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Isolation and Characterization of Bioactive Moiety from Leaf Extract of *Ipomoea mauritiana* and Evaluation of Anti-Inflammatory Activity by Carrageenan Induced Rat Paw Edema Test

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Abstract

Ipomoea mauritiana Jacq. is a member of family Convolvulaceae and commonly called as Ksheervidari is widely used in various traditional medicines for the treatment of many diseases. In the present work the column fractions of leaf extracts of I. mauritiana obtained by Soxhlet exrtaction was subjected to TLC, column chromatography, HPLC and Liquid Chromatography and Mass Spectroscopy (LC-MS). The compounds like caffeic acid and β-amyric acetate were tentatively identified to be present in one of the purified fraction. The column fraction was also tested for anti-inflammatory activity by carrageenan induced rat paw edema assay. The column fraction tested showed a very good anti inflammatory activity.

Keywords: Anti Inflammatory Activity, Carrageenan Induced Rat Paw Edema Column, Chromatography, *Ipomoea Mauritiana*

1. Introduction

Ipomoea belongs to the family Convolvulaceae including about 40 genera and 1000 species and they are found in all the warmer parts of the world but well developed mostly in tropical Asia, Africa, Australia and America. The genus *Ipomoea* is characterized by herbaceous nature and climbing habit in majority of the species. As far as the species available in Indian sub-continent is concerned, except *I. aquatica* Forsk all others are typical climbing plants and mostly are cultivated for showy flowers[1].

Ipomoea mauritiana Jacq is a member of family Convolvulaceae and called as Ksheervidari, Payasvini, Bhui-Khola, Bhumi-Kumra, Bhumi-kushmanda in various languages. Various species of *Ipomoea* have been extensively used in local traditional medicine in many countries for the treatment of several diseases[2].

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Plant extracts have been used since ancient times for the treatment of many diseases in many of our traditional medicinal methods. The plant extracts often contain many numbers of plant secondary metabolites, which make the plant extract a complex mixture[3]. These chemicals isolated from plants have been the source of many present day drugs.

The genus *Ipomoea* has been used for various purposes, such as, nutritional, medicinal, ritual and agricultural since time immemorial[4]. Different species of *Ipomoea* have been used extensively for the treatment of several diseases in the traditional medicine. One of the most common use is of the roots of *Ipomoea* species which is used I the treatment of constipation[4].

Ipomoea mauritiana also called as vidari is useful as diuretic, cardiotonic, demulcent, aphrodisiac and galactogogue[5]. In traditional medicine it is also used in the treatement of enteric fever and spermatorrhea[6].

2. Material and Methods

2.1 Preparation of the Plant Extract

Plant selected for this study, *Ipomoea mauritiana* is based on its traditional medicinal use. The plant material is cleaned thoroughly and shade dried material are cut into small pieces and powdered in a grinder. The plant material (500g) is extracted with methanol using Soxhlet apparatus for 24h at a temperature not exceeding the boiling point of the respective solvent. The obtained extract is filtered by using Whatmann No. 1 filter paper and then concentrated under vacuum at 400°C by using a rotary evaporator.

2.2 Column Chromatography

The column chromatography was performed to elute out individual components from the crude plant extract. The column was washed cleanly and then rinsed with hexane. It was completely dried before use. The column was packed with silica gel (60-120 mesh size). The silica gel was activated by heating before packing into the column. The silica gel was poured gently on the top of the column with constant tapping to avoid air bubbles and cracks after mixing with hexane. Methanolic extract which was evaporated to dryness was re-dissolved in methanol. It was subjected to chromatography on silica gel (60–120 mesh, Merck) eluted with hexane and Methanol:Chloroform (7:3) solvent system. Different fractions (5mL) was collected and based on preliminary TLC analysis pooled in to two major fractions.

2.3 Thin Layer Chromatography (TLC)

Thin Layer chromatographyis one of the most commonly used technique for separation of mixture of plant compounds. The TLC plate (Merck, Germany - T LC Silicagel 60F254) was used to observe the separation of individual compounds as a from the selected crude extract. The extract was filtered through 0.45 micrometer filter and diluted to 10mg/mL. 5 µl of sample was used for TLC analysis. The solvent system used for the separation was Toluene:Ethyl acetate: Glacial acetic acid in the ratio 6.5:3.5:0.2 respectively.

