SCREENING OF ANTI-INFLAMMATORY ACTIVITY OF *MESUA FERREA* LINN FLOWER

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ABSTRACT

Mesua ferrea Linn is used traditional medicine in many parts of the world for the treatment of various diseases viz. cutaneous affections, sores, scabies, wounds, etc. and as an embrocation in rheumatism. It is claimed in traditional medicine that the root and bark of the plant are use in the treatment of gastritis and bronchitis. In the present study, the ethanolic extract of flower of *Mesua ferrea* Linn was screened for its anti-inflammatory activity using carrageenan induced rat paw edema using rat model. The extract was administered orally in the dose of 100mg/kg body wt., 200mg/kg body wt. and 400mg/kg body wt. The ethanolic extract of 400mg/kg body wt. shows the maximum anti-inflammatory action with the comparison to the standard anti-inflammatory agents.

Keywords: Anti-inflammatory activity, Carrageenan induced rat paw oedema, ethanolic extract of *Mesua ferrea* Linn flower

1. Introduction

Mesua or Iron wood tree, commonly known as Nagapushpam is an important medicinal plant which finds varied uses in Ayurveda, Siddha and Unani. Leaves are used in the form of poultice which is applied to head in severe colds. Bark and roots in decoction or infusion or tincture is a better tonic and are useful in gastritis and bronchitis. Fixed oil expressed from seeds is used as an application for cutaneous affections, sores, scabies, wounds, etc. and as an embrocation in rheumatism. Dried flowers powdered and mixed with ghee, or a paste made of flowers with addition of butter and sugar, are given in bleeding piles as well as dysentery with mucus. They are also useful in thirst, irritability of the stomach, excessive perspiration, cough with much expectoration, dyspepsia, etc. Leaves and flowers are used in scorpion stings. Syrup of the flower buds is given for the cure of dysentery. In Ayurveda, it is an ingredient of "Nagakeshara-adi-Churna", used for bacillary dysentery and in "Naga Keshara Yoga", for piles. In Unani system, the drug is an ingredient of large number of recipes like, "Jawarish Shehryaran" a stomach and liver tonic, "Hab Pachaluna", an appetiser, "Halwa-i-supari pack" a general tonic, etc[1-3].

Inflammation in the body response to noxious or injurious stimuli, characterized by warmth, redness of the skin, pain, swelling and loss of function. Inflammation is a part of host defense mechanism. There are several tissue factors that are known to be involved in the inflammatory reactions such as release of histamines, bradykinin and prostaglandins[4].

However this plant has not been studied for anti-inflammatory activity. This study was aimed at providing pharmacologic basis for its folkloric use in inflammation. Based on this an attempt has been made to evaluate the inflammatory potency of *M. ferrea* flower as well as isolation of the active compound.

2. Material and Methods

2.1. Plant material: The fresh flowers was collected during the month of August 2008, from the Market in Jhansi U.P., The plant materials was identified and authenticated by Dr. Gaurav Nigam, Department of Botany, Bundelkhand University, Jhansi ref. no. BU/BOT/370/ 17-01-09.

Picture representing the Mesua ferrea Linn flower

2.2. Extraction of plant material: The shade dried and powdered flower of *M. ferrea* was subjected to successive extraction using ethanol in a soxhlet apparatus. The extract was concentrated under reduced pressure using rotatory evaporator at temperature not exceeding 40°C and then dried in vacuum oven. The extract was stored in desiccators at cool place.

2.2.1. Isolation[5-7]:

2.2.2. Thin Layer Chromatography of Ethanolic Extract: 100 mg of ethanolic extract was weighted and dissolved in 10 ml of ethanol and filtered. Filterate was taken as sample for TLC.

2.2.3. High Performance Thin Layer Chromatography: High performance thin layer chromatography also known under the synonym planar chromatography which is a modern, powerful analytical technique with separation power and reproducibility super-ior to TLC.

2.2.4. Column Chromatography of Ethanolic extract

2.2.4.1. Preparation of Sample: Ehanolic extract was dried in reduced pressure and dissolve in minimum quantity of ethanol, mixed with silica gel, then dried, and applied in the column and eluted with Hexane: Diethyl-ether.

