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Phytochemical Screening and Anti-inflammatory activity of *Murraya Koenigii & Ficus Lacor roots* in Carrageenan, Histamine and Serotonin induced paw edema in albino Wistar rats

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Abstract

Objective: To perform the Phytochemical Screening & Anti-inflammatory activity of *Murraya Koenigii & Ficus Lacor roots* in Albino Wistar rats. **Methods:** The different Pharmacognostical parameters were evaluated as per standard procedure. The crude drug was evaluated for organoleptic properties shape, size, color, odor, taste. Preliminary Phytochemical Screening was carried out & finally Anti-inflammatory activity was evaluated by adopting different methods. **Results:** The extracts showed a marked Anti-inflammatory effect. The FLET fraction from *Ficus lacor* aerial roots showed maximum inhibition (75%) of Carrageenan induced edema (Table 3.12B), followed by FLPE and MKCF from *Murraya koenigii* roots (74 % and 70% approx) ($p < 0.001$ for all). The inhibition of inflammation was comparable for all the fractions for any change in extent & percentage of inhibition at 30 min, 1hr, 2hr and 3hr. The anti-inflammatory effect induced via indomethacin gradually increased and reached at higher level (80.8%) after 3 hrs. It was maintained up to six hours. For, MKPE the inhibition was observed to be maximum at the end of 2 hrs and then tapered. MKEA, FLEA and FLCF showed minimum response that was constant throughout and insignificant. MKPE was slightly significant (55.1 %, $p<0.05$) which was more effective. **Conclusion:** The results of the study indicate that the extract of *Murraya koenigii* roots and *Ficus lacor* possesses strong Anti-inflammatory activity. This study also describes therapeutic effect of *Murraya koenigii* roots and *Ficus lacor* aerial roots in inflammation and arthritis which will give a new direction for the future scientific research.

Keywords: *Murraya koenigii*, *Ficus lacor*, Phytochemical, Pharmacological, Anti-inflammatory.

Introduction

Inflammation is normal and necessary protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents, and ischemia. It is caused by a variety of stimuli, including physical damage, UV irradiation, microbial attack, and immune reactions. The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as chronic asthma, arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis. Many of these diseases are debilitating and are becoming increasingly common in our ageing society. Rheumatoid arthritis and degenerative arthritis are the major inflammatory diseases affecting people worldwide [1,2]. Rheumatoid arthritis is an inflammatory term that usually involves multiple joints. It affects 0.3–1.0% of the worldwide population and is more predominant among women in developed nations. The continual inflammation leads to joint damage; however the disease can be inhibited with drugs uses. Degenerative joint disease, which is considered by trouncing of joint cartilage that leads to pain loss and damage the function primarily in the hips and, affects 9.6% of adult males and 18% of women aged more than 60 years.

Typical morphological features of acute and chronic inflammation:

Acute inflammation shows large neutrophilic infiltration, edema and vascular changes, and then complete resolution of the inflamed area by removal of the chemical mediators, necrotic debris & inflammatory cells by lymphatic drainage and phagocytic action of macrophages. Occasionaly, there may be scarring and fibrosis or abscess formation or progression to chronic inflammation[3].

Chronic inflammation, on the other hand, arises in the following settings:

- Persistent infections.
- Prolonged exposure to potentially toxic agents.
- Autoimmune diseases.

It shows infiltration with mononuclear inflammatory cells, including macrophages, lymphocytes and plasma cells which continue for a long time (Thomas and Lipsky 1997),tissue destruction induced by inflammatory cells, and then fibrosis after repair process may be a general conclusion in chronic inflammation. Granuloma formationmay also be a prominent feature in most cases of chronic inflammation [4].

Murraya genus had 14 known species are available in world, but only two species are in India these are *Murraya koenigii and Murraya paniculata* L. Jack. *Murraya koenigii* Linn., commonly kenned as Meetha neem, odor aromatic more or less deciduous shrub or a minuscule tree up to 6 m in height found all over India up to an elevation of 1500 m.

Classification:

Kingdom	:	Plantae
Division	:	*Magnoliophyta*
Class	:	*Magnoliopsida*
Order	:	*Sapindales*
Family	:	*Rutaceae*
Genus	:	*Murraya*
Species	:	*Koenigii*

Synonyms: *Hindi: Mitha neem, Curry patta, Sanskrit: Krishna nimbi, Assamese: Narsinghs, Bisharhari, Bengali: Barsanga, Kariphulli, Punjabi: Curry patta; Marathi: Poospala, Karhinimb, Gandla.* Ficus genus also called fig genus, consists of over 800 species and about 40 genera of the mulberry family. All Ficus species possess latex-like material within their vasculatures, affording protection and self-healing from physical assaults (Lansky *et al.*,2008). *Ficus lacor* Buch – Ham, pilkhan is local name and it is a large deciduous, rapidly grown foliaceous plant, about 20 meters in height, with fine shape crown. It is widely distributed in subtropical and tropical areas of the world. It also grown in India's various humid regions [5].