2.4 High Profile Liquid Chromatography (HPLC)

HPLC profiling of the column fraction 1 and 2 was done using a Shimadzhu LC- Prominence 20AT system. The mobile phase used for the separation was HPLC grade Water (A) and HPLC grade Acetonitrile (B) in the ratio of 60: 40 (V/V). The column used C18 column (250 mm x 4.6 mm, 5u particle). The injection volumes of the samples were 10 µL. The flow rate was maintained to 1 mL/min for a total run time of 15 minutes. The PDA signal was recorded at 320 nm.

2.5 Liquid Chromatography - Mass Spectrophotometry (LC-MS)

The column fraction number 2 (1µg/mL) was taken for the LC-MS analysis. The MS analysis was performed using ESI in the positive mode. The MS analysis was carried out using TSQ Quantum Access MAX Triple-Stage Quadrupole Mass Spectrometer. The mass spectrometry parameters were: curtain gas 10, gas1 20 and gas2 0, needle voltage 5000 V, and declustering potential 100 V.

2.6 Carrageenan-Induced Rat Paw Edema Test

Animals for the present research work were obtained from animal house from University of Mysore. Institutional animal ethics committee approval was

obtained for carrying out the present research work (UOM/IAEC/06/2016). The Carrageenan-induced rat paw edema test was conducted according to method based on previous study[7]. 0.1 ml carrageenan was injected into the sub-plantar region of the left hind-paw of each rat. Edema was observed in the paw of rats soon after the injection of carrageenan. The rats were then randomly divided into six groups of six (n = 6) rats each. The *Ipomoea* plant extracts of concentrations 25, 50, 75 and 100 mg/kg were injected intraperitoneally to the first four groups of rats and standard drug indomethacin 10 mg/kg (positive control) was injected intraperitoneally to the fifth group of rats one hour before the injection of carrageenan. The sixth group of rats was kept as negative control. The rats were then observed for the reduction in edema every one hour for six hours.

2.7 Histopathology of Liver and Kidney

The animals were sacrificed for the histopathological studies since it is the golden standard for evaluating treatment related pathological changes in tissues and organs. Kidneys and liver samples were isolated from each individual and dehydrated by serial ethanol solution. They were then enclosed with paraffin wax. Micrometer sections (5 µm) cut were taken using microtome and were then stained with hematoxylin and eosin. They were then examined under a light microscope, photographs of the samples were recorded.

The microscopic images of the sections of liver and kidney (Figure 8 and 9) show very few differences between the control and test group. The microscopic examination revealed that liver and kidney from the extract treated rats did not show considerable alteration in cells structure when viewed under the light microscope under high magnification power. Samples of organs isolated from each individual were dehydrated by serial ethanol solution and enclosed with paraffin wax. Micrometer sections (5 µm) were stained with hematoxylin and eosin and were examined under a light microscope and photographs of the samples were recorded.

2.8 Statistics

The anti-inflammatory activity of *Ipomoea mauritiana* tested by Carrageenan induced rat paw edema assay was analyzed for the statistical significance by Cramer's V method.

3. Results

Ipomoea is one of the common medicinal plant used in many traditional medicinal preparations. In the present research work, the leaves of *Ipomoea mauritiana* were powdered and extracted using Soxhlet extraction. The extracts obtained were further subjected to column chromatography. The fractions similar in pigmentation were pooled and were tested for antibacterial activity against uropathogens like *Escherischia coli*, *Klebsiella* Sp, *Proteus mirabilis* and *Enterococcus faecalis*. The column fractions which showed the bioactivity against these pathogens were selected and subjected to preliminary thin layer chromatography (Figure 1) and the rf values were noted (Table 1). Two column fractions which were selected were further analysed by HPLC (Table 2). The HPLC results showed that the column fraction 1 showed four major peaks whereas column fraction 2 showed only two major peaks (Figure 2 and Figure 3). The column fraction 2 was further analyzed by LC-MS in positive mode where compounds with masses of 148.77, 165.08, 180.96, 217.98, 297.12, 327.28, 413.14, 469.26, 521.67, 611.7, 716.76, 749.72, 797.58, 843.62, 889.32, 917.45, 928.22, 976.79 were obtained (Figure 4 and Figure 5), of which the compounds with most abundant mass found were 180.96 and 928.22 (Table 3). Based on the previous works and the current research work, two compounds with molecular mass of 180.15 and 469.75 were tentatively identified as Caffeic acid and β-amyrin acetate respectively.