2.2.4.2. Collection of Samples in Volumetric: First of all prepared solvent (Hexane: Diethyl-ether) according to increasing polarity ratio as shown in Table 5.4 Fraction of eluting column was collected in 25 ml. Volumetric flask and TLC was performed for each volumetric flask. The samples showing the same TLC pattern were mixed as shown in **Table 3**.

2.2.5. Characterization of isolated compound: The compound which is isolated in column chromatography is characterized by the analytical techniques such as infrared spectroscopy, NMR spectroscopy and Mass spectroscopy.

2.2.5.1. Infrared Spectroscopy: Infrared spectroscopy is generally sensitive to the presence of functional groups in the samples. The most powerful aspects of infrared spectroscopy is that it allows identification of unknown compound. IR spectroscopy of compound was performed in CDRI, Lucknow. Spectra of compound have shown in figure. The interpretation that can be made from spectra has shown in **Table 4**.

2.2.5.2. NMR spectroscopy: NMR spectroscopy is a technique that enables us to study the shape and structure of molecules. It reveals the different chemicals environments of the various forms of hydrogen present in the molecules, NMR spectroscopy of compound was performed in CDRI, Lucknow. The standard data of Kaemferol 3-neohesperi-doside, and the data obtained from isolated compound sepctra of NMR has shown in **Table 5**.

2.2.5.3. Mass Spectroscopy: The mass spectroscopy of compound was performed at C.D.R.I. Lucknow, the mass spectra is used to determine the possible fragmentation in the compound. The mass spectra of compo-und have shown in the spectra exhibited various peeks suggesting fragmentation pattern.

2.3. Animals: Mice (20-30gm) of either sex (Bred in Central Drug Research Institute, Lucknow) were used. The animals were obtained from animal house of the institute of Pharmacy, Bundelkhand University, Jhansi, India. The animals were housed in standard cages with free access of food (standard laboratory rodent's chow) and water. The animal's house temperature was maintained at 23 ± 3.0 ºC with a 12-h light / dark cycle (light on from 6.00A.M. to 6.00P.M.). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) of the institute (approved by CPCSEA Regd No. 716/02/a/CPCSEA) and BU/Pharm/IAEC/008/027)

2.4. Acute Toxicity Study: An acute toxicity study relating to the determination of the LD50 value was performed with different doses of SAP M into different group of mice, each containing ten animals, as per the method discussed by Litchfield and Wilcoxon5. The median lethal dose of the extract having anticonvulsant activity was determined by administering 300, 2000, 5000mg/kg i.p. dose and percent mortality was observed 24 h later.

2.5.

Carrageenan induced rat paw oedema: The animals were weighed and numbered. The right hind paw just beyond tibio tarsal junction was marked, so that every time the paw was dipped into water column upto the fixed mark to ensure constant paw volume. The initial paw volume of each rat was measured by water displacement method. The animals were divided into five groups each comprising of six rats.

Group I: Control animals were received normal saline solution at the dose of 10ml/kg.

Group II: Animals received standard Diclofenac sodium at the dose of 10mg/kg body wt.

Group III: Animals received ethanolic extract at the dose of 100mg/kg body wt.
Group IV: Animals received ethanolic extract at the dose of 200mg/kg body wt.
Group V: Animals received ethanolic extract at the dose of 400mg/kg body wt.
All groups received intra peritoneal injection. After 30 mins 0.1 ml of 1% (w/v) caregeenan was injected in the plantar region of the right paw of each rats. The paw volume was measured after 30, 60,120 and 180 minutes of administration of carrageenan. Compare the mean percentage change in paw volume in control, extract, and diclofenac treated animals and expressed as percent oedema inhibition.

2.6. Statistical analysis: The results of the duration of seizures in electrically induced seizures were analyzed using the paired Student's t-test, while the proportion of animals that exhibited tonic seizures in electrically induced seizures was analyzed using Chi-squared test. A *p* value of <0.05 was considered as statistically significant[8, 9].

