WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 03, 1623-1636. Research Article ISSN 2277–7105

PHYTOCHEMICAL SCREENING AND *IN VITRO* HEPATOPROTECTIVE ACTIVITY OF *IPOMOEA OBSCURA*

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Article Received on
20 Dec. 2017,

Revised on 10 Jan. 2018,
Accepted on 31 Jan. 2018

DOI: 10.20959/wjpr20183-10996

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ABSTRACT

The World Health Organization (WHO) estimates that 80% of the people of developing countries rely on traditional medicines, mostly plant derived drugs for their primary health needs. Medicinal and preventing specific ailments are considered to play a significant role in health care. The use of plants in traditional systems is an indispensable source of medicinal preparations. Hundreds of species recognized as having medicinal value. Indeed, 'phytomedicines ' to link traditional and modern medicines. In the present study to investigate the phytochemicals, histochemical, fluorescence, GCMS analysis and hepatoprotective activity of *Ipomoea obscura* leaves. The results of the present study concluded that *I. obscura* leaves may be a good source of phytochemicals, histochemical, fluorescence, GCMS. Over all the leaves of *I. obscura* is a source of phytochemicals and possess hepatoprotective activity that can be important in oxidative stress mediated diseases diabetic, cancer, arthritis etc.

KEYWORDS: *Ipomoea obscura*, phytochemicals, histochemical, fluorescence and GCMS.

INTRODUCTION

India is well-known for its rich traditional systems of medicine, i.e. Ayurvedic, siddha, Unani and Amchi (Tibetan) besides a vast reservoir of living traditional of ethnomedicine. In India systems medicine, generally the medicines of animal origin, due to the presence of abundant natural flora. The basic concept of disease prevention has existed in the ancient Vedic scripture and has been practiced in Indian traditional medicine, the Ayurveda, for many

centuries. The emphasis on the maintenance of positive health or Swastha Vrutta,is a distinguishing feature of positive health or Swastha Vrutta, is a distinguishing of Ayurveda. The World Health Organization (WHO) estimates that 80% of the people of developing countries rely on traditional medicines, mostly plant derived drugs for their primary health needs. Medicinal and preventing specific ailments are considered to play a significant role in health care. The use of plants in traditional systems is an indispensable source of medicinal preparations. Hundreds of species recognized as having medicinal value. Indeed, 'phytomedicines ' to link traditional and modern medicines (Finar,1975).

Owing to the global trend towards improved 'quality of life, there is considerable evidence of an increase in demand for medicinal plant. Use of plants for treating various ailments is as old practice as man himself. India ia richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk remainds or indirectly as pharmaceutical preparation of modern medicine. In recent times focus on plant research has increased all over the world and a large body of evidence has collected to show immence potential of medicinal plants used in various traditional systems (Ayurveds, Siddha and unani). Medicinal plants are a major source of biodynamic compounds of therapeutic values (IWU and Chiori, 1986).

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well documented uses of plantproducts is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent (Ward and Daly, 1999). In the present study to investigate the phytochemicals, histochemical, fluorescence, GCMS analysis and hepatoprotective activity of *Ipomoea obscura* leaves

MATERIALS AND METHODS

Collection and preparation of alcoholic extract

The leaves of *Ipomoea obscura* were collected from kadukaval, Thanjavur district of Tamil nadu, India. The plants were identified with the help of flora of karnatic TamilNadu. The leaves of *I.obscura* washed well in order to remove dust. The leaves were dried at room

temperature and coarsely powdered. The powder was extracted with aqueous and 70% methanol for 24 hours. The extract was stored in refrigerator until used.

Phytochemical screening

Phytochemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Quantitative analysis of phytochemicals

Total phenols estimated by the method of Edeoga *et al.*, (2005), Alkaloid determine by the method of Harborne (1973), Tannin determination by method of Van-Burden and Robinson (1981), Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994) and Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

Histochemical tests

The powder of leaf I. obscura leaf was treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope. The I.obscura leaf treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H2SO4 gave yellow colour indicates flavonoids and treated with Dragant draft reagent gave brown colour indicates alkaloids.

Determination of Fluorescence behaviuor of plant powder (Rao et al., 2011)

Fluorescence analysis of entire root of *I.obscura* has been carried out in daylight and under U.V light. Fluorescence analysis of leaf powder of *I.obscura* leaf was carried out by the treatment of different chemical reagents such as methanol, H2SO4, HCl, HNO3, NaOH, acetone, hexane, chloroform and distilled water. The powders were observed in normal daylight and under short (245nm) and long U.V. light (365 nm).