Classification:

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Urticales
Family	**:**	Moraceae
Genus	**:**	*Ficus*
Species	**:**	*lacor*

Synonyms

Ficus lacor Buch - Ham synonym *Ficus infectoria* Roxb. The various speies of ficus viz. *Ficus aspera, Ficus auriculata, Ficus benghalensis* (Indian banyan), *Ficus binnendykii, Ficus carica, Ficus deltoidea, Ficus elastica* (Indian rubber tree), *Ficus lingua, Ficus lyrata.*

Material and Method

Plant material

Collection and authentication of plant materials

The aerial root of *Ficus lacor* was collected from Panchkula (Haryana) and the roots of *Murraya koenigii* was collected from campus Chitkara University, Punjab, India. The taxonomically, authenticated and identified plant material by Dr. H.B. Singh, HRMHM department. The specimens of voucher have been submitted at the NISCAIR, Delhi for further reference in herbarium section. The roots dried, sliced into small pieces, using a mechanical grinder, coarse powder made and in tight container stored for further use.

Animals

The Wistar rats of any sex were used as per experimental protocols (IAEC/CCP/12/PR-005) after consent from the Institutional Animal Ethical Committee and Chitkara College of Pharmacy, Chitkara University, Rajpura, Punjab, India.

Chemicals

Petroleum ether, Chloroform, Methanol, Paracetamol, Ethyl acetate, Glacial acetic acid, Acetone, Formic acid, Benzene, N-propanol, Ethanol, Tween-80, Dimethylsulphoxide (DMSO), Glycerine, conc. Sulphuric acid, Hydrochloric acid, Benzene, N-Butanol, Dichloromethane, n-hexane, pyridine, toluene, xylene.

Preparation of extracts

Powdered (10 g) roots of *F. lacor* and *M. koenigii* were weighed and macerated with 100 ml of water and left overnight. The supernatant clear liquid was filtered. The extraction was repeated for three consecutive days so as to exhaust the root of all water soluble extractives. The combined filtrates were concentrated on a water bath and the proteins precipitated by addition of alcohol (95%) were washed with ethanol to remove unbound amino acids. The extract was filtered and filtrate was concentrated by vacuum distillation. Percentage yield of methanolic extract was found to be 13.8%. The mother liquor obtained after removing the proteins was concentrated for detection of amino acids in Free State and carbohydrates [6].

Anti-inflammatory activity

Grouping: In most models, the animals were divided into groups on the following basis**:**

- Vehicle treated Normal control Group
- Untreated diseased control Group
- Reference group treated with standard drugs
- Test Groups depending on number of doses and fractions for study.

Screening for anti-inflammatory

Inhibition of Carrageenan induced, paw edema in rats

Rats were divided into groups of 06 each (120-150gm).

- Normal saline treated control
- Untreated diseased animals
- Reference group treated with indomethacin before carrageenan
- Experimental Groups

Control group I was given normal saline one hour before the carrageenan infusion. Experimental groups were given doses of different portions in 0.5ml of ordinary saline, infused intraperitonealy one hour prior to infusion of 0.1 ml of 1% carrageenan arrangement in the right rear paw under the plantar aponeurosis (s.c) for affectation of edema. The volume of paw edema was controlled by Plethysmometer and a measurement reaction relationship was built for both oral and i.p. dosage and a connection built between i.p. furthermore,

oral dosages delivering most extreme mitigating impact. Reference gathering was given Indomethacine

Inhibition of histamine and serotonin induced paw edema in rats

In another set of experiments serotonin and histamine (0.1ml 0f 1mg/ml of both) were used as phlogistic agents. Groups of 6 animals were made as earlier;

- Vehicle treated normal control
- Untreated diseased animals
- Reference group treated with indomethacin before carrageenan
- Experimental Groups depending on the number of extracts and fractions.

The extract, various fractions, standard pyrilamine and control vehicle (arrangement of 2.5% DMSO and 2.5% Tween 20) were regulated intraperitonealy one hour prior to the infusion of incendiary arbiters in their particular gatherings. Various doses of concentrate or fractions were infused intraperitonealy in vehicle to discover dosage reaction relationship. 0.1ml Serotonin (1mg/ml) or histamine (1mg/ml) was infused and reaction noted at 30 mins for Serotonin and 60 mins for Histamine bunches. Pyrilamine maleate (1 mg/kg) was utilized as the enemy (reference) of histamine and as a standard medication in the reference bunch. The volume of paw edema was dictated by plethysmometer [8]

2.5 mg/kg 1hr preceding the carrageenan infusion [7].