3. Results and Discussion

3.1. According to combination tried above it was found that Cyclohexane: Ethyl acetate: Formic acid, n-hexane: Ethyl-acetate: Formic acid, n-hexane: Diethyl ether: Formic acid may be the best solvent system and select best solvent system of n-hexane: Diethyl ether: Formic acid. **(Figure 1)**

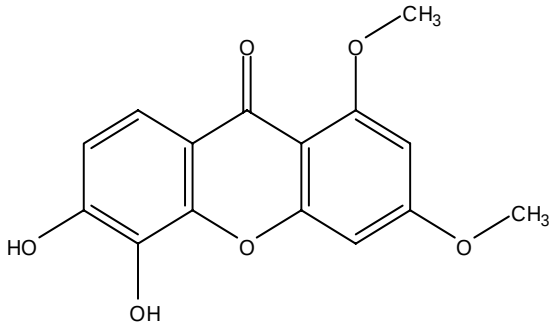
3.2. HPTLC of extract show the ten peaks so the ten peaks give confirmation that the ten compounds may be present in the ethanolic extract of flower *Mesua ferrea.* **(Figure 2).**

3.3. The entire fractions were subjected to TLC using solvent system (n- Hexane, Diethyl-ether) Fraction P-1, P-2, P-3 and P-5 showed single spot but P-5 and P-1 was not in sufficient amount; hence P-2 was selected for further characterization. Other fraction P, P-8 showed no any spot and P-6, P-7 showed two or more spots.

3.4. The infra red spectroscopy, NMR and mass spectroscopy is shown in **Table 4, 5, 6 and Figure 3, 4, 5**

In mass spectroscopy, fragmentation data of isolated compound P-2: 95, 149, 157, 205, 295, 552.

According to above study, the isolated compound (Melting Point 290 ^{0}C) may be Ferroxanthone.



1,3-dimethoxy-6,5 dihydroxyxanthone

Melting Point-294-295^{0}C
Chemical Name- Ferroxanthone

3.5. Anti inflammatory activity: An inflammatory response has been associated with various manifestations such as elevated body temperature and pain. Hence, a drug having anti-inflammatory activity may also show antipyretic and antibacterial properties. Preliminary pharmacological screening experiments were conducted (data not shown) with crude *Mesua ferrea* flower extracts found to exhibit significant anti inflammatory activity whose effect is comparable to that of standard drug-Diclofenac reported in this study.

Extract caused significant ($P < 0.001$) reduction in paw edema from the second hour at the 200mg/kg and 400mg/kg dose level, whereas significant ($P < 0.001$) reduction in paw edema was not observed from the second hour at the 100mg/kg dose level. There is good evidence that the early or first phase of transient permeability is due to the release of histamine and can thus be suppressed by antihistamines. The mediation of the delayed or second phase of exudation is more controversial and complex, and has been attributed in part to kinins, prostaglandins, neutrophils, and lipoxygenase products of arachidonic acid metabolism[10-13]. The probable mechanism of anti-inflammatory action of Extract may be due to its influence on the second phase of inflammation, the cyclooxygenase pathway rather than the lipoxygenase pathway. This is evident by the maximal inhibition of inflammation at the end of the third hour after the challenge with carrageenan.

4. Conclusion

In conclusion, the present study demonstrated that extracts of the flower of *M. ferrea* has anti-inflammatory effect, without any serious toxic effect. Further detailed investigation is

underway to determine the exact mechanisms, which are responsible for the anti-inflammatory activity. In conclusion, the present results provide a new way for further study in the light of developing new anti-inflammatory compounds.

Acknowledgement

The authors are grateful to Prof. S.K. Prajapati (Head, Institute of pharmacy, Bundelkhand University, Jhansi, India) Vivek Jain, Vivekananda Katare, Abhishek Dwivedi and O. P. Gautam for providing the necessary laboratory facilities

Table 1: TLC of Ethanolic flower extract of *M. ferrea*.

S. No	Solvent system	Ratio	N. of spots	Resolution
1	Cyclohexane: Ethyl acetate: Formic acid.	78:20:2	6 spots	Good
2	n-hezane: Ethyl-acetate: Formic acid.	80:18:2	6 spot	Good
3	n-hexane: Diethylether: Formic acid	75: 20:5	7 spot	Excellent

Adsorbent – Activated Silicagel-G
Detecting agent: Iodine chamber

Table 2: Representing the R_f value of the spots of Ethanolic flower extract of *M. ferrea*.

