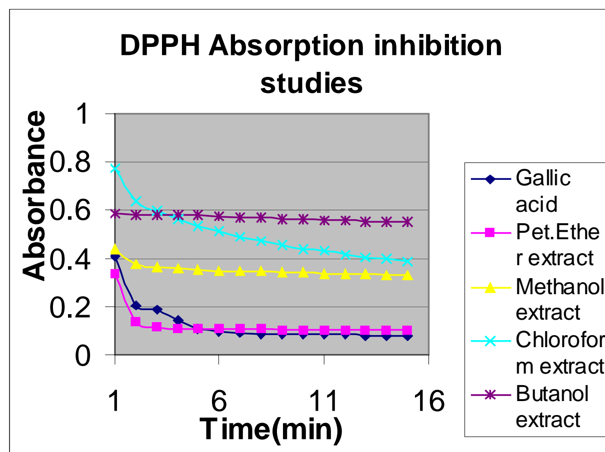



**Fig. 1**: DPPH Absorption Inhibition Studies of different extracts of Berberis lyceum.

**Table 1**: Antioxidant Activities of Extracts Assessed with DPPH Assay

| Extract | DPPH inhibition % |
|---|---|
| Pet.ether | 82.0 |
| Methanol | 49.7 |
| Chloroform | 57.1 |
| n-Butanol | 7.5 |
| Gallic acid | 86.0 |

***Butyl-3-hydroxypropyl phthalate***

Mass spectrum at low resolution showed molecular ion peak at m/z 279.1 (which was close to the calculated 280.1) corresponding to the molecular formula $C_{15}H_{20}O_5$ indicating six degrees of unsaturation in the molecule. On the basis of above mentioned structural data Compound was identified as butyl-3-hydroxypropyl phthalate.

***3-(4'-(6-methyl butyl) phenyl)propan-1-ol***

The mass spectrum at low resolution showed molecular ion peak at m/z 206.12 corresponding to molecular formula $C_{14}H_{22}O$ (Calculated mass=206.33) showed four degrees of unsaturation in the molecule. The above structural data of compound was identified 3-(4'-(6-methyl butyl) phenyl) Propan-1-ol.

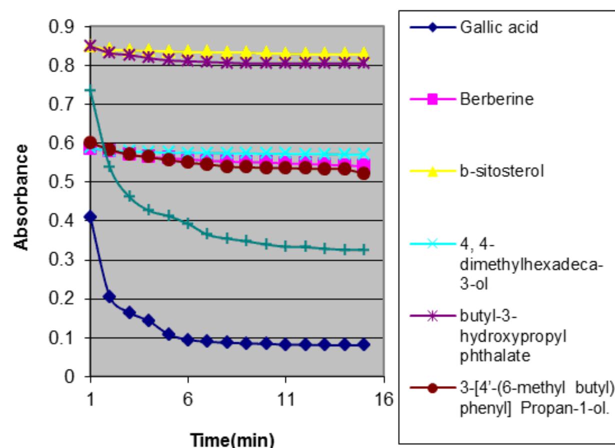



**Fig. 2**: DPPH Absorption Inhibition Studies of pure compounds obtained from *Berberis lycium.*

***4-methyl-7-hydroxycoumarin***

The mass spectrum at low resolution of the compound showed molecular ion peak at m/z 172 corresponding to the molecular formula $C_{10}H_8O_3$, indicating seven degrees of unsaturation in the molecule. This spectral data verified the reported values in literature (Amarendra, P.*et al*, 2004) of the structure 4-methyl-7-hydroxycoumarin.

**Table 2**: Antioxidant Activities of Extracts Assessed with DPPH Assay

| Compound | DPPH inhibition % |
|---|---|
| Gallic acid | 86.0 |
| Berberine | 8.9 |
| ß-sitosterol | 3.71 |
| 4, 4-dimethylhexadeca-3-ol | 8.0 |
| butyl-3-hydroxypropyl phthalate | 7.2 |
| 3-(4'-(6-methyl butyl) phenyl) Propan-1-ol. | 18.2 |
| 4-methyl, 7-hydroxy coumarin | 49.4 |

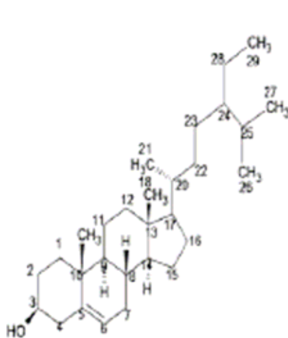
***Antioxidant Activity of Berberis lycium extracts***

The method is based on ability of an antioxidant which allows it to scavenge free radical (DPPH$^{\bullet}$) through donation of hydrogen to form DPPH-H molecule the course of reaction is monitored colorimetrically. The absorbance decreases as a result color changes from purple to yellow. This color change is due to the radicals scavenged by antioxidants. The more rapidly the absorbance decreases, the more potent will be the antiradical activity of the compounds in terms of hydrogen donating ability (Gadow *et al.,* 1997).