Result and Discussion

Pharmacognostic studies

Morphological Studies

The *Ficus lacor* has well developed aerial root system. The fresh roots have a light green colour which turned brown on drying. The bark of the root was thin. The aerial roots are thick,long, cylindrical, and hard, approximately 1.3-2 cm in diameter having rough surface and secondary rootlets also present.

The roots of *Murraya koenigii* is a typical root and in transverse section (T.S.) shows the characteristics of a dicot root, i.e. Epidermis, Cortex, Endodermis, Phloem and Xylem. The microscopic characteristics of the powder showed the presence of xylem vessel, fiber, parenchymatous cell and cork cells.

Extractive values

Extractive values are useful for evaluation of crude drugs and give an idea about the nature of chemical constituents present in them. The amount of extractive a drug yields to a given solvent is often an approximate measure of a certain constituent or group of related constituents the drug contains. [9] [Table 1]

Table 1: Extractive values of roots powder with various solvents

Sr. No.	Solvent	Extraction period (h)	FL Extractive values (%) w/w	MK Extractive values (%) w/w
1.	Petroleum ether (60-80°C)	24	5.7	3.5
2.	Chloroform	24	10.0	5.3
3.	Ethyl acetate	24	5.5	3.2
4.	Ethanol	24	4.5	8.5
5.	Aqueous	24	10.5	4.5

Table 2: Crude fiber content, Loss on drying, Swelling index, Foaming index, Tanins contents, Bitterness value, Haemolytic value [10]

Parameter	Observation in FL	Observation in MK
Crude fiber content	9.45 %	7.53 %
Loss on drying	14 %	10 %
Swelling index	No significant result	No significant result
Foaming index	124.6	No significant result
Tannins	22	19
Bitterness value	1.9 unit / g	2.5 unit / g
Haemolytic activity	23.45 %	30.34 %

The extracts showed a marked anti-inflammatory effect (Fig. 1) by causing a reduction in carrageenan histamine and serotonin paw inflammation. Methanolic extract (400mg/kg) showed the effect to the same degree as paracetamol (20 mg/kg, i.p.). The experimentally induced laboratory model was employed in evaluating the antipyretic activities of methanolic extracts of *Cassia occidentalis L.* The extract caused a better hypothermal activity against yeast-induced pyrexia in rats [11].[Table 2]

Pharmacological screening

Various extracts were subjected to detailed pharmacological investigations for anti- inflammatory, anti-arthritic and antioxidant activities along with determination of toxicity.

Screening for Anti-inflammatory Activity

Inhibition of Carrageenan induced paw edema in rats

The separated fractions from the two plants were screened for their capacity to inhibit carrageenan induced edema at different doses given intraperitonealy. The observed inhibitions are tabulated in Table 3,4,5,6.

Table 3: Inhibition of Carragenan induced paw edema in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor*

Groups	Isolated Fractions	Dose mg/kg i.p.	M.E.V and SEM	P.I. (%inhibition)
I	Normal	(Normal saline) Arthritic control	0.37±0.02	--
II.	MKPE	50	0.25± 0.056	32.4
III.	MKEA	50	0.24± 0.049	35.8
IV.	MKCF	50	0.14± 0.051 ***	66.4
V.	FLPE	50	0.21± 0.058 **	41.9
VI.	FLEA	50	0.26±0.048	30.7
VII.	FLCF	50	0.25± 0.058	35.9
VIII.	FLET	50	0.12±0.049 ***	68.3
IX.	Indomethacin	2.5 mg	0.07±0.057 ***	81.8

MEV (Mean Edema volume) Values represent Mean± SEM; & P.I. = Percentage inhibition; Group I- (Saline) Edema Control; Group II- Treated with fraction MKPE; Group III- Treated with fraction MKEA; Group IV- Treated with fraction MKCF, isolated from *Murraya koenigii;* Group V - Treated with fraction FLPE; Group VI- Treated with fraction FLEA; Group VII- Treated with fraction FLCF; Group VIII- Treated with fraction FLET, isolated from *Ficus lacor*, Group IX- Treated with standard drug Indomethacin at the dose of 2.5mg/kg body weight; *p<0.05; **p<0.01;*** p <0.001, as compared with arthritic control.