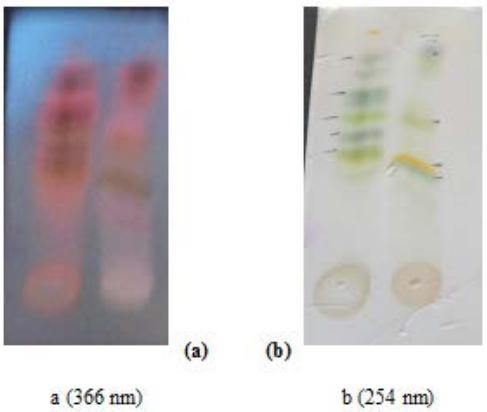


Fig. 1. TLC chromatogram of purified fraction. **(a)** Fraction 1. **(b)** Fraction 2.

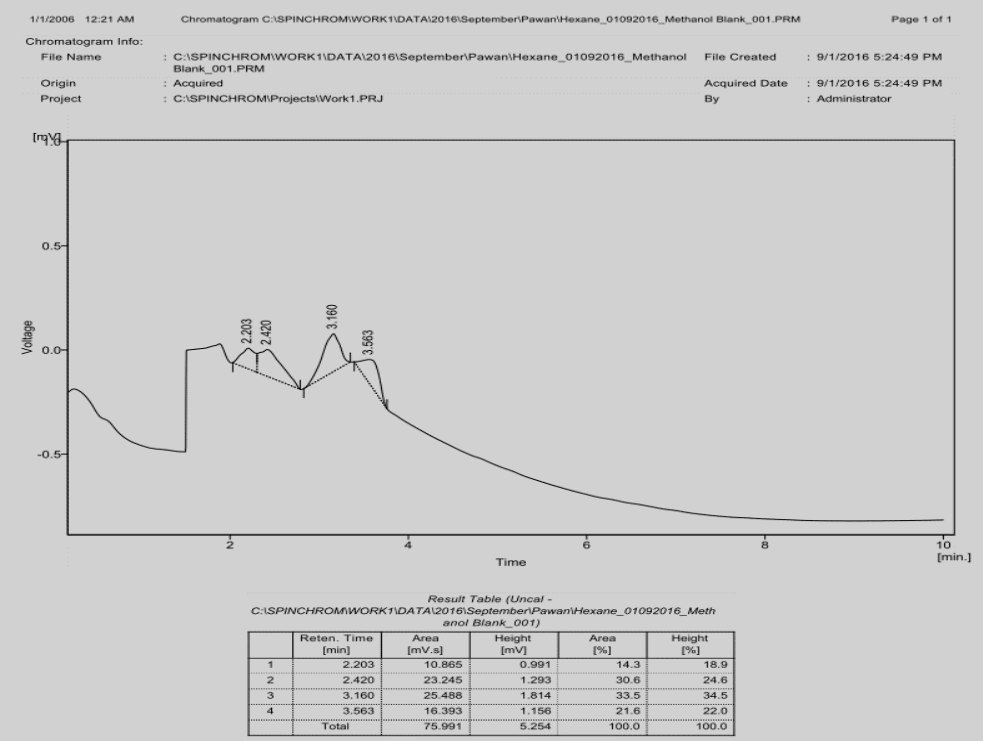


Fig. 2. HPLC sample of purified fraction no 1.

Table 1: TLC characteristics of column fractions

Sample code	TLC Band	Retention Factor	TLC Profile characteristics	
			Shortwave UV 254 nm (Figure 1b)	Longwave UV 366 nm (Figure 1a)
Fraction 1	1	0.3	Green	Pink
	2	0.4	Yellow	Red
	3	0.6	Light Green	Red
	4	0.9	Dark Green	Greenish yellow
Fraction 2	1	0.5	Dark Green	Brown
	2	0.6	Blue	Brown
	3	0.7	Green	Brown
	4	0.8	Dark Green	Brown

