Determination of in Vivo Biological Activities of Dodonaea Viscosa Flowers Against CCL4 Toxicity in Albino Mice With Bioactive Compound Detection

Hina Gul
PMAS UAAR Rawalpindi
Muhammad Awais
PMAS UAAR Rawalpindi
Salina Saddick
King Abdulaziz University
Falak Sher Khan
University of Sialkot
Muhammad Gulfraz
PMAS UAAR Rawalpindi
Khizar Nazir
University of Wah
Sami Ullah Khan
COMSATS University Islamabad
M. Ijaz Khan (mikhan@math.qau.edu.pk)
Riphah International University

Research Article

Keywords: Dodonaea viscosa flower, Protective assay, Histopathology, Phytochemical screening, Antioxidant, Antituberculosis

Posted Date: April 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-275991/v1

Determination of in vivo Biological activities of Dodonaea viscosa flowers against CCL4 toxicity in Albino mice with bioactive compound detection

Hina Gul[1], Muhammad Awais[1], Salina Saddick[2], Falak Sher Khan[3], Muhammad Gulfraz[1], Khizar Nazir[4], Sami Ullah Khan[5], M. Ijaz Khan[6]

[1]University institute of Biochemistry and Biotechnology (UIBB), PMAS UAAR Rawalpindi. Pakistan

[2]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia

[3]Department of Biotechnology, University of Sialkot, Pakistan

[4]Department of Biosciences, University of Wah, 47040, Wah Cantt, 47040, Pakistan

[5]Department of Mathematics, COMSATS University Islamabad, Sahiwal 57000, Pakistan

[6]Department of Mathematics and Statistics, Riphah International University I-14, Islamabad 44000, Pakistan

([*]Corresponding author: mikhan@math.qau.edu.pk (M. I. Khan))

Abstract

Dodonaea viscosa L. Jacq. is an evergreen shrub and native to Asia, Africa and Australia. It has been used as traditional medicine in different countries. The foremost objective of the current study was to discover protective potential of *D. Viscosa* flowers Methanol (DVM) and Chloroform (DVC) extracts against CCL_4 induced toxicity in mice. This study was intended to identify phytochemicals through HPLC, GCMS and FT-IR as well as *in vitro* antioxidant and *in vitro* antituberculosis activity. Our comprehensive findings indicate that *Dodonaea viscosa* is valuable and widespread herbal medicines through therapeutic potentials for curing various ailments. *Dodonaea viscosa* flowers are found to have protective effect against oxidative stress produced by CCL_4 in liver, kidney and spleen. The level of hepatic enzymes (ALP, AST ALT and Direct bilirubin), hematological parameters (RBCs, WBCs and Platelets), total protein and liver antioxidant enzymes (SOD, GPx and CAT) were restored by the intake of DV extracts after

decline in levels by CCL_4. Histopathological results discovered the defensive effect of 300mg/kg of DVM extract against CCL_4 induced damage, thus having improved protective effect as compared to DVC and control. As a result of analysis total flavonoids and total phenolics were also revealed. Phytochemical investigation by HPLC identified gallic acid, epicatechin, cumeric acid, flavonoids while Oleic acid (Octadecenoic acid) ($C_{18}H_{34}O_2$), Stearic acid ($C_{18}H_{36}O_2$), Ricinoleic acid ($C_{18}H_{34}O_3$) and Cedrol ($C_{15}H_{26}O$) was estimated by GCMS. DVM extract exhibited resistance against in vitro Mycobacterium tuberculosis strains. This study proposed that protective effect of DV against oxidative damage induced in Liver, Kidney and Spleen can possibly be correlated to their antioxidant as well as free radical scavenging property.

Keywords: *Dodonaea viscosa* flower: Protective assay: Histopathology: Phytochemical screening: Antioxidant: Antituberculosis.

1. INTRODUCTION

The fueling of different biological processes is governed by the oxidation reduction reactions [1]. Usually, overproduction of free radicals like superoxide (O^-_2), hydroxyl (OH), and peroxyl (ROO) is classified as oxidative stress that may result in aging, cellular destruction and partial process attenuation [2]. High grade oxidant accumulation is considered responsible for as many as 100 different human diseases [3]. Out of these the liver damages and diseases are regarded as global health issue of serious cadre [4].

Human body has many responsibilities that are assigned to liver such as synthesis, storage, conversion and detoxification of various biological, natural and synthetic molecules [5-6]. Another, small organ responsible for accessory duties is spleen which can act as organ of immune system, blood cell destruction and blood filtration with iron storage occasionally [7-8]. Most of the liver carcinoma, cirrhosis, fibrosis and cellular attenuation is due to Hepatitis C virus (HCV) [9]. Moreover, the radiation damages during, diagnosis, treatment and working at a medical facility are other damages that contribute to liver tissue impairment [10]. Reactive oxygen species are generated by X-rays and other radiation that later on cause a damage to the biological molecule and oxidize most of them. Liver disorders are usually classified with increasing serum levels of bilirubin, ALP, AST and ALT and decline in liver function with cirrhosis mostly [11-12].

The effects posed by most of the hepatotoxins are reversed or counteracted by natural or derive antioxidants [13]. The free radicals produced by metabolic activities are neutralized by antioxidants that usually scavenge these radicals [14]. There has been a lot of research going on to find and utilize the antioxidants for potential radical scavenging and impeding the cellular damage [15]. Redox signaling and antioxidant potential of plant species has always fascinated mankind to explore their potential to fight aging and many medical conditions [16]. Many plant species have been reported as a remedy to liver problems [17] due to their phenolic constituents that have potential for free radical scavenging, anti- inflammatory activities and antioxidant response that may contribute to altered cellular gene expression and other metabolic adjustments [18].

Pulmonary Tuberculosis (PTB) is another global health issue with 56% of patients being reported from South East Asia. PTB is very complex in its spread, contagiousness and treatment therefore, a need to develop an effective medicine against it to help its cure and spread. Furthermore, the multi-drug-resistant tuberculosis (MDRTB) is growing more concerns in medical science to look for a short-term and effective treatment measure. One of the targets of mission 2035 by WHO is to make the world TB free by the end of 2035 leaving no patient with persistent TB [19]. Many plant species have always shown potential in ancient history to treat the medical disorder with promising therapeutic agents [20]. The knowledge about single chemical component encompassed by medicinal plant plays a critical role from method of extraction to understanding pharmacological assay and possible toxicity. Generally, plant species consists of intermingling of phenolics, flavonoids, di and triterpenes and, saponins. Curative activity of plants is accompanied by pharmacological possessions carried through respective plant compounds that brought synergistic amalgamation as compared with single compound [21].

Belonging to the family Sapindaceae *Dodonaea viscosa* L. Jacq. (DV) is mostly a shrub with evergreen leave and sometimes it occurs as small tree. This plant is native to Asia, Africa and Australia and in South East Asia it is locally known as vilayati Mehndi. DV has been reported previously with many application s in traditional medicine in various countries [22] where it is given as an oral medicine or as bandage medicine applied externally to cure many ailments and inflammations. It has also been observed that many African and Asian countries have history of administering DV dried leaves to people with stomachaches arising from liver or spleen, stomach ulcer and liver ache [23-24]. There are reports where DV has been put forward as a remedial

medicine, local anesthetic and smooth muscle relaxant. Moreover, its potentials to treat ulcer, malaria, tooth pain, cold, itching, throat soreness, diarrhea, fractures and inflammation were also observed in the past [25-26]. More non-traditional usage reports of DV include its therapeutic potentials as wound healer, antioxidant, anti-inflammatory, antibacterial, anti-insecticidal, antifungal and hypolipidaemic [27-31]. Some reported biological activities are antidiarrhoeal [32], antispasmodics, hypolipidaemic and anti-diabetic [29]. Though this plant contains diversity of phytochemicals and maximum research has emphasized on flavonoid compounds [33-35, 24]. Isolated compounds from DV includes coumarins along with lignocoumarins, tannins [36] and saponins [37] as well as.

Inspired by the above literature the present study was conducted to validate and advance the potential therapeutic activity of DV against liver disorders. The foremost objective of the current study was to discover protective potential of *D. Viscosa* flowers Methanol (DVM) and Chloroform (DVC) extracts against CCL_4 induced toxicity in mice. This study was intended to identify phytochemicals through HPLC, GCMS and FT-IR as well as *in vitro* antioxidant and *in vitro* antituberculosis and hepato-protection activity.

2. MATERIAL AND METHODS

2.1 Sample collection and preparation

The collection of DV **(wild)** was carried out during March and April from Murree hills, District Rawalpindi, Punjab, Pakistan **according to the plant collection guidelines of herbarium of Pakistan (ISL), Quaid-i-Azam University, Islamabad, Pakistan. Collection of plants was done with the permission of local inhabitants.** By the Help of Department of Botany Quaid-I-Azam University Islamabad, Pakistan, the plant species was detailed identified before further processing **by Prof. Dr. Mushtaq Ahmad (Plant taxonomist), Department of Botany, Quaid-i-Azam University, Islamabad, Pakistan** and **a plant voucher specimen (HG052) was submitted to the herbarium of Pakistan (ISL) for the future record**. Cleaned specimens were subjected to shade drying followed by grinding and sifting. Next to sieving, placed the plant sample in heating oven (37◦C) to eradicate moisture for absolute drying and the pulverized material was prepared for the further examination. 500 grams of plant powder was dissolved in Methyl alcohol and Chloroform for 5 days and filtration of extracts were done with Whatman

filter paper. Both extracts were evaporated with the help of rotary evaporator and dried crude extracts were stored in air fitted vials for more operations.

2.2 In vivo Study

2.2.1 Selection and Purchase of Animals

50 male albino mice (body weight 55.2±2.5g) were purchased from NIH (National institute for health) Islamabad. The experimental study was approved by National Veterinary Lab Islamabad ethical committee adhering to the guidelines of institution (2015:16). **All the protocols used in animals study were in acquiescence with the ARRIVE guide lines**. The mice were housed under controlled conditions and animals had free access to mouse chow (Feed Mills, Islamabad) and water ad libitum. Animals were cautiously monitored and kept up in standard house conditions.

2.2.2 Acute oral toxicity study

An acute toxicity study was directed to select suitable doses of plant extracts for animals as earlier reported by [38]. Body weight of animals were recorded before and after study. Plant extracts were orally gavage to mice at the dose of 100-300mg/kg. After the dose animals were meticulously observed after 24, 48 and 72hrs days for the development of any toxicological symptoms. Slaughtering was done on 21 day of experiment.

2.2.3 Experimental design

Animals were divided randomly into 10 groups of 5 animals in each group. i: Normal control group were given normal feed up to 21 days. ii: Olive oil group received 1 ml of olive oil with their feed up to 21 days. iii: CCl4 (1 cc/kg b. w) was induced by intraperitoneal way for 21 days. iv: This group was administrated 100 mg/kg b. w of the methanolic flower extract after induction of CCl4. v: Animals of this group were fed 200 mg/kg b. w of methanolic extract after CCl4 induction. vi: Animals were provided 300 mg/kg b. w of methanolic extract after CCl4 given. vii: This group was fed 100 mg/kg b. w of chloroform extract after CCl4 administration. viii: The group was nourished with 200 mg/kg b. w of chloroform extract after CCl4. ix: 300 mg/kg b. w (chloroform extract) was given after CCl4 induction. x: Animals were administrated 100 mg/kg body weight/day of Silymarin (standard drug) after induction of CCl4. At 20 day of experiment mice were kept for fasting for 12 h. After anesthesia (21 day) whole blood was

obtained from the heart by cardiac puncture and to get serum, stand the blood to clot for 30 mins followed by centrifugation (3000 rpm for 10 mins). Animals were sacrificed through cervical dislocation and required body parts (Liver, kidney and Spleen) were collected, rinsed by using ice cold saline solution and for further analysis kept in -20°C freeze. Weight of collected organs from all the groups were recorded. For biochemical analysis, phosphate buffer saline was used to homogenized liver (one part), centrifuged at 3000rpm for 20mins and stored supernatant at -20°C. For histopathological study, liver, kidney and spleen was stored in formalin solution (10%).

2.2.4 Analysis of Blood samples

The serum biomarkers alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin were examined by using autoanalyzer with AMS diagnostic kits (Italy). RBC (red blood cells), WBC (White blood cells) and platelets in blood sample were evaluated by utilizing the method reported by [39].

2.2.5 Oxidative stress parameters

Liver homogenates were subjected to oxidative stress parameters (antioxidant enzymes). Catalase (CAT) was estimated, Superoxide dismutase (SOD) activity was measured by using [40], Glutathione peroxidase (GPx) was calculated by the method of [41] and total protein were evaluated by the method suggested by [42].