Table 4: Inhibition of Carrageenan induced edema in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor*

Groups	Isolated Fractions	Dose mg/kg i.p.	M.E.V and SEM	P.I. (% Inhibition)
I	Normal	(Normal saline 1ml) Arthritic control	0.37±0.02	--
II.	MKPE	100	0.20±0.058 **	55.10
III.	MKEA	100	0.22± 0.050 *	38.80
IV.	MKCF	100	0.10±0.050 ***	73.12
V.	MKAF-1	100	0.09±0.043	74.68

VI.	FLPE	100	0.10±0.040 ***	73.50
VII.	FLEA	100	0.26±0.048	30.70
VIII.	FLCF	100	0.21± 0.058 *	41.90
IX.	FLET	100	0.10±0.037 ***	72.42
X	FLTP-1	100	0.09±0.050	75.68
XI	Indomethacin	2.5 mg	0.07±0.057	81.8

MEV (Mean Edema volume) Values represent Mean± SEM; & P.I. = % inhibition, Group I- (Saline) Edema Control, Group II- Treated with fraction MKPE, Group III- Treated with fraction MKEA, Group IV- Treated with fraction MKCF, Group V - Treated with fraction MKAF-1 from *Murraya koenigii*, Group VI- Treated with fraction FLPE, Group VII- Treated with fraction FLEA, Group VIII- Treated with fraction FLCF, Group IX- Treated with fraction FLCF, Group X- Treated with fraction FLET, isolated from *Ficus lacor*, Group XI- Treated with standard drug Indomethacin at the dose of 2.5mg/kg body weight; as compared with arthritic control.
MKPE = *Murraya koenigii* Petroleum Ether, MKEA= *Murraya koenigii* Ethyl Acetate, MKCF = *Murraya koenigii* Chloroform, MKAF-1 (alkaloidal fractions), FLPE = *Ficus lacor* Petroleum Ether, FLEA = *Ficus lacor* Ethyl Acetate, FLCF = *Ficus lacor* Chloroform, FLET = *Ficus lacor* ethanol, FLTP-1 (Terpenoidal fractions).

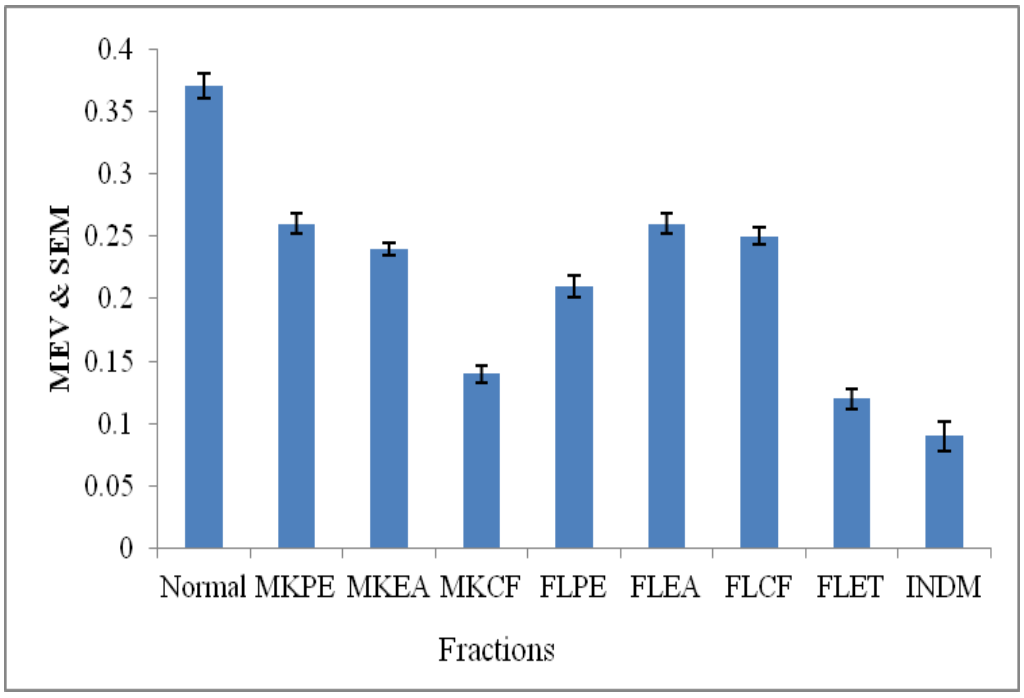

Figure 1: Inhibition of Carragenan induced paw edema in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at the dose of 50mg/kg, body weight

