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ISSN: 0975-7538
Research Article

Phytochemical screening and evaluation of hepatoprotective activity of *Mimusops elengi linn*., bark

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ABSTRACT

Liver diseases are one among the deadly diseases in the world today. Since modern medicines are less effective in treating the liver diseases and it is the plant preparations which are most commonly and effectively used for the treatment of hepatic diseases, there is a need to develop some novel, potent plant preparations for treating the same. With this objective our present study includes phytochemical screening and evaluation of *In-vivo* hepatoprotective activity of *Mimusops elengi* L bark. Here the powdered bark was subjected to successive Soxhlet extraction using different solvents of increasing polarity. The successive methanol and aqueous extracts were subjected for *In-vivo* hepatoprotective evaluation. The methanolic extract was found more effective. So it was further subjected for preliminary phytochemical and chromatographic examination. Attempt was made to isolate the compounds using isocratic elution technique. The isolated compounds was characterized by UV, FT-IR, ^{1}H-NMR,^{13}C-NMR and mass spectroscopy.

Keywords: Silymarin; carbon tetrachloride; hepatotoxicity; *Mimusops elengi* L bark; Sapotaceae

INTRODUCTION

Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base.

Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity.

Liver diseases remain one of the serious health problems (Epp J., 1986). Since, we do not have satisfactory hepatoprotective drugs in allopathic system of medicine for serious liver disorders, herbal drugs play a role in the management of various liver disorders, which act by fastening the natural healing processes of liver. Many medicinal plants and their preparations are used for liver disorder in ethno medical practices and also in traditional system of medicine in the world.

Mimusops elengi L. is an evergreen tree, belonging to family Sapotaceae, cultivated in gardens as an ornamental tree. It is popularly known as bakula or Spanish cherry or bullet wood. The bark is acrid and sweet in taste. The plant is wildly grown in the south India, Pakistan and Burma. It is one of the important medicinal plant since each and every part of the plant is used for curing many of the human diseases. The bark has shown anti-ulcer activity (Payal et al., 2003). It is also useful in treating high fever (Kirtikar K.R and Basu B.D., 1999 & Chopra R.N., et al, 2000), Diuretic activity (Koti.B.C and Purnima.A., 2010), calcium-channelblocking, antimicrobial (Mundhada S.S and Tatke P.A., 2005) anti-anxiety(Gayatri G., et al, 2011), antibacterial activity (Murudkar A., et al, 2007), anthelmintic activity (Mali R.G., et al, 2007), anti-hyperlipidemic activity (Ghaisas M.M., et al, 2008)

In traditional medicine, *Mimusops elengi* bark used for treating dental disease, uterine disorders, cardiac diseases and also as astringent, aphrodisiac (Kirtikar K.R and Basu B.D., 1999 & Yoganarashiman S.N., 1996). Although the *Mimusops elengi* L bark is traditionally used for treating liver diseases, it has not been investigated for hepatoprotective activity. Hence, this study was carried out to evaluate the potent bioactive consti-

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Received on: 21-09-2014
Revised on: 25-10-2014
Accepted on: 30-10-2014

tuents for hepatoprotective activity in *Mimusops elengi* L bark.

MATERIALS AND METHODS

The bark of *Mimusops elengi* L. were collected from the local areas of AshokaVan (Gokarn), Karnataka and were authenticated by Dr. B. D. Huddar, Head, Department of Botany, Shri Kadasiddheshwar Arts College and H. S. Kotambari Science Institute, Vidyanagar, Hubli (Annexure No:10.1). A voucher specimen (08PG356/ RVB/Ph-2010) has been deposited in the PG Pharmacognosy laboratory of KLES's college of pharmacy for future reference.