2.2.6 Histopathological Study

Mice organs (liver, kidney and spleen) were removed carefully after sacrifice and preserved in formalin (10 %). Dehydration of testing specimen after fixation was done in alcohol, cleaned in xylene and inserted into molten paraffin wax. Sections of paraffin were cut down in 5µm thickness by using microtome and obtain tissues were mounted on slides and deparaffinized. Staining of tissue sections were performed with the help of Ehrlich's hematoxylin and eosin counter stained (H&E) and examined under light microscope [43-44].

2.3 Determination of total phenolic and total flavonoid content

Total phenolic content of extracts was measured by using Folin -Ciocalteu reagent [45]. Results were expressed as grams of gallic acid equivalents per 500g/dry weight. Total flavonoid content

of the extracts was measured using colorimetric assay [46]. Results were expressed by using grams of quercetin equivalents per 500g/dry weight.

2.4 Antioxidant Activity

2.4.1 Chemicals required

Methyl alcohol, ethyl alcohol, chloroform, DPPH, ABTS, Hydrogen peroxide, EDTA, Formalin, Xylene, Heamotoxylin, Essen, KH_2PO_4 buffer, ALT Alanine aminotransferase, AST Aspartate aminotransferase, ALP alkaline phosphatase and bilirubin were obtained. Analytical grade solvents and reagents were purchased from local dealers of Sigma Aldrich and Merck.

2.4.2 DPPH Assay

This process is used to calculate scavenging capacity of sample by the protocol with some modifications [47]. DPPH (2,2-diphenyl-1-picrylhydrazyl) aliquot (2ml) was poured 1ml to each concentration of plant sample ranged from 20-100 µg/ml. This mixture was incubated at 37ºC for 30 minutes in darkness. Standard or Positive controls were Ascorbic and Gallic acid. DPPH solution was taken as negative control. Reading was taken recorded at 517nm and results were expressed in ascorbic acid equivalent AAE and gallic acid equivalent GAE. The experiment was done in triplicate manner and the inhibition percentage is obtained by following formula

$$\text{DPPH } \% = [A^a - A^h/A^a] * 100$$

A^a: absorbance of reaction mixture except plant extract. A^h: absorbance of reaction mixture comprising plant extract. IC 50 (µg /mg) was measured in by plotting scavenging percentage against extract concentration.

2.4.3 Iron Chelating Assay

Iron Chelating method was described by the [48]. Antioxidant potential of plant extract was accomplished through incubation of reaction mixture comprising of plant sample extracts (20-100 µg/ml), 2mM ferrous sulfate (1ml) and 0.25mM Ferrozine (1ml). After stirring, let the mixture stand for10 mins and absorbance was read at 517 nm.

$$\text{Chelating rate } \% = [A^a - A^h /A^a] * 100$$

A^a: Mixture absorbance lacking plant extract. A^h: Absorbance of mixture with plant extract. Standard solutions or Positive controls (Ascorbic and Gallic acid) were used to make the calibration curve. IC_{50} was stated as µg AAE/mg and GAE/mg.

2.4.4 Hydroxyl Radical Scavenging Assay

Hydroxyl Radical method was investigated by the protocol of [50]. Plants extract concentrations 20 to100 µg/ml were investigated by adding 0.2 M Sodium phosphate buffer (7 pH), 2deoxyribose (10mM), FeSO4 -EDTA (10mM), H_2O_2 (10mM) and 525µl of H_2O. Put all the mixture into TCA (2.8%) and TBA (1%) and incubate at 90°C for color development. Spectrophotometric reading was observed at 520nm. Standard drugs (Ascorbic and Gallic acids) were taken as Positive control and results were measured in AAE and GAE µg/mg.

$$\text{Scavenging activity} = [1 - A^h / A^a] * 100$$

Where A^a: mixture absorbance (without plant sample extracts) and A^h: absorbance of mixture containing plant sample.

2.4.5 ABTS (2,2- azinobis [3- ethylbenzothiazoline-6- sulfonate]) Radical Cation Decolorization Assay

Plant extracts were analyzed through the enhanced ABTS+ radical cation scavenging capacity by some modification [50-51]. ABTS+ mixture was prepared by adding 3mM ABTS (2,2-azinobis [3- ethylbenzothiazoline-6- sulfonate]) and potassium persulfate (2.5mM). Leave the solution in dark for 12 hours. To measure ABTS+ activity, ABTS + solution (3ml) was taken with different concentrations of plant extract (20 to100 µg/ml). Optical density was measured at 734nm. Standard drug used was Ascorbic and Gallic acids.

$$\text{Percent Scavenging potential} = [A^a - A^h / A^a] * 100$$

A^a: absorbance of control.

A^h: absorbance of plant extract.

2.4.6 Reducing Power Assay

FRAP (Ferric ion reducing power) was determined by the method involved the blending of each plant sample concentrations (20-100) µg/ml), phosphate buffer (0.2M) and potassium ferricyanide (0.1%). Allow the mixture to incubate in water bath for 20 minutes. Subsequently

add trichloroacetic acid (10%) and mixture was centrifuged at 3000 rpm for 10 mins. Supernatant was mixed in distilled water (2ml) succeeded by ferric chloride (0.01%) and set it down for incubation. Blank and samples were interpreted at 700nm. Standard compounds i.e. Ascorbic and Gallic acid were utilized as positive control. Results were quantified as AAE and GAE (µg/mg) [52-53].

2.4.7 Hydrogen Peroxide Scavenging Activity (H_2O_2)

Hydrogen Peroxide Scavenging Activity was described according to the protocol of [54]. Reaction mixture comprised of H_2O_2 solution 4Mm (prepared in phosphate buffer) with different plant concentrations (20-100) µg/ml) followed by incubation for 10 minutes at room temperature. The reading was observed at 230nm against blank solution comprising phosphate buffer with 7.4 pH. Gallic acid and Ascorbic acid were used as standard and expressed in GAE and AAE (µg/mg).

Scavenging activity% = $[A^a - A^h / A^a] * 100$

A^a absorbance of H_2O_2 and A^h is mixture absorbance with plant extract.

2.4.8 Superoxide Assay

The activity was determined by NBT reduction as per the method of Beauchamp and Fridovich, 1971 [55]. PMS (phenazine methosulfate) and NADH (nicotinamide adenine dinucleotide) system produce superoxide radicals, that condenses nitro blue tetrazolium (NBT) to a purple formazan. Add up 50mM Phosphate buffer, 0.73mM NADH, 20mM PMS and 0.5Mm NBT in numerous concentrations of sample (20µg/ml) and incubate them for 20 mins. Optical density was documented at 560nm against blank to determine generated formazan. The positive control was Ascorbic acid and Gallic acid. Inhibition concentration was obtained from the formula:

$$\text{Scavenging percentage} = [1 - A^h / A^a] * 100$$

Where A^a: absorbance except plant concentrate and A^h: absorbance of mixture with plant distillate.

2.5 Analysis of plant with High Performance Liquid Chromatography (HPLC)

Crude extracts analysis was performed by using a Shimadzu HPLC (high performance liquid chromatography) system, Tokyo, Japan equipped with a C18 column (250 mm × 4.5 mm, 5 m)

used for the separation at the flow rate of 1 ml/min. Column temperature was sustained at 40◦C followed by gradient pump and UV/Visible detector. HPLC grade methanol was used for extraction of crude plant to prepare tested sample. Before injection, filtration of samples was done by using 0.2µm PTFE filter and the injection volume was 10µl. The compounds were eluted by means of gradient elution of mobile phases A and B (Acetonitrile and 0.1% phosphoric acid; 36:64). Separation steps are as follow: 0 mins-5% B, 15 min-15% B, 15 min-45% B, 5 min-90%B, and Conditioning cycle for 5mins along with the analysis of next initial conditions. The UV-vis detection was recorded at 280 nm-285 nm at a current rate of 1 ml/min per 20 min retention time. Quercetin was utilized as standard and all findings were completed in triplicates.

2.6 GC-MS

2.6.1 Gas chromatography and Mass Spectrometry Analysis

GC-MS system QP2010 model (Shimadzu®) equipped with Mass selective Detector and split–split-less system of injection. The instrument was fitted with capillary column RTx- 5MS (cross bond 5% diphenyl – 95% dimethylpolysiloxane) with 30m x 0.25millimeter with 0.25 µm film thickness. At the rate of 1.2 ml/min, helium was being used as carrier gas. The temperature program of column was started at 150 ºC (1 min) then programmed at 4 ºC / min to 150 ºC (10 min). Temperature of the injector was 275 ºC while detector was 250 ºC. 0.2 µl volume was injected in split mode. A split ratio was 1:50 and the mass spectra was operated electron ionization at 70 eV in Selected Ion Monitoring (SIM) mode, were maintained. The run time of machine was 40 mins. Relative percentage of the plant extract compounds were expressed in terms of percentage with normalization of peak area.

2.6.2 Compounds identification

GC mass spectrum interpretation was conducted by employing database of National Institute Standard and Technology (NIST). Compound name, molecular weight and structure of the test materials were determined. The percentage (%) of each compound was calculated by comparison of average area to total area. Spectrum of unknown constituents was compared with the version 2005, software, and Turbo mass 5.2. The aim was to discover the individual compound or group of compounds might showed its current commercial and traditional roles [56-57].

2.7 Fourier Transform Infrared Spectrophotometer analysis

The plant extract was analyzed for infrared spectrum analysis by FT-IR (Fourier transform Infrared) spectroscopy shimadzu machine, IR affinity 1, Japan. At first loaded samples were grounded by KBr (1:100 w/w) with scan range (400-4000cm) and $4cm^{-1}$ resolution. Samples components were subjected to structural characterization and used to indicate functional groups which are chemical bonds types [58].

2.8 Anti-tuberculosis activity

The antimycobacterial activity of *Dodonaea viscosa* L. was measured using the REMA method [59]. *Mycobacterium tuberculosis* strains bug 206 and bug 1972 and H37Rv were grown in 7H9 broth. Sample stock solutions were diluted by using DMSO to get final concentration ranging 0.98 to 250µg/mL. Rifampicin was used as positive control drug ranges 0.004 to 1 µg/mL. Add the bacterial culture (5×10^5 CFU/mL) to each well of 96-well plate and incubate at 37°C. Viability was tested by using resazurin and the color change and fluorescence were examined in plates by using SPECTRAfluor Plus microfluorimeter (TECAN). Experiments were performed in triplicates. The lowest concentration resulting in 90% growth inhibition of M. tuberculosis. The MIC was defined as the lowest concentration results in inhibition of 90% growth of M. tuberculosis.

2.9 Statistical Analysis

All the data was obtained in triplicate manner and results are presented as mean ± standard deviation. One-way ANOVA was used for the processing of results. Statistical analysis (Mean, standard deviation, probability and Pearson coefficient correlation) was obtained via statistical software (Prism pad 7). The level of significance was considered at <0.05.

3. RESULTS

3.1 **In vivo study**

3.1.1 **Acute toxicity and effect on weight**

According to the results, acute toxicity manifested significant noticeable signs on the mice body weight. The observed change was shown in Table 1 and Table 2. In present study DV flowers with methanol and chloroform extracts were found no devastating effect on mice and no mortality was found at highest dose of 2000mg/kg as it is considered as the highest dose by

OECD guidelines for any acute toxicity assay. Three plant concentrations 100 mg/kg, 200 mg/kg and 300 mg/kg were selected for the study. In case of normal and olive oil group body weight along with body organ weight was increased. While the group treated with CCL_4, body weight followed by weight of liver and kidneys were decreased and spleen weight was increased. However adverse changes were found in weight due to CCL_4 that was further recovered by plant methanol and chloroform extracts. Changes observed in weight are closer to the weight of normal group.