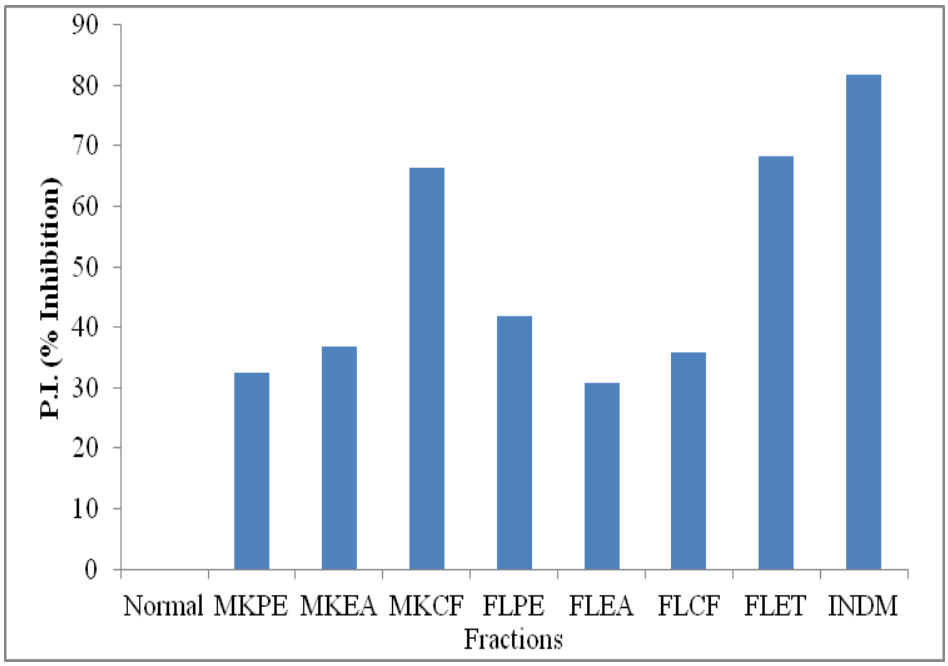

Figure 2: Percentage Inhibition of Carrageenan induced edema in rats by various fractions isolated from roots *Murraya koenigii* and aerial roots of *Ficus lacor* at the dose of 50mg/kg body weight

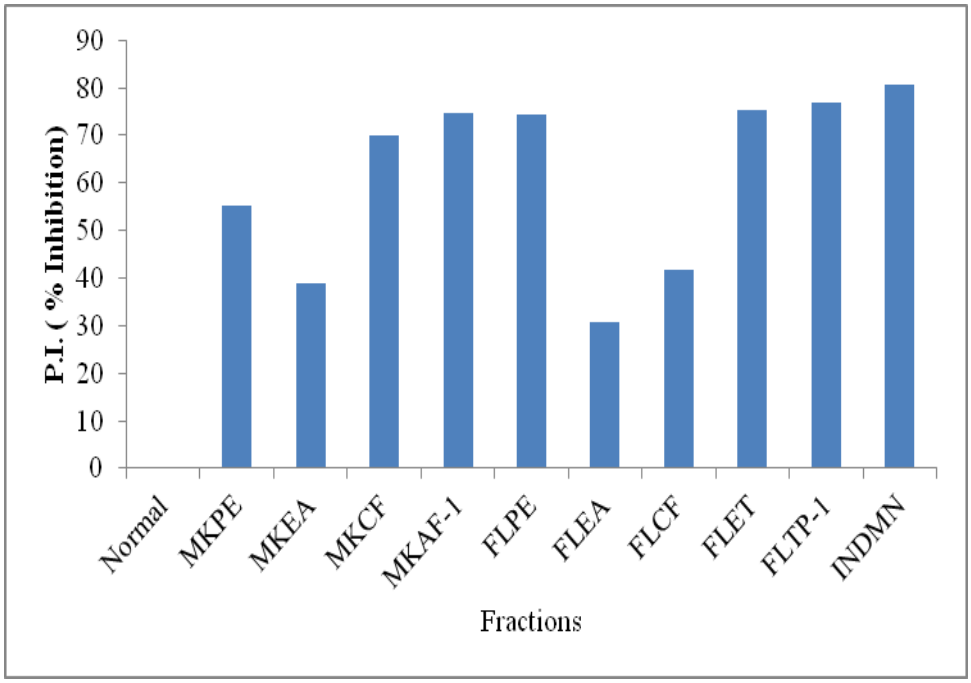

Figure 3: Percentage Inhibition of paw edema, induced via Carrageenan in rats by various fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at the dose of 100mg/kg.