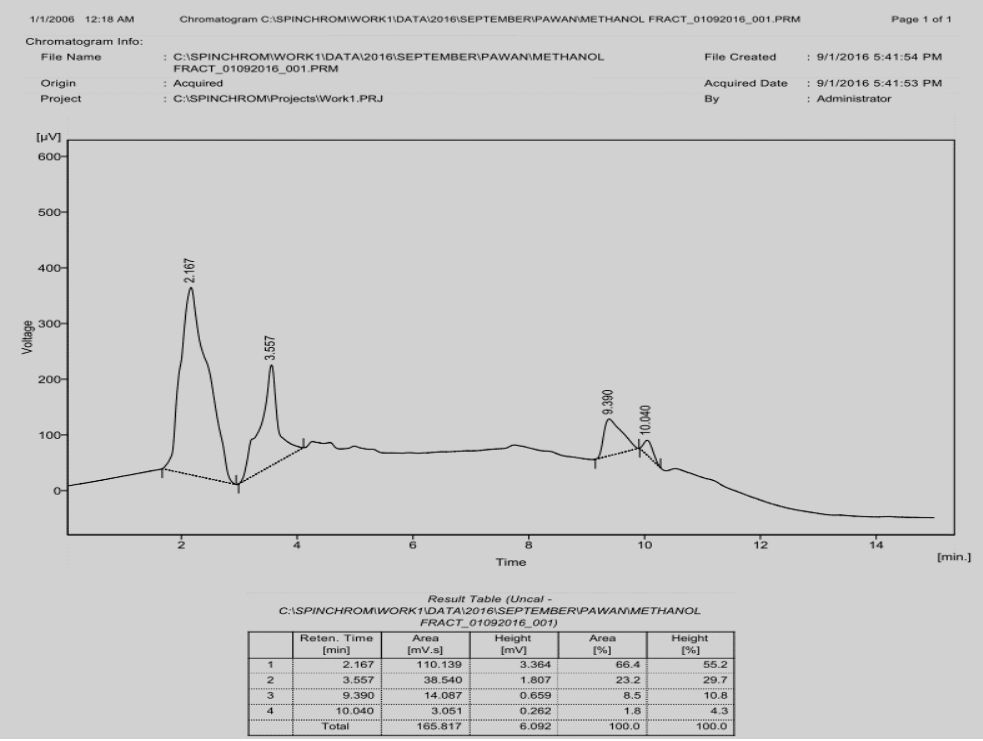
Table 2: HPLC summary of column fractions

Sl. No	Sample	Major Peaks	Retention Times (min)	% Area	Chromatogram reference
1	Pooled Fraction 1	4	2.203	14.3	Fig. 2
			2.403	30.6	
			3.160	33.5	
			3.563	21.6	
2	Pooled Fraction 2	2	2.167	66.4	Fig. 3
			3.557	23.2	

Table 3: Ion mass detected in positive mode

Retention Time	Positive Mode $[M+H]^{+1}$ Ion Mass	Chromatogram Reference	Compounds identified	
			M.W	Compound
2.50	148.77, 165.08, **180.96,** 217.98, 297.12, 327.28, 413.14, 469.26, 521.67, 611.7, 716.76, 749.72, 797.58, 843.62, 889.32, 917.45, **928.22**, 976.79	Fig.4 & 5	180.15	Caffeic acid
			469.75	Beta Amyrin acetate.

Note: Abundant mass highlighted



Result Table (Uncal - C:\SPINCHROM\WORK1\DATA\2016\SEPTEMBER\PAWAN\METHANOL FRACT_01092016_001)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	2.167	110.139	3.364	66.4	55.2
2	3.557	38.540	1.807	23.2	29.7
3	9.390	14.087	0.659	8.5	10.8
4	10.040	3.051	0.262	1.8	4.3
	Total	165.817	6.092	100.0	100.0

Fig. 3. HPLC sample of column fraction no. 2.