S.N.	R_f value	Detecting in Iodine chamber
1.	0.18	Dark Brown
2.	0.48	Dark Brown
3.	0.54	Reddish Brown
4.	0.62	Brown
5.	0.69	Reddish Brown
6.	0.78	Light Brown
7.	0.91	Light Brown

Figure 1: Showing the TLC of detection in iodine chamber by using Solvent system: (n- Hexane: Diethyl ether: Formic Acid) (75:20:5)

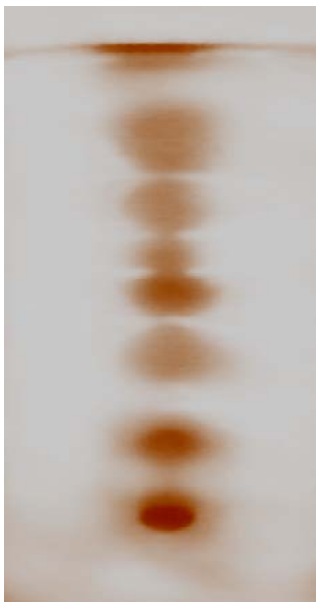
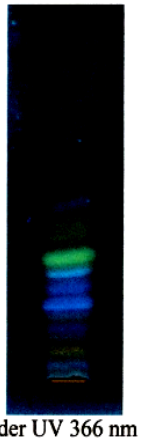


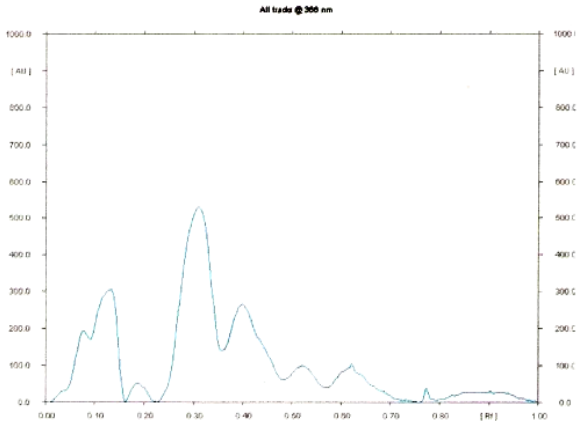
Figure 2: Showing the Spectra for HPTLC Chromatogram of ethanolic extract of of *M. ferrea*.

Prepared the sample extract 10mg/ml and applied 10 µl

CIF-250



Under UV 366 nm



Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area%	Assigned Substance
1	1	0.01 Rf	0.8 AU	0.08 Rf	193.2 AU	11.81%	0.09 Rf	170.3 AU	4112.6 AU	7.13%	unknown *
1	2	0.09 Rf	171.8 AU	0.13 Rf	304.6 AU	18.62%	0.16 Rf	0.6 AU	8822.5 AU	15.29%	unknown *
1	3	0.16 Rf	2.9 AU	0.19 Rf	50.8 AU	3.10%	0.22 Rf	0.5 AU	1007.1 AU	1.74%	unknown *
1	4	0.23 Rf	0.0 AU	0.31 Rf	531.2 AU	32.46%	0.36 Rf	139.1 AU	21681.7 AU	37.56%	unknown *
1	5	0.36 Rf	139.7 AU	0.40 Rf	264.6 AU	16.17%	0.48 Rf	59.7 AU	12334.2 AU	21.37%	unknown *
1	6	0.48 Rf	60.2 AU	0.52 Rf	98.1 AU	6.00%	0.57 Rf	39.1 AU	3771.1 AU	6.53%	unknown *
1	7	0.57 Rf	39.3 AU	0.62 Rf	102.8 AU	6.28%	0.72 Rf	8.1 AU	4573.5 AU	7.92%	unknown *
1	8	0.76 Rf	0.2 AU	0.77 Rf	37.7 AU	2.30%	0.80 Rf	5.7 AU	265.8 AU	0.46%	unknown *
1	9	0.81 Rf	7.2 AU	0.85 Rf	27.1 AU	1.65%	0.86 Rf	26.0 AU	563.2 AU	0.98%	unknown *
1	10	0.92 Rf	26.0 AU	0.92 Rf	26.3 AU	1.61%	0.96 Rf	10.1 AU	587.7 AU	1.02%	unknown *