Table.1. Protective effect of Methanol extract of *Dodonaea Viscosa* flowers

Treatments	Liver Weight (g)	Kidney Weight (g)	Spleen Weight (g)	Rise (%) in body weight
Normal	5.60±1.21	0.48±0.1	2.080±0.19	16±2.9
Olive oil Control	5.82±0.94	0.51±0.08	2.15±0.2	20±3.10
CCL_4	3.96±1.18	0.34±0.12	3.34±0.5	7.41±2.05
Silymarin+CCL_4	6.02±0.749	0.5±0.17	2.150±0.31	22.27±0.09
DV (100mg/kg) +CCL_4	4.016 ±0.83	0.25±0.045	3.11±0.77	6.03±0.67
DV (200mg/kg) +CCL_4	4.67±0.71	0.33±0.01	2.85±0.3	10.060±1.04
DV (300mg/kg) +CCL_4	5.105±0.51	0.41±0.1	2.49±0.5	15.52±1.75

Results were expressed in triplicate manner with mean ± SD

Table.2. Protective effect of Chloroform extract of *Dodonaea Viscosa* flowers

Treatments	Liver weight (g)	Kidneyweight (g)	Spleen Weight (g)	Rise (%) in body weight (g)
Normal	5.60 ± 1.21	0.48±0.05	2.080±0.1	16±2.9
Olive oil Control	5.82±0.94	0.51±0.02	2.15±0.5	20±3.10
CCL_4	3.96±1.18	0.34±0.08	3.34±0.2	7.41±2.05
Silymarin+CCL_4	6.02±0.749	0.5±0.1	2.150±0.16	22.27±0.09
DV (100mg/kg) +CCL_4	4.15±0.92	0.24±0.03	3.06±0.8	7.22±0.92
DV (200mg/kg)+CCL_4	4.62±1.23	0.28±0.14	2.76±0.5	10.24±2.31

DV (300mg/kg)+CCL_4	5.16±0.92	0.36±0.02	2.3±0.33	12.94±2.08

All the values are obtained in triplicate (mean ± standard deviation)

Table.3. Estimation of liver enzymes from mice blood serum

	ALT	**AST**	**ALP**	**Direct bilirubin**
Normal control	38±0.83	80.2±4.1	110±9.2	0.2±0.001
Olive oil control	40±5.2	63±2	175±20	0.32±0.06
CCl_4 Control 24 hrs	74.5±7.7	90±11	208±5.1	1.0±0.2
CCl_4 Control 48 hrs	85±12	95±3.5	225±11	1.25±0.04
CCl_4 Control 72 hrs	89±14	109±9.2	247±2	1.7±0.45
CCl_4 Control 21days	120±9	127.8±8.6	294±15	1.91±0.08
Silymarine drug	66.4±7.2	76.1±12	185±31	0.53±0.09
DVM 100mg+ CCl4	62.5±8	104±20	264.8±11	0.7±0.08
DVM 200mg+ CCl4	61.8±4.5	88±11	236±14	0.6±0.01
DVM 300mg+ CCl4	53±2	74.5±6	197±9.8	0.56±0.02
DVC 100mg+ CCl4	77±5	126±7.1	357±12.4	1.1±0.2
DVC 200mg+ CCl4	72±3	104.3±5	283.6±3.2	0.98±0.05
DVC 300mg+ CCl4	69±6	96±4.3	229±11	0.81±0.07

Results are taken in triplicate way with mean ± SD. Level of significance at <0.05

3.1.2 Liver enzymes

DV flower extracts was tested for protective effect on liver enzymes in albino mice, substitute to synthetic medicine. Group 1 and 2 showed normal enzymes level with no treatment. (Group 3) Treatment of CCL_4 presented slight to elevated changes after 24, 48, 72hrs and 21 days in liver enzymes as compared to other groups. (Group 4 to 9) DVM showed more restorative effect on liver enzymes in relation to chloroform extract. The biochemical parameters found near to normal group. Group 10 indicated hepatoprotective effect by silymarin as compared with CCL_4. (Table 3)

3.1.3 Hematological parameters

CCL_4 injection resulted in decline in RBCs, WBCs and platelets. DV methanol and chloroform extracts significantly increase in parameters (Table 4). DV methanol depicted greater RBCs and WBCs values which are closer to the normal control with significant results ($p<0.05$).

Table.4. Hematological parameters of different groups of mice

Groups	RBCs	WBCs	Platelets
Normal group	4.80±0.06	5.02±0.47	240±6.9
Olive oil control	4.93±0.2	5.50±0.008	252±10.1
CCl_4 Control 24 rs	3.05±0.081	3.68±0.03	170±5.03
CCl_4 Control 48 rs	2.73±0.03	3.25±0.15	155.2±0.25
CCl_4 Control 72 hrs	2.40±0.009	2.913±0.092	140.01±0.17
CCl_4 Control 7days	1.88±0.005	2.351±0.11	100.59±2.9
Silymarin drug	3.97±0.12	4.802±0.060	227.3±4.12
DVM 100mg+ CCl4	3.7±0.016	4.67±0.04	240.3±0.15
DVM 200mg+ CCl4	4.181±0.004	5.002±0.12	247.09±0.21
DVM 300mg+ CCl4	4.62±0.21	5.56±0.01	256.6±0.82
DVC 100mg+ CCl4	2.612±0.093	3.205±0.83	174.1±0.26
DVC 200mg+ CCl4	3.05±0.02	3.84±0.131	198.3±0.03
DVC 300mg+ CCl4	3.73±0.1	4.291±0.20	224.20±0.19

3.1.4 Antioxidant enzymes

The results showed that normal level of enzymes altered due to CCL_4 administration. After 24 hours of carbon tetra chloride induction, antioxidant enzymes were observed to extreme level CAT 5.94 m mol /min / mg / protein, SOD 6.89 m mol / min / mg / protein and GPx 22.40µ mol/ min / mg / protein. Toxicity level reduced after 21 days of CCL_4 induction. Silymarin drug (positive control) and plant extracts revealed positive results. Results obtained in triplicate manner along with coefficient variation was <0.05 (Table 5).

Table.5. Effects of *Dodonaea Viscosa* flowers on Antioxidant enzymes and total proteins

Groups	CAT (m mol /min/mg protein)	SOD (U SOD/mg protein)	GPx (µmol/min/mg protein)	Protein tissue
Normal control	8.2±2	10.15±1.4	32.10±3	3.15±0.4
Olive oil	7.9±0.5	10.23±2	34.7±4.5	3.2±0.25
CCL4 24 hours	5.94±1.3	6.89±0.7	22.40±3.7	1.2±0.11
CCL4 48 Hours	5.28±1.7	6.24±1	20.47±1.9	1.03±0.2
CCL4 72 Hours	5.03±0.43	5.98±1.4	18.16±2	0.98±0.3
CCL4 21 days	4.75±1.9	5.75±0.6	17.83±3	0.75±0.08
DVM100 mg + CCl4	6.8±0.2	7.79±1	19.4±1.2	1.58±0.05
DVM200 mg + CCl4	7.3±0.8	8.58±2.3	23.0±3.5	2.1±0.4
DVM300 mg+ CCl4	7.8±1.03	10.7±0.5	29.4±2	2.8±0.08
DVC100 mg+CCl4	5.9±1.5	5.86±1.09	20.1±3	1.02±0.5
DVC200 mg+ CCl4	6.2±2	6.9±1.5	23.4±0.7	1.71±0.9
DVC300 mg+ CCl4	6.57±1	8.02±1	25.8±1	2.1±0.71
Silymarin + CCl4	8.11±1.2	11.62±0.6	33.7±1.5	3.22±0.8

All the results were obtained in triplicate with mean and standard deviation.

3.1.5 Histopathology

Morphological changes in organ (Liver, kidney and spleen) tissues were investigated by histopathological microscopy. CCL_4 intoxication damaged normal architecture of cells and after 21 days of DV treatment, cell structure of mice tissues was integrated. Several concentrations of plant extracts exhibited protective effect on tissues by attenuating injuries while methanol extract presented more supportive evidence on cellular organization as compared to DVC extract. So, for the further analysis DVM extract will be used for different in vitro studies (Figure 1).

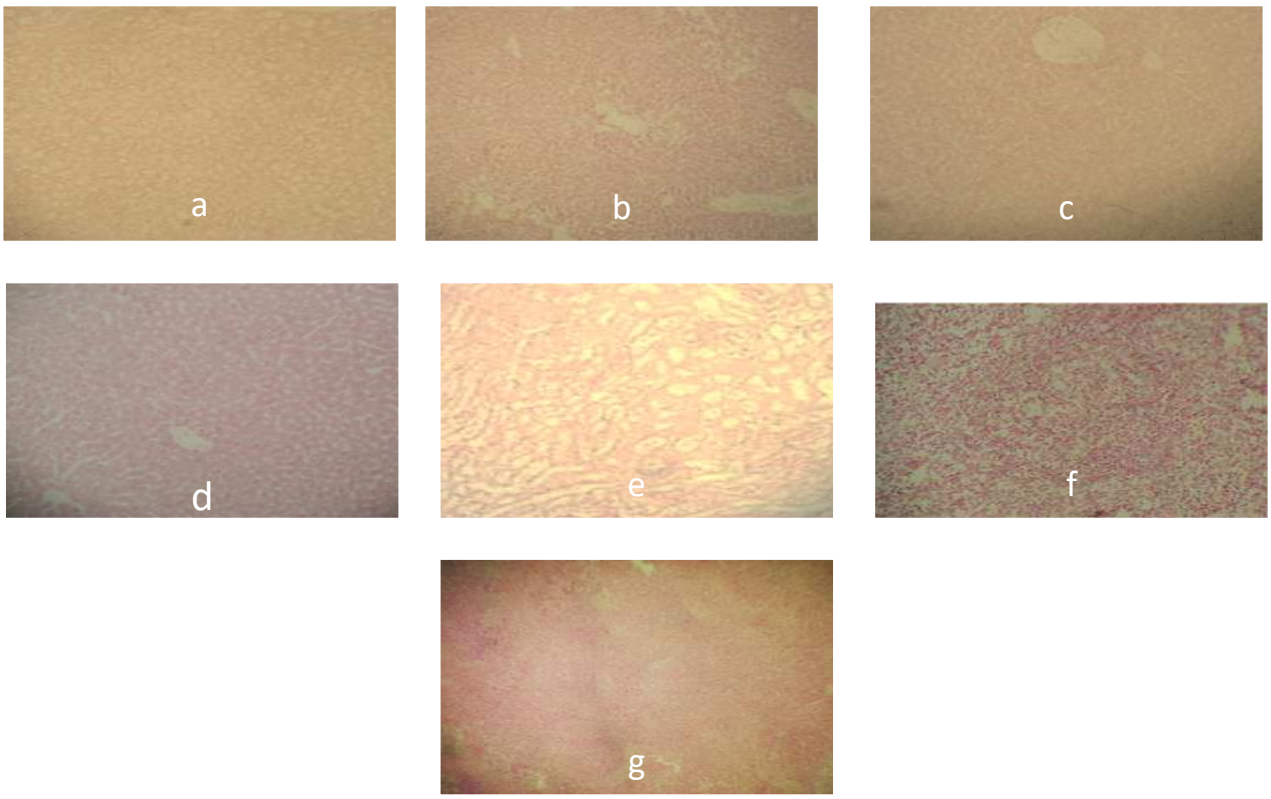


Figure1. "a" showed the normal mice liver tissues followed by Figure "b" showed the damaged liver tissues. Figure "c" presents recovery of normal cellular organization caused by CCl_4 induction. Figure "d" showed normal kidney Figure "e" DVM treated kidney. Figure "f" presents normal spleen while Figure "g" depict DVC treated spleen.

Normal liver architecture depicts central vein, hepatic sinusoids as well as portal veins with normal appearance. Fibrosis and vascular irregularities for instance liver sinusoids alteration and central veins congestion were seen in carbon tetrachloride mice. Renal histology revealed normal features like intact glomerular, tubular structure, bowman's space and capillary tufts. Treated spleen showed white pulp containing normal lymphoid masses followed by extremely vascular red pulp, which was similar to normal mice histology.

3.2 Phytochemical analysis and antioxidant assays

Dodonaea viscosa flower possessed total phenolic content 174±4mg/g dry weight and total flavonoid content 98±7mg/g , where the results are significantly different $p<0.05$. DVM extract was evaluated against different antioxidant activities and the lowest IC_{50} was found in hydrogen

peroxide assay 11.37±0.4mg/g dry weight and hydroxyl radical scavenging assay 19±0.56mg/g dry weight which is greater than Ascorbic and Gallic acid. DVM also showed good activity to quench free radicals in DPPH free radical scavenging assay (Table 6).