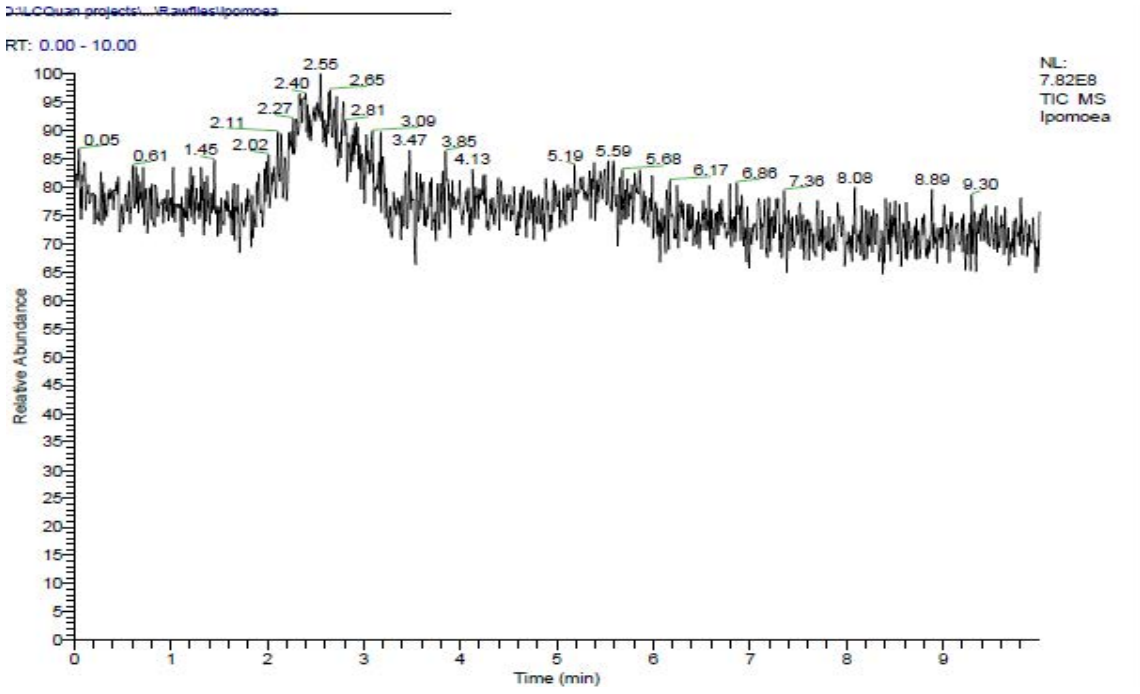


Fig. 4. Total ion chromatogram of sample (positive mode).

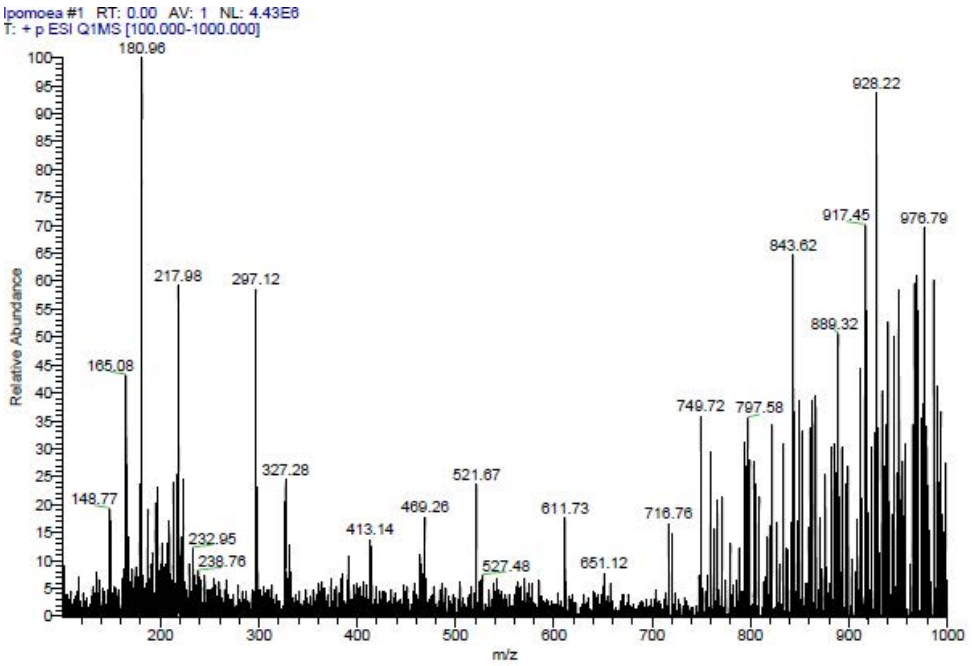


Fig. 5. Extracted ion chromatogram of RT 2.50 min.