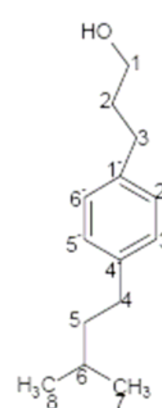
The absorbance of DPPH in presences of different extracts as compared to Gallic acid with one-minute time intervals is summarized in table 1. Antioxidant activity was calculated in terms of % inhibition. Pet ether extract showed maximum antioxidant activity (82% inhibition), methanol (49.7% inhibition) and chloroform (57.1%). n-Butanol showed minimum antioxidant activity (7.5%) which is not significant.

***Antioxidant activity of pure fractions of different extracts***

Berberine has no OH group in the molecule. Although there was conjugation in the form of aromatic rings, this structure cannot donate hydrogen to the free radical. So contribute low antioxidant activity (8.9% inhibition.

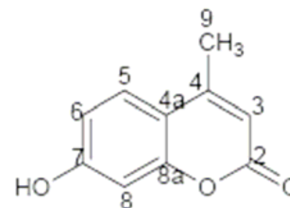


ß-sitosterol

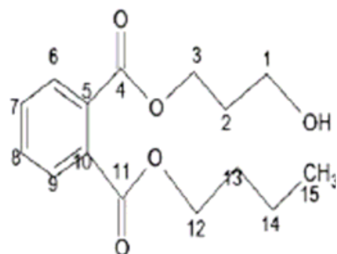


3-(4'-(6-methyl- butyl) phenyl)propan-1-ol

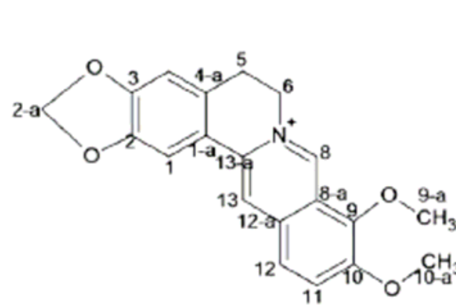
4, 4-dimethylhexadeca-3-ol.



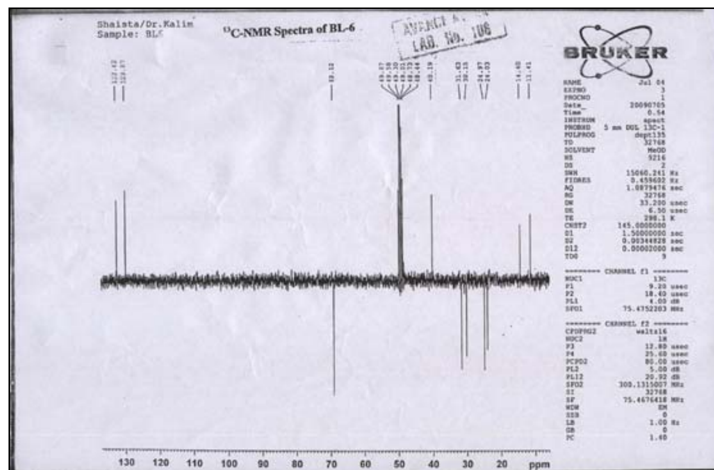
4-methyl- 7-hydroxycoumarin.



Butyl-3-hydroxypropyl phthalate



Berberine




Shaista/Dr.Kalim
Sample: BL6 (A.I.O.U.)
$^{1}$H-NMR Spectra of BL-6
BRUKER
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

Relative Abundance
148.0
176.0
91.0
74.0
120.0
131.0
100 150 200 250 300
MS of BL-9

Shaista/Dr.Kalim
Sample: BL8 (A.I.O.U.)
$^{1}$H-NMR Spectra of BL-9
AVANCE AV-300
BRUKER
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

β-sitosterol represented the presence of one OH group attached to the cyclohexane ring. It cannot donate this hydrogen because ($O^{\cdot}$) is unstable radical and showed very low antioxidant activity (3.7% inhibition). 3, 4, 4-Dimethylhexadeca-3-ol was also subjected to antioxidant activity assay and also proved as weak antioxidant (8.0% inhibition). Compounds obtained from chloroform extracts were also checked for their antioxidant activity. Butyl-3-hydroxypropyl phthalate, contained OH group attached to the aliphatic systems. But this OH group cannot donate hydrogen to free radical to form stable molecule. Compound-5 showed 20.4% inhibition. The chloroform fraction that represented maximum antioxidant activity was 4-methyl, 7-hydroxy coumarin was also subjected to antioxidant activity assay. Charact-erization proposed coumarin structure (4-methyl, umbelliferone). Coumarin is the class of natural products that has close structure to the phenolics. Some coumarins possess lipid peroxidation scavenging while others show no activity in this respect but are active for other antioxidation such as $OH^{\cdot}$. For example umbelliferone (7-hydroxycoumarin) showed no activity for lipid peroxidation while showed antioxidant activity for $OH^{\cdot}$ (Ramesh *et al* 2006). The maximum antioxidant activity was shown by 6, 7 dihydroxy substituted coumarins (Gadow, A, *et al,* 1997). As the isolated compound had a structure of umbellifer-one, which in literature showed free radical scavenging activity for $OH^{\cdot}$. That is the reason that isolated compound showed maximum antioxidant activity (49.4% inhibition).

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