Inhibition of histamine induced paw edema in rats

Table 5: Inhibition of paw edema, induced via histamine in rats by various fractions from roots of *Murraya koenigii* and aerial roots of *Ficus lacor*

Group	Isolated Fractions	Dose mg/kg	Histamine	
			MEV	PI
I.	Normal	(Normal saline 1ml) Arthritic control	0.37±0.02	--
II.	MKPE	50	0.42±0.04	13.66
III.	MKEA	50	0.27±0.10	24.1
IV.	MKCF	50	0.15±0.04**	64
V.	FLPE	50.	0.10±0.04***	67.01
VI.	FLEA	50	0.22±0.04 **	43.46
VII.	FLCF	50	0.24±0.01	38.66
VIII.	FLET	50	0.12±0.04***	68.02
IX	Pyrilamine	1mg/kg	0.08±0.04***	79.01

MEV (Mean Edema volume) Values represent Mean ± SEM; P.I. = % inhibition; Group I- (Saline) Edema Control, Group II- Treated with fraction MKPE; Group III- Treated with fraction MKEA; Group IV- Treated with fraction MKCF, isolated from *Murraya koenigii;* Group V - Treated with fraction FLPE; Group VI- Treated with fraction FLEA; Group VII- Treated with fraction FLCF; Group VIII- Treated with fraction FLET, isolated from *Ficus lacor,* Group IX- Treated with standard drug Pyrilamine at the dose of 1mg/kg body weight, *p<0.05; **p<0.01; *** p <0.001, as compared with arthritic control.

Table 6: Inhibition of histamine induced paw edema in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Group	Isolated Fractions	Dose mg/kg	Histamine	
			MEV	PI
I	Normal	(Normal saline 1ml) Arthritic control	0.37±0.02	--
II.	MKPE	100	0.23±0.04*	25.10
III.	MKEA	100	0.22±0.10	35.66
IV.	MKCF	100	0.15±0.06**	60.00
V.	MKAF-1	100	0.12±0.05***	67.25
VI.	FLPE	100	0.10±0.04***	70.01
VII.	FLEA	100	0.21±0.04 **	41.66
VIII.	FLCF	100	0.25±0.01	38.66
IX.	FLET	100	0.9±0.04***	74.01
X	FLTP-1	100	0.08±0.04***	78.28
XI	Pyrilamine	1mg/kg	0.06±0.04**	82.01

MEV (Mean Edema volume) Values represent Mean± SEM; & P.I. = % inhibition, Group I- (saline) Edema Control, Group II- Treated with fraction MKPE, Group III- Treated with fraction MKEA, Group IV- Treated with fraction MKCF, Group V - Treated with fraction MKAF-1 from *Murraya koenigii*, Group VI- Treated with fraction FLPE, Group VII- Treated with fraction FLEA, Group VIII- Treated with fraction FLCF, Group IX- Treated with fraction FLCF, Group X- Treated with fraction FLET, isolated from *Ficus lacor*, Group XI- Treated with standard drug Pyrilamine at the dose of 1mg/kg body weight,, *p<0.05; **p<0.01; *** p <0.001, as compared with arthritic control.

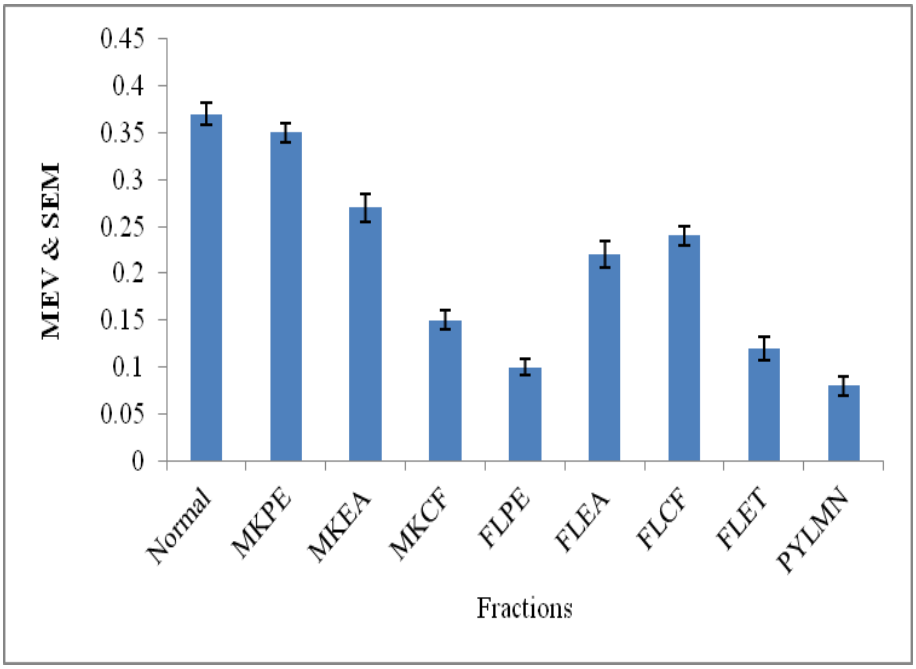

Figure 4: Inhibition of paw edema, induced via histamine in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 50mg/kg body weight