The physico-chemical parameters of Mimusops elengi linn., barkThe *Mimusops elengi* L. bark was shade dried at room temperature, pulverized and 200g of coarse powder was successively extracted with chloroform, methanol and water respectively in increasing order of polarity. The extracts were concentrated under reduced pressure using rotary flash evaporator and the residues were dried in dessicator. After drying, the respective extracts were weighed and percentage yield was calculated. All the extracts were further subjected for preliminary phytochemical investigations by qualitative chemical tests.

Hepatoprotective activity: (Prabhat Kumar Das., et al, 2008).

Carbontetrachloride induced hepatotoxicity model.

Chronic administration of carbon tetrachloride to rats induces severe disturbances of hepatic function together with histologically observable liver disturbances.

Hepatoprotective activity was carried out using male Albino rats (150-180gm). The animals are grouped into five of six animals each & maintained on standard diet & water ad libitum.

Group I: serve as normal control and received 1% Tween-80 (1ml/kg; p.o) daily for 7 days.

Group II: serve as toxic control and received CCl_4: olive oil (1:1, 2ml/kg of body wt; i.p.) on day 1 and day 7 of the experiment.

Group III: Animals treated with standard drug Liv-52 (5ml/kg; p.o) daily for 7 days and received CCl_4: olive oil (1:1, 2ml/kg of body wt; i.p.) on day 1 and day 7, 30 min after administration of Liv-52.

Table 1: Physico-chemical parameters of *Mimusops elengi* Linn., bark

Sl.No	Parameter	Determined Value % w/w
1	Alcohol soluble extractive value	22.00
2	Water soluble extractive value	28.00
3	Pet ether soluble extractive value	1.00
4	Moisture content	12.75
5	Total ash	6.5
6	Water soluble ash	1.00
7	Acid insoluble ash	1.5
8	Sulphated ash	4.5

Table 2: Percentage yield and physical characteristics of various extracts of *Mimusops elengi* L bark

Extract	% Dry weight in gms	Colour	Odour	Consistency
Chloroform	2.85	Brownish Black	Characteristic	Sticky mass
Methanol	23.9	Brown	Characteristic	Powder
Aqueous	3.35	Reddish Brown	Characteristic	Powder

Table 3: Physical characteristics of methanolic extract which was further fractionated with ethyl acetate

Fraction	% Dry weight in gms	Colour	Odour	Consistency
Ethyl acetate	4.00	Reddish Brown	Characteristic	Powder

Table 4: Preliminary phytochemical tests of various successive extracts of *Mimusops elengi* L.,

NATURE	CHLOROFORM	METHANOL	AQUEOUS	ETHYL ACETATE
Alkaloids	+ve	-ve	-ve	-ve
Steroids	+ve	-ve	-ve	-ve
Carbohydrates	-ve	-ve	-ve	-ve
Phenolic compounds	-ve	+ve	+ve	-ve
Flavonoids	-ve	+ve	-ve	+ve
Glycoside	-ve	-ve	-ve	-ve
Triterpenoid	+ve	-ve	-ve	-ve
Tannins	-ve	+ve	+ve	+ve

Note: +ve = Present; -ve = Absent

Table 5: Chemical tests of isolated Compound-A

Chemical Test	Observation	Inference
$FeCl_3$	Greenish precipitate	Flavonoids may be present
Shinoda Test	Pink Color	Flavonoids may be present
Zinc-HCl test	Red Color	Flavonoids may be present

Table 6: Physical parameters of isolated Compound-A

Parameters	Compound-A
Physical state	Solid
Colour	Brown
Odour	Characteristic
Solubility	Methanol, DMSO

Table 7: Effect of Methanol and Aqueous extracts on SGPT levels in hepatotoxic rats

Animals	Normal (N)	CCl_4 (C)	Standard (S)	Methanol (M)	Aqueous (A)
1	47.42	164.42	68.15	78.65	125.43
2	50.12	154.35	84.35	94.16	122.41
3	53.46	146.32	61.35	80.46	112.41
4	48.16	156.35	60.43	74.31	126.48
5	54.68	134.32	57.64	62.46	135.31
6	55.12	164.56	75.12	58.46	125.24
Mean	51.49	153.4	67.84***	74.75***	124.5**
SEM	3.369	11.57	10.26	12.97	7.385
SD	1.376	4.725	4.190	5.295	3.015