GC-MS Analysis

Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dukes, 2013). The relative percentage amount of each component was

calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan et al., 2013).

In vitro hepatoprotective activity

Experimental Design: Five different groups taken for the study.

Group I : Normal.

Group II : Carbon tetrachloride treated alone.

Group III : Carbon tetrachloride + 100mg leaves of *I.obscur* treated.

Group IV : Carbon tetrachloride + 250mg leaves of *I. obscura* treated.

Group V : Carbon tetrachloride + 500mg leaves of *I.obscura* treated.

Biochemical estimations

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). The GOT and GPT were estimated by the method of Reitman and Frankel (1957). Reduced glutathione was estimated by method of Moron *et al*.,(1979). Copper-zinc superoxide dismutase activity was determined by the procedure of Kakkar et al. (1984) in plasma. The activity of catalase was assayed by the method of Beers and Sizer (1952).

Statistical Analysis

The results were presented as Mean ± SD. Data was statistically analyzed using student "t" test. P.values set as lower than 0.05 were considered as statistically significant.

RESULTS

Quantitative phytochemical analysis

The qualitative phytochemical screening of the leaves of *Ipomoea obscura* is given in Table 1 tannin, phlobatannins, saponin, steroids, terpenoids, alkaloids, protein, polyphenol and glycoside were further confirmed by histochemical analysis Table 2 & Plate.1. Triterpenes, carbohydrate and anthroquinone were absent in aqueous extracts. Fluorescence behaviour of the powdered leaves were given in Table 3.

Table 1: Phytochemical screening of the leaves of Ipomoea *obscura.*

S.No	Phytochemical analysis	Aqueous	70% Methanol	Quantitative analysis (mg/gm)
1	Tannin	+	+	18
2	Phlobatannins	+	-	---
3	Saponin	+	+	---
4	Flavonoids	+	+	25
5	Steroids	++	+	---
6	Terpenoids	+	++	---
7	Tritrpenoids	-	+	---
8	Alkaloids	+	+	30
9	Carbohydrate	-	+	---
10	Protein	+	+	---
11	Anthroquinone	-	+	---
12	Polyphenol	+	+	100
13	Glycoside	+	+	---

(+) Presence (-) Absence

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *I.obscura* leaf plant investigated and summarized in Table-1. The phytochemical screening aqueous extract of leaves of *I.obscura* leaf showed that the presence of tannin, carbohydrate, saponins, terepenoids, phenolics, phlobatannins, and protein Flavonoids, alkaloids, glycosides and steroids while triterpenoids, carbohydrate and anthriquinone were absent. Methanol extract of *Ipomoea obscura* leaf showed that the presence of alkaloids, steroids, saponins, triterpenoids, phenolics, anthriquinone, glycosides flavonoids, tannin, terepenoids, carbohydrate and protein while phlobatannins was absent. Significant amount of Flavonoids (25 mg/gm), terpenoids (20mg/gm), alkaloids (30mg/gm), tannin (18mg/gm) and phenol (100 mg/gm) present.

Falodun *etal*., (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla*. Raghavendra *etal*., (2006) examined the powdered flower material of different solvent of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins. Awoyinka *etal*., (2007) extracted eight bioactive compounds from dry flower of *Cnidoscolus aconitifolius* using water and ethanol. Different extracts of *Semecarpus anacardium* were analysed by Mohanta *etal.*, (2007) for its phytochemical properties.