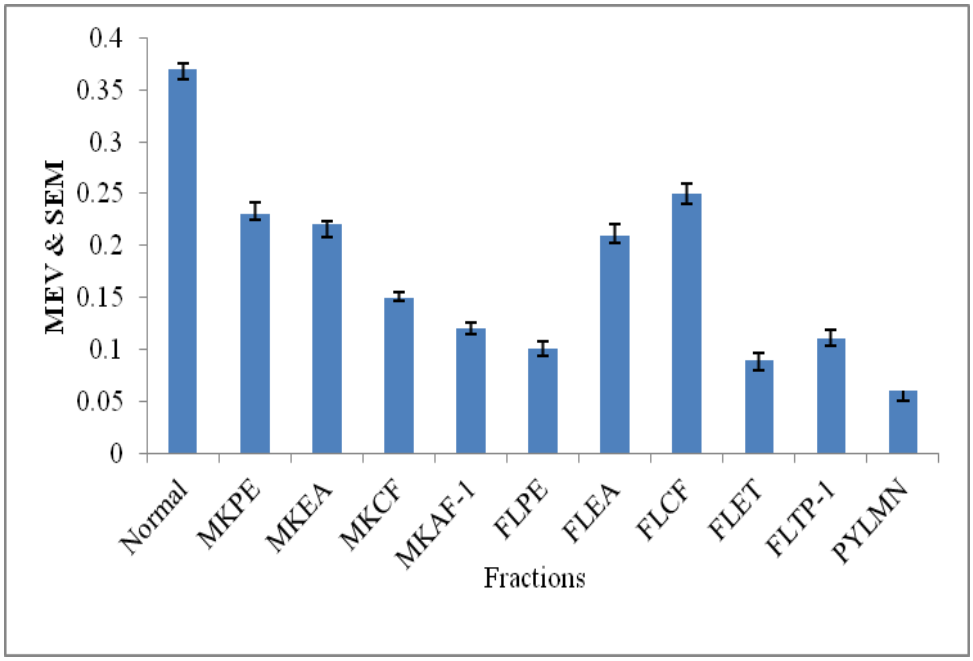

Figure 5: Percentage Inhibition of paw edema, induced via histamine in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 50mg/kg body weight

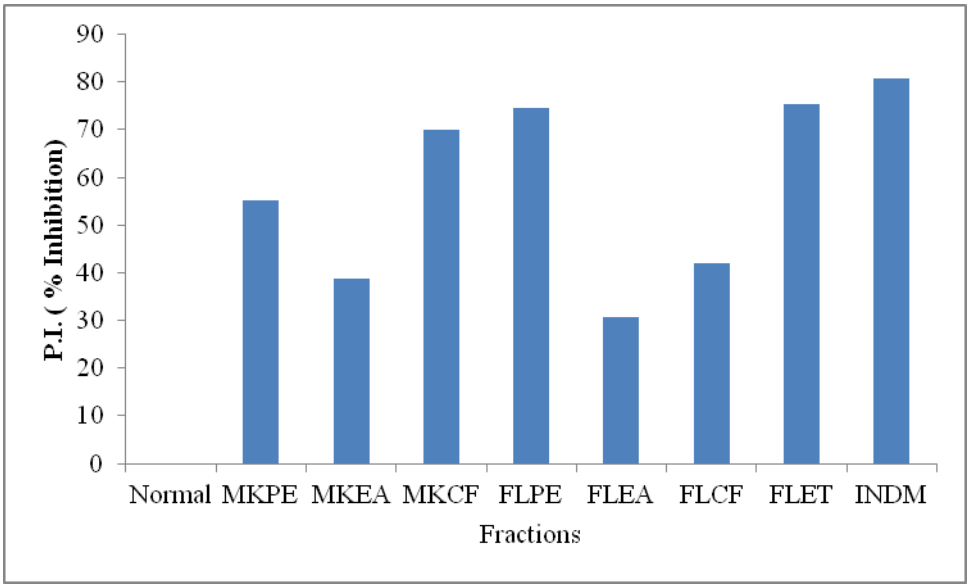

Figure 6: Inhibition of paw edema, induced via histamine in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 100mg/kg body weight