Sample Preparation- 10mg/ml
Application-Linomat 5 Applicator (Camag)
Volume applied-10 µl
Solvent System-n-Hexane: Diethyl ether: Formic acid (75:20:5)
TLC plate Development-Presaturated Camag Twin Trough Chamber

Table 3: Column Chromatography of isolated compounds ethanolic extract of flower *Mesua ferrea.*

S.N.	Elute	Volume collected (ml)	No of spot	Code
1.	Hexane(100)	--	--	P
2.	Hexane: Diethyl ether (95:5)	1-5	One spot	P-1
3.	Hexane: Diethyl ether (85:15)	6-10	One spot	P-2
4.	Hexane: Diethyl ether (75:25)	11-20	One spot	P-3
5.	Hexane: Diethyl ether (50:50)	21-30	Two spot	P-4
6.	Hexane: Diethyl ether (30:70)	30-40	One spot	P-5
7.	Hexane :Diethyl ether (20:80)	41-50	Three spot	P-6
8	Hexane :Diethyl ether (10:90)	50-55	Three spot	P-7

Figure 3: Showing the IR spectra of isolated compound P-2 of ethanolic extract of of *M. ferrea*

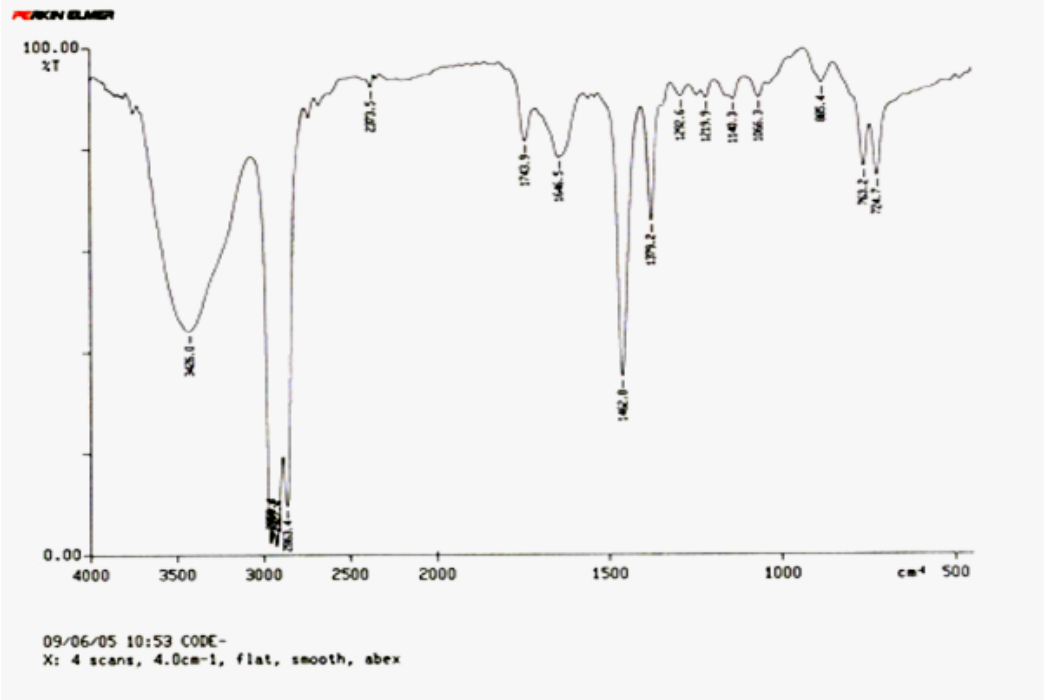


Table 4: Interpretation of I.R. Spectroscopy ethanolic extract of flower *Mesua ferrea.*