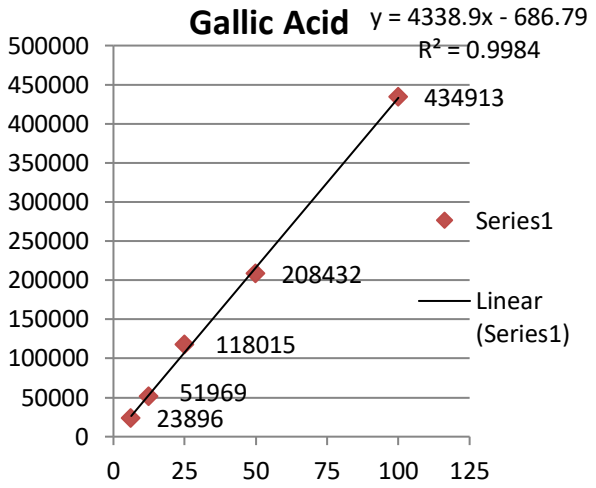
Table.6. IC_{50} of different antioxidant assays

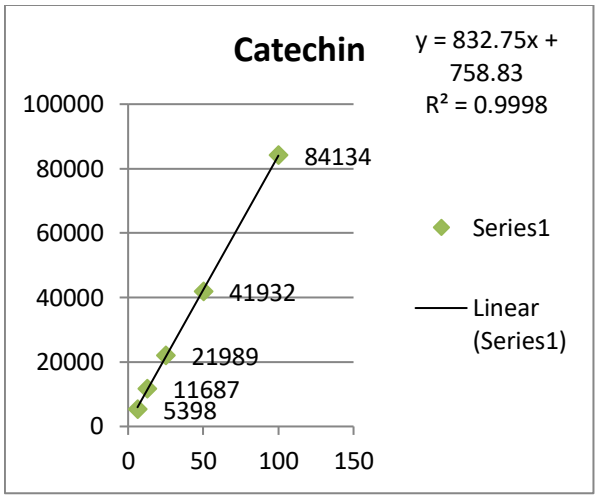
	ABTS Assay	**Reducing power Assay**	**DPPH Assay**	**Iron chelating Assay**	**Hydrogen peroxide Assay**	**Hydroxyl scavenging Assay**	**Superoxide Assay**
D. viscosa	107.1±11.4	75.59±4	54.95±2.1	20.7±1.3	11.37±0.4	19±0.56	111.6±2.1
Ascorbic acid	119±7.9	25.7±2	15.7±3	29.2±.7	16.8±2.1	24.5±0.84	116.6±2.8
Gallic acid	229±15	39.2±1	24.7±2	34.8±2	13.1±1	26.2±1	134.2±5.6

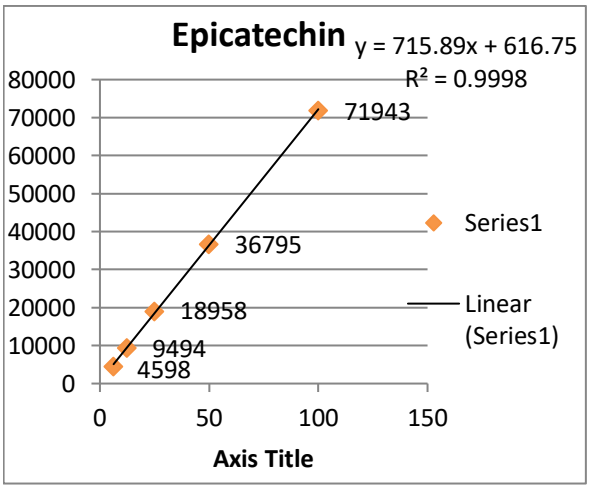
The values were measured in µg/g

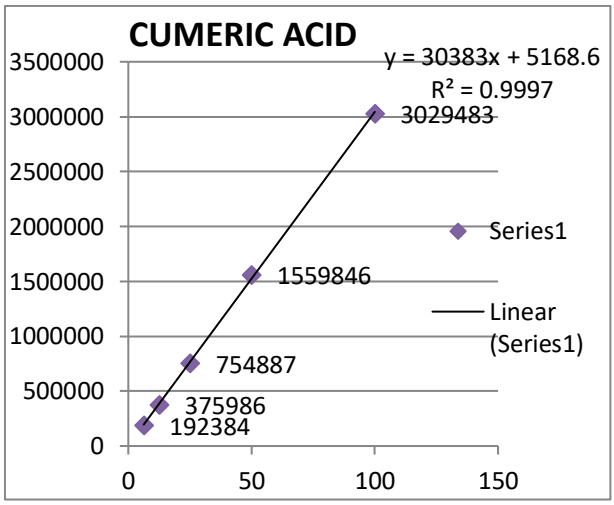
3.3 High Performance Liquid Chromatography

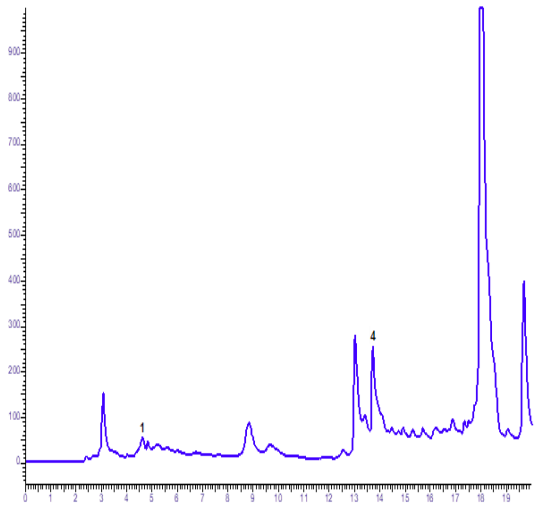
For the detection of some important medicinal compounds, HPLC analysis was carried out. Identification of peaks were done by comparing retention time of *Dodonaea viscosa* flower with standard compounds. The resulting peaks correspond to each compound were proportioned (Fig.2). HPLC quantification of DVM flower extract identified the presence of gallic acid, epicatechin, cumeric acid and quercetin compound. Whereas catechin has not been quantified in DV extract (Table 7). Gallic acid was the highest content observed with the quantity of 196.78mg/kg.

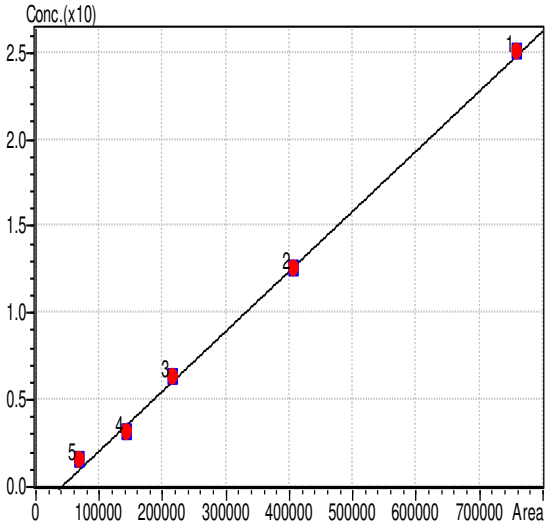

Gallic Acid
y = 4338.9x - 686.79
R² = 0.9984
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
25
50
75
100
125
434913
208432
118015
51969
23896
Series1
Linear (Series1)

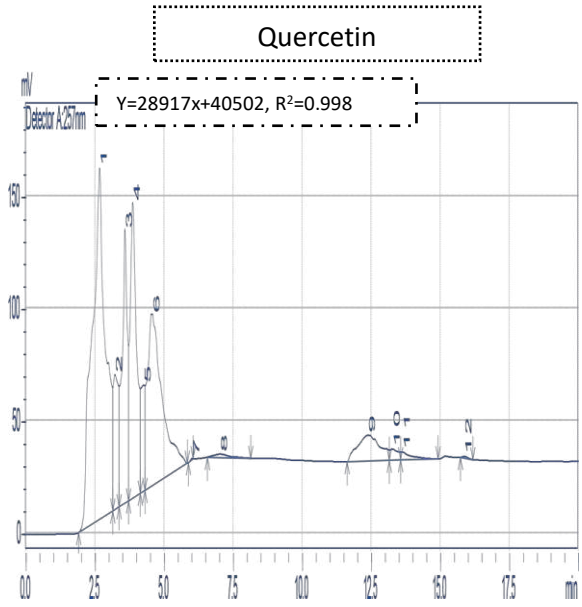

Catechin
y = 832.75x + 758.83
R² = 0.9998
100000
80000
60000
40000
20000
0
0
50
100
150
84134
41932
21989
11687
5398
Series1
Linear (Series1)


Epicatechin
y = 715.89x + 616.75
R² = 0.9998
80000
70000
60000
50000
40000
30000
20000
10000
0
0
50
100
150
Axis Title
71943
36795
18958
9494
4598
Series1
Linear (Series1)


CUMERIC ACID
y = 30383x + 5168.6
R² = 0.9997
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
50
100
150
3029483
1559846
754887
375986
192384
Series1
Linear (Series1)







a: Gallic acid standard graph

b: Catechin standard graph

c: Epicatechin standard graph

d: Cumeric acid standard graph

e: Peaks showing gallic acid, epicatechin and cumeric acid from DVM

f: Quercetin Calliberation curve

g: Chromatogram Peaks exhibited Quercetin from DVM

Fig.2. Chromatograms depicting different peaks detecting different compounds

Table.7. HPLC analysis of *Dodonaea viscosa* flower Methanol Extract

Compounds	**Area**	**Quantity (mg/kg)**
Gallic Acid	690219	196.78
Catechin	0	-1.04
Epichatechin	101583	140.76
Cumeric acid	3374640	110.85
Quercetin	1760828	59.49

3.4 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) Analysis

DVM extract is composed of volatile based organic compounds mainly fatty acids. Numerous compounds were identified by GC-MS and the compound list followed by corresponding GC-MS spectrum was presented in (Table 8) (**Fig 3**). Among all compounds, the most significant were Ascorbic acid ($C_{38}H_{68}O_8$), Octadecenoic acid ($C_{18}H_{34}O_2$), Ricinoleic acid ($C_{18}H_{34}O_3$), Carboxylic acid ($C_{23}H_{32}O_4$), Stearic acid ($C_{18}H_{36}O_2$) and Cedrol ($C_{15}H_{26}O$).

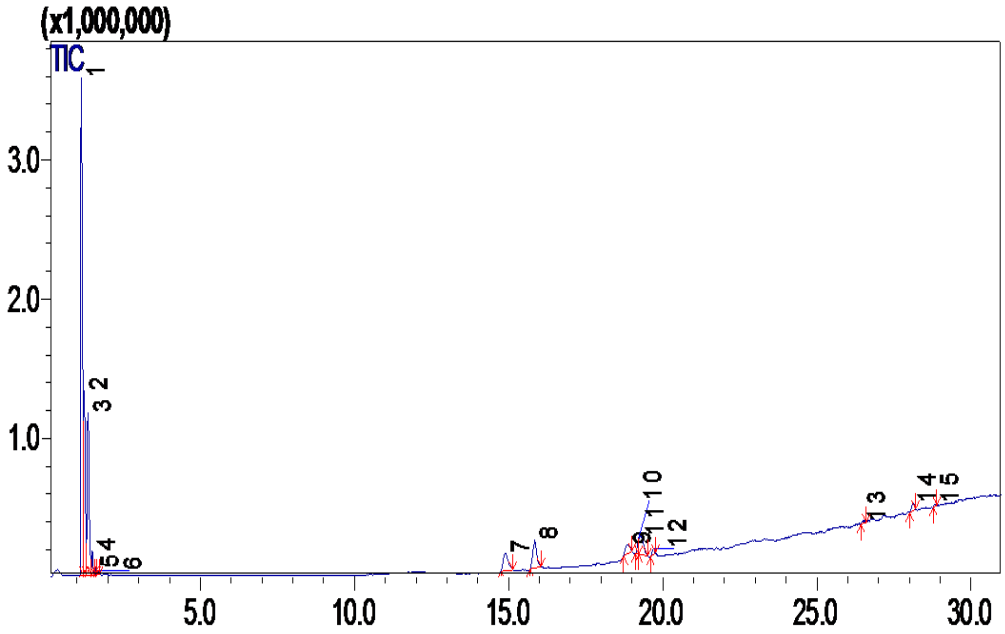


Fig.3. GCMS Chromatogram of *Dodonaea viscosa* flowers

Table.8. GCMS of *Dodonaea viscosa* flowers compounds

Peaks	**Compounds name**	**Formula**	**Molecular weight (amu)**	**Area %**	**Retention time (mins)**
1	Ethyl fluoride	C_2H_5F	48	44.66	1.132
2	Isobutyl alcohol	C4H10O	74	16.36	1.218
3	Isopentyl alcohol	C5H12O	88	16.78	1.350
4	Furanone	C5H8O2	100	1.58	1.488
5	Dimethyl Sulfoxonium formylmethylide	C4H8O2S	120	0.34	1.612
6	Isopentyl alcohol	C7H14O2	130	0.30	1.660
7	Ascorbic acid	C38H68O8	652	4.56	14.900
8	Ascorbic acid	C38H68O8	652	5.56	15.846
9	Octadecenoic acid	C18H34O2	282	2.11	18.858
10	Ricinoleic acid	C18H34O3	298	1.02	19.205
11	Octadecenoic acid	C18H34O2	282	4.30	19.278
12	Stearic acid	C18H36O2	284	0.74	19.697

13	Carboxylic acid	C23H32O4	372	0.40	26.460
14	Cyclopentanone	C15H20O	216	1.08	28.112
15	Cedrol	C15H26O	222	0.21	28.862

Data was obtained by triplicate readings with mean and standard deviation.