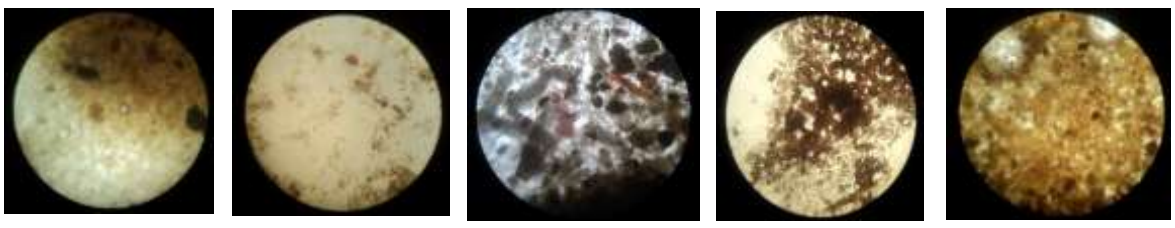
Histochemical studies

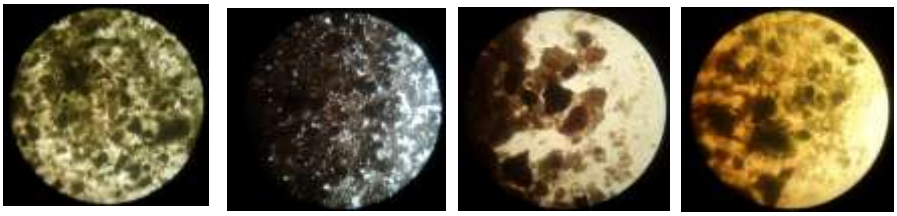
Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues (Krishnamurthy, 1998). Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron (Krishnan *et al*., 2001). The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. Histochemical studies of *I.obscura* leaf powder. Shows the presence of phytochemicals like lignin, flavonoids, alkaloids, tannin. In the present work histochemical study shows the presents of lignin, flavonoid, alkaloids and tannins in the leaves of I.obscura.

Table 2: Histochemical studies of powdered leaves of *Ipomoea obscura.*

S.No.	Secondary metabolites	Chemical reaction	Observation	Result
1	Lignin	Phloroglucinol + concentrate Hcl	Red/Pink	+
2	Flavonoids	Dilute Ammonia + H_2SO_4	Yellow	+
3	Alkaloids	Mayers Reagent	Reddish Brown	+
4	Tannin	$Fecl_3$ Solution	Dark Blue to Black	+
5	Starch grain	Iodine	Blue	+
6	Steroids	Liberman (5 drops of acetic anhydride + 5 drops of H_2SO_4	Violet to Blue (or) Green	+
7	Poly phenol	Toludine blue	Blue green/Red	+
8	Terpenoids	Dinitrophenol hydrazine (few drops)	Orange	+
9	Saponin	$H_2S\ O_4$ (few drops)	Yellow	+

(+) Presence ; (-) Absence





Lignin, Flavonoids, Alkaloids, Tannin, Starch grain, Steroids, Poly phenol, Terpenoids and Saponin

Plate: 1 Histochemical studies of the powdered leaves of *Ipomoea obscura.*

Fluorescence behavior

Fluorescence is the phenomenon exhibited by various chemical constituents present in the *Ipomoea obscura* leaf. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many products, which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Kokashi *et al*., 1957).

Table 3: Fluorescence analysis of powdered leaves of *Ipomoea obscura.*

S.NO	Test	Visible Light	Short UV
1	Powdered leaf (pp)	Green	Green
2	PP + water	Light Green	Green
3	PP + Hexane	Green	Green
4	PP +Chloroform	Green	Green
5	PP + Methanol	Light Green	Green
6	PP + acetone	Green	Green
7	PP + IN Sodium hydroxide in water	Dark Green	Dark Green
8	PP + IN Hydrochloric acid	Light Green	Green
9	PP + 50% H_2so_4	Yellow	Light Green
10	PP + Nitric acid	Dark Green	Pale Green

Identification of bioactive compounds in *Ipomoea obscura* leaves extract by GC MS analysis

The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results.