Wave number (cm-1)	Assignment
3426.0	O-H stretching in phenol group
2958.4	C-H stretching in alkane
2863.4	C-H stretching
1743.9	C=O stretching
1462.8	C-H bending in alkane CH_3

Figure 4: Showing the ^{1}HNMR of isolated compound P-2 of ethanolic extract of *M. ferrea.*

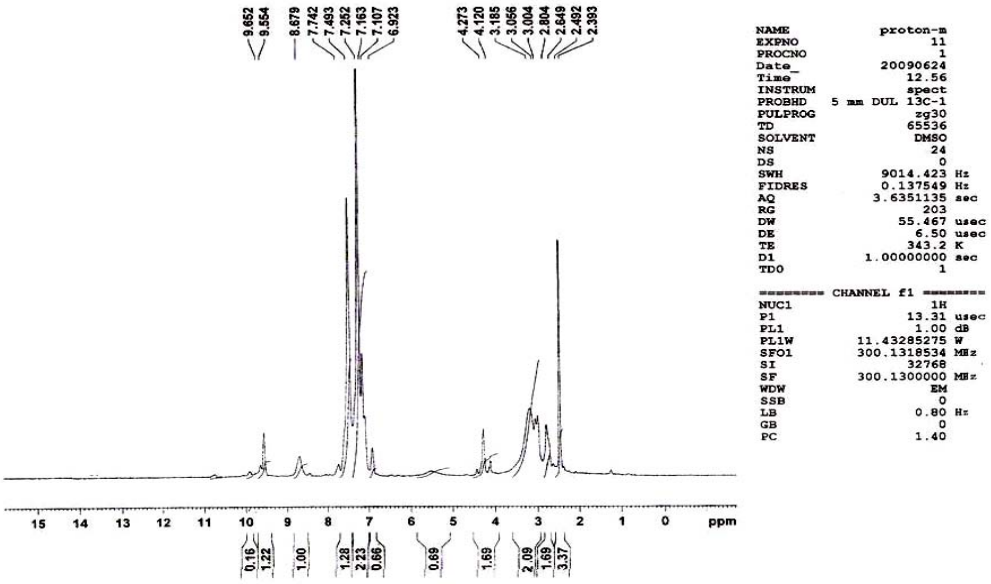


Table 5: Interpretation of Proton NMR Spectroscopy ethanolic extract of flower *Mesua ferrea.*

Assignment	δ Value(ppm)
$-CH_3$	2.393-4.273 (d,6H)
Ar-H	6.923-7.742 (m,4H)
-OH	8.879-9.652 (s,2H)

Figure 5: Showing the Mass Spectra of Isolated compound P-2 of ethanolic extract of *M. ferrea*