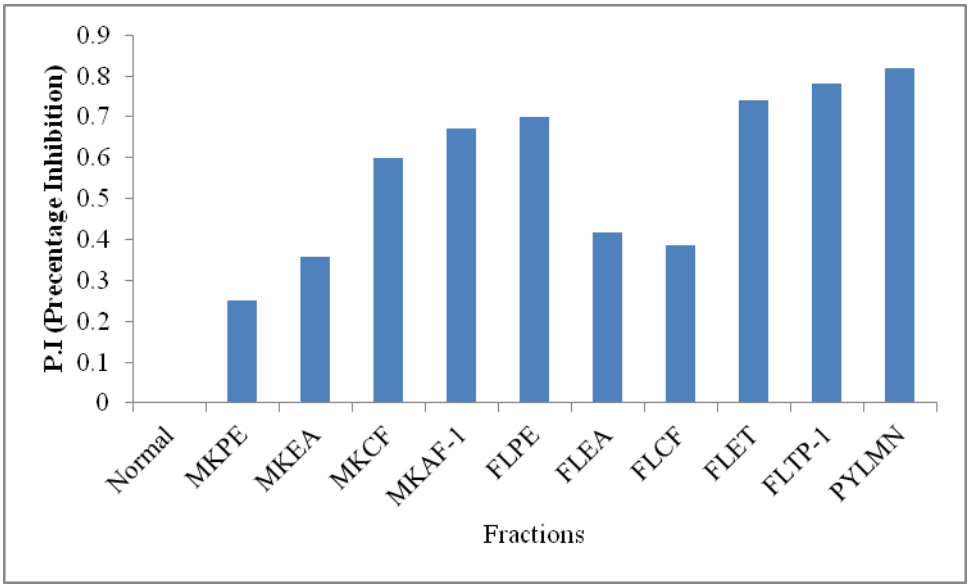

Figure 7: Percentage inhibition of paw edema, induced via histamine in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 100mg/kg body weight.

Carrageenan induced edema: The FLET fraction from *Ficus lacor* aerial roots showed maximum inhibition (75%) of Carrgeenan induced edema (Table 3), followed by FLPE and MKCF from *Murraya koenigii* roots (74 % and 70% approx) ($p < 0.001$ for all) (Tables 3.12 A and 3.12 B. The inhibition of inflammation was

comparable for all the fractions for any change in extent & percentage of inhibition at 30 min, 1hr, 2hr and 3hr. The anti-inflammatory effect induced via indomethacin gradually increased and reached at higher level (80.8%) after 3 hrs. It was maintained up to six hours. For, MKPE the inhibition was observed to be maximum at the end of 2 hrs and then tapered. MKEA, FLEA and FLCF showed minimum response that was constant throughout and insignificant. MKPE was slightly significant (55.1 %, $p<0.05$) which was more effective in first phase [12, 13, 14].

Histamine and Serotonin induced paw edema: *Murraya koenigii* roots: The MKCF fraction (60%, $p<0.01$) shows greater inhibition of histamine induced edema as compared to MKPE and MKEA . MKEA shows slightly significant inhibition of Histamine induced edema (35.66%, $p<0.05$) and even for serotonin induced edema (31.32, $p<0.05$). But MKPE and MKEA did not show any significant response. The alkaloidal fraction (MKAF-1) showed significant ($p<0.001$) inhibition as compared with standard drug. For both, the response is dose dependent. *Ficus lacor* aerial roots: FLET fraction from *Ficus lacor* shows greatest dose dependent inhibition of histamine (74.01%, $p<0.001$) and serotonin induced edema (68.01%, $p<0.001$) as compared to FLPE[Figures1-7].

Conclusion

From the result we conclude that The present study is first reported study for aerial roots of *Ficus Lacor* and the roots of *Murraya koenigii* and demonstrates by pharmacognostical, phytochemical and in vivo model that various factions can significantly repress the development of inflammation and arthritis, which is evident from its effect on inhibition of inflammation, decreasing the arthritic scores and suppressing the articular cartilage damage [15, 16, 17]. The mechanisms involved can be partly explained by its decreasing serum level of some important inflammatory cytokines including IL-1 and TNF-*α*. Furthermore, our investigation provides some evidences that MKPE, MKCF, MKAF- 1, FLPE, FLET and FLTP-1 are the important fractions significantly ($p< 0.05$-0.001) responsible for maintaining body weight, body organ weight, Hb, RBCs count, WBCs count, ESR, maintaining lysosomal enzyme level and producing antioxidant activity[18-22]. Therapeutic effect of *Murraya koenigii* roots and *Ficus lacor* aerial roots on inflammation and arthritis is reported.

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