3.5 Fourier transform infrared spectroscopy

The most notable peaks in DVM were observed between 3500 to 2800nm. Peak at 2926.68nm belong to O-H stretch, Carboxylic acids and then at 3399.20nm correspond to O-H stretch, H-bonded which signifies Alcohols, phenols. Peaks ranging from 2900 to 700nm belong to C-H stretch, C=O stretch, C-N stretch and -C=C- stretch (**Fig.4**) (Table.9).

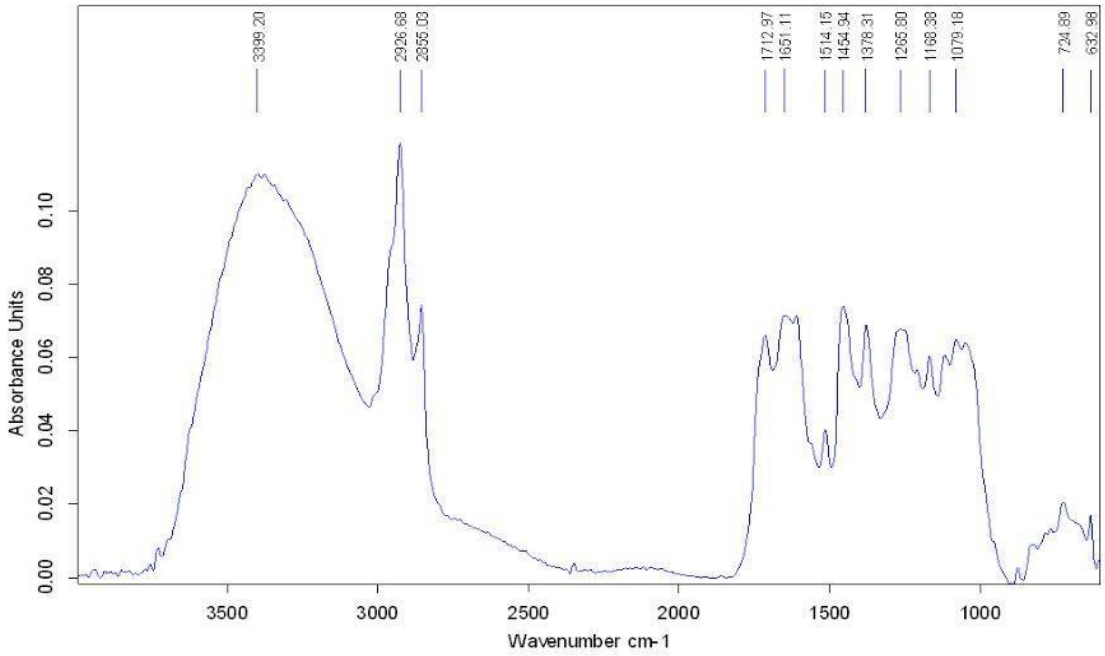


Fig.4. FT-IR analysis of *Dodonaea viscosa* flowers methanolic extract

Table.9. FT-IR analysis of flowers of *Dodonaea Viscosa*

Peaks	Wavelength	Bond	Functional group
1	3399.20	O-H stretch, H-bonded	Alcohols, phenols
2	2926.68	O-H stretch	Carboxylic acids

3	2855.03	C-H stretch	Alkanes
4	1712.97	C=O stretch	Carbonyl (general)
5	1651.11	-C=C- stretch	Alkenes
6	1514.15	N-O asymmetric stretch	Nitro compounds
7	1454.94	C-H Bend	Alkanes
9	1265.80	C-H wag (-CH_2X)	Alkyl halides
10	1168.38	C-N stretch	Aliphatic amines
11	1079.18	C-N stretch	Aliphatic amines
12	724.89	C-H rock	Alkanes
13	632.98	C-Br stretch	Alkyl halides

3.6 Anti-tuberculosis assay of *Dodonaea Viscosa* flowers

Anti-tuberculosis assay of DVM extract was screened out against Mycobacterium tuberculosis 3 strains bg 1972, bg 206 and H37Rv. 5 mg, 10 mg and 50 mg/ml concentrations were used, and tuberculosis % inhibition was increased with rise in concentrations. Plant extract showed resistance against all strains but highest activity was found against H37Rv strain (Table 9). Minimum inhibitory concentration (MIC) was determined at 25mg against H37Rv and bg 206. While standard drug (Rifampicin) exhibited MIC at 0.125mg against H37Rv strain.

Table.9. Anti-tuberculosis activity of DV Methanol against different strains

	Mean CFU on media			Percentage Inhibition		
Isolates	bg1972	H37Rv	Bg206	bg1972	H37Rv	Bg206
Control	130	140	150	130	140	150
5mg/ml extracts	80	40	53	38	71	65
10mg/ml	55	4	26	58	97	83
50mg/ml	0	0	0	100	100	100

% Inhibition = Ccn – Ct/Ccn x100 Where Ccn = No of colonies in the control media slope,

Ct = No of colonies in the Test media slope

Discussion

The present study demonstrated the *in vitro* and *in vivo* biological activities of *Dodonaea viscosa* flowers. Liver, kidney and spleen are the important parts of our body and involved in different pivotal functions. Liver is one of the most important organs and played an important role in detoxification of toxins [60,38]. CCL_4 is the toxin produce trichloromethyl radical (CCl_3) and linked with CCl_4 induced damage in organs leads to lipid peroxidation of membrane and necrosis of cell [2].

Mice weight was reduced with the variation in weight of organs (Liver, Kidney and Spleen). By ingesting plant extracts, increased in animal weight was observed followed by rise in the liver and kidney weight while weight of spleen reduced to their standard weight. 300mg/kg remarkably presented significant outcomes as witness in normal group. Increase in body weight owing to *Dodonaea viscosa* exhibited its protective effect. *Dodonaea viscosa* shown innocuous and protective to the mice as reported earlier [61]. Level of liver enzymes ALT, ALP, AST and direct bilirubin aimed to determine the sternness of damage tissue [62]. Biochemical markers altered or elevated by carbon tetra chloride and then restored by the treatment with plant extracts and standard drug silymarin indicated the protection of *Dodonaea viscosa* flowers against liver injury. CCL_4 also increased liver enzymes levels in some other biochemical study [63-64]. When an unbalanced increase observed in the ALP level, it causes liver diseases [65]. Harmful change

in enzymes reflected the development of tissue necrosis, weaken the liver capacity (biosynthetic and catabolic) and hepatocyte degenerative alterations [66].

Hematological parameters such as RBCs, WBCs and platelets were also altered by CCL_4 administration. Methanol and chloroform extracts of DV flower revealed positive effects on hematological parameters which specifies its suitability for the management of blood cells disorders [67]. Endogenous enzymes CAT, SOD and GPx involves in deactivating of free radicals. Decline in enzyme levels indicate hepatic damage [68]. CCL_4 declines the level of antioxidant enzymes (CAT, SOD and GPx) and total protein when compared with normal group, proved the liver injury [69] whereas the above factors were reinstated after dealing by plant extracts. Intoxication of CCL_4 in antioxidant enzymes can be improved by using Medicinal plants [70-71].

Exposure of CCL_4 leads to damage in Liver (Necrosis, Fibrosis and Central vein alteration), Kidney (Renal fibrosis, Glomerular and Tubular changes) and Spleen (deterioration in White and Red pulp) [72-74]. As a result of CCL_4, cellular structure and function of Kidney relies on Liver functional state [75]. Small improvement was observed in the cellular organization of animal tissues when treated with 100 and 200 mg/kg of DVM or DVC extracts. Highest defense shown by 300 mg/kg body weight of DVM extract than the 300 mg/kg body weight of DVC, that is more comparable to silymarin drug. Structural integrity of Liver, Kidney and Spleen was chiefly restored by DVM extract, proved its defensive properties. Liver, kidney and spleen seemed normal with no visible gross morphological and histopathological modifications at high doses. Similar results were documented by Agbaje *et al*., [76]. Changes observed in mice bodyweight, liver enzymes, antioxidant enzymes and microscopic examination are comparable with the other studies of *Dodonaea viscosa* on rat. Studies showed that process of excessive oxidation which is responsible for the deterioration of body tissues can be prevented by *D. viscosa* extract [77].

Methanol solvent was selected for the further activities on the basis of best results in *in vivo* study as compared to chloroform. Preliminary screening of secondary metabolites resulted significant amount of Total phenolic compounds and Total flavonoids content. Phenolics and Flavonoids are considered as singlet oxygen quenchers, radical scavenger, reducing agent and hydrogen donors [78]. So, the analysis of total flavonoids and phenolic compounds of plant is important to measure its antioxidant capacity. The results of experiment presented strong

antioxidant activities of *D. Viscosa* flowers. The highest antioxidant activity of DVM was shown against hydrogen peroxide assay. DVM manifested great radical scavenging ability as follows Hydroxyl radical assay>iron chelating assay>DDPH assay>Reducing power assay>ABTS radical assay>Superoxide assay. In the Current study, the reducing capacity of *D. viscosa* significantly decrease the complex of ferric cyanide to ferrous. Occurrence of antioxidants were determined by evaluating the ability of plant extract to form ferrous by reducing ferric cyanide complex [79]. Reducing power of plant compound specifies its potential antioxidant capacity. High reducing power in a sample have great ability of donating the electron and free radicals, and produce stable elements through acceptance of donated electron, that terminates the free radical reaction [80]. Hydroxyl radical are highly reactive free radicals in biological systems and there are no specific enzyme present in human to protect against them. Their presence in human body cause oxidative DNA damage. Therefore, there is a need of a solution to scavenge ROS with natural products having scavenging activity. Due to high reactivity of OH radical, the antioxidant activity of scavenging hydroxyl radical is important [81-82]. The most commonly used method for evaluation of antioxidant is DDPH assay. The quenching of DPPH measurement relies on discoloration of purple colored 2,2-diphen-yl-2-picryl-hydrazyl compound by antioxidant. DPPH comprises odd electron gives absorbance at 515nm. Donor antioxidant decolorizes DPPH radical by electron acceptance, can be measured quantitatively from variations in absorbance [83]. Furthermore, D. *Viscosa* expressed significant radical scavenging activity against ABTS assay with low value of $IC_{50.}$ All the assays are positively as well as significantly correlated with phenols and flavonoids.

HPLC quantified four compounds in DVM i.e. gallic acid, epicatechin, quercetin and cumeric acid. Quercetin is an iso flavonoid and flavonoid content (rutin and quercetin) was identified in the stem of *dodonaea viscosa*. The remedial aptitudes of *Dodonaea viscosa* is associated by means of pharmacological effects which is brought through the synergistic action of numerous constitutes, i.e. flavonoids, saponins, di and triterpenes along with combination of phenolics existing in the plant [84]. Flavonoids and diterpenoids are the richest secondary metabolites that was previously identified and isolated from Dodonaea [85]. These phenolic and flavonoid compounds revealed anticancer, antiallergic, antibacterial, antiviral and anti-inflammatory activities [86]. The chemical compounds elucidated by GCMS were Oleic acid (Octadecenoic acid), Ascorbic acid, Ricinoleic acid, Stearic acid, Carboxylic acid, Cyclopentanone and Cedrol.

Fatty acids (Oleic, linoleic and linolenic acids) enriched food showed pleiotropic effects and used for the management of inflammation, hypertension, cardiovascular diseases, hyperlipidemia, reproductive ailments, immune system and aggregation of platelets [87-88]. Research studies showed that Oleic acid exert remedial effect on human body such as cancer, anti-inflammatory and autoimmune diseases, and also play vital role in wound healing [89]. Ricinoleic acid is significant unsaturated and hydroxylated fatty acid, that depicts antipathogenic activity by deterring bacteria, virus, mold and yeast [90]. DVM showed very good activity against Tuberculosis strains. Mycobacterium tuberculosis is responsible for tuberculosis, which is among the most fatal diseases. *Dodonaea Viscosa* has been locally used in traditional medicines for the treatment of tuberculosis [91-93]. Tested plant extract of DV flowers exhibited stronger resistance from all tested strains of *Mycobacterium tuberculosis* owing to the occurrence of bioactive components among the different concentrations of plant methanol extract that are probably antimycobacterial metabolites. Tuberculosis remains accountable for numerous mortalities around the world. During treatment, TB patients require extensive chemical analysis and eventually generate antagonistic effects to patient wellbeing. To diminish the use of resistant unnatural drugs, medicinally important plants contribute to a great sureness as a potential reason for bioactive antimycobacterial metabolites [94]. A limited distinct species of genus Dodonaea have extensively examined both by chemically and pharmacologically. The most known specie of genus Dodonaea is *D. Viscosa* in literature [95].