The anti-inflammatory activity of *Ipomoea mauritiana* (column fraction 2) was tested by carrageenan induced rat paw edema test method. The concentrations 25, 50, 75 and 100 mg/kg of extracts were injected intraperitoneally one hour before the administration of carrageenan. After inducing edema by the injection of carrageenan, the rats were then observed for the reduction of edema every hour for six hours. There was no reduction in the edema observed in all rats during the first two hours. Reduction in edema was seen only in the rats injected with standard drug indomethacin after three hours. After four hours reduction in edema was observed in rats injected with 75 and 100 mg/kg of plant extracts. After fifth and sixth hours reduction in edema was observed in rats injected with 25, 50, 75 and 100 mg/kg of plant extracts (Figure 6 and Figure 7) (Table 4).

Table 4: Anti-inflammatory activity observed at different intervals of time

Extract		3 h		4 h		5 h		6 h		Total	
		-	+	-	+	-	+	-	+	-	+
Control	F	6	0	6	0	6	0	6	0	24	0
	%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%
Std	F	0	6	0	6	0	6	0	6	0	24
	%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%
25 mg/kg	F	6	0	6	0	6	0	6	0	24	0
	%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%
50 mg/kg	F	6	0	6	0	6	0	6	0	24	0
	%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%
75 mg/kg	F	6	0	0	6	0	6	0	6	6	18
	%	100.0%	0.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	25.0%	75.0%
100 mg/kg	F	6	0	0	6	0	6	0	6	6	18
	%	100.0%	0.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	25.0%	75.0%
Total	F	30	6	18	18	18	18	18	18	84	60
	%	83.3%	16.7%	50.0%	50.0%	50.0%	50.0%	50.0%	50.0%	58.3%	41.7%
Statistics		CV=1.000									
P value		P=.001									

Fig. 6. Edema induced by Carrageenan in the left paw of rat.

Fig. 7. Decrease in edema after 5 hours.

The rats were later sacrificed for the histopathological studies. The microscopic examination revealed that liver and kidney from the extract treated rats did not show considerable alteration in cells structure or any unfavorable effects when viewed under the light microscope under high magnification power (Figure 8 and Figure 9).

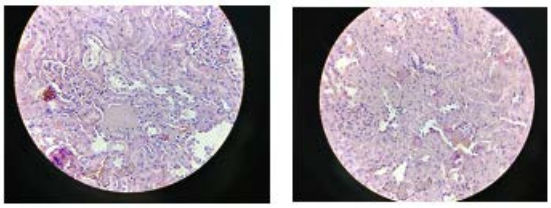


Fig. 8. Histological sections of Kidney of Rat. **(a)** Section of kidney of rat treated with extract. **(b)** Section of kidney of control rat.

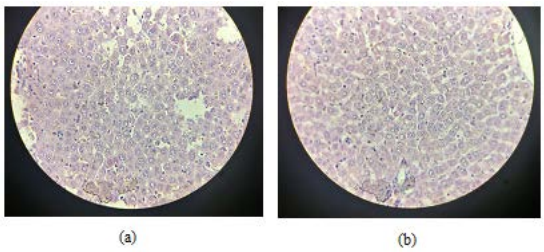


Fig. 9. Histological sections of liver of rat. **(a)** Section of liver of rat treated with *I. mauritiana* extract. **(b)** Section of liver of control rat.

4. Discussion and Conclusion

The phytochemistry of the *Ipomoea* genus has been widely studied since 1950. Some species of *Ipomoea* showed antimicrobial, analgesic, spasmolitic, spasmogenic, hypotensive, psychotomimetic and anticancer activities. Phytochemical, antimicrobial and anti-inflammatory studies have been conducted on many of the *Ipomoea* species. Phytochemicals such as taraxerol, taraxerol acetate, β-sitosterol, scopoletin, and 7-O-β-Dglycopyranosyl scopoletin were found in I. mauritiana (Khan et al., 2009)[8]. Phytochemicals such as caffeic acid (*The Wealth of India, Raw Materials*)[9], ethyl caffeate (Kritikar and Basu, 2000)[10], have been isolated from *I. hederacea*. In the present study, two compounds caffeic acid and β-amyrin acetate have been tentatively identified from the leaf extracts of *Ipomoea mauritiana*.

Anti inflammatory activities of *Ipomoea mauritiana* was tested by Carrageenan induced rat paw edema assay method where the edema induced by carrageenan in the left hind paw gradually decreased in the rats administered with the test extracts. The histological studies also showed no considerable changes in the cell structures of kidney and liver.

Hence from the current research findings it can be concluded that *I. mauritiana* leaf extracts shows a considerably good anti inflammatory activity.

5. Acknowledgements

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6. References

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