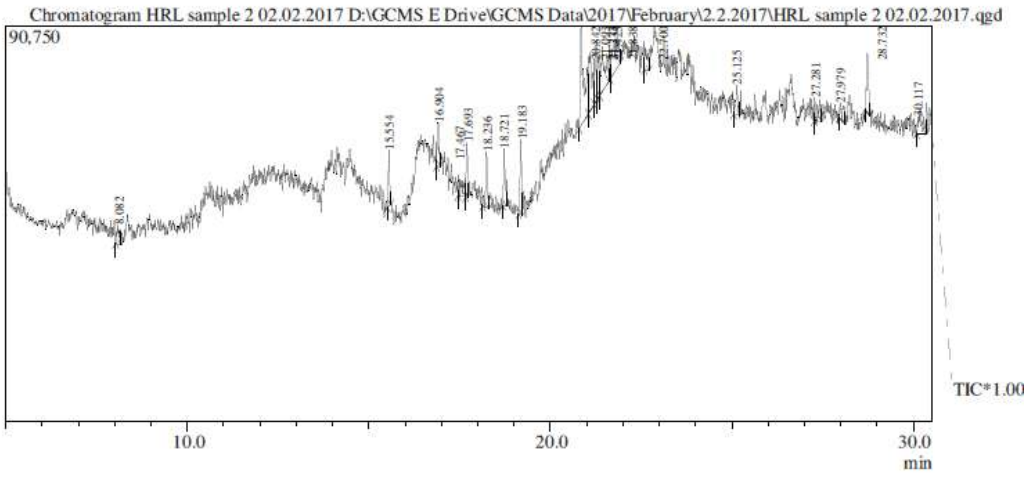


Fig 1: Chromatogram of the leaves of *Ipomoea obscura.*

Table 4: Identification of active compounds in the leaves of *Ipomoea obscura* using GCMS.

Peak	R.Time	Area %	Height %	Molecular Formula	Name	Molecular Weight
1	8.082	2.33	2.15	$C_3H_4O_4$	Propanedioic acid	104
2	15.554	3.25	6.36	$C_{18}H_{16}N_2O_3S$	(2-Phenyl-1,3-dioxolan-2-yl)methy	340
3	16.904	3.36	5.06	$C_{19}H_{32}O_2S$	2-Thiopheneacetic acid, 2-tridecyl	324
4	17.467	3.44	2.36	$C_{15}H_{24}O_2Si$	Benzenepropanoic acid, tert-butyldimethylsilyl	264
5	17.693	3.36	6.19	$C_4H_{10}O_4$	dl-Threitol	122
6	18.236	3.90	6.43	$C_{10}H_{20}O$	3,7-Dimethyl-7-octen-1-ol	156
7	18.721	4.07	6.56	$C_8H_{17}Br$	2-Bromo-6-methylheptane	192
8	19.183	6.15	8.59	$C_{20}H_{40}O_2$	Nonadecanoic acid, methyl ester	312
9	20.842	15.46	11.05	$C_{36}H_{75}O_3P$	Phosphonic acid, dioctadecyl ester	586
10	21.093	9.34	6.69	$C_9H_7NO_2S$	3,4-Methylenedioxybenzyl isothiocya	193
11	21.273	5.51	6.95	$C_7H_{14}O_5$	.alpha.-L-Galactopyranoside, methyl 6	178
12	21.333	3.21	4.68	$C_4H_{10}O_4$	Butane-1,2,3,4-tetraol	122
13	21.425	8.29	5.21	$C_4H_9NO_2$	L-Alanine, N-methyl-	103
14	21.838	8.60	3.45	$C_6H_{14}O_2$	2,2-Dimethyl-1,3-butanediol	118
15	22.700	2.53	1.84	$C_5H_{10}O_2$	3-Methoxy-2,2-dimethyloxirane	102
16	25.125	2.74	3.41	$C_8H_{22}OSSi_2$	3-Oxa-6-thia-2,7-disilaoctane, 2,2,7,7-	222
17	27.281	2.69	2.60	$C_{10}H_{24}O_4Si_2$	1,4-Dioxane-2,3-diol, bis(trimethylsily	264
18	27.979	2.17	1.68	$C_{24}H_{45}N_3O_{12}$	2,4,6-Tris(1,4,7,10-tetraoxaundecyl)	567
19	28.732	5.57	6.84	$C_{10}H_{20}O_3$	Carbonic acid, isobutyl 2-methylbutyl	188
20	30.117	4.03	1.90	$C_4H_6O_3$	Butanoic acid, 2-oxo-	102

Table 5: Biological activity of selected compounds present in the leaves of *I.obscura.*

S.No	R.Time	Area %	Name	Biological activity
1.	15.554	3.25	(2-Phenyl-1,3-dioxolan-2-yl)methy	Antimicrobial activity, anti-inflammatory
2.	16.904	3.36	2-Thiopheneacetic acid, 2-tridecyl ester	Antimicrobial activity
3.	17.693	3.36	dl-Threitol	Insecticidal activity
4.	19.183	6.15	Nonadecanoic acid, methyl ester	Antimicrobial, anti inflammatory, anti tumor, anti hyperpigmentative, anti proliferative, anti acne, cytotoxic, Anti leukemic, oxy radical scavenging activity
5.	21.838	8.60	2,2-Dimethyl-1,3-butanediol	Pesticide, Antrepellent, Nematicide
6.	22.700	2.53	3-Methoxy-2,2-dimethyloxirane	Nematicide, Pesticide
7.	25.125	2.74	3-Oxa-6-thia-2,7-disilaoctane, 2,2,7,7-tetramethyl	Nematicide, Antialopecic
8.	27.281	2.69	1,4-Dioxane-2,3-diol, bis(trimethylsilyl) ether	Nematicide, Insectifuge Antihistaminic, Antieczemic