Conclusion

Dodonaea viscosa is well known plant species and widely possess so many biological activities. Results showed the potential pharmacological effect of *Dodonaea Viscosa* against acute toxicity in albino mice which specifies its use against different diseases, most of all liver diseases. This plant showed significant biological activities such as antioxidant and anti-tuberculosis. Chemical composition of the plant is rich in antioxidant compounds flavonoids and phenols and rich source of Fatty acids mainly oleic acid. These compounds could probably protect elevated hepatic enzymes caused by carbon tetra chloride and chronic tuberculosis. These curative effects are linked with traditional use of this plant against different diseases. This plant might be used for the extraction of promising drug for the management of liver and multiple organs injury. The active compounds and their action mechanism, pharmacokinetics, toxicology, efficacy along

with molecular mechanisms that are still need to be explored to attain integration into remedial practice.

References

[1] Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: a state-of-the-art review. *International journal of food microbiology*, *144*(1), 51-63.

[2] Kandimalla, R., Dash, S., Kalita, S., Choudhury, B., Malampati, S., Kalita, K., & Kotoky, J. (2016). Bioactive guided fractions of Annona reticulata L. bark: protection against liver toxicity and inflammation through inhibiting oxidative stress and proinflammatory cytokines. *Frontiers in pharmacology*, *7*, 168.

[3] Kosecik, M., Erel, O., Sevinc, E., & Selek, S. (2005). Increased oxidative stress in children exposed to passive smoking. *International journal of cardiology*, *100*(1), 61-64.

[4] Huang, W., Metlakunta, A., Dedousis, N., Zhang, P., Sipula, I., Dube, J. J., & O'Doherty, R. M. (2010). Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. *diabetes*, *59*(2), 347-357.

[5] Baratta, J. L., Ngo, A., Lopez, B., Kasabwalla, N., Longmuir, K. J., & Robertson, R. T. (2009). Cellular organization of normal mouse liver: a histological, quantitative immunocytochemical, and fine structural analysis. *Histochemistry and cell biology*, *131*(6), 713-726.

[6] KP, S. G., Satish, S., & Mahesh, C. M. (2009). Study on the diuretic activity of Cynodon dactylon root stalk extract in albino rats. *Evaluation*, *7*, 11.

[7] Cesta, M. F. (2006). Normal structure, function, and histology of the spleen. *Toxicologic pathology*, *34*(5), 455-465.

[8] Steiniger, B. S. (2015). Human spleen microanatomy: why mice do not suffice. *Immunology*, *145*(3), 334-346.

[9] El-Zayadi, A. R., Abe, K., Selim, O., Naito, H., Hess, G., & Ahdy, A. (1999). Prevalence of GBV-C/hepatitis G virus viraemia among blood donors, health care personnel, chronic non-

B non-C hepatitis, chronic hepatitis C and hemodialysis patients in Egypt. *Journal of virological methods*, *80*(1), 53-58.

[10] Vedi, M. A. H. I. M. A., Kalaiselvan, S., rasool, M., & sabina, E. P. (2013). Protective effects of blue green algae Spirulina fusiformis against galactosamine-induced hepatotoxicity in mice. *Asian J. Pharm. Clin. Res*, *6*(3), 150-154.

[11] Liu, C. M., Ma, J. Q., & Sun, Y. Z. (2012). Puerarin protects the rat liver against oxidative stress-mediated DNA damage and apoptosis induced by lead. *Experimental and Toxicologic Pathology*, *64*(6), 575-582.

[12] Lin, H., Liu, X. B., Yu, J. J., Hua, F., & Hu, Z. W. (2013). Antioxidant N-acetylcysteine attenuates hepatocarcinogenesis by inhibiting ROS/ER stress in TLR2 deficient mouse. *PLoS One*, *8*(10), e74130.

[13] Sundararajan, R., Haja, N. A., Venkatesan, K., Mukherjee, K., Saha, B. P., Bandyopadhyay, A., & Mukherjee, P. K. (2006). Cytisus scoparius link-A natural antioxidant. *BMC complementary and alternative medicine*, *6*(1), 8.

[14] Fu, S. Y., Lau, W. Y., Li, A. J., Yang, Y., Pan, Z. Y., Sun, Y. M., ... & Wu, M. C. (2010). Liver resection under total vascular exclusion with or without preceding Pringle manoeuvre. *British Journal of Surgery*, *97*(1), 50-55.

[15] Pareek, A., Godavarthi, A., Issarani, R., & Nagori, B. P. (2013). Antioxidant and hepatoprotective activity of Fagonia schweinfurthii (Hadidi) Hadidi extract in carbon tetrachloride induced hepatotoxicity in HepG2 cell line and rats. *Journal of ethnopharmacology*, *150*(3), 973-981.

[16] Stevenson, D. E., & Hurst, R. D. (2007). Polyphenolic phytochemicals–just antioxidants or much more?. *Cellular and Molecular Life Sciences*, *64*(22), 2900-2916.

[17] Nayak, B. S., Marshall, J. R., Isitor, G., & Adogwa, A. (2011). Hypoglycemic and hepatoprotective activity of fermented fruit juice of Morinda citrifolia (Noni) in diabetic rats. *Evidence-Based Complementary and Alternative Medicine*, *2011*.

[18] Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., & Bren, U. (2016). Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules*, *21*(7), 901.

[19] Fauziyah, P. N., Sukandar, E. Y., & Ayuningtyas, D. K. (2017). Combination effect of antituberculosis drugs and ethanolic extract of selected medicinal plants against multi-drug resistant Mycobacterium tuberculosis isolates. *Scientia pharmaceutica*, *85*(1), 14.

[20] Sahu, L., Jena, S., Swain, S. S., Sahoo, S., & Chand, P. K. (2013). Agrobacterium rhizogenes-mediated transformation of a multi-medicinal herb, Boerhaavia diffusa L.: optimization of the process and anti-microbial activity against bacterial pathogens causing urinary tract infections. *Frontiers in Life Science*, *7*(3-4), 197-209.

[21] Wagner. H. In Handbook of Medicinal plants (Ed, Yaniv, Z. B., U.) *Haworth press*, New York, 2005.

[22] Anilreddy, B. (2009). Preparation, characterization and biological evaluation of some overview of Dodonaea viscosa Linn. *J Pharm Sci Technol*, *1*(1), 1-9.

[23] Perry, L. M., & Metzger, J. (1980). *Medicinal plants of east and southeast Asia: attributed properties and uses*. MIT press.

[24] Pengelly, A. R. (2008). Flavonoid Profile and Bioactivity of Dodonaea Viscosa (Australian Hop Bush)-an Indigenous Shrub. University of Newcastle.

[25] Kishore, K. K., & Sasidharan, N. (2002). A case study from the shola forests of Kerala, India. In *Recent progress in medicinal plants* (pp. 201-214). Science Tech Publishing Inc. Texas, USA.

[26] Jawahar, N., Manivannan, R., Jubie, S., & Saiganesh, E. (2004). Pharmacognostical and phytochemical studies on leaves of Dodonaea Viscosa Linn. *Ancient science of life*, *23*(3), 46.

[27] Getie, M., Gebre-Mariam, T., Rietz, R., Höhne, C., Huschka, C., Schmidtke, M., & Neubert, R. H. H. (2003). Evaluation of the anti-microbial and anti-inflammatory activities of the medicinal plants Dodonaea viscosa, Rumex nervosus and Rumex abyssinicus. *Fitoterapia*, *74*(1-2), 139-143.

[28] Khalil, N. M., Sperotto, J. S., & Manfron, M. P. (2006). Antiinflammatory activity and acute toxicity of Dodonaea viscosa. *Fitoterapia*, *77*(6), 478-480.

[29] Veerapur, V. P., Prabhakar, K. R., Thippeswamy, B. S., Bansal, P., Srinivasan, K. K., & Unnikrishnan, M. K. (2010). Antidiabetic effect of Dodonaea viscosa (L). Lacq. aerial parts in high fructose-fed insulin resistant rats: a mechanism-based study.

[30] Ramamurthy, V., Rajeswari, D. M., Gowri, R., Vadivazhagi, M. K., Jayanthi, G., & Raveendran, S. (2013). Study of the phytochemical analysis and antimicrobial activity of Dodonaea viscosa. *International Journal of Pure and Applied Zoology*, *1*(2).

[31] Akhalwaya, S., van Vuuren, S., & Patel, M. (2018). An in vitro investigation of indigenous South African medicinal plants used to treat oral infections. *Journal of ethnopharmacology*, *210*, 359-371.

[32] Rajamanickam, V., Rajasekaran, A., Anandarajagopal, K., Sridharan, D., Selvakumar, K., & Rathinaraj, B. S. (2010). Anti-diarrheal activity of Dodonaea viscosa root extracts. *International Journal of Pharma and Bio Sciences*, *1*(4), 182-185.

[33] Sachdev, K., & Kulshreshtha, D. K. (1983). Flavonoids from Dodonaea viscosa. *Phytochemistry*, *22*(5), 1253-1256.

[34] Ghisalberti, E. L. (1998). Ethnopharmacology and phytochemistry of Dodonaea species. *Fitoterapia*, *69*, 99-113.

[34] Sachdev, K., & Kulshreshtha, D. K. (1986). Viscosol, a C-3′ prenylated flavonoid from Dodonaea viscosa. *Phytochemistry*, *25*(8), 1967-1969.

[36] Sastry, K. N. S., & Nayudamma, Y. (1966). Leucocyanidin from Dodonaea viscosa bark. *Leather Sciences*, *13*, 174-176.

[37] Wagner, C., Ludwig, L., Grotjahn, M.S., Y. Khan. *Phytochemistry*, 1987; 26: 697-702.

[38] Li, M., He, Y., Zhou, Z., Ramirez, T., Gao, Y., Gao, Y., & Feng, D. (2017). MicroRNA-223 ameliorates alcoholic liver injury by inhibiting the IL-6–p47phox–oxidative stress pathway in neutrophils. *Gut*, *66*(4), 705-715.

[39] Dacie, J. W., And S. M. Lewis. 1991. Practical Haematology. 7th Edition. Churchill Livingstone, New York, New York, 556 pp.

[40] Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*, *247*(10), 3170-3175.

[41] Flohé, L., & Günzler, W. A. (1984). Assays of glutathione peroxidase. In *Methods in enzymology* (Vol. 105, pp. 114-120). Academic Press.

[42] Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, *193*(1), 265-275.

[43] Yakubu, M. T., Akanji, M. A., & Oladiji, A. T. (2007). Evaluation of antiandrogenic potentials of aqueous extract of Chromolaena odoratum (L.) KR leaves in male rats. *Andrologia*, *39*(6), 235-243.

[44] Giribabu, N., Karim, K., Kilari, E. K., & Salleh, N. (2017). Phyllanthus niruri leaves aqueous extract improves kidney functions, ameliorates kidney oxidative stress, inflammation, fibrosis and apoptosis and enhances kidney cell proliferation in adult male rats with diabetes mellitus. *Journal of ethnopharmacology*, *205*, 123-137.

[45] Sabir, S. M., & Rocha, J. B. T. (2008). Antioxidant and hepatoprotective activity of aqueous extract of Solanum fastigiatum (false "Jurubeba") against paracetamol-induced liver damage in mice. *Journal of ethnopharmacology*, *120*(2), 226-232.

[46] Moreno, M. I. N., Isla, M. I., Sampietro, A. R., & Vattuone, M. A. (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *Journal of ethnopharmacology*, *71*(1-2), 109-114.

[47] Moon, J. K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of agricultural and food chemistry*, *57*(5), 1655-1666.

[48] Dinis, T. C., Madeira, V. M., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of biochemistry and biophysics*, *315*(1), 161-169.

[49] Nagai, T., Nagashima, T., Suzuki, N., & Inoue, R. (2005). Antioxidant activity and angiotensin, I-converting enzyme inhibition by enzymatic hydrolysates from bee bread. *Zeitschrift für Naturforschung C*, *60*(1-2), 133-138.