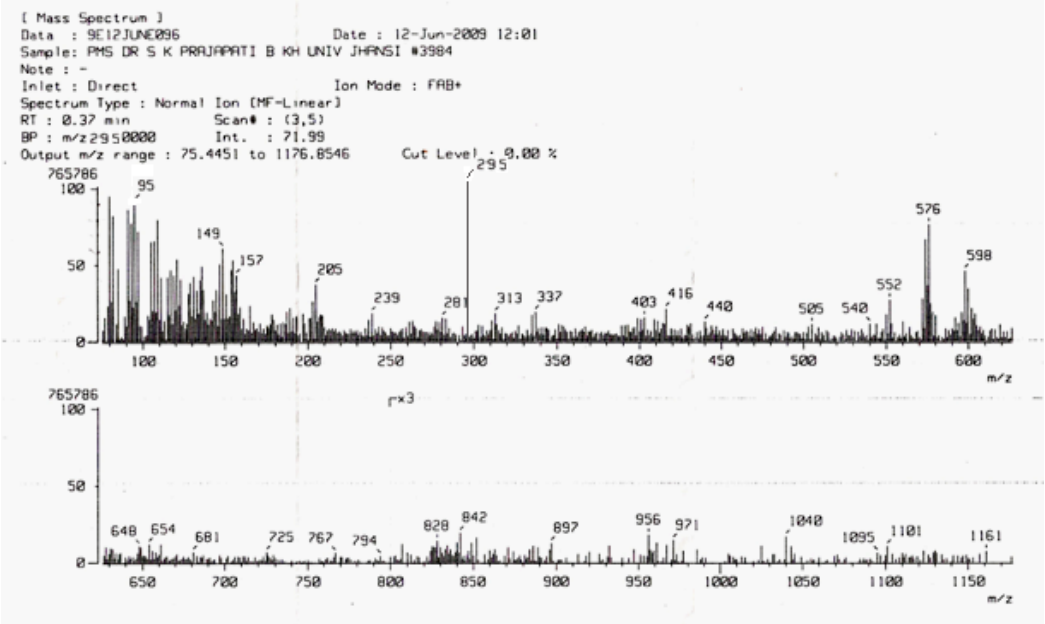


Table 6: Interpretation of Mass Spectra of ethanolic extract of flower *Mesua ferrea.*

m/z value	Relative intensity
95	88
149	63
157	48
205	38
295	100
552	24

Table 7: % Inhibition of Paw Volume (ml) by the ethanolic extract of *Mesua ferrea* on rats

Sl.No	Dose	Initial volume	30 min	60min	120min	180min
1.	**Control**	0.5±0.01	0.7±0.02	0.84±0.01	1.1±0.04	1.22±0.05
2.	**Standard (10mg/kg b.w)**	0.38±0.04	0.46±0.02a** (34.29%)	0.55±0.01a** (34.53%)	0.48±0.02a** (56.37%)	0.46±0.03a** (62.3%)
3.	**Extract (100mg/kg b.w)**	0.40±0.03	0.48±0.02[a]** (31.43%)	0.54±0.04[a]** (35.72%)	0.73±0.04 (33.64%)	0.87±0.07[a]** (28.69%)
4.	**Extract (200mg/kg b.w)**	0.30±0.04	0.42±0.02[a]** (40%)	0.50±0.01[a]** (40.48%)	0.70±0.02[a]** (36.37%)	0.57±0.01[a]** (53.28%)
5.	**Extract(400mg/kg b.w)**	0.35±0.02	0.40±0.04a** (42.86%)	0.53±0.02[a]** (36.91%)	0.65±0.01[a]** (40.91%)	0.50±0.02[a]** (59.02%)

Values are ±SEM;n=6 in each groups; a represents comparisons between Group2-5 with Group 1; statistical test done by t-test; symbols represents statistical significance : * P < 0.05. ** P < 0.01

Figure 5: Representing the paw volume changes with time interval by different concentration of *Mesua ferrea* extract.

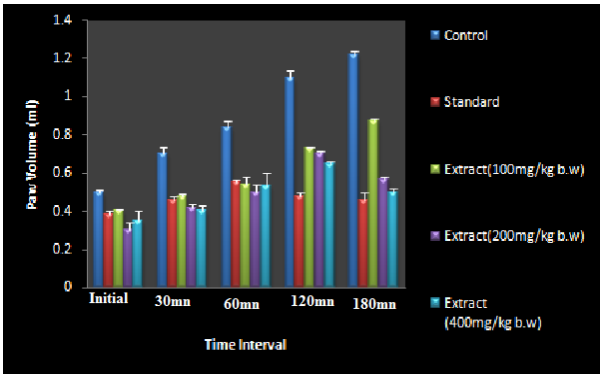
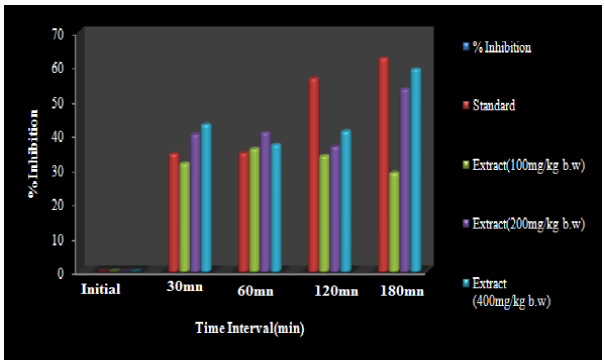


Figure 6: Representing the percentage inhibition changes with time interval by different concentration of *Mesua ferrea* extract.



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