***In vitro* hepatoprotective activity**

The present study was carried out to evaluate the *In vitro* antioxidant activity of *Ipomoea obscura* leaf on CCl_4 induced hepatotoxicity. The observations made on different groups of experimental and control animals were compared as follows.

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning complementary and alternative medicine (CAM). Many traditional remedies employ herbal drugs for the treatment of liver ailments (Wolf, 1999). To the best of our knowledge, there is no scientific report available in support of the hepatoprotective activity of *I.Obscura* leaf. Hence, to justify the herbal claims we have evaluated the hepatoprotective effects of *I.Obscura* leaf on CCl_4 exposed hepatotoxicity in hepatocyte. The hepatoprotective activity of the plant reported in this study would provide scientific evidence of its claimed medicinal properties.

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce

trichloromethyl free radical ($^{\bullet}CCl_3$). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloro methylperoxyl free radical leads to initiate the process of lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death (De Groot and Noll, 1986; Clawson, 1989). These result in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver damage (Recknagel and Glende, 1973; Reckengel *et al*., 1991). MDA is a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions. Hepatotoxic compounds like CCl_4 are known to cause marked elevation in SOD, CAT and GPx activities. In the present study, treatment with *I. obscura* has increased content of MDA and activities of SOD, CAT and GPx in hepatocyte. The antioxidant acitivity of *I.obscura* leaf extract was dose dependent. The highest dose has 500mg potential antioxidant activity.

In the assessment of liver damage by carbon tetrachloride, the determination of enzyme activities such as aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) is largely used. Activities of AST and ALT are the most frequently utilized indicators of hepatocellular injury. Necrosis or membrane damage releases the enzymes into circulation; and therefore, they can be measured in hepatocyte. Elevated levels of hepatocyte enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver (Wolf, 1999). The mechanism by which transaminase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions and the other hypothesis is that the damaged liver fails to excrete transaminase made in the liver (Thapa and Walia, 2007). Total protein level on other hand, are related to the function of hepatic cells i.e they reveal the functional status of the hepatic cells. Decreased levels of total protein and albumin are indicative of the failure of the biosynthetic function of the hepatocyte (Crawford, 2004).

Table 6: Effect of *Ipomoea obscura* leaves on MDA and Protein in experimental hepatocyte.

Groups	MDA (nmole/dl)	SOD(U/dl)	CAT(U/dl)	GPx (U/dl)
I	0.90±0.45	0.82±0.26	13.65±1.13	0.93±0.34
II	4.39±0.69	1.37±0.16	18.41±0.98	2.26±0.39
III	2.72±0.32	1.01±0.05	15.82±2.04	1.19±0.25
IV	0.90±0.45	0.90±0.10	14.08±2.63	1.06±0.45
V	0.87±0.73	0.88±0.16	13.17±0.99	0.79±1.32

Values were expressed as mean ± SD for triplicate in each group.

Table 7: Effect of leaves of *I.obscura* leaves on GOT and GPT activities in experimental hepatocyte.

Groups	GOT (IU/L)	GPT (IU/L)	GSH (mg/dl)
I	15.17±18.97	49.25±3.23	0.47±0.07
II	55.01±2.39	61.18±3.23	0.25±0.1
III	44.65±2.48	48.21±1.55	0.57±0.04
IV	40.24±1.93	39.92±2.37	0.43±0.21
V	37.84±2.48	27.48±3.91	0.58±0.10

Values were expressed as mean ± SD for triplicate in each group

Group-I **Group-II** **Group-III** **Group-IV** **Group-V**

Plate. 2: In vitro hepattoprotectiive activity of leaves of *I. obscura.*

CONCLUSION

The results of the present study concluded that *I. obscura* leaves may be a good source of phytochemicals, histochemical, fluorescence and GCMS. Over all the leaves of *I.obscura* is a source of phytochemicals and possess hepatoprotective activity that can be important in oxidative stress mediated diseases diabetic, cancer, arthritis etc.

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