[50] Ashafa, A. O. T., Grierson, D. S., & Afolayan, A. K. (2010). In vitro antioxidant activity of extracts from the leaves of Felicia muricata Thunb. an underutilized medicinal plant in the eastern cape province, South Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, *7*(4).

[51] Dehghan, G., & Khoshkam, Z. (2012). Tin (II)–quercetin complex: Synthesis, spectral characterization and antioxidant activity. *Food Chemistry*, *131*(2), 422-426.

[52] Hazra, B., Biswas, S., & Mandal, N. (2008). Antioxidant and free radical scavenging activity of Spondias pinnata. *BMC complementary and Alternative Medicine*, *8*(1), 63.

[53] Adedapo, A. A., Jimoh, F. O., Afolayan, A. J., & Masika, P. J. (2009). Antioxidant Properties of the Methanol Extracts of the Leaves and Stems of Celtis africana. *Records of Natural Products*, *3*(1).

[54] Aiyegoro, O. A., & Okoh, A. I. (2010). Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. *BMC Complementary and Alternative medicine*, *10*(1), 21.

[55] Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, *44*(1), 276-287.

[56] Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2000). GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicillium digitatum. *Journal of agricultural and food chemistry*, *48*(6), 2576-2581.

[57] Upadhyay, R. K. (2015). GC-MS analysis and in vitro antimicrobial susceptibility of Foeniculum vulgare seed essential oil. *American Journal of Plant Sciences*, *6*(07), 1058.

[58] Zargar, M., Shameli, K., Najafi, G. R., & Farahani, F. (2014). Plant mediated green biosynthesis of silver nanoparticles using Vitex negundo L. extract. *Journal of Industrial and Engineering Chemistry*, *20*(6), 4169-4175.

[59] Palomino, J. C., Martin, A., Camacho, M., Guerra, H., Swings, J., & Portaels, F. (2002). Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis. *Antimicrobial agents and chemotherapy*, *46*(8), 2720-2722.

[60] Gallagher, E. J., LeRoith, D., Stasinopoulos, M., Zelenko, Z., & Shiloach, J. (2016). Polyol accumulation in muscle and liver in a mouse model of type 2 diabetes. *Journal of diabetes and its complications*, *30*(6), 999-1007.

[61] Naidoo, R., Patel, M., Gulube, Z., & Fenyvesi, I. (2012). Inhibitory activity of Dodonaea viscosa var. angustifolia extract against Streptococcus mutans and its biofilm. *Journal of ethnopharmacology*, *144*(1), 171-174.

[62] Tu, X., Zheng, X., Li, H., Cao, Z., Chang, H., Luan, S., & Zhang, J. (2015). MicroRNA-30 protects against carbon tetrachloride-induced liver fibrosis by attenuating transforming growth factor beta signaling in hepatic stellate cells. *Toxicological Sciences*, *146*(1), 157-169.

[63] Zhang, H., Yu, C. H., Jiang, Y. P., Peng, C., He, K., Tang, J. Y., & Xin, H. L. (2012). Protective effects of polydatin from Polygonum cuspidatum against carbon tetrachloride-induced liver injury in mice. *PLoS One*, *7*(9), e46574.

[64] Dong, D., Zhang, S., Yin, L., Tang, X., Xu, Y., Han, X., & Peng, J. (2013). Protective effects of the total saponins from Rosa laevigata Michx fruit against carbon tetrachloride-induced acute liver injury in mice. *Food and chemical toxicology*, *62*, 120-130.

[65] Jose, J. K., & Kuttan, R. (2000). Hepatoprotective activity of Emblica officinalis and Chyavanaprash. *Journal of Ethnopharmacology*, *72*(1-2), 135-140.

[66] Nigatu, T. A., Afework, M., Urga, K., Ergete, W., & Makonnen, E. (2017). Toxicological investigation of acute and chronic treatment with Gnidia stenophylla Gilg root extract on some blood parameters and histopathology of spleen, liver and kidney in mice. *BMC research notes*, *10*(1), 625.

[67] Middleton, E. (1998). Effect of plant flavonoids on immune and inflammatory cell function. In *Flavonoids in the living system* (pp. 175-182). Springer, Boston, MA.

[68] Beji, S. R., Abidi, A., Zemni, R., & Jameleddine, B. K. S. (2013). Antifibrosis effects of all-trans-retinoic acid in a rat model of bleomycininduced pulmonary fibrosis. *Fundamental and Clinical Pharmacology*, *27*, 46-47.

[69] Simeonova, R., Kondeva-Burdina, M., Vitcheva, V., Krasteva, I., Manov, V., & Mitcheva, M. (2014). Protective effects of the apigenin-O/C-diglucoside saponarin from Gypsophila

trichotoma on carbone tetrachloride-induced hepatotoxicity in vitro/in vivo in rats. *Phytomedicine*, *21*(2), 148-154.

[70] Gul, H., Ahmad, M., Zafar, M., Sheeraz Ahmad, M., Abid, A., Hira, S., & Gulfraz, M. (2017). The In Vitro and In Vivo Biological Activities of the Leaf of Cape Myrtle, Myrsine africana L. *Phytotherapy Research*, *31*(9), 1305-1309.

[71] Safhi, M. M. (2018). Nephroprotective effect of Zingerone against CCl4-induced renal toxicity in Swiss albino mice: molecular mechanism. *Oxidative medicine and cellular longevity*, *2018*.

[72] Ozturk, F., Ucar, M., Ozturk, I. C., Vardi, N., & Batcioglu, K. (2003). Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. *Urology*, *62*(2), 353-356.

[73] Jiang, J. X., Chen, X., Serizawa, N., Szyndralewiez, C., Page, P., Schröder, K., & Török, N. J. (2012). Liver fibrosis and hepatocyte apoptosis are attenuated by GKT137831, a novel NOX4/NOX1 inhibitor in vivo. *Free Radical Biology and Medicine*, *53*(2), 289-296.

[74] Seniutkin, O., Furuya, S., Luo, Y. S., Cichocki, J. A., Fukushima, H., Kato, Y., & Rusyn, I. (2018). Effects of pirfenidone in acute and sub-chronic liver fibrosis, and an initiation-promotion cancer model in the mouse. *Toxicology and applied pharmacology*, *339*, 1-9.

[75] Rincón, A. R., Covarrubias, A., Pedraza-Chaverrí, J., Poo, J. L., Armendáriz-Borunda, J., & Panduro, A. (1999). Differential effect of CCl4 on renal function in cirrhotic and non-cirrhotic rats. *Experimental and Toxicologic Pathology*, *51*(3), 199-205.

[76] Agbaje, E. O., Adeneye, A. A., & Daramola, A. O. (2009). Biochemical and toxicological studies of aqueous extract of Syzigium aromaticum (L.) Merr. & Perry (Myrtaceae) in rodents. *African Journal of Traditional, Complementary and Alternative Medicines*, *6*(3).

[77] Arun, M., & Asha, V. V. (2008). Gastroprotective effect of Dodonaea viscosa on various experimental ulcer models. *Journal of ethnopharmacology*, *118*(3), 460-465.

[78] Motamed, S. M., & Naghibi, F. (2010). Antioxidant activity of some edible plants of the Turkmen Sahra region in northern Iran. *Food Chemistry*, *119*(4), 1637-1642.

[79] Ahmed, R. G., Incerpi, S., Ahmed, F., & Gaber, A. (2013). The developmental and physiological interactions between free radicals and antioxidant: Effect of environmental pollutants. *J. of Natural Sci. Res*, *3*(13), 74-110.

[80] Kumari, S., Elancheran, R., Kotoky, J., & Devi, R. (2016). Rapid screening and identification of phenolic antioxidants in Hydrocotyle sibthorpioides Lam. by UPLC–ESI-MS/MS. *Food chemistry*, *203*, 521-529.

[81] Liu, R. H., & Finley, J. (2005). Potential cell culture models for antioxidant research. *Journal of agricultural and food chemistry*, *53*(10), 4311-4314.

[82] Wang, H., Gao, X. D., Zhou, G. C., Cai, L., & Yao, W. B. (2008). In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit. *Food Chemistry*, *106*(3), 888-895.

[83] Khan, A., Anand, V., Badrinarayanan, V., Thirunethiran, K., & Natarajan, P. (2017). In vitro Antioxidant and Cytotoxicity Analysis of Leaves of Ficus racemosa. *Free Radicals & Antioxidants*, *7*(1).

[84] Wagner, C., Fachinetto, R., Dalla Corte, C. L., Brito, V. B., Severo, D., Dias, G. D. O. C., ... & Rocha, J. B. (2006). Quercitrin, a glycoside form of quercetin, prevents lipid peroxidation in vitro. *Brain research*, *1107*(1), 192-198.

[85] Simpson, B. S., Claudie, D. J., Smith, N. M., Gerber, J. P., McKinnon, R. A., & Semple, S. J. (2011). Flavonoids from the leaves and stems of Dodonaea polyandra: A Northern Kaanju medicinal plant. *Phytochemistry*, *72*(14-15), 1883-1888.

[86] Maurya, P. M. (2017). Phytochemical and Pharmacological examination of Achyranthes aspera Linn. *Journal of Pharmacognosy and Phytochemistry*, *6*(6), 1866-1871.

[87] Eromosele, C. O., & Eromosele, I. C. (2002). Fatty acid compositions of seed oils of Haematostaphis barteri and Ximenia americana. *Bioresource technology*, *82*(3), 303-304.

[88] Riediger, N. D., Othman, R. A., Suh, M., & Moghadasian, M. H. (2009). A systemic review of the roles of n-3 fatty acids in health and disease. *Journal of the American Dietetic Association*, *109*(4), 668-679.

[89] Campos, S. H., Reis de Souza, P., Crema Peghini, B., Santana da Silva, J., & Ribeiro Cardoso, C. (2013). An overview of the modulatory effects of oleic acid in health and disease. *Mini reviews in medicinal chemistry*, *13*(2), 201-210.

[90] Tunaru, S., Althoff, T. F., Nüsing, R. M., Diener, M., & Offermanns, S. (2012). Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors. *Proceedings of the National Academy of Sciences*, *109*(23), 9179-9184.

[91] Asres, K., Bucar, F., Edelsbrunner, S., Kartnig, T., Höger, G., & Thiel, W. (2001). Investigations on antimycobacterial activity of some Ethiopian medicinal plants. *Phytotherapy Research*, *15*(4), 323-326.

[92] McGaw, L. J., Lall, N., Meyer, J. J. M., & Eloff, J. N. (2008). The potential of South African plants against Mycobacterium infections. *Journal of Ethnopharmacology*, *119*(3), 482-500.

[93] Sankaranarayanan, S., Bama, P., Ramach, J., Kalaichelvan, P. T., Deccaraman, M., Vijayalakshimi, M., & Bama, S. S. (2010). Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. *Journal of Medicinal Plants Research*, *4*(12), 1089-1101.

[94] Gemechu, A., Giday, M., Worku, A., & Ameni, G. (2013). In vitro Anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and Mycobacterium bovis Strains. *BMC complementary and alternative medicine*, *13*(1), 291.

[95] Simpson, B., Claudie, D., Smith, N., Wang, J., McKinnon, R., & Semple, S. (2010). Evaluation of the anti-inflammatory properties of Dodonaea polyandra, a Kaanju traditional medicine. *Journal of ethnopharmacology*, *132*(1), 340-343.