***P < 0.0001, **P < 0.001, when compared to CCl_4 control

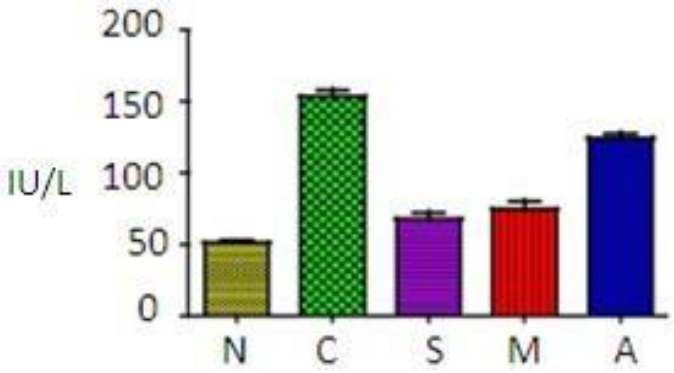

Figure 1: Effect of Methanol and Aqueous extracts on SGPT levels in hepatotoxic rats

Group IV: Animals treated with Successive Methanolic extract (200mg/kg b.w, p.o) daily for 7 days and received CCl_4: olive oil (1:1, 2ml/kg b.w, i.p.) on day 1 and day 7, 30 min after administration of extract.

Group V: Animals treated with Successive Aqueous extract (200mg/kg b.w, p.o) daily for 7 days and received CCl_4: olive oil (1:1, 2ml/kg b.w, i.p.) on day 1 and day 7, 30 min after administration of extract.

On the 8th day of the experiment, the animals were anaesthetized with mild ether and 1ml blood was collected into the Eppindrof tube by retro-orbital vein puncture. The blood collected in Eppindrof tube was centrifuged to separate serum and used for the estimation of various biochemical parameters like SGOT, SGPT, SALP and Bilirubin (total and direct). Livers were excised and fixed in formalin for assessment of Histopathological studies.

Assessment of liver function

Biochemical parameters such as Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Alkaline Phospatase (SALP) and bilirubin (total & direct) were determined according to the standard procedure prescribed by manufacturer (Transasia Biomedicals Ltd., Daman, India).

Estimation of Serum Alanine Transaminase (SALT)

SALT is also called as Serum Glutamate Pyruvate Transaminase (SGPT), which is located in the cytosol of the liver cell. During liver cell inflammation and break down of liver cells, they are released into circulation due to increased permeability of cell membrane. Hence determination of SALT is an index of the extent of liver damage. Its normal serum is 0-40 IU/L. Alanine amino transferase catalyses the transfer of the amino group from Alanine to α-ketoglutarate (α-KG) with the forma-

Table 8: Effect of Methanol and Aqueous extracts on SGOT levels in hepatotoxic rats

Animals	Normal (N)	CCl_4 (C)	Standard (S)	Methanol (M)	Aqueous (A)
1	64.67	165.31	87.23	106.36	124.14
2	55.57	176.26	91.65	85.64	134.56
3	57.74	174.26	86.15	98.68	104.23
4	62.17	184.21	78.69	101.45	126.25
5	58.43	176.25	82.13	112.81	117.31
6	56.12	165.14	94.58	88.47	121.24
Mean	59.12	173.6	86.74 ***	98.90 ***	121.3***
SEM	3.580	7.313	5.869	10.40	10.16
SD	1.462	2.986	2.396	4.244	4.146

***P < 0.0001, when compared to CCl_4 control

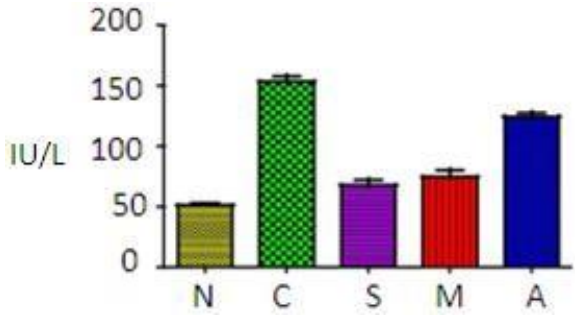

Figure 2: Effect of Methanol and Aqueous extracts on SGOT levels in hepatotoxic rats

Table 9: Effect of Methanol and Aqueous extracts on SALP levels in hepatotoxic rats

Animals	Normal (N)	CCl_4 (C)	Standard (S)	Methanol (M)	Aqueous (A)
1	146.15	346.14	167.01	198.16	214.64
2	138.23	421.35	156.46	194.35	248.34
3	158.16	384.68	178.16	187.64	201.46
4	162.14	425.47	164.58	201.65	224.64
5	159.46	412.06	182.15	227.16	253.18
6	147.25	304.31	177.16	175.14	204.13
Mean	151.9	382.3	170.9***	197.4***	224.4**
SEM	9.410	48.31	9.819	17.35	22.06
SD	3.842	19.72	4.009	7.081	9.007

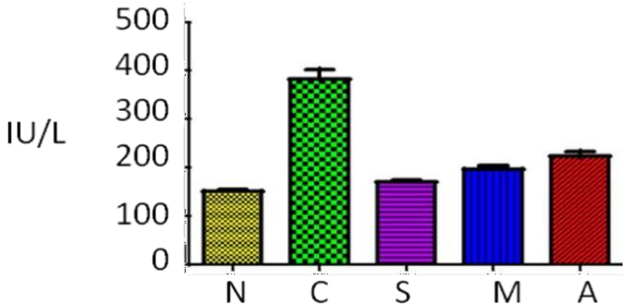

Figure 3: Effect of Methanol and Aqueous extracts on SALP levels in hepatotoxic rats

tion of Glutamate and Pyruvate. The liberated Pyruvate reduced to lactate, by lactate dehydrogenase (LDH) in the same reaction an equivalent amount of NADH is oxidized to NAD. Results are in Table 7.

The reaction can thus be written as;

$$L-Alanine + \alpha-ketoglutarate \xrightarrow{GPT} Pyruvate + L-glutamate$$

$$Pyruvate + NADH \xrightarrow{LDH} Lactate + NAD$$

Estimation of Serum Asparate amino transaminase (SAST)

SAST is located on the cytosol of liver. In addition, it is also found in the mitochondria and in many tissues heart, liver, skeletal muscle and kidney. The hepatic cell damage leads to increased level of SGOT in blood serum. Its normal serum is 5-34 IU/L.

Serum Asparate amino Transferase (AST) also known as Serum Glutamate Oxaloacetate Transaminase (SGOT) is

Table 10: Effect of Methanol and Aqueous extracts on Total Bilirubin levels in hepatotoxic rats

Animals	Normal (N)	CCl_4 (C)	Standard(S)	Methanol(M)	Aqueous (A)
1	0.07	3.12	1.12	1.43	2.45
2	0.14	3.46	0.77	2.01	3.01
3	0.21	4.64	0.84	2.12	2.14
4	0.15	4.31	1.05	0.85	2.16
5	0.16	3.42	1.13	1.56	3.05
6	0.18	4.71	1.41	2.13	3.41
Mean	0.1517	4.110	1.053	1.683	2.703
SEM	0.04708	0.8899	0.2295***	0.5041***	0.5273***
SD	0.01922	0.3633	0.09369	0.2058	0.2153

***P < 0.0001, when compared to CCl_4 control

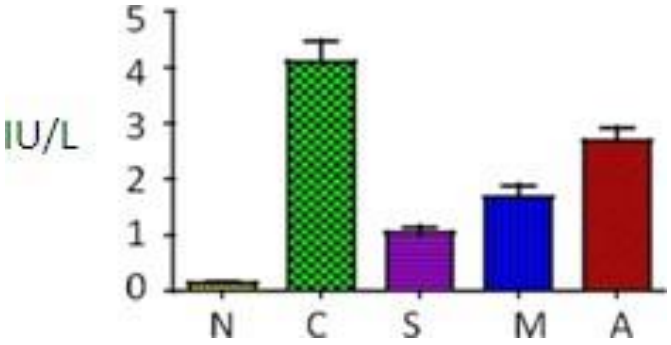

Figure 4: Effect of Methanol and Aqueous extracts on Total Bilirubin levels in hepatotoxic rats

Table 11: Effect of methanol and aqueous extracts on direct bilirubin levels in hepatotoxic rats

Animals	Normal (N)	CCl_4 (C)	Standard (S)	Methanol (M)	Aqueous (A)
1	0.084	0.41	0.06	0.16	0.27
2	0.051	0.74	0.10	0.24	0.34
3	0.074	0.64	0.14	0.34	0.36
4	0.064	0.35	0.08	0.42	0.37
5	0.056	0.42	0.21	0.31	0.21
6	0.070	0.57	0.23	0.17	0.32
Mean	0.0665	0.5217	0.1367***	0.2733***	0.3117**
SEM	0.01210	0.1525	0.07005	0.1019	0.06113
SD	0.004938	0.06226	0.02860	0.04161	0.02496

***P < 0.0001, **P < 0.001when compared to CCl_4 control

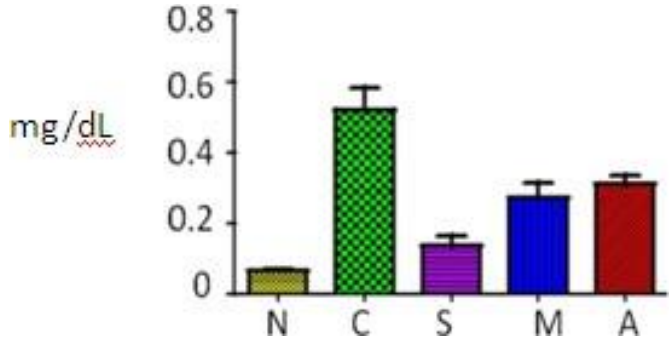

Figure 5: Effect of Methanol and Aqueous extracts on Direct Bilirubin levels in hepatotoxic rats

a tissue enzyme that catalyses the exchange of amino and keto groups between alpha amino acids. AST is widely distributed in tissue principally cardiac, hepatic, muscle and kidney, injury to these tissues result in increase in the AST (SGOT) enzyme level into general circulation. Hepatobilary diseases, such as cirrhosis, metastatic carcinoma and viral hepatitis will also increase serum AST levels. Results are in Table 8.

Estimation of Serum Alkaline Phospatase

Many tissues produce serum alkaline Phospatase, especially bone, liver, intestine and placenta and excreted in the bile. Most of the normal serum alkaline Phospatase (range 15 – 112 IU/L) is derived from bone. Elevation in activity of this can be found in the disease of bone, liver and in pregnancy. In the absence of bone disease and pregnancy, an elevated serum alkaline

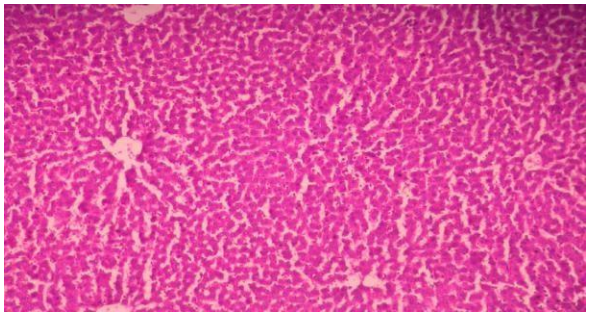

Figure 6: Photograph of normal liver biopsy (Normal group)

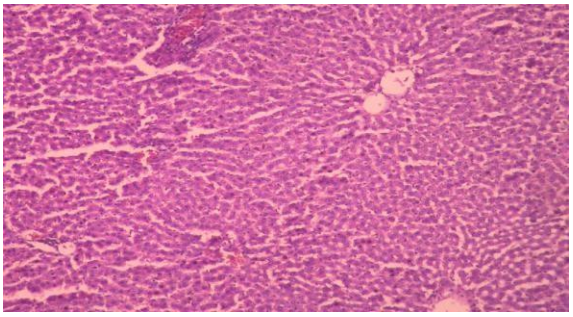

Figure 7: Photograph showing necrosis of hepatocytes (CCl4 group)

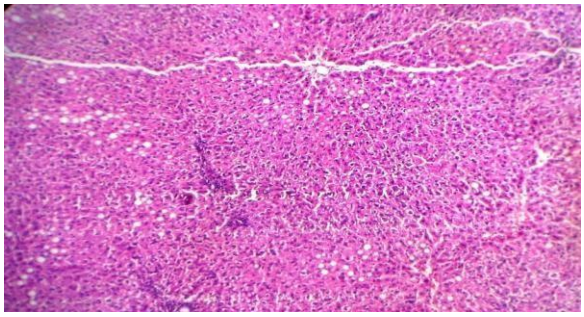

Figure 8: Photograph showing effect of liv-52 on regeneration of Hepatocytes

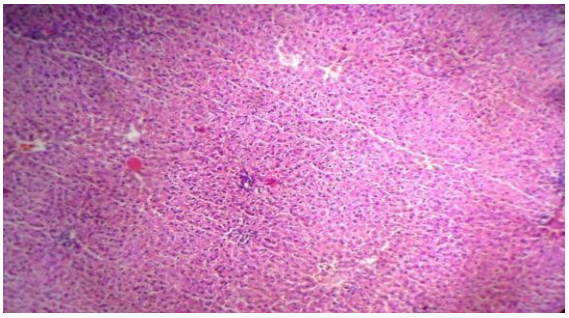

Figure 9: Photograph showing effect of Successive Methanol extract on

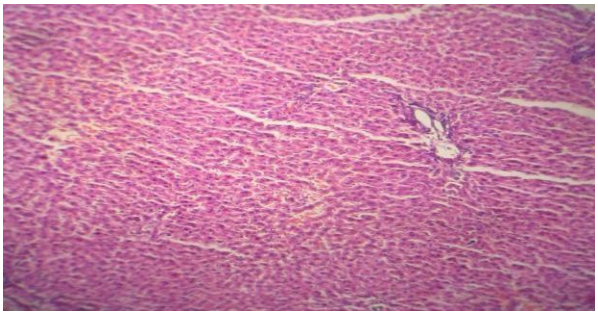

Figure 10: Photograph showing effect of Successive Aqueous extract on

Phospatase level generally reflects hepatobiliary disease. The greatest elevation (3 to 4 times normal) occurs in biliary tract obstruction. Slight to moderate increase is seen in parenchymal liver diseases such as in hepatitis and cirrhosis and in metastatic liver disease. Results are in Table 9.

Estimation of (Total and Direct) Bilirubin in blood serum

Bile is produced by the liver, stored in the gall bladder and secreted via biliary ducts into duodenum. Bile consists of bile salts and bile pigments. To understand the mechanisms underlying biliary pathology, jaundice will develop if bilirubin is excessively produced, or there is impaired hepatic uptake and conjugation of bilirubin or it is insufficiently excreted into the duodenum. Tests are employed to assess the synthesis and elimination of bilirubin pigment. Results are in Table no-10 and 11

After collecting the blood for the biochemical parameter the liver was isolated, washed with alcohol and preserved in 10% neutral formalin solution for histopathological studies.

Physico-chemical parameters of *Mimusops elengi* Linn., bark are as shown in Table 1. Percentage yield and physical characteristics of various extracts of *Mimusops elengi* L bark are as shown in Table 2. Physical characteristics of methanolic extract which was further fractionated with ethyl acetate are as shown in Table 3. Preliminary phytochemical tests of various extracts of *Mimusops elengi L.,* bark are as shown in Table 4. Chemical tests of isolated Compound-A are as shown in Table 5. Physical parameters of isolated Compound-A are as shown in Table 6.

The successive methanolic and aqueous extracts of bark of *Mimusops elengi* L. were evaluated for Hepatoprotective studies in Wistar Albino rats by CCl_4 induced hepatotoxicity.

Initially due to CCl_4 induced hepatotoxicity an increase in levels of SGPT, SGOT, SALP and Bilirubin (Total and Direct) in blood serum were observed. The results indicate that the elevated levels of SGPT, SGOT, SALP and Bilirubin (Total and Direct) in blood serum were significantly reduced in successive methanol and aqueous extracts. The reduction was significant in the following

range: successive methanol extract > successive aqueous extract.

Successive methanol extract treated group: sections from liver show minimal hepatocytes necrosis. Good number of binucleate regenerating hepatocytes are also seen. Successive aqueous extract treated group: liver section showed dense foci of necrosis. Hepatocytes showing fatty change and few regenerating hepatocytes are also seen.

From the above studies it is evident that successive methanolic and aqueous extracts of bark of *Mimusops elengi* L. plays a promising role in the treatment of Liver disease and worth for further investigations for isolation of more bioactive phytoconstituents for the same.

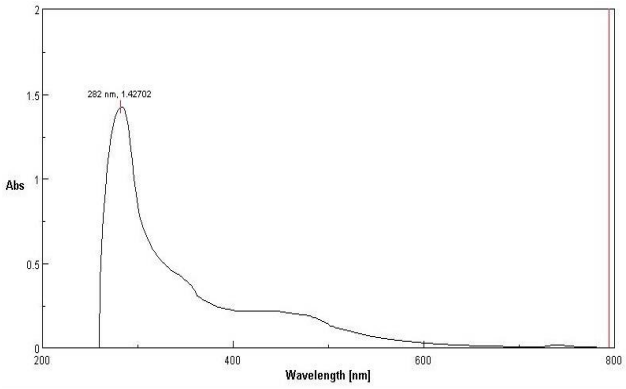

Figure 11: UV Spectra of Compound-A

CONCLUSION

Preliminary phytochemical investigations of chloroform, methanol, aqueous extracts and ethyl acetate soluble fraction of methanolic extract of *Mimusops elengi* L. bark have revealed the presence of alkaloids, steroids, triterpenoids, tannins, flavonoids and phenolic compounds.

The successive methanol and aqueous extracts exhibited more promising hepatoprotective activity at the dose of 200mg/kg body weight which is supported by histopathological data. Collectively these natural flavonoids and tannins of *M. elengi* barks are promising in this research. However further investigations are needed to give some more evidences to support this research.

The isolated COMP-A was characterized by UV (Figure 11), FT-IR, H^1-NMR, ^{13}C-NMR spectral data co-relates with member of natural flavonoids. The product of acid butanol reaction yields cyanidin. This confirms the proanthocyanidin presence in *Mimusops elengi* L. bark.

ACKNOWLEDGEMENT

The authors are thankful to the management and Principal, KLE's College of Pharmacy, Hubli and SET's College of Pharmacy, Dharwad, Karnataka, for providing necessary facilities to carry out this research work. We are also grateful to The Director, SAIF, Punjab University, Chandigarh and The Chairman, USIC, Karnataka University, Dharwad for providing NMR and Mass spectral data.

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