Figures

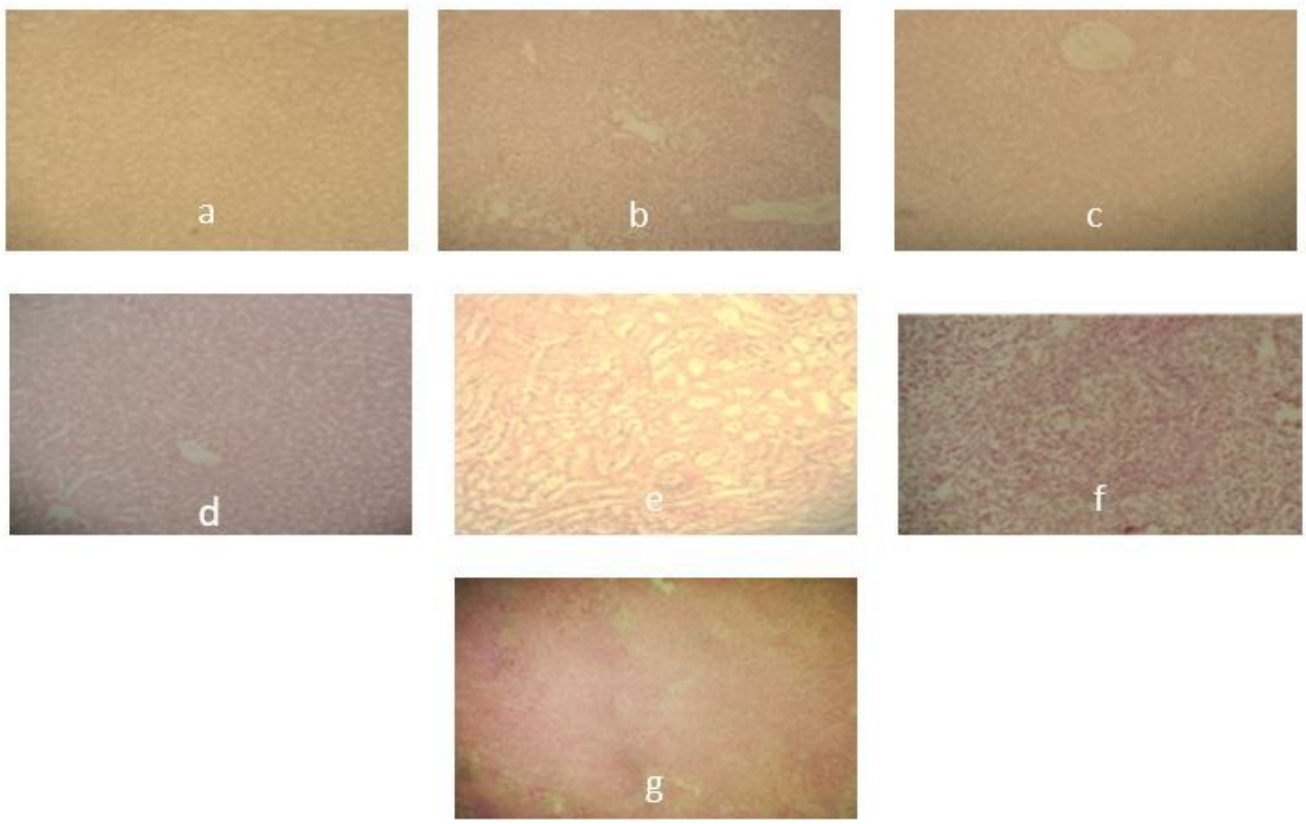


Figure 1

“a” showed the normal mice liver tissues followed by Figure “b” showed the damaged liver tissues. Figure “c” presents recovery of normal cellular organization caused by CCl4 induction. Figure “d” showed normal kidney Figure “e” DVM treated kidney. Figure “f” presents normal spleen while Figure “g” depict DVC treated spleen.

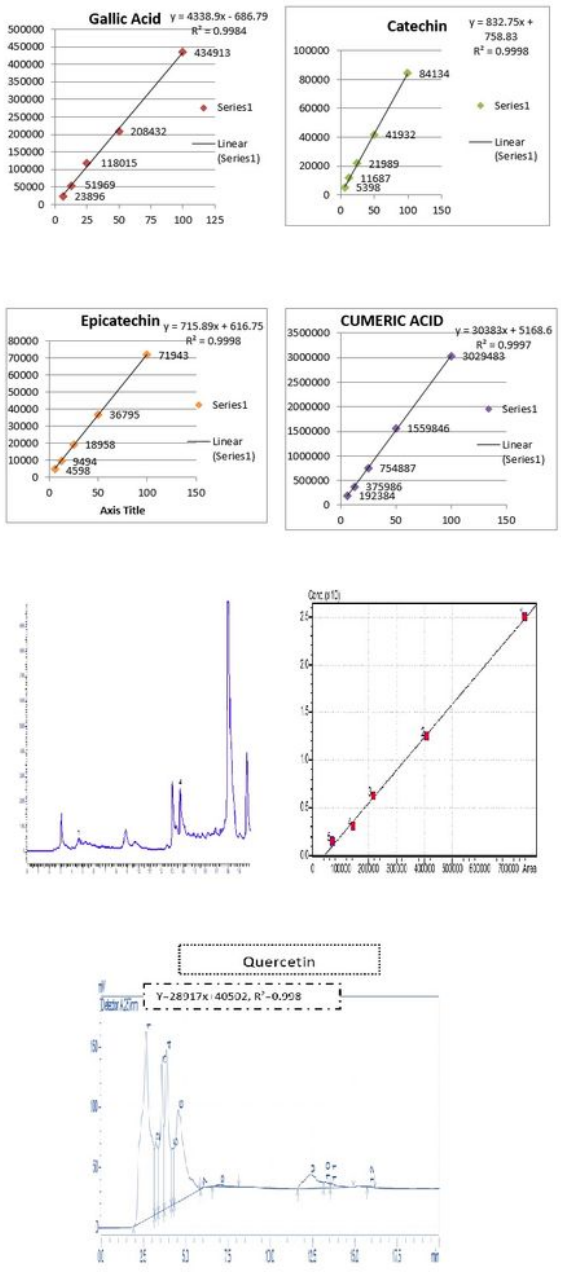


Figure 2

a: Gallic acid standard graph b: Catechin standard graph c: Epicatechin standard graph d: Cumeric acid standard graph e: Peaks showing gallic acid, epicatechin and cumeric acid from DVM f: Quercetin Calliberation curve g: Chromatogram Peaks exhibited Quercetin from DVM Chromatograms depicting different peaks detecting different compounds

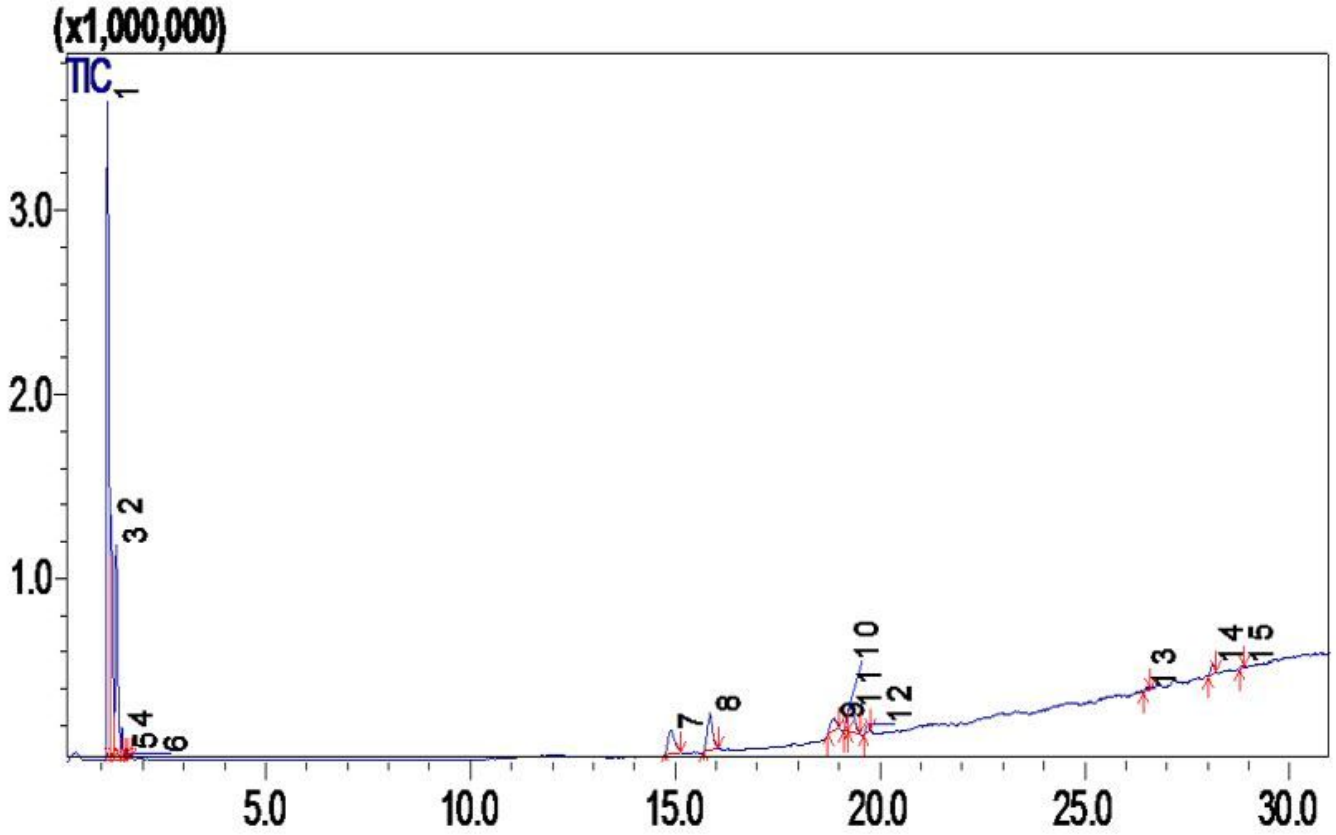


Figure 3

GCMS Chromatogram of Dodonaea viscosa flowers

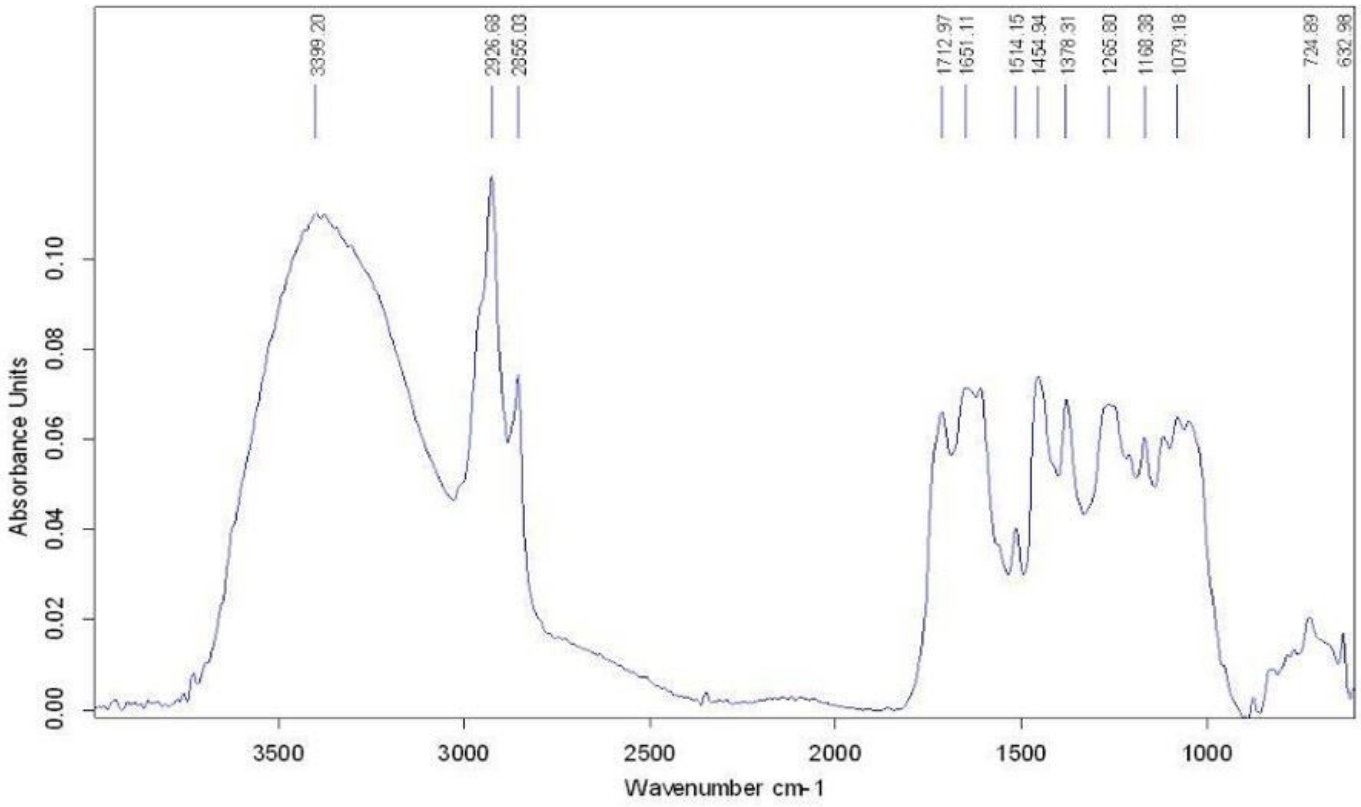


Figure 4

FT-IR analysis of Dodonaea viscosa flowers